NITROGEN BIOGEOCHEMISTRY AND ANCIENT OCEANIC ANOXIA

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by
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This study is an exploration of the links between nitrogen biogeochemistry and ancient oceanic anoxia. The goal of this dissertation is to answer the question: Is enhanced N₂-fixation a necessary response to widespread oceanic anoxia? Understanding the N-cycle is important because N is one of the primary nutrients limiting carbon fixation on Earth. Over geologic time scales, N availability, along with that of P and Fe, impacts the regulation of atmospheric CO₂ and climate through the limitation of carbon fixation by photoautotrophs in the oceans and on land. This work focuses on understanding the geologic record of the nitrogen cycle during episodes of ancient oceanic oxygen deprivation during the mid-Cretaceous and Neoproterozoic, and the processes controlling the preservation of N-cycle proxies in Holocene surface sediments of the Peru Margin. Under anoxic conditions, nutrient N is lost from the ocean through microbial metabolic processes but P is more efficiently recycled (Ingall and Janke, 1993). It could be envisioned that intervals of more widespread marine anoxia would significantly impact the balance of the marine nutrient cycles, affecting biological productivity. As geoscientists, we provide a unique perspective that can help answer some of the most important questions regarding the evolution of the N-cycle through time, the biological evolution of the earth, and the potential impacts of natural and anthropogenic climate change.

To assess the state of the ancient nitrogen cycle I have focused on the isolation and N-isotopic analysis of chlorophyll derivatives (e.g. porphyrins and chlorins), and bulk organic extracts. Utilization of porphyrins and chlorins for compound-specific
nitrogen isotope analysis requires an in-depth analysis of the processes that control their transformation and preservation over geologic time and in modern environments. A significant proportion of this work focuses on the abundances and distribution of porphyrins and chlorins in addition to N-isotopic analysis.

In this study, initial investigation focused on the preserved chlorophyll derivatives of the Cretaceous strata recovered from the Demerara Rise. This work yielded unexpected discoveries of high abundances of bicycloalkanoporphyrins (BiCAPs), present as free bases (metal free) and Zn and VO\(^{2+}\) complexes. The occurrence of Zn bicycloalkanoporphyrins represents the first occurrence of primary Zn porphyrins found in the geologic record. Structural confirmation of the chlorin mesochlorophyllone in the Demerara Rise black shales represents the oldest such occurrence in the geologic record by over 70 million years; its presence suggests that the abundant bicycloalkanoporphyrins in the Demerara Rise sediments are derived from chlorophyll \(a\), the only possible precursor for mesochlorophyllone.

The stratigraphic distribution of BiCAPS is controlled, foremost, by metal availability in the water column and sediments rather than early diagenesis Eh/pH conditions, or post depositional thermal maturity. Titration of the local water-column metal reservoir by sulfide during Oceanic Anoxic Event II (OAE II) resulted in high concentrations of FB BiCAPs and very low concentrations of metallo-BiCAPs. The highest total concentrations of porphyrins are found where metal concentrations are highest, suggesting that porphyrin preservation is enhanced by the increased stability that results from formation of metal complexes. Paradoxically, the total concentration of
porphyrins is lowest during the heart of OAE II, in an interval of higher TOC where enhanced organic matter preservation would be expected; this may be the result of decreased preservation of tetrapyrroles in the absence of the stabilizing effect of metals.

The nitrogen isotopic composition of BiCAPs confirms that the $\delta^{15}N$ of dissolved inorganic nitrogen becomes $^{15}N$-depleted probably in response to expanded nitrogen fixation during Oceanic Anoxic Event II. These data support a strong spatial and temporal link between nitrogen fixation and loss of nutrient nitrogen via suboxic metabolisms. I have also found that the $\delta^{15}N$ values of the three porphyrins are systematically different despite a common chlorophyll source; the origin of this difference is related to nitrogen isotopic effects associated with the formation of metal complexes. These results demonstrate that direct reconstruction of primary phototroph biomass from porphyrins can be misleading without a full assessment of the $\delta^{15}N$ of the range of structures present in ancient sediments.

Analysis of the $\delta^{15}N$ record of bulk sediments and co-occurring chlorins from Peru Margin surface sediments demonstrates that downslope transport and degradation of organic matter results in an isotopic depletion of bulk sedimentary nitrogen. Despite an order of magnitude decrease in the sedimentary concentration of chlorins downslope, their $\delta^{15}N$ values remain constant, demonstrating that chlorin degradation causes no significant nitrogen isotopic effects. These data suggest that studies that utilize bulk $\delta^{15}N$ for paleoceanographic studies in dynamic environments need to account for possible diagenetic effects even in low oxygen settings.
The factors controlling carbon burial in the Neoproterozoic are illustrated by the range of processes associated with the deposition of the Kwagunt Formation sediments. Shallow epicratonic rift basins associated with the break-up of Rodinia may have been significant depocenters for burial of organic carbon and aiding in the drawdown of CO$_2$ prior to the Snowball Earth glaciations. Microbial mat communities played an integral role in this process by providing efficient burial of carbon in shallow environments. The $\delta^{15}$N record does not confirm the presence of a euxinic deep ocean during the mid-Neoproterozoic but it suggests that the range of nutrient regimes inferred by $\delta^{15}$N record can be put into the context of modern of modern processes.
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Chapter 1: Introduction

1-1 Why Nitrogen?

Nitrogen is one of the primary nutrients limiting carbon fixation on Earth. The impact of N limitation on biological productivity is clearly seen in the distribution of photosynthetic organisms in the surface ocean exemplified density of chlorophyll \( a \) in surface waters as seen by satellites (Figure 1-1). High latitudes, equatorial regions, and upwelling zones off the West Coasts of the Americas, Africa and in the Arabian Sea are highly productive. The high biological productivity is due in large part to the high flux of N, as nitrate, to surface waters. By contrast, the central gyre regions of the major ocean basins have comparatively low chlorophyll density (Figure 1-1); here, of low fluxes of nitrate and phosphate to the photic-zone limit photosynthetic productivity.

Over geologic time scales, N availability, along with that of P and Fe, impacts the regulation of atmospheric CO\(_2\) and climate through the limitation of carbon fixation by photoautotrophs in the oceans and on land (Tyrell, 1998; Falkowski, 1997). This work focuses on understanding the geologic record of the nitrogen cycle during episodes of oceanic oxygen deprivation (anoxia, defined as the lack of molecular oxygen). Under anoxic conditions, nutrient N species (“fixed” N: primarily nitrate and ammonium) are lost from the ocean through microbial metabolic processes (Brandes et al., 2007; Galbraith et al., 2008) but P is more efficiently recycled (Ingall and Janke, 1993). It could be envisioned that intervals of more widespread marine anoxia would significantly impact the balance of the marine nutrient cycles, affecting biological productivity. As geoscientists, we provide a unique perspective that can help answer some of the most important questions regarding the evolution of the nitrogen cycle through time, the biological evolution of the earth, and potential impacts of natural and anthropogenic climate change.

1-2 The Marine N-Cycle

The dissolved nutrient distribution of the modern ocean is captured by the concept of the Redfield Ratio (16N:1P) (Redfield, 1936). It is, in general, the average stoichiometry of dissolved N and P in the water column and is interpreted to reflect the metabolic requirements of plankton communities. Variations in the Redfield Ratio in a pre-industrial ocean would have arisen largely from the loss of N, ultimately as N\(_2\) or
N$_2$O as products of microbial metabolic processes that reduce nitrate (denitrification), and the oxidize ammonium using nitrite (anaerobic ammonium oxidation, referred to as anammox) (Figure 1-2) (Brandes et al., 2004 and references therein). These two processes constitute the most important sinks for biologically available N in the marine environment (e.g. Gruber and Sarmiento, 1997; Kuypers et al., 2005) and occur exclusively under anoxic conditions in sediments and the water column. (Figure 1-2). Denitrification and anammox act to alter the Redfield N:P balance by reducing the oceanic inventory of N resulting in Redfield Ratios that are lower than 16. In a Redfield world, phytoplankton utilize the available, dissolved N and P at a ratio of 16:1. In situations where the N:P is below Redfield, as is the case in many of the oligotrophic regions of the ocean (e.g. Karl et al., 2002), N will be consumed before P resulting in N-limitation.

It should be noted that there are large variations in the modern marine N:P ratio for phytoplankton biomass and dissolved N and P. N:P is highly dependent on a range of geologic and biological factors including basin size, redox state and the dominant phytoplankton species (Quan and Falkowski, 2008). For example, not all phytoplankton have Redfield stoichiometries (Zohary et al., 2005; Sanudo-Wilhelmy et al., 2004). Additionally, N loss is not the only control of the N:P ratio; partitioning of P onto mineral surfaces (Ingall and Jahnke, 1993), addition of P from riverine sources, dust (Kump et al., 2000), or regeneration of mineral and organic P under anoxic conditions (cf. Van Cappellan and Ingall, 1994) can also alter the N:P balance. For example, low N:P ratios are observed in The Black Sea. Euxinia (the presence of free sulfide under anoxic conditions) in deep waters foster denitrification and efficient recycling of P from organic and mineral phases (Fuchsman et al., 2008). As we have come to learn, Redfield Ratios are a simplification, but a cursory assessment of the processes governing N-cycling in the modern ocean benefit from a ‘Redfieldian’ perspective.

In the situations where N is limiting or absent but P and Fe are available, organisms capable of N$_2$-fixation (diazotrophy), primarily cyanobacteria, will produce ammonium from atmospheric N$_2$, meeting their metabolic needs. However, N$_2$-fixation comes at great energetic cost; 16 moles of ATP are required per mole of NH$_4^+$. Additionally, nitrogenase, the enzyme used for the reduction of N$_2$ is inactivated by the
presence of O₂, requiring either dedicated cells for N₂-fixation (heterocysts) or diel variation of photosynthesis and nitrogenase production (Herrero et al., 2001). Diazotrophy is most commonly observed in the low-nutrient, low-productivity mid-latitudes where dissolved inorganic nitrogen (DIN) is absent, or in extremely low concentration (Mulholland et al., 1999). In fact, the presence of NH₄⁺ inhibits expression of the genes that code for nitrogenase production (Herrero et al., 2001) limiting N₂-fixation to low-N regions. The trace metal requirements for nitrogenase are substantial, 1 mole of nitrogenase contains 18 moles of Fe, which is typically in very low concentration where N₂-fixation is most common (Karl et al., 2002). Despite the energetic costs and comparatively narrow geochemical niche that diazotrophs inhabit, the supply of biologically available N they produce is the ultimate source of N for primary production in the ocean. Other classes of phytoplankton, such as calcareous nannoplankton and diatoms, lack the ability to reduce N₂ and subsist on the nutrient N (nitrate, nitrite, ammonium, amino acids) that was ultimately produced by diazotrophic organisms.

N₂-fixation is a process that has proven extremely difficult to quantify globally, but new estimates on the basis of P uptake in the surface ocean suggest that rates of N₂-fixation (250 Tg*yr⁻¹) are on the order of denitrification (350 Tg*yr⁻¹) (Deutsch et al., 2007). Additionally, the highest rates of N₂-fixation are found adjacent to major oxygen minimum zones where reductive N-loss rates are high. These results are important because they suggest that the distribution of N₂-fixation is linked spatially and temporally to denitrification (Deutsch et al., 2007), and that variation in the rates of denitrification through time will be compensated by increases or decreases in N₂-fixation (Deutsch et al., 2004; Ren et al., 2009).

1-3 The N-cycle and N-isotopes

Denitrification and N₂-fixation are the two most important processes controlling the size and isotopic composition of the DIN reservoir, and both processes have distinct δ¹⁵N signatures (e.g. Deutsch et al., 2004; Sigman et al, 2009; Carpenter et al., 1997 and references therein). This allows one to estimate the relative importance of the two processes on the basis of the δ¹⁵N of DIN. Denitrification has a strong, negative isotope fractionation (ε = -15 to -25‰) (Barford et al., 1999; Mariotti et al., 1981), leaving the remaining NO₃⁻¹⁵N-enriched (Figure 1-3). Subsediment denitrification accounts for
~80% of the global denitrification balance (Galbraith et al., 2008), but it is diffusion limited and the NO$_3^-$ is utilized completely, erasing the associated isotope effect on the overlying water column. Water column denitrification in the modern ocean rarely consumes all of the available NO$_3^-$; the isotope effect of incomplete denitrification is observed clearly in modern marine oxygen minimum zones where the NO$_3^-$ $\delta^{15}$N values are as high as +15‰ (Sigman et al., 2009). The global average NO$_3^-$ $\delta^{15}$N value (+5‰), is $^{15}$N-enriched largely due to the influence of water column denitrification (Sigman et al., 1999).

Diazotrophs utilize the atmospheric N$_2$ reservoir, which by definition is 0‰. Average diazotroph biomass is -1‰ and $^{15}$N abundance varies (-3 to +1‰) but remains near 0‰ (Carpenter et al., 1997; Karl et al., 2002). The range of $\delta^{15}$N values is associated with small fractionations during sea-air gas exchange, assimilation of N$_2$ by diazotrophs and the concentration of available Fe (Zerkle et al., 2008). Degradation of diazotroph biomass results in a return of organic N to the ocean reservoir as NH$_4^+$, which is subsequently oxidized by nitrifying bacteria to NO$_3^-$. Large N-isotope effects are associated with nitrification, however it is generally a complete conversion of NH$_4^+$ to NO$_3^-$ or NO$_2^-$, and phytoplankton in water columns with active diazotrophy typically have $\delta^{15}$N values that are near 0‰.

Recent estimates of the importance of anammox suggest that it may be the dominant source of reductive N-loss and ultimate sink for DIN species in the ocean (Kuypers et al., 2006). Estimates of the N isotopic fractionation associated with anammox are not yet understood. Despite its importance as a sink for DIN, it may not be significant for the isotope mass balance. Anammox bacteria utilize NO$_2^-$ to oxidize NH$_4^+$ and do not have the ability to reduce NO$_3^-$. Dissimilatory nitrate reduction (metabolism where N is not assimilated into biomass, but NO$_3^-$ is used to oxidize organic matter or to fix inorganic carbon) is a process that is only performed by denitrifying bacteria of which a product can be NO$_2^-$. The large fractionation associated with denitrification occurs in the conversion of NO$_3^-$ to NO$_2^-$ (Galbraith et al., 2008). The residual available NO$_2^-$ is utilized completely by anammox bacteria, thus erasing any possible isotopic effects associated with the anammox process.
Intermediate water (region of water column below mixed layer (~100 to 1000m) oxygen deficits in the modern ocean foster denitrification, clearly observed in $^{15}$N-enrichment of NO$_3^-$, Under more strongly reducing conditions, nitrate is consumed, erasing the $^{15}$N-enrichment associated with incomplete denitrification. Following NO$_3^-$ consumption chemolithotrophic bacteria utilize SO$_4^{2-}$ for organic matter oxidation, resulting in the build up of H$_2$S in deep waters. The euxinic water-column of the Black Sea has N/P ratios that are substantially lower than the Redfield Ratio (<16; Fuchsman et al., 2008) resulting from denitrification and anaerobic oxidation of ammonium coupled with the release of P from authigenic and organic phases (van Capellen and Ingall, 1994). The low N/P should make N$_2$-fixation a favorable process in the Black Sea, but widespread N$_2$-fixation has not been directly observed on a large scale in modern environments (McCarthy et al., 2007), but may have been more important in the past (Fulton, 2010). Modern euxinic and anoxic systems present a problem in that they do not yield substantial data indicating extensive diazotrophic communities. Tight coupling of N$_2$-fixation and denitrification is predicted (Deutsch et al., 2007) but not yet directly observed on a large scale in the modern ocean. It is the $\delta^{15}$N record of ancient black shales (referred to as black shales in reference to their color and finely layered structure and have greater than ~2% organic carbon by mass) lends strong supports for a spatial and temporal link between N$_2$-fixation, denitrification and euxinia. Episodic euxinia is uncommon in the modern open ocean but occurs in restricted basins such as the Black Sea or Baltic Sea. Geochemical evidence suggests that widespread, open-ocean euxinia was a more common feature during intervals of Earth’s past, such as the Mesozoic (250-65 Ma).

From an N-isotope perspective we can assess the balance between N$_2$-fixation and denitrification. The principle proxy that has been used for understanding past changes in the N-cycle are stable isotope ratios of N as preserved in whole sediments. The basis for the connection between the $\delta^{15}$N of DIN and organic matter is illustrated by sediment trap data that demonstrate correspondence between the $\delta^{15}$N of sub-euphotic zone DIN and the sinking flux of organic matter (Thunnel et al., 2004; Galbraith et al., 2008) (Figure 1-4). These observations provide a basis for using $\delta^{15}$N of organic matter preserved in sediments to interpret changes in the modern and ancient N-cycle.
1-4 Reconstructing the Ancient N-Cycle

Many studies over the past 25 years have utilized bulk sedimentary $\delta^{15}$N as a N-cycle proxy (e.g. Rau et al., 1987; Altabet et al., 1995; Ganeshram et al., 2002; Haug et al., 1998; Sachs et al., 1999; Kuypers et al., 2004). One of the most important questions that many of these studies have worked to address is whether standard, bulk sediment $\delta^{15}$N techniques accurately record primary processes (e.g. Sachs et al., 1999; Altabet et al., 1999; Junium and Arthur, 2007). Nitrogen is present in many phases in sediments, not just organic matter. Assuming that the only source for this N is from surface water primary production is not always correct. Terrestrial organic matter and clay-bound nitrogen derived from soils may be a significant fraction of sedimentary nitrogen in coastal sequences (e.g. Freudenthal et al., 2001). Biomass supplied from organisms other than oxygenic photoautotrophs is also a concern. For example, molecular biomarker evidence from some ancient sequences indicates significant populations of phototrophic sulfide oxidizing bacteria or archaea (Kuypers et al., 2002; Kuypers et al., 2001). Chemocline bacteria and archaea exist in different nutrient regimes than oxygenic photoautotrophs; these communities have the potential to greatly alter the nitrogen isotopic composition of bulk sedimentary organic matter if their biomass is significantly $^{15}$N-enriched or depleted (e.g. Valinsky and Fogel, 1999).

Internal cycling of N from primary producers can also alter $\delta^{15}$N signals. Organic matter from primary production can undergo a range of diagenetic processes mediated by bacteria in the water column and sediments (diagenesis encompasses the chemical changes that occur from origin of organic material in the photic zone, through sinking, early burial, and over geologic time). $C_{org}/N_{total}$ ratios in ancient black shales are significantly higher (as high as 60) than primary biomass (4-10). There is also an apparent correlation of C/N with $\delta^{15}$N values in black shales (Figure 1-5) that suggests the possibility of diagenetic alteration (Junium and Arthur, 2007). When one considers the many possibilities, $\delta^{15}$N values can appear ambiguous in ancient sequences because of the unconstrained nature of bulk sedimentary nitrogen.

Recognizing the limitation of bulk $\delta^{15}$N has led to method development aimed at isolating diagenetically resistant N-fractions attributed to a specific source. The presence of nitrogenous chlorophyll derivatives (chlorins, porphyrins, maleimides) in ancient
sequences is well known (Treibs, 1936; Gibbison et al., 1995; Keely, 2006) and have been the target of compound specific δ^{15}N analyses (Chicarelli et al., 1987; Sachs et al., 1999; Kashiyama et al., 2008). Additionally, N isolated from biogenic fractions such as diatom associated organic matter (Sigman et al., 1995) or foraminifera (Ren et al., 2009) have been very useful for isolating N-cycle signals in Pleistocene Age sediments. This type of approach has great appeal for deeper time studies.

Ancient organic matter-rich sediments from open marine settings are unusual in that their nitrogen isotopic compositions are almost exclusively ^{15}N-depleted (Figure 1-5). This suggests that DIN was supplied by diazotrophs, and that incomplete denitrification did not have a significant impact on the δ^{15}N of DIN. The link that ties many of these ancient black shales is presence of more widespread water-column euxinia, (the presence of free sulfide in the water column produced by sulfate reducing bacteria) (e.g. Kuypers et al., 2002). As the quantity of δ^{15}N data through time increases, there appears to be a consistent relationship in earth history between euxinic basins, widespread black shale deposition, and δ^{15}N values below 0‰ (e.g. Junium and Arthur, 2007; Kuypers et al., 2002, 2004; Jenkyns et al., 2001; Sachs et al., 1999; Beaumont et al., 1999; Papineau et al., 2005; Meyer and Kump, 2008). These data suggest that nitrogen cycling and dominant primary producer communities may have been markedly different during intervals of widespread anoxia.

Bulk δ^{15}N analyses of sediments are the basis for these hypotheses. The ambiguities of bulk analyses and the potential for diagenetic alteration demonstrates that N-isotope studies in ancient sediment require a more rigorous approach. A substantial part of this work has focused on developing techniques to isolate primary N-cycle signals through analysis of N-containing chlorophyll derivatives as well as assessing the processes that control the preservation of δ^{15}N signals in sedimentary N. The goal of this thesis is to answer the question: Is enhanced nitrogen fixation a necessary response to widespread oceanic anoxia? To help answer this question I have focused on an interval of global black shale deposition during the mid-Cretaceous, Cenomanian-Turonian, Oceanic Anoxic Event II on which this hypothesis was initially developed. I then extended my work to the mid-Neoproterozoic (~750Ma), an interval of time where deep-water anoxia may have been much more widespread than in the modern ocean (e.g.
Canfield et al., 2008). In an effort to understand the processes that control the nitrogen isotopic composition of bulk sediments also I examined organic matter rich sediments from the modern Peru Margin. This work is designed to provide a framework that is a guide for future studies that are attempting to perform \( \delta^{15}N \) analyses in the troubled waters that are ancient sediments.

**1-5 Organization of the Thesis**

This work was written over the course of my doctoral study at Penn State from the Fall of 2004 to present under the supervision of Dr. Michael A. Arthur. Not all studies performed during my time in the Department of Geosciences are included in this document. The thesis comprises 3 main topics and 7 chapters of original research. All chapters have been written as publishable units.

*Chapters 2-5:* The Cenomanian-Turonian record from ODP Leg 207, Site 1261 at Demerara Rise has provided the bulk of the work presented in this thesis. Results from investigations of the controls on chlorophyll biomarker distributions and compound-specific nitrogen isotope records have resulted in two unexpected manuscripts, one of which was published in 2008 in *Organic Geochemistry*, with co-authors Deborah Mawson, Michael A. Arthur, Katherine H. Freeman, and Brendan J. Keely. The first two chapters cover the ‘discovery’ and distribution of chlorophyll derivatives. Chapter 2 focuses on the unexpected occurrence of high abundances of Zn bicycloalkanoporphyrins in the Demerara Rise black shales. Chapter 3 discusses the oldest occurrence of chlorins in the geologic record, found in Demerara Rise sediments and the implications their presence has for the sources and formation of bicycloalkanoporphyrins in sediments. Chapter 4 discusses the factors that control the stratigraphic distribution of bicycloalkanoporphyrins and their carbon and nitrogen isotopic composition. Chapter 5 considers global \( \delta^{15}N \) record through Oceanic Anoxic Event II combining bulk sediment records with the knowledge gained from the compound specific \( \delta^{15}N \) record. The culmination of this work demonstrates that the expansion of anoxia at the Cenomanian-Turonian boundary resulted in a global expansion of marine nitrogen fixation.

*Chapter 6* is concerned with the factors that control the \( \delta^{15}N \) of bulk sediments in the modern environment and the implications for ancient studies. This study focuses on the Peru Margin, a locality where organic matter is rather poorly preserved despite low
oxygen conditions. Chlorophyll derivative $\delta^{15}$N values show no change from the inner shelf through the upper slope but bulk $\delta^{15}$N values decrease over that interval; transport of organic matter down-slope degrades organic N phases altering bulk $\delta^{15}$N signatures.

Chapter 7 examines the controls on black shale deposition in the mid-Neoproterozoic Kwagunt Formation of the Chuar Group prior to the first Snowball Earth interval. For the vast majority of the Kwagunt Formation deposition, organic matter was associated with benthic microbial mat communities. Two pronounced intervals of organic matter-rich deposition are related to transgression and deepening of the Chuar Basin which fosters elevated productivity.

Chapter 8 provides a discussion of the larger implications of this work with respect to nitrogen analyses in sediments and is aimed at guiding those interested in performing similar $\delta^{15}$N studies. Directions for future research are also discussed.

1-6 Anticipated Publications Arising from this Work

Chapter 2: was published in 2008 in *Organic Geochemistry* with co-authors Deborah Mawson, Michael A. Arthur, Katherine H. Freeman, and Brendan J. Keely

Chapter 3: Chlorins in mid-Cretaceous black shales of the Demerara Rise: the oldest known occurrence, will be submitted to *Organic Geochemistry* with co-authors Michael A. Arthur, Katherine H. Freeman, and Brendan J. Keely.

Chapter 4: Controls on the stratigraphic distribution and nitrogen isotopic composition of porphyrins from OAE II of Demerara Rise, will be submitted to *Geochimica et Cosmochimica Acta* with co-authors Michael A. Arthur, Katherine H. Freeman, and Brendan J. Keely.

Chapter 5: Global expansion of $N_2$-fixation supported primary productivity during mid-Cretaceous Oceanic Anoxic Event II will be submitted to *Nature Geoscience* with co-authors Michael A. Arthur and Katherine H. Freeman.

Chapter 6: Controls on bulk and compound specific $\delta^{15}$N and pigment distributions in surface sediments of the Peru Margin, will be submitted to *Paleoceanography*, with co-authors Michael A. Arthur and Katherine H. Freeman.

Chapter 7: Biogeochemical controls on black shale deposition in the Neoproterozoic Kwagunt Formation, Chuar Group, Grand Canyon, USA, will be
submitted to *Precambrian Research*, with co-authors Michael A. Arthur and Kevin M. Bohacs.

1-7 References


Jenkyns, H. C., et al. (2001), Nitrogen isotope evidence for water mass denitrification during the early Toarcian (Jurassic) oceanic anoxic event, *Paleoceanography, 16*, 593-603.


Kump, L. R., et al. (2005), Massive release of hydrogen sulfide to the surface ocean and atmosphere during intervals of oceanic anoxia, *Geology*, 33, 397-400.


Figure 1-1. The time integrated chlorophyll $a$ concentration as seen from the NASA SeaWIFS Ocean Color satellite. High concentrations are marked by warmer colors (greens, yellows and reds). Low concentrations are observed in the blues hues of central gyres in the mid-latitudes of the Atlantic and Pacific. Image is courtesy of the NASA Ocean Color program.
Figure 1-2. The major, microbially-mediated transformations in the N-cycle and the corresponding oxidation states of the major and intermediate species. Chemical equations are for the major processes that most concern this work. This figure is adapted from a figure provided by Don Canfield and Bo Thamdrup for the Agouron Institute N meeting.
Figure 1-3. Description of the fractionations associated with major N-cycle transformations relative to the fractional nitrate concentration. Arrows describe the trajectory of the concentration and nitrogen isotopic composition of nitrate reservoirs during the dominant N-cycle processes. Water column denitrification and nitrate uptake have strong fractionations, however, nitrate uptake is typically complete, thus the fractionation is not expressed. This figure is adapted from Galbraith et al., (2008).
Figure 1-4. Correspondence between sub-euphotic zone nitrate $\delta^{15}\text{N}$ and bulk $\delta^{15}\text{N}$ of underlying surface sediments. The strong correlation between both measurements suggests that bulk $\delta^{15}\text{N}$ values reflect primary processes and accurately reflect the $\delta^{15}\text{N}$ of DIN. Adapted from Galbraith et al., 2008.
Figure 1-5. A composite of $\delta^{15}\text{N}$ vs. C/N of bulk marine OM in modern and ancient black shales. Cretaceous data are from ODP Leg 207 Black Shales; Sapropel units S5, ODP site 969 and T1 ODP Site 974 (Milder et al., 1999); Framvaren Fjord (Velinsky and Fogel, 1999); Holocene Black Sea and Green lake sediments (Fulton et al., in preparation); Baltic Sea data (Bianchi et al., 2000); Devonian (Calvert et al., 1996). The Cretaceous, Devonian and Mediterranean black shale $\delta^{15}\text{N}$ show a negative correlation with C/N, suggesting that processes controlling the loss of N relative to C may be impacting $\delta^{15}\text{N}$ (for a full discussion see Junium and Arthur, 2007).
Chapter 2: Unexpected occurrence and significance of zinc alkyl porphyrins in Cenomanian-Turonian black shales of the Demerara Rise


Abstract
Alkylporphyrins in acetone extracts of Cenomanian-Turonian black shales from the Demerara Rise have been analyzed by reverse phase high performance liquid chromatography and liquid chromatography-tandem mass spectrometry. The major alkylporphyrins comprise mixtures of free-base and metalloporphyrins of the C_{33} bicycloalkanoporphyrin (BiCAP) structural type. Typically, the most abundant porphyrins in the sediments are vanadyl complexes, occurring with varying relative amounts of free-base porphyrins, nickel complexes and, unexpectedly, zinc complexed C_{33} alkylporphyrins. The geochemical conditions that favor production of vanadyl, zinc/nickel and free-base porphyrins are very different. Although the geochemical conditions that controlled metal availability were highly variable over the sampling interval, the dominant precursor chlorophyll(s) appears to have remained constant giving rise to limited structural variation with dominance of BiCAP structures.

2-1 Introduction
Geoporphyrins (Figure 1) are biomarkers that result from the transformation of tetrapyrroles including chlorophylls, bacteriochlorophylls and hemes. Cycloalkanoporphyrins (CAPs), are chlorophyll derivatives that originate from photosynthetic organisms and represent the largest fraction of tetrapyrroles within sediments. The presence of a five-membered exocyclic ring (Figure 1, I) between carbons 13 and 15 confirms a chlorophyll source for such porphyrins (Fookes, 1983). Porphyrin data can contribute greatly to paleoenvironmental studies, providing clues to the precursor photosynthetic organisms and, in some cases, providing unambiguous identification of the biological sources. For example, porphyrins derived from bacteriochlorophyll d are produced only by photoautotrophic green sulfur bacteria and possess side-chain alkylation patterns that are diagnostic of their source (Ocampo et al., 1985, 1992; Eckardt et al., 1991; Keely et al., 1993; Gibbison et al., 1995; Rosell-Melé et al., 1999; Mawson et al., 2004). As such, porphyrin analyses can aid the development and testing of hypotheses about ancient photosynthetic communities and water column chemistry.
The reactions that convert chlorophylls to chlorins and geoporphyrins are initiated in the water column and sediments during early diagenesis and are dependent on a range of variables including, but not limited to, water column redox state, biological activity, sedimentary geochemical conditions and time (Keely, 2006). In more recent sediments it is common to find the more functionalized chlorins (Keely et al., 1990; Harris et al., 1995; Airs et al., 2000), which are diagenetic intermediates between chlorophylls and porphyrins. Porphyrins are most often found complexed with metals (typically Ni and VO) but can also be found as free-bases (metal free). Here, we present data documenting the major porphyrins identified in the Cenomanian-Turonian black shales of the Demerara Rise recovered during ODP Leg 207 and the geochemical implications of their presence.

2-2 Geologic Setting

During Cenomanian-Turonian time the Demerara Rise was located in the circum-equatorial region of the proto-Atlantic. It is a gently northward-sloping portion of continental crust that presently ranges from 800 to 3000 m water depth. By the mid-Cenomanian (95 Ma) the northern edge of the rise was ~2000 m (Arthur and Natland, 1979). Paleodepths of the ODP Leg 207 sites are considered to be representative of continental slope depths (~1000 m) although exact paleodepths are not well constrained (Erbacher et al., 2004). Enhanced productivity is believed to have existed during the Cenomanian-Turonian (93.5 Ma) OAE II in the circum-equatorial region that included the Demerara Rise, based on elevated organic matter accumulation rates (e.g. Kuhnt et al., 1990). Notably, unlike regions elsewhere during this time period, the mid-Cenomanian to basal Campanian record of black shales at Demerara Rise demonstrates that conditions conducive to the deposition of organic matter-rich strata and preservation of labile organic matter were not limited to the OAE II interval. Biomarker evidence for the presence of green sulfur bacteria (Sinninghe-Damsté and Köster, 1998; Kuypers et al., 2002; Pancost et al., 2004) confirms at least episodic presence of sulfide within the photic zone during OAE II. High concentrations of 2-methylhopanes (Kuypers et al., 2004) and low nitrogen isotope values (Kuypers et al., 2004; Junium and Arthur, 2007) also suggest a greater proportion of organic matter derived from nitrogen-fixing cyanobacteria over much of the Atlantic basin.
2-3 Experimental

2-3-1 Materials

Sediments recovered from the mid-Cretaceous interval of ODP Leg 207 are very finely laminated organic matter and biogenic carbonate-rich black shales (see Erbacher et al., 2004 for detailed sedimentology). A suite of 12, 2.5 cm thick samples (1 sample per 1.5 meters) through OAE II were targeted for pigment analyses. The samples and data presented are representative of the pigment distributions observed from the 12 samples. Sediments were frozen shortly after sampling, freeze-dried and powdered prior to solvent extraction. The fragile nature of the sediments and high extract yields allowed for simple solvent extraction by sonication in HPLC-grade acetone of relatively small samples sizes (5-6 g). Centrifuged extracts were filtered through solvent-washed cotton wool. This process was repeated until extracts were clear; the resultant extract was evaporated to dryness.

2-3-2 HPLC and LC-MS

Reverse phase HPLC analysis of total acetone extracts was conducted at York University, Department of Chemistry using a Waters system (Milford, MAUSA) comprising of a 717 autosampler, 600 MS system controller and 966 photodiode array (PDA) detector. The system was controlled, and data recorded and processed using Waters Millenium 2010 software. All solvents were degassed by sparging with helium or by vacuum degassing. Separations were achieved using two Waters Spherisorb ODS2 3 μm columns (4.6 x 150 mm i.d.) in series. Aliquots of acetone extracts were analysed using a quaternary gradient elution program comprising acetonitrile, methanol, water and ethyl acetate over 85 min with a flow rate of 0.7 ml min⁻¹ (Airs et al., 2001). Determination of complexing metal was achieved by examination of online UV/vis-PDA spectra, which are diagnostic of metal type.

LC–MSⁿ analysis was performed using a Finnigan LCQ system comprising a Thermo Separations AS3000 autosampler, P4000 gradient pump, UV2000 UV/Vis detector and a Finnigan MAT LCQ ion trap mass spectrometer equipped with an atmospheric pressure chemical ionisation (APCI) source. Concentrated formic acid was infused into the eluent following chromatographic separation at the rate of 7 μl min⁻¹ immediately prior to introduction into the LC-MS source to prevent metallation of free-
base porphyrins and chlorins within the source (cf Airs and Keely, 2000). The interface conditions were as follows: vaporiser 450°C; capillary 150°C; discharge current 50 μA; sheath gas flow 40 (arbitrary units); auxiliary gas flow 10 (arbitrary units), collision energy 40%. Structural determinations were based on multi-stage mass spectra and comparison to spectra of authentic standards where possible.

2-4 Results

HPLC-PDA and LC-MS analysis of acetone extracts of Cenomanian-Turonian black shales from the Demerara Rise reveal porphyrin distributions comprising mixtures of free-base and metallo porphyrins and chlorins. The on-line UV/vis spectra confirm three closely eluting peaks (Figure 1, I, II and III) as vanadyl complexes (absorbance bands: Soret 407 nm, β 533 nm, α 572 nm). Typically, the vanadyl complexes are the most abundant metalloporphyrins in the sediments examined. The full mass spectra of I, II and III are dominated by a single ion at m/z 554 (Figure 2), consistent with protonated molecules, [M+H+] of C33 VO BiCAP porphyrins. The corresponding free-base porphyrin molecular mass is 488. The multistage mass spectra (MS² to MS⁶) of I, II and III (Figure, 2), generated from collision induced dissociation (CID) of [M+H+] and subsequently from the base peak in the preceding spectrum, are very similar to those of an authentic C33 free-base BiCAP isolated from Pliocene lacustrine sediments of Willershausen, Germany (Keely et al., 1994). Observed differences in the relative abundances of the product ions in MSⁿ spectra are likely to result from increased planarity in the metalloporphyrin macrocycle compared to the free-base counterparts. Small differences exist in the relative abundances of product ions in the MSⁿ spectra of structures I-III. We speculate that these result from small differences associated with structural isomerism and stereoisomerism between the observed peaks, though the origin of these differences is beyond the scope of this work. It is possible that additional vanadyl BiCAP structures are present but are in too low abundance for identification. One nickel C33 BiCAP porphyrin (Figure 1, peak IV), present in very low abundance, was identified from its on-line UV/vis (absorbance bands: Soret 390 nm, β 512 nm, α 550 nm) and MSⁿ spectra; [M+H⁺] at m/z 545, corresponding to a Ni complex of free-base porphyrin with a molecular mass of 488.
The on-line UV/vis spectrum for Peak V (absorbance bands: Soret 407 nm, β 538 nm, α 572 nm) matches the UV/vis spectra of zinc complexed porphyrins (Buchler and Puppe, 1970). The spectra for peaks VI and VII show similar relative intensities of the α and β bands but are red shifted by approximately 20 nm (absorbance bands: Soret 422 nm, β 557 nm, α 597 nm). The full mass spectra of peaks V, VI and VII all show clusters of ions in which the major species occur at m/z 551 (V) and 549 (VI and VII) (Figure 3). The ion intensities and profiles within the clusters correspond to Zn complexed counterparts of free-base porphyrins with a molecular mass 488 for peak V and 486 for peaks VI and VII. Thus, peaks V, VI, VII (Figure 1) correspond to a suite of C₃₃ zinc BiCAPs. The full mass spectra of the Zn porphyrins show additional ions at m/z 489 for peak V and m/z 487 for peak VI, corresponding to the [M+H⁺] of free-base BiCAP porphyrin counterparts formed by demetallation as a result of the post-column addition of concentrated formic acid that was employed to prevent mettallation of free base porphyrins within the ion source (Mawson et al., 2008).

The MS² to MS⁴ spectra for peaks V, VI, VII (Figure 3) are very similar to the corresponding spectra of the C₃₃ VO BiCAPs and authentic C₃₃ BiCAP standards. Only small differences in the relative abundances of product ions occur in the MS⁴ spectra of peak V and peaks VI, VII. The two Da difference between peak V and peaks VI, VII is attributed to the presence of an additional double bond in the last pair of structures. The similarity in the MSⁿ spectra for peaks V, and VI and VII suggest that the mass difference is not associated with differences in peripheral alkyl substituents that are lost in the last stages of CID (Mawson et al., 2008). Thus, the double bond is most likely to be present in the seven-membered ring of the C₃₃ BiCAP structure, consistent with the structure of a sulfur-linked porphyrin proposed by Shaeffer et al., (1994). The red shift in the on-line UV/vis spectra of VI and VII is consistent with the presence of a double bond in conjugation with the macrocycle (cf. Spooner et al., 1994). The retention time difference between VI and VII suggests only a small difference in structure, possibly in the position of the double bond.

Multiple lines of evidence suggest that the Zn porphyrins were not formed during extraction. The relative abundances of Zn porphyrins vary stratigraphically, including samples where all metalloporphyrins are in very low abundance (Junium, unpublished
data). None of the late-eluting free-base non-BiCAP porphyrins (the series small unlabelled peaks from 40-70 minutes in Figure 1) have detectable Zn-complexed counterparts. The possibility that Zn porphyrins were formed during extraction was examined by spiking sediment with Zn acetate prior to extraction. Metallation of all free base porphyrins occurred without the structural bias that exists in unspiked samples where only BiCAP forms exist as Zn complexes. Thus, the difference in distribution between the Zn and free-base porphyrins indicates that the Zn porphyrins were not formed by metallation of the latter during extraction.

The most abundant free-base porphyrin in the Demerara Rise black shales (peak VIII, Figure 1) corresponds to a C_{33} BiCAP having a protonated molecule at m/z 489 (Figure 4). The MS^2 to MS^4 spectra (Figure 4) are very similar (Mawson et al., 2008) to the C_{33} BiCAP structure isolated from Willershausen sediment and confirmed by NMR structural studies (Keely et al., 1994). A small quantity of a free-base C_{33} BiCAP having a protonated molecule at m/z 487 (Figure 1, Peak IX) is also apparent. Similar to the m/z 549 Zn BiCAPs, the diode array absorbance bands of Peak IX are red-shifted by approximately 20 nm. This may be explained by the presence of an additional double bond within the porphyrin macrocycle similar to structures with a double bond located within the 7-membered ring proposed by Schaeffer et al., (1994) and is the most likely precursor for peaks VI and VII.

2-5 Discussion

The C_{33} BiCAP porphyrins are common in modern and ancient sediments and are often found as free-bases and metal-complexes (Chicarelli et al, 1987; Callot, 1990; Schaeffer et al., 1993; Keely et al., 1995; Ocampo et al., 1999; Sachs et al., 1999). In most sediments, the BiCAPs are secondary constituents to more common porphyrin macrocycles, such as DPEP, which lack the seven-membered ring. The formation of the BiCAP structure is proposed to be a result of the cyclization of the C-17 propionic acid substituent during early diagenesis (e.g. Chicarelli et al., 1984) possibly mediated by invertebrate grazing as indicated by its isolation from fecal pellets and benthic macroinvertebrates (e.g. Goericke et al., 2000). Significant sedimentary concentrations of BiCAP forms are most commonly found in carbonate-rich sediments characterized by inferred reducing and high alkalinity depositional environments (Shaeffer et al., 1993;
Keely et al., 1995; Mawson et al., 2008). The black shales of the Demerara rise are in concordance with this circumstantial trend, having calcium carbonate concentrations often in excess of 50 wt. %, sub mm-scale laminations (Erbacher et al., 2004) and trace metal abundances that indicate reducing sedimentary conditions (Brumsack, 2006). In spite of these congruencies, the extraordinary abundances of BiCAP structures is highly unusual and merits further examination.

The predominance of vanadyl relative to nickel as the complexing metal in the Demerara Rise black shales is typical for marine sediments (e.g. Callot and Ocampo, 2000). The relative abundances of nickel and VO porphyrins is a function of the Eh/pH conditions which govern stability ranges of vanadyl species and the activity of nickel. In marine environments where sulfide is present nickel is effectively removed by precipitation of nickel sulfides, favoring vanadyl complexation of porphyrins (cf. Lewan, 1984). In situations where metal inventories are depleted, or the Eh/pH conditions are such that metal ions are not available for complexation, preserved porphyrins will remain as free bases (e.g. Schaeffer et al., 1993). Based on a wide range of geochemical proxies reducing sedimentary geochemical conditions are inferred during deposition of the black shales of the Demerara Rise (Brumsack, 2006) and within the water column during the Cenomanian-Turonian OAE II (e.g. Kuypers et al., 2002). Thus, the occurrence of vanadyl complexes as the most abundant CAPs together with high concentrations of free base CAPs is not unexpected.

The occurrence of high abundances of Zn CAPs has not been previously described for any marine sedimentary sequence. Zinc is a chalcophile element, similar to nickel. Hence, its presence and relatively high abundance is unexpected. Metal enrichments in Cenomanian-Turonian black shale sequences have been known for some time, and similar enrichments have been described for the Demerara Rise (e.g. Brumsack, 2006). The high metal concentrations can, in part, be attributed to episodic euxinic conditions and high organic matter accumulation rates that should reduce zinc activity and limit its incorporation into porphyrins. It is, however, apparent that the geochemical conditions that allowed for the formation of Zn complexes must have resulted in appreciable availability of Zn despite the episodic sulfide-rich conditions.
The distribution of the different structures and complexing metals observed from the Demerara Rise black shales suggests two possibilities for their occurrence: 1. different sources produced the different pools of metalloporphyrins observed; 2. they reflect different geochemical conditions that were variable spatially and temporally. Clearly, these possibilities are not mutually exclusive, particularly since the sampling interval employed here (ca. 1-2.5 cm) integrates significant periods of time over which large changes in geochemical conditions may have occurred. Given that the geochemical conditions which favor formation of vanadyl, zinc and free base porphyrins are very different, it is evident that there were significant changes in local paleoenvironments over the sampling interval. It is, therefore, somewhat surprising that the dominant precursor chlorophyll pool appears to have remained constant, giving rise to limited structural variation with dominance of BiCAP structures. These observations are important for evaluating the timing and nature of environmental changes that occurred during deposition of the Cenomanian-Turonian sediments of the Demerara Rise.

2-6 Bibliography


2-7 Figures

Figure 2-1. UV/Vis maximum absorbance (max plot) chromatograms of total acetone extracts with proposed porphyrin structures. The distribution of peaks is representative of the samples analyzed. Samples are Cenomanian-Turonian black shales from ODP Site 1261a on the Demerara Rise.
Figure 2-2: Representative full MS to MS$^4$ spectra for the C33 VO BiCAP porphyrin, I-III.
Figure 2-3: Representative full MS to MS⁴ spectra for C33 m/z 551 and m/z 549 Zn BiCAP porphyrins, V-VII.
Figure 2-4: Representative full MS to MS$^4$ spectra for C33 free-base BiCAP porphyrin, VIII.
Chapter 3: Chlorins in mid-Cretaceous black shales of the Demerara Rise: the oldest known occurrence.

Abstract

Liquid chromatography, multi-stage mass spectrometry (LC-MS^n) of acetone extracts confirms the presence of the mesochlorophyllone in the mid-Cretaceous black shales of Demerara Rise. This finding represents the oldest molecular confirmation of primary chlorins in the geologic record and is evidence for a chlorophyll a source for bicycloalkano porphyrins in the Demerara Rise black shales.

3-1 Introduction

The utilization of sedimentary porphyrins as chlorophyll biomarkers and for compound-specific stable isotopic analyses in paleoenvironmental studies have long been an important part of the organic geochemical toolbox (e.g. Chicarelli et al., 1987; Sachs and Repeta, 1999) and recent advances in structure and isotopic analysis have renewed interest in pigment isotopic biogeochemistry (Mawson et al., 2004; Kashiyama et al., 2008; Polissar et al., 2009). Characterizing the conditions that control the formation of porphyrins is of great importance as we link chlorophylls to their geologic counterparts. Chlorins are the intermediates in the transformation of chlorophylls to geoporphyrins (Figure 3-1). The processes that control the chlorophyll to porphyrin transition occur via a series of defunctionalization reactions that are initiated in the water column and continue after burial (e.g. Keely, 2006; Callot and Ocampo, 2000). Characterization of chlorin structures in sediments serves in identifying specific precursor chlorophylls, an integral step in understanding how porphyrins form in the sedimentary environment.

In modern environments and recent sediments chlorins are common constituents of organic extracts (Keely et al., 1990; Harris et al., 1995; Airs et al., 2001). However, chlorin occurrence in pre-Quaternary sediments is rare (Mawson and Keely, 2008; Baker and Louda, 1986), and tetrapyrrole moieties are typically dominated by cyclo-alkano porphyrins. Here we present molecular data confirming the presence of chlorins in 95 Ma Cenomanian-Turonian black shales of the Demerara Rise recovered during ODP Leg 207. This finding represents the oldest confirmed chlorin structures preserved in the sedimentary record and has significant implications concerning the processes that control conversion of chlorophylls to their geologic counterparts. The presence of mesochlorophyllone also provides strong evidence of the ecological dominance of
chlorophyll \(\alpha\)-producing organisms at Demerara Rise.

3-2 Experimental

3-2-1 Materials

Sediments were sub-sampled from refrigerated cores 4 months after core retrieval during Ocean Drilling Program Leg 207. Samples were freeze-dried and powdered prior to solvent extraction. The labile nature of organic matter and high extract yields allowed for simple solvent extraction by sonication in HPLC-grade acetone. Centrifuged extracts were filtered through solvent-washed cotton wool. This process was repeated until the solvent remained clear; the resulting extracts were rotary evaporated to dryness. Analysis of black shale samples focused on Site 1261 encompassing the time interval from mid-Cenomanian to the upper Santonian (97-83.5 Ma).

3-2-2 HPLC and LC-MS

Reverse phase HPLC of total acetone extracts was conducted using a Waters system (Milford, MA USA) comprising a 717 autosampler, 600 MS system controller and 966 photodiode array (PDA) detector. The system was controlled, and data recorded and processed using Waters Millenium 2010 software. All solvents were degassed by helium sparging or vacuum degassing. Separations were achieved using two identical Spherisorb ODS2 3\(\mu\)m columns (4.6 x 150 mm) linked in series. Aliquots of acetone extracts were analyzed using a quaternary gradient elution program comprised of acetonitrile, methanol, water and ethyl acetate over 85 min with a flow rate of 0.7 ml min\(^{-1}\) (Method B of Airs and Keely, 2001). Confirmation of tetrapyrroles as free-base constituents was achieved by examination of online UV/Vis-PDA spectra which are diagnostic of presence or absence of complexing metals (e.g. Junium et al, 2008).

Liquid chromatography multi-stage mass spectrometry (LC–MS\(^{n}\)) was performed using a Finnigan LCQ system comprising a Thermo Separations AS3000 autosampler, P4000 gradient pump, UV2000 UV/Vis detector and a Finnigan MAT LCQ ion trap mass spectrometer equipped with an atmospheric pressure chemical ionisation (APCI) source. APCI source conditions were as follows: vaporizer 450 \(^{\circ}\)C; capillary 150\(^{\circ}\)C; discharge current 50 \(\mu\)A; sheath gas flow 40 (arbitrary units); auxiliary gas flow 10 (arbitrary units), collision energy 40\%. Structural determination was based on multi-stage mass spectra, and comparison to spectra of known structures and PDA data (Mawson and Keely, 2008).
3-3 Results

HPLC-PDA and LC-MSn analyses of acetone extracts of black shales from the Demerara Rise reveal tetrapyrrole distributions comprising mixtures of free-base and metalloporphyrins (Zn, VO and Ni) that are dominated by the bi-cyloalkanoporphyrin macrocycle (BiCAP) (Junium et al., 2008). BiCAPs are distinguished from cycloalkanoporphyins of chlorophyll origin in that they bear an additional, 7-membered exocyclic ring between C-15 and C-17 (Figure 1, structure II). On-line UV/Vis spectra of two early eluting peaks (Figure 3-2; Peaks I and II) have PDA spectra consistent with chlorophyll a-type dihydroporphyrins (chlorins), displaying Soret bands at 405nm and Qy bands at 658nm (Figure 3-2). Full mass spectra of peaks I and II contain protonated masses, [M+H]+, at m/z 535 (Figure 3-1; Peaks I and II). Collision induced dissociations (CID) of the m/z 535 ions of peaks I and II through MS4 indicate the presence of three oxygen functional groups (Figure 3-3), consistent with the loss of oxygen as CO and H2O. The CID of the Demerara Rise peaks I and II are nearly identical to those found within the Miocene Vena del Gesso (Figure 3-3) (Mawson and Keely, 2008) with small differences evident only as minor differences in the relative abundances of fragments in tertiary or quaternary MSn spectra. Peaks I and II contain the m/z 489 ion in MS3 (Figure 3-3) which indicates a bi-cyclo macrocycle for the two chlorins, consistent with high abundances of BiCAPs in the Demerara Rise black shales. The strong similarity of CID, UV/Vis spectra and relative abundances to those found in the Vena del Gesso suggests that peaks I and II are diastereomers of mesochlorophyllone a. On the basis UV/Vis data, Peaks I and II also display relative abundance differences that are typical of chlorophyllone and related compounds (Aydin et al., 2003). This series of peaks is present in nearly every sample analyzed (n > 40) from the Cenomanian through to the Santonian, indicating that chlorins are ubiquitous constituents of the Demerara Rise black shales.

3-4 Discussion

The presence of functionalized chlorins within Demerara Rise black shales represents the oldest confirmed chlorins by more than 70 Ma (mid-Miocene, Vena del Gesso) (Mawson and Keely, 2008) and supports a primary origin for dihydroporphyrins (chlorins) indicated by UV/Vis data in Cretaceous sediments of DSDP Site 367 (Baker et
identification of chlorins in sediments greater than 90 Ma is important because it demonstrates that the conversion of chlorophylls to porphyrins and defunctionalization reactions are less a function of time than of the geologic and geochemical conditions. It is clear that the shallow burial and geochemical conditions in the Demerara Rise black shales were exceptional for the preservation of tetrapyrroles, and may hold promise for the preservation of other highly functionalized biomarkers.

Chlorins of the bi-cyclo form (e.g. chlorophyllone, structure II, Figure 3-1) are common constituents of water-column particulates and modern core-top sediments (Walker and Keely, 2004; Sachs and Repeta, 2000; Ocampo et al, 1999). Their formation results from the cyclization of the propionic acid side-chain present at C-17 (Figure 1) associated with the loss of the esterifying alcohol (Keely, 2006). Chlorophyllone is the most abundant of the bicyclo-compounds found in modern environments; its formation has been linked directly to heterotrophy (Goericke et al., 2000), and presumably, the chemical conditions that are present in the digestive tract of the consuming organisms facilitate the condensation reactions that produce the BiCAP structure.

In ancient sediments, bicycloalkano-porphyrins and chlorins are most often found in calcareous, organic matter-rich sediments deposited under water columns with inferred reducing conditions (Mawson et al., 2004; Mawson and Keely, 2008; Schaffer et al., 1993). In some instances, the BiCAP form is present in very high concentrations (Junium et al., 2008; Shaeffer et al., 1993; Mawson and Keely, 2008) and it is possible that the majority of the preserved tetrapyrroles are the products of structural re-organization during heterotrophy (Georicke et al., 2000). However, there are no modern environments where the tetrapyrroles are exclusively of the bicyclo-form even where well-documented heterotrophic communities provide the balance of organic matter delivered to the sediment water interface ((Walker and Keely, 2004; Junium, Chapter 6). Therefore it seems likely that euxinic conditions in calcareous, organic matter-rich sediments (Shaeffer et al., 1993; Shaeffer et al, 1994; Mawson and Keely, 2008) foster the formation of the bi-cyclo forms. Recent work suggests that the reaction mechanism resulting in the reduction of the C3-vinyl substituent of chlorophyll a and chlorophyllone occurs under euxinic conditions either by anaerobic microbial communities or directly by hydrogen sulfide (Shaeffer et al., 1993; Mawson and Keely 2008; Pickering and Keely,
2008). In this study, the presence of the meso form of chlorophyllone and retention of an ethyl group at C-3 in an overwhelming majority of the porphyrins supports the presence of a strongly reducing depositional environment.

The possible precursor chlorophylls for BiCAPs include chlorophylls \( a \) and \( c \), however the only rational precursor for bicylo-chlorins is chlorophyll \( a \) (Keely, 2006). Fossils of calcareous nannoplankton represent the majority of the biogenic calcite present in these sediments suggesting that it is possible that chlorophyll \( c \) may have supplied some of the BiCAPs, however, the concentration of chlorophyll \( c \) in modern Chromista algae is typically less the 30% (Dougherty et al, 1970). The primary form of Chlorophyll \( c \) has a double bond between C-17 and C-18 and is, by definition, a porphyrin. It is not likely that the mild geochemical conditions present in the Demerara Rise black shales were conducive to saturation reactions at C-18 resulting in the formation of secondary dihydroporphyrins. Formation of dihydroporphyrins is a process that is associated with oil generation and high temperatures (Baker and Louda, 1986), and under these conditions it is not likely that the high functionalization that we observe in mesochlorophyllone would be retained. Additionally, if chlorophyll \( c \) were a significant component of the source chlorophylls in these sediments we might expect to find a tetrapyrrole compound that is similar to mesochlorophyllone but retains the primary double bond between C-17 and C-18.

3-5 Conclusions

The identification of mesochlorophyllone in Demerara Rise sediments is the oldest confirmation of primary chlorins in the geologic record. This finding demonstrates that defunctionalization reactions for chlorophylls are less dependent on time than of the geochemical conditions of the sedimentary environment. The presence of mesochlorophyllone, high concentrations of BiCAPs, and retention of an ethyl group at C-3 in the majority of the porphyrins (Junium et al., 2008) supports the presence of a strongly reducing, sulfidic depositional environment where similar compound distributions are found. The likelihood that chlorophyll \( c \) was not an important component of the primary chlorophyll moiety suggests that the BiCAPs in the Demerara Rise sediments were derived from chlorophyll \( a \), and that mesochlorophyllone was an important intermediate in BiCAP formation.
3-6 References


3.7 Figures

Figure 3-1. A schematic diagram of the proposed synthesis of bicycloalkanoporphyrin (BiCAP) from chlorophyll \textit{a} (adapted from Callot and Ocampo, 2000) The conversion of chlorophyll \textit{a} (I) to chlorophyllone (II) proceeds via the cyclization of the propionic acid chain following the loss of phytol. Defunctionalization reactions in the sedimentary environment cleave oxygen functional groups resulting in the BiCAP of structure III.
Figure 3-2. Online Uv/Vis data at 658nm and PDA spectra for the proposed chlorins found in the Demerara Rise black shales. The presence of two peaks is interpreted to represent an enantiomeric pair with stereochemical differences proposed to exist associated with the OH group at C-15\(^2\), marked by the wavy bond on the mesochlorophyllone structure. The difference in relative abundances for peaks I and II is typical of the Demerara Rise black shales. The accompanying structure is the meso form of chlorophyllone found in the sediments of the Miocene Vena Del Gesso (Mawson and Keely, 2008).
Figure 3-3. (A) Multi-stage mass spectra for MS$^2$-MS$^4$ for the meso form of chlorophyllone (MS$^1$ at $m/z$ 535) from the Miocene Vena Del Gesso (VDG) (Mawson and Keely, 2008). (B) A representative multi-stage mass spectra for MS$^2$-MS$^4$ for peaks I and II of Figure 1 (MS$^1$ at $m/z$ 535). Arrows mark the proposed mass losses from the parent ions. Minor differences are observed in the relative abundance of the $m/z$ 507 fragment in the MS$^2$, the relative abundance of the $m/z$ 488 and 489 fragments in MS$^3$. 
Chapter 4: Controls on the stratigraphic distribution and nitrogen isotopic composition of porphyrins from OAE II of Demerara Rise.

Abstract

The Cenomanian-Turonian sediments of the Demerara Rise contain a unique distribution of tetrapyrroles and provide an excellent opportunity to study the factors that control the stratigraphic distribution and nitrogen isotopic composition of porphyrins. Three C33 bicycloalkanoporphryins (BiCAP) are present as free bases (metal free; FB) and as complexes with Zn and VO in quantities sufficient for compound-specific isotopic analysis. The stratigraphic distribution of BiCAPS is controlled, foremost, by metal availability in the water column and sediments rather than early diagenesis Eh/pH conditions or post depositional thermal maturity. Titration of the local water-column metal reservoir by sulfide during Oceanic Anoxic Event II (OAE II) resulted in high concentrations of FB BiCAPs and very low concentrations of metallo-BiCAPs. Conversely, high metals concentrations are found in sediments above and below the OAE, and Zn and VO porphyrin abundances mirror bulk metal concentrations. The highest total concentrations of porphyrins are found where metal concentrations are highest, suggesting that porphyrin preservation is enhanced by the increased stability that results from formation of metal complexes. Paradoxically, the total concentration of porphyrins is lowest during the heart of OAE II, in an interval of higher TOC where enhanced preservation would be expected; this may be the result of decreased preservation of tetrapyrroles in the absence of available metals. We might infer that high reactivity of Zn$^{2+}$ with sulfide limited the formation of Zn complexes to the non-sulfidic region of the Demerara Rise water column or at the sediment/water interface. Vanadyl complexes are not found in recent sediments or water columns. The vanadyl ion remains stable under sulfidic sedimentary conditions and suggest that formation of the formation of vanadyl porphyrins occurs exclusively within the sediments from available FBs or through transmetallation reactions with other metallo-BiCAPS (Zn, Ni, Cu).

The formation of metal complexes involves bonding with the N atoms of the tetrapyrrole center and may have associated N-isotopic fractionations. We observe significant differences in the $\delta^{15}N$ of the three most abundant BiCAPs. Vanadyl BiCAPs are systematically $^{15}N$-depleted by an average of 2.5‰ +/- 1.5‰ relative to FBs, and Zn BiCAPs are equivalent to FB BiCAPs within error ($^{15}N$-enriched by +0.1‰) but are variable (+/- 1.5‰). The $\delta^{13}C$ values of the Zn, VO and FB BiCAPs are equivalent, suggesting that the three compounds share a common chlorophyll source, in agreement with structural data. The significant difference in $\delta^{15}N$ values between the VO and FB BiCAPs may be diagenetically controlled, either by N-isotopic effects during VO complex formation. It is now standard practice to reconstruct the $^{15}N$-abundance of primary phototrophic biomass by applying the empirically derived addition of 5‰ to chlorophyll and porphyrin $\delta^{15}N$. However, application of the ‘+5‰ rule’ results in very different values for primary biomass in Demerara Rise and raises the question of whether we can use the +5‰ rule for porphyrin $\delta^{15}N$ records. Despite the complexities, the covariance of bulk and BiCAP $\delta^{15}N$ through OAE II suggests that the observed variability in the bulk $\delta^{15}N$ record represents primary variability in the N-cycle.
4-1 Introduction

Cycloalkanoporphyrins are the geologically stable transformation products of chlorophylls and chlorins (Keely, 2006; Callot and Ocampo, 2000; Baker and Louda, 1986; Treibs, 1936) (Figure 1). They have proven very useful as biomarkers for ancient photosynthetic communities (e.g. Junium et al., 2008; Mawson et al., 2004; Gibbison et al., 1999) and are the targets of many recent studies utilizing porphyrins for compound-specific $\delta^{15}$N analyses (Chicarelli et al., 1993; Sachs et al., 1999; Ohkouchi; Kashiyama et al., 2008). The utility for porphyrins as isotopic biomarkers is clear, they have a definitive phototrophic source. Beyond biological information, porphyrins can be useful in deciphering the redox history of the diagenetic environment. In ancient sequences and oils, extractable porphyrins are found as complexes with divalent metals (VO, Ni, Cu, Fe, Zn); their formation is likely controlled by the Eh/pH conditions of the early diagenetic environment (Lewan, 1984), and or by the kinetics of metal sulfide formation (Morse and Luther, 1999) and the limitation of metal availability.

As we expand our use of porphyrins as biomarkers and for $\delta^{15}$N studies we aim to understand the range of processes that may affect their isotopic values. Few studies have systematically described the factors that control the stratigraphic distribution of metallo and FB porphyrins (e.g. Sundaraman et al., 1993) and none have investigated the stable isotopic composition of porphyrins of the same structure but with different complexing metals in detail. The sediments of Demerara Rise contain sufficient quantities of bicycloalkanoporphyrin (BiCAP), present as Zn or VO complexes or as FBs (Junium et al., 2008) to allow for $\delta^{15}$N analyses of the three in most samples. The abundance of the BiCAPs in the Demerara Rise black shales provides an ideal situation that will allow for better understanding of the controls on porphyrin $\delta^{15}$N and stratigraphic variability in metalloporphyrin abundances.

Here we present porphyrin abundances and compound-specific $\delta^{15}$N and $\delta^{13}$C analyses of the three most abundant C33 BiCAP porphyrins from the Cenomanian-Turonian sediments of the mid-Cretaceous of Demerara Rise. The goal of this study is to determine the controls on porphyrin abundance and stable isotopic composition with respect to biogeochemical changes through Oceanic Anoxic Event II. Of particular interest is the origin of isotopic differences that exist between the Zn, VO and FB
BiCAPs that are so abundant in the Demerara Rise sediments. All BiCAPs present in the Demerara sediments are derived from the same parent chlorophyll (Junium et al., 2008). Temporal or spatial variations in redox state may have impacted the N-cycle and metal availability, resulting in the observed δ^{15}N differences. However, observed differences in the δ^{15}N of Zn, VO and FB porphyrins of the same structure may be artifacts of diagenesis. Metal complexation and mineral-organic interactions directly involve the N atoms of the tetrapyrrole allowing for the possibility for different N-isotopic fractionations.

4-2 Methods

4-2-1 Compound Identification

Reverse phase HPLC analysis of total acetone extracts was conducted at York University, Department of Chemistry using a Waters system (Milford, MAUSA) comprising of a 717 autosampler, 600 MS system controller and 966 photodiode array (PDA) detector. The system was controlled, and data recorded and processed using Waters Millenium 2010 software. All solvents were degassed by sparging with helium or by vacuum degassing. Separations were achieved using two Waters Spherisorb ODS2 3 μm columns (4.6 x 150 mm i.d.) in series. Aliquots of acetone extracts were analysed using a quaternary gradient elution program comprising acetonitrile, methanol, water and ethyl acetate over 85 min with a flow rate of 0.7 ml min^{-1} (Airs et al., 2001). Determination of complexing metal was achieved by examination of online UV/vis-PDA spectra, which are diagnostic of metal type.

LC–MS^n analysis was performed using a Finnigan LCQ system comprising a Thermo Separations AS3000 autosampler, P4000 gradient pump, UV2000 UV/Vis detector and a Finnigan MAT LCQ ion trap mass spectrometer equipped with an atmospheric pressure chemical ionisation (APCI) source. Concentrated formic acid was infused into the eluent following chromatographic separation at the rate of 7 μl min^{-1} immediately prior to introduction into the LC-MS source to prevent metallation of free-base porphyrins and chlorins within the source (cf Airs and Keely, 2000). The interface conditions were as follows: vaporiser 450°C; capillary 150°C; discharge current 50 μA; sheath gas flow 40 (arbitrary units); auxiliary gas flow 10 (arbitrary units), collision
energy 40%. Structural determinations were based on multi-stage mass spectra and comparison to spectra of authentic standards where possible.

4-2-2 Porphyrin Quantification

Porphyrin quantification was achieved on the basis of UV/Vis absorbances calibrated with known quantities of commercially available free-base and VO octaethylporphyrin (OEP) (Frontier Scientific). Reversed phase purification of the commercial standard was required to isolate pure octaethlyporphyrin for purposes of quantification. Zn porphyrin was prepared from purified free-base OEP. Dilution series for each of the three porphyrins were prepared and calibration curves were used to quantify porphyrin concentrations from online UV/Vis data.

4-2-3 Porphyrin Purification for Isotopic analysis

Preparation of porphyrins for isotopic analysis was adapted from 2-dimensional (reversed/normal phase) HPLC methods developed by Sachs and Repeta, (1999) and are similar to those detailed in Kashiyama et al., (2007). Porphyrin fractions were collected from analytical reverse phase effluent (Method B of Airs et al., 2001) and dried under N₂ stream and stored at -20°C until normal phase purification. The isolated reversed phase BiCAP porphyrin aliquot is diluted in a small volume of 1:2 DCM:Hexane, typically 40 μl, but adjusted based on porphyrin concentration of individual samples. Small volumes (10 μl) of the highly concentrated reversed phase fraction are injected to maintain the baseline resolution necessary for effective tetrapyrrole purification (cf. Kashiyama et al., 2007). Normal phase purification is achieved with 2, 250 mm, 5 μm, 4.6 mm ID Agilent Sil HPLC columns linked in series under isocratic elution at 2 ml*min⁻¹ (Figures 4-4) (Table 4-1).

4-2-4 Isotopic Analyses

Isotopic analyses of porphyrins were conducted using a modified elemental analysis, isotope ratio mass spectrometer (EA-IRMS) system that employs a cyro-trapping/capillary-column focusing method that increases the proportion of analyte gas sampled by the IRMS, and effectively increases sample peak height. Details of this method, the analytical system and its capabilities are detailed in Polissar et al., (2009) (Figure 5). All data are reported using standard, delta notation and calibrated within
individual runs to octaethylporphyrin (Frontier Scientific), amino acids (methionine and alanine) house standards and IAEA N1, N2 and ANU-Sucrose.

Recent analytical improvements have resulted in a reduction in the size of procedural N-blank from ~80 to 20 nanomoles. Bypassing of the stock Costech-EA He regulator with He flow regulated directly from the He tank resulted in the largest decrease in the procedural blank. This allows for the use of stock EA oxidation furnaces and quartz inserts. The addition of inserts allow use of smooth-walled tin boats which are sonically cleaned in dichloromethane and methanol. This produces a precision of +/-1.0‰ for as little as 5 nanomoles of N and better than +/-0.5‰ for samples of 10 nanomoles N and greater for single samples, quantities that are easily isolated using analytical HPLC given sufficient porphyrin concentrations in samples. The drawback associated with use of smooth-walled tin cups, as opposed to roasted silver boats, is an increase in the size and variability of the procedural-C blank. However, the high C peaks largely reduces the influence of the C blank on the isotopic composition of samples and standards. Precision is reduced from the system described in Polissar et al., (2009) to +/-1.0‰ for single samples of 100 nanomoles. Though this is largely overcome through multiple analyses and the use of Keeling style plots (Keeling, 1958; Polissar et al., 2009), we are conservatively estimating the error for multiple analyses at +/- 1‰ for porphyrin $\delta^{13}C$ measurements.

4-2-5 Zinc metallation experiments

Octaethylporphyrin in acetone was prepared by gentle heating and sonication to bring OEP into solution. 500 nanomoles of OEP in solution were reacted with Zn-acetate dissolved in methanol to form Zn-OEP. The reaction is quantitative and rapid and the metallation process occurs within minutes and can be seen in a color change from deep red to scarlet. Zn-acetate was added to OEP to achieve incomplete conversion over a range of Zn-OEP concentrations as a fraction of total molar concentration of porphyrin (i.e., 0.4, 0.6 F Zn-OEP). These experiments were performed to explore whether fractionation occurs during the metallation process. Zn-OEP/OEP mixtures were purified using normal phase HPLC under isocratic solvent conditions (97% Hex/Acetone, 1 ml*min$^{-1}$) using 2 Agilient Sil columns (4.6 mm i.d. x 250 mm) linked in series.
4-3 Results

4-3-1 BiCAP Concentrations

We quantified the three most abundant porphyrins in the Demerara Rise, Cenomanian-Turonian black shales (Zn, VO and FB BiCAPs) and data are normalized to total organic carbon on a decarbonated basis. Total BiCAP concentrations are highest in the sediments immediately above and below the heart of OAE II, as defined by the plateau in $\delta^{13}C$ values, from 641 to 633 mbsf (Figure 6). Minimum porphyrin concentrations (900-1200 nmol/g TOC) are found within the $\delta^{13}C$ plateau. Metallo-BiCAP concentrations match maxima in bulk sedimentary metal concentrations (c.f. Hetzel et al., 2009) (Figure 7) and are absent or in low concentration during the $\delta^{13}C$ plateau. FB BiCAP concentrations rise during the $\delta^{13}C$ plateau, and have a secondary peak immediately after the drop in $\delta^{13}C$ values.

4-3-2 BiCAP $\delta^{15}N$ and $\delta^{13}C$

$\delta^{15}N_{\text{porphyrin}}$ values are $^{15}N$-depleted compared to $\delta^{15}N_{\text{bulk}}$ (Figure 8). N-isotope effects during the biosynthesis of chlorophyll $a$ result in an offset between $\delta^{15}N_{\text{biomass}}$ and $\delta^{15}N_{\text{chlorin}}$ (Sachs et al., 2000) and accounts, in part, for the observed differences between $\delta^{15}N_{\text{bulk}}$ and $\delta^{15}N_{\text{porphyrin}}$. The $\Delta^{15}N_{\text{biomass-chlorin}}$ in modern algae averages $+5\%e$, as determined by cultures and collected algae; this value has been confirmed in modern sediments between bulk sediments and sedimentary chlorins (Sachs and Repeta, 1999). Variability in $\Delta^{15}N_{\text{biomass-chlorin}}$ values exist between different strains of algae (Sachs and Repeta, 1999) and cyanobacteria (e.g. Beaumont et al., 2006) and differences in the dominant phototrophic community may contribute to differences in the $\Delta^{15}N_{\text{biomass-chlorin}}$.

The $\Delta^{15}N_{\text{bulk-porphyrin}}$ values in the Demerara Rise sediments are consistent for VO, Zn and FB BiCAPs but offset between the different compounds (Figure). The VO porphyrins are consistently $^{15}N$-depleted relative to the Zn and free-base BiCAPs and have $\Delta^{15}N_{\text{bulk-porphyrin}}$ values that are in agreement with modern estimates for $\Delta^{15}N_{\text{biomass-chlorin}}$ (Figure 8). Stratigraphic variability in $\delta^{15}N_{\text{bulk}}$ values is largely reflected by all three compounds (Figures 8 and 9). $\delta^{13}C_{\text{porphyrin}}$ data record the prominent, positive excursion in $\delta^{13}C$ (Figure 10). The offset between $\delta^{13}C_{\text{porphyrin}}$ and $\delta^{13}C_{\text{bulk}}$ is the result of the loss the $^{13}C$-depleted esterifying alcohol tail from primary chlorophylls, and typically results in
approximately a 2‰ $^{13}$C-enrichment of the residual tetrapyrrole. The basis of the $\delta^{13}$C difference is in the separate biosynthetic pathways for tetrapyrrols and the estyrifying alcohols (Ohkouchi et al., 2008).

4-3-3 Experimental preparation of Zn-Octaethylporphyrin

Zn-OEP formed by the addition of Zn-acetate to FB-OEP is $^{15}$N-depleted relative to the residual OEP (average $\Delta^{15}$N=-2.4‰, n=3, Table 2). Mass balance of Zn and FB-OEP $^{15}$N values yield the accepted value for unreacted OEP. Regression of $\delta^{15}$N of Zn-OEP on [f/(1-f)]*ln(f), where f is the fraction of undreacted FB-OEP yields an $\varepsilon_{p/K}$ value of -3.9‰ (Figure 14) , following the approximations described in Mariotti et al. (1981).

4-4 Discussion

4-4-1 Porphyrins in the Sedimentary Environment

Porphyrins in ancient sedimentary sequences and oils are found almost exclusively as complexes with divalent metals (Baker and Louda, 1986; Callot and Ocampo, 2000 and references therein). The loss of Mg$^{2+}$ from chlorophylls and the rearrangement and defunctionalization to porphyrins through diagenesis (Treibs et al., 1936; Keely et al., 1990; Keely, 2006) leaves the reactive center of the tetrapyrrole that bond with appropriately sized divalent cations (Table 2 and Figure 1). The most common metal complexes in marine sediments are Ni and VO; Cu, Fe, and Zn are typically found in low abundance or under unique circumstances (Baker and Louda, 1986; Callot and Ocampo, 2000; Junium et al., 2008). The metal complexation process results in a flattening of the porphyrin structure (Quirke, 1987) and greatly limits their reactivity (Foster et al, 2002), producing geologically stable molecules (c.f. Buchler, 1975) (Table 2). Preservation of porphyrins as FBs in ancient sediments is less common, and is generally viewed as a function of low maturity (Baker and Louda, 1986; Callot and Ocampo, 2000), or lack of metal available for complexation (Schaeffer et al., 1994). Here we will discuss the range of factors that control the distribution of porphyrins in the organic-rich sediments of the Cenomanian-Turonian sequence of the Demerara Rise, ODP Site 1261, to provide a framework from which to discuss the stratigraphic distribution and $\delta^{15}$N of BiCAPs.
4-4-2 Nickel and Zinc

Thermodynamic calculations suggest that the abundance of Ni porphyrins is limited by sulfide (Lewan, 1984), as Ni$^{2+}$ is insoluble in its presence (Figure 11). Indeed, there is an inverse correlation between the sulfur content of oils and abundance of Ni porphyrins (Lewan and Maynard, 1982). However, evidence for an early diagenetic source for Ni complexes is scant (Prowse et al., 1990) suggesting that their formation occurring later in diagenesis. However, the kinetics of NiS formation are slow (Morse and Luther, 1999), allowing formation of some Ni-porphyrins in the sulfidic region of sediments despite thermodynamic considerations. Dissolved Ni is present as NiCO$_3$ or sorbed to organic acids in the water column and is released during decomposition (Algeo and Maynard, 2004; Achterberg et al., 1997), providing a source of Ni$^{2+}$ to the sediments even under euxinic conditions. The C/T Demerara Rise black shales have low abundances of Ni-BiCAPs (Junium et al., 2008) which can be attributed to the sulfidic sedimentary conditions that were present during black shale deposition despite noted Ni enrichments in bulk sediments (Brumsack, 2006; Hetzel et al., 2008).

In the water column, Zn is present as Zn$^{2+}$, ZnCl or sorbed to humic and fulvic acids and is released to the pore waters during OM degradation (Algeo and Maynard, 2004; Achterberg et al., 1997). Like Ni$^{2+}$, Zn$^{2+}$ is insoluble in the presence of sulfide, and the formation of Zn-porphyrins is thermodynamically incompatible with the euxinic conditions present in the sedimentary environment of the Demerara Rise, and many black shales (Figure 11). However, unlike Ni$^{2+}$, the Zn$^{2+}$ reaction kinetics with sulfide are very rapid (Morse and Luther, 1999) and limit the high Zn$^{2+}$ activity to a sulfide-free zone. With respect to tetrapyrroles, Zn$^{2+}$ is considered a labile metal; it bonds easily and rapidly with tetrapyrroles under neutral laboratory conditions and Zn-chlorins may have formed in the water-column. Formation of metal complexes (Cu, Ni, Zn) in the water-column with chlorophylls and chlorins has been found in modern environments under elevated heavy metal concentrations (Kupper et al., 1996). The high abundance of Zn-BiCAPS (Figure 6), which have not been observed in other ancient marine sequences appear to be a function of the Zn enrichment that was present in the Demerara Rise water column as recorded in sediments (Hetzel et al., 2008).
4-4-3 Vanadium

Vanadate (VO$_4^{3-}$) is reduced to the diivalent vanadyl ion (VO$_{2}^{+}$) under reducing conditions. V(IV) species (VO$_{2}^{+}$, VOOH$^{1+}$) are known to form strong associations with dissolved organic matter (Tribovillard et al., 2006; Breit and Wanty, 1991) and covariance of V with TOC in marine settings supports an organic association (Brumsack, 1982), and not with sulfide minerals (Algeo and Maynard, 2004). V-enrichments in sediments, such as those that are found in Mediterranean Sapropels or Cretaceous black shales are the result of the reduction of VO$_4^{3-}$ to VO$_{2}^{+}$ and complexation with sinking and dissolved organic matter in the water column under anoxic conditions.

Formation of VO complexes is described as a late diagenetic process occurring after defunctionalization and aromatization of the macrocycle with time and heating (Baker and Louda, 1986; 1981; Filby et al., 1987). No evidence exists for the formation of VO-tetrapyrrrole complexes in the water column or early diagenetic environment and there are presently no described occurrences of VO-chlorins in the literature. However, the relative abundance of the two VO$_{2}^{+}$ BiCAP enantiomers in the Demerara sediments suggests that complexation may have occurred when the precursor tetrapyrrrole retained the hydroxyl functional group present at C-15$^{2}$ of mesochlorophyllone (Figure 2, Chapter 2). The VO BiCAPs are present as an enantiomeric pair with the oxygen of the vanadyl ligand α or β (facing out or back) to the planar tetrapyrrrole. The relative abundances of the two BiCAPs (~3:1) is similar to the relative abundances of α and β mesochlorophyllone. We are proposing that the α position of the hydroxyl group on mesochlorophyllone favors the formation of βVO BiCAP$^{+}$. If this is correct, this suggests that the formation of vanadyl complexes can occur with chlorins, during early diagenesis.

Laboratory preparation of VO porphyrins requires reflux above 100°C in glacial acetic acid and pyridine (Erdman et al., 1958), conditions that are hardly reflective of geologic systems. This suggests that energetic or kinetic barriers exist, impeding the formation of VO complexes in the sedimentary environment, or that complexation is catalyzed by mineral surfaces or organic matter (Filby et al., 1987). Several mechanisms have been proposed for the formation of VO porphyrins; the breaking of V-OM bonds during OM degradation yielding free VO$_{2}^{+}$, mediation of VO$_{2}^{+}$ bonding with sulfur compounds complexed with porphyrin nitrogens (Yen et al., 1969), transmetallation
reactions where less stable metal complexes such as Cu are replaced by VO$^{2+}$ (Quirke, 1987), or addition of VO$^{2+}$ at clay surfaces (Filby et al., 1987). Under these models, VO$^{2+}$ porphyrins would be drawn from the available pool of free-base porphyrins present in sedimentary OM or from weakly bonded metal complexes such as Cu porphyrins.

A vanadyl porphyrin predominance relative to Ni is used to infer anoxic or euxinic conditions during the generation of oil shales; indeed, the concentration of VO porphyrins is higher in high-sulfur oils (Lewan, 1984) (Figure 11). The reduction of V(IV) to its lowest valence state, V(III), can occur under euxinic conditions (Wanty, 1986) resulting in the precipitation of authigenic V phases (Lewan, 1984) and incorporation into clays (Convey et al., 1987; Breit, 1991). This process could limit the formation of VO-porphyrin complexes in euxinic sedimentary settings such as the Demerara Rise blacks shales.

4-4-4 Free Bases and Chlorins

The preservation of FBs and chlorins in sedimentary sequences is typically viewed as a function of low maturity (Baker and Louda, 1981; Junium, Chapter 3). Indeed, FBs are significantly less stable than metalloporphyrins (Foster et al., 2002). They are more susceptible to electrophilic attack at the meso positions (see Chlorophyll a in Figure 1) of the porphyrin structure that results in opening of the porphyrin ring (Quirke, 1987). Clay sorption experiments demonstrate that FBs are also more efficiently retained on acidic clay surfaces because of the availability of basic pyrrole nitrogens (Foster et al., 2002), a process that may catalyze incorporation into an insoluble organic phase or formation of metal complexes (Quirke, 1987).

4-4-5 OAE II and Metalloporphyrin Abundance

The carbon cycle response that defines OAE II is observed in the prominent rise in the $\delta^{13}C$ of carbonate (+2 to 3‰) and organic carbon (+4 to 7‰) in response to the enhanced fractional burial of organic carbon over an interval of ~540 Ka over which $\delta^{13}C$ values remain high (the “plateau phase”) (Arthur et al., 1988; Sageman et al., 2006). The geologic response to OAE II is expressed in the quasi-global deposition of organic matter-rich black shales (Schlanger et al., 1987) under anoxic and episodically euxinic water column conditions; many of these sediments are enriched in redox sensitive metals,
fixed as metal sulfides (Ni, Cu, Zn) or associated with organic matter (V, Co) (e.g. Brumsack, 2006).

At Demerara Rise, the concentrations of V and Zn in bulk sediments peaks immediately prior to the rise in δ^{13}C where metal concentrations are approximately an order of magnitude greater than average marine shale (Figure 7). Coincident with the rise in δ^{13}C, metal concentrations (V, Zn, Cu, Mn, Mo) drop precipitously during the plateau phase of the δ^{13}C record (Figure 6). This is attributed to the stripping of water-column trace metal reservoirs as sulfides and organic complexes elsewhere, associated with expansion of water-column euxinia and organic matter burial during OAE II (Hetzel et al., 2009). Sedimentary metal supply is most likely the primary control on the abundance of metalloporphyrins through OAE II at Demerara Rise (Figures 6 and 7). Following the OAE, δ^{13}C values decrease and trace metal concentrations return to significantly enriched values. The metalloporphyrin concentrations respond in step with the sedimentary trace metal concentrations and reflect the evolution of the OAE control on metal availability at Demerara Rise.

The highest concentrations of porphyrins do not correspond to the highest total organic carbon percentages present during the δ^{13}C plateau phase (Figure 6), under euxinic water column conditions (Van Bentum et al., 2009) during the height of OAE II. Rather, the highest concentrations of porphyrins correspond to the intervals with the greatest metal enrichment. We attribute this to enhanced stability of metal complexes and increased probability of preservation over geologic time. Surprisingly, the lowest total BiCAP concentrations are found within OAE II, where TOC values are as high as 30% on a decarbonated basis, but metal concentrations are significantly lower than adjacent strata. One explanation is that a significant proportion of the more reactive FB BiCAPs are sulfurized or ether-linked (Schaeffer et al., 1993; 1994; Huseby and Ocampo, 1997). However this is not the case, as neither nickel-boride desulfurization nor hydrolysis yield higher concentrations of BiCAPs or additional structures (Appendix Figure). Clay-mediated sorption and degradation, and a lack of metal-enhanced preservation could explain the low concentrations of BiCAPs during the height of the OAE, despite significant TOC-enrichment (Figure 6).
4-4-6 Reconsidering Treibs Scheme

The distribution of tetrapyrroles in the Demerara Rise black shales presents a more complex picture of the Treibs Scheme, as revised over the last 70 years (e.g. Keely, 2006; Callot and Ocampo, 2000) since its brilliant conception (Triebs, 1936). It presents a continuum in the structural transition from chlorophyll and chlorins found at the sediment water interface (e.g. pheophytins and pheophorbides) to deoxyphylloethroetioporphyrin (DPEP), related structures and metalloporphyrins (Figure 1). Chlorins and metalloporphyrins typically do not co-occur (Prowse et al., 1990), and there is no previously reported occurrence of VO$_2^+$ porphyrins and chlorins in the same sediments. Therefore, the previous assertion that vanadyl porphyrins are present only as extractable compounds from sediments where temperatures were greater than 65°C (Baker and Louda, 1986) is not correct. The Demerara Rise black shales are well below oil window thermal maturities. Pyrolysis hydrogen index (HI) values are often as high as 900 (mg hydrocarbon* g TOC$^{-1}$ and T$_{max}$ values are well below 425°C (Erbacher et al., 2004; Junium and Arthur et al., 2007) indicating that heating during burial was minimal. The distribution and relative abundance of VO BiCAPs in the Demerara Rise sediments supports formation and release of VO-porphrins from organic matrices in immature sediments and may in fact occur during early diagenesis.

In the Demerara Rise black shales, initial Zn complexation occurred prior to defunetionalization and in the water column or at the sediment water interface. We have not identified Zn-chlorins, but the formation of metallochlorins is possible in water columns with high metal concentrations (Kupper et al., 1996). The presence of Zn complexes in these sediments is a special case, and it is likely that the Zn porphyrins are a product of unusual metal enrichments that may have been present in the Cretaceous, Demerara Rise water column (Hetzel et al., 2008).

The co-occurrence of functionalized chlorins (Chapter 3), free-bases and vanadyl porphyrins in thermally immature sediments suggests that the distributions of sedimentary tetrapyrroles do not simply follow the “dictum of time and temperature interdependence” (Baker and Louda, 1981). Other factors, including the sedimentary concentration of metals are important to the formation and sedimentary concentration of
metalloporphyrins, and that time is not the primary component in defunctionalization reactions.

**4-4-7 Nitrogen isotopic composition of porphyrins**

The values of $\Delta^{15}N_{cell-chla}$ have been determined using laboratory cultures and modern algal populations and extended to sedimentary chlorins and porphyrins (Sachs et al., 1999; Kashiyama et al., 2008). Paleoenvironmental studies that utilize chlorophyll derivatives reconstruct the $\delta^{15}N$ of biomass by the addition of $\sim +5\%e$ to chlorophylls, chlorins and porphyrins to reconstruct the $\delta^{15}N$ of algal biomass from chlorophyll $\delta^{15}N$ (Sachs et al., 1999; Ohkouchi et al., 2006; Kashiyama et al., 2008) (Figure 12). However, the factors that control the $\delta^{15}N$ of different porphyrins and bulk N have yet to be thoroughly explored.

As applied to ancient sediments, $\delta^{15}N$ data for Ni-DPEP from the Cenomanian-Turonian, Furlo Bonarelli (-3 to -6‰) agree reasonably with bulk values (-1 to -3‰) and support hypotheses that N$_2$-fixation was the source for dissolved inorganic nitrogen (DIN) during OAE II in the central Tethys (Kashiyama et al, 2008). $\Delta^{15}N_{bulk-porphyrin}$ data are slightly lower than +5‰ (c.f. Ohkouchi et al., 2006), a trend that is also observed from the Triassic, Serpiano Marl (Chicarelli et al., 1993). It is possible that the $\Delta^{15}N_{bulk-porphyrin}$ values reflect a lower value for $\Delta^{15}N_{cell-chla}$, minor diagenetic alteration of bulk $\delta^{15}N$ values or nitrogen isotopic effects associated with the formation of metal complexes.

The BiCAPs of Demerara Rise sediments exhibit differences in the average $\Delta^{15}N_{bulk-porphyrin}$ for Zn, VO and freebase BiCAPs. Vanadyl BiCAPs are systematically $^{15}N$-depleted relative to the FBs, whereas Zn BiCAPs are on average equivalent in $^{15}N$-abundance to FB, but are variable (Figures 8 and 9). If the three BiCAP porphyrins were derived from different biological sources, one may expect to observe significant and systematic differences in $^{13}C$-abundance, but this is not the case. The $\delta^{13}C$ values of the Zn, VO and FB porphyrins display some deviation between structures, but are equivalent within error (Figure 10), supporting structural evidence for a single chlorophyll source. As expected, $\delta^{13}C_{porphyrin}$ values record the positive $^{13}C$ excursion that defines OAE II (Arthur et al., 1987; Sageman et al., 2006) and are $^{13}C$-enriched relative to bulk organic matter by 2-3‰, resulting from the loss of the $^{13}C$-depleted estyrifying alcohol during
early diagenesis. $^{13}$C-enrichment has been described for modern chlorophylls (Sachs and Repeta, 2000; Ohkouchi et al., 2008) and observed in coretops of recent sediments (Junium, Chapter 6). $\delta^{13}$C$_{\text{porphyrin}}$ data suggests that the observed porphyrin $\delta^{15}$N values are not the result of differences in $\Delta^{15}$N$_{\text{bulk-porphyrin}}$ related to different organisms and that biological sources are not responsible for the observed differences in the $\delta^{15}$N of the BiCAPs.

We performed a simple bench-top experiment producing incomplete formation of Zn-octaethylporphyrin (OEP) by the addition of Zn-acetate to FB-OEP (Table 4). Results from the Zn experiments demonstrated a systematic $^{15}$N-depletion of Zn-OEP by an average $\Delta^{15}$N$_{\text{fb-Zn}} = 2.4\%e$, and yield an $\varepsilon_{\text{p/r}}$ value of -3.9\%e (Figure 14). These simple, bench-top experiments, in well-mixed solvent solutions may not replicate the geochemistry involved in the formation of metal complexes. For example, it is unknown whether the Zn metallation reactions are reversible under the experimental or natural conditions, or if the rate of Zn-OEP formation acts to limit the maximum fractionation. However, these results demonstrate that fractionation during the formation of metal complexes can occur. Similar experiments were attempted using OEP and VO-sulfate, however, formation of VO-OEP was at concentrations that were too low for isotopic analysis and conversions were not quantitative. Kashiyama et al., (2008) report no observable fractionation in the formation of Ni-DPEP from FB-DPEP. It is possible that the formation of different metal complexes may have different isotopic effects and this is something that needs to be explored more fully in the future.

We have established that the Demerara Rise FB and metallo BiCAPs are sourced from the same precursor chlorophyll, thus, the observed differences in $^{15}$N-abundance of the FB and metallo-BiCAPs are likely to be of diagenetic origin. If the formation of metalloporphyrins in the Demerara Rise sediments behaves as closed system, our Zn-OEP experiments can provide a model from which we can assess the observed N-isotopic differences in the BiCAPs. The simplest scenario is a sequential formation of Zn complexes resulting in a $^{15}$N-enrichment FB pool (Figure 14). VO-BiCAPs would have formed from the remaining FB reservoir. If there is a fractionation during the formation of VO BiCAPS similar to that observed in the Zn-OEP experiments, the residual FB-BiCAPs should be most $^{15}$N-enriched fraction. Zn-BiCAPs would have the lowest $\delta^{15}$N
values, and the enrichment should be proportional to the concentration of metalloporphyrins. However, this is not what we observe, FB-BiCAP $\delta^{15}N$ values are on average equivalent to Zn BiCAPs, and VO BiCAPs $^{15}N$-depleted by 2.7‰. These data also exclude the situation where there is no fractionation during formation of Zn-BiCAPs, but a large, negative fractionation during the formation of VO-BiCAPs because the FB-BiCAPs pool would be $^{15}N$-enriched relative to the Zn-BiCAPs. Therefore, the processes controlling the observed $\delta^{15}N_{\text{BiCAP}}$ values of are more complex and include the possibility that the Zn and VO BiCAPs are formed from separate FB-BiCAP pools.

Weak metal-N bonds such as those present in Zn and Cu porphyrins can be broken under mildly acidic conditions (Quirke, 1987) or catalyzed at mineral surfaces and replaced with the more stable VO$^{2+}$ bonds (Hodgson et al., 1967; Foster et al., 2002). Transmetallation reactions present an additional avenue that may explain the observed fractionation in the formation of Zn-OEP and the VO-BiCAP data. The conversion of Zn-BiCAPs to VO$^{2+}$ complexes through transmetallation reactions may also favor the breaking of $^{14}N$-ligand bonds resulting in observed $^{15}N$-depletion in the VO$^{2+}$ BiCAPs and increasing the $\delta^{15}N$ values of Zn-BiCAPs to values closer to the FB-BiCAPs.

Additional complexities also may contribute to the observed $^{15}N$-abundances. FB porphyrins sorb more strongly to acidic clay surfaces and organic matter than their metal complexed counterparts due to the availability of basic pyrrole nitrogens (Foster et al., 2002; Bergaya and Van Damme, 1992) (Figure 15). For example, sorption reactions under equilibrium conditions favor retention of isotopically enriched Fe isotopes on mineral surfaces (Icopini et al., 2004). Similar processes could result in the $^{15}N$-enrichment of FB BiCAPs through the preferential retention of $^{15}N$-enriched FB BiCAPs, decreasing their activity and limiting the formation of metal complexes.

Regardless of the processes that control the isotopic partitioning of porphyrin-N in the BiCAPs it is apparent that analysis of only one class of compound (i.e. VO$^{2+}$ porphyrins) in the Demerara black shales would result in a biased paleoenvironmental reconstruction. One can reconstruct the estimated primary phototrophic biomass on the basis of a $\Delta^{15}N_{\text{cell-chla}}$ of 5‰ in the manner of Sachs et al., (1999) or Kashiyama et al, (2008) for VO$^{2+}$ and FB BiCAPs. Application of a $\Delta^{15}N_{\text{cell-chla}}$ of 5‰ results in significantly different $\delta^{15}N$ records through OAE II (Figure 16). The FB $\delta^{15}N$ (+5‰)
record suggests a change from a denitrification source for DIN prior to and after OAE II, with N$_2$-fixation providing DIN during the OAE. By contrast, the VO BiCAP (+5‰) record indicates that the pre-OAE II interval is characterized by a largely N$_2$-fixation source for DIN. During OAE II the VO BiCAP (+5‰) record suggests that chemocline production, or upwelling of ammonium from anoxic deep waters supplied $^{15}$N-depleted ammonium to the photic zone resulting in $\delta^{15}$N values that are lower than diazotroph biomass.

In many sedimentary systems, porphyrins are found complexed with one metal (typically VO$^{2+}$ or Ni) and FBs are generally absent or in low concentration. What is unclear is if the porphyrins are converted quantitatively from their precursor FBs to metal complexes, or if this process is incomplete and the less stable FBs were degraded during diagenesis. This is an important unknown that has particular significance to the N-isotopic compositions of porphyrins in ancient sequences. If the formation of VO$^{2+}$ complexes does indeed have a significant N-isotopic effect and the formation of VO porphyrins is incomplete, faithful reconstructions of primary biomass (c.f. Kashiyama et al., 2008) may be not be possible.

4-4-8 Nitrogen isotopic composition of porphyrins: Implications

The overall trend and sample-to-sample variability in the bulk $\delta^{15}$N record is confirmed by the three BiCAPs (Figures 8 and 9). We have outlined a range of processes that could explain the differences in the $\delta^{15}$N of the different BiCAPs in the Demerara Rise sediments. However, it is evident from the covariance between the bulk and BiCAP $\delta^{15}$N records (Figure 9) that the decrease in $\delta^{15}$N through OAE II represents primary changes in the $\delta^{15}$N of phototrophic biomass. This is an important finding because it demonstrates the utility of bulk and compound-specific $\delta^{15}$N in settings where well preserved marine organic matter of low thermal maturity is the dominant source of N. It also demonstrates that in situations where multiple sources of N may be present, such as continental margins and lakes, that variability in the N-cycle can be reconstructed using chlorophyll derivatives.

One of the limitations of porphyrin $\delta^{15}$N is illustrated here in the difference between metallo and FB BiCAPs. Targeted analysis of only one porphyrin in these sediments would yield reconstructed phototrophic biomass that suggests a very different
state of the nitrogen cycle through OAE II (Figure 16). Where multiple porphyrins are present, it is extremely important to assess any potential $\delta^{15}N$ differences between structures and their complexing metals. Where only one type of metalloporphyrin is present in sufficient abundance for routine $\delta^{15}N$ analysis, the variability in $\delta^{15}N$ may be correct, but reconstructed values may not directly reflect the $\delta^{15}N$ of primary, phototrophic biomass and DIN.

4-5 Conclusions

The stratigraphic distribution of FB and metallo BiCAPS is controlled primarily by metal availability in the water column and sediments. Titration of the local water-column metal reservoir by sulfide and organic matter during Oceanic Anoxic Event II (OAE II) resulted in high concentrations of FB BiCAPs and very low concentrations of metallo-BiCAPs. Conversely, high metals concentrations are found in sediments above and below the OAE, and Zn and VO porphyrin abundances mirror bulk metal concentrations in the Demerara Rise sediments (Hetzel et al, 2009). The highest total concentrations of porphyrins are found where metal concentrations are highest, suggesting that porphyrin preservation is enhanced by the increased stability that results from formation of metal complexes. The total concentration of porphyrins is lowest during the heart of OAE II, in an interval of higher TOC where enhanced preservation would be expected; this is the result of decreased preservation of tetrapyroles in the absence of metals and a potential increase in sorptive retention of free bases on mineral surfaces and to organic matter.

The high reactivity of Zn$^{2+}$ with sulfide limits the formation of Zn complexes to the non-sulfidic region of the Demerara Rise water column or at the sediment/water interface. Vanadyl complexes are not found in recent sediments or water columns. The vanadyl ion remains stable under sulfidic sedimentary conditions and suggest that formation of the formation of vanadyl porphyrins occurs exclusively within the sediments from available FBs or through transmetallation reactions with other metallo-BiCAPS (Zn, Ni, Cu). The relative abundance of the two VO$^{2+}$ BiCAP enantiomers in the Demerara sediments suggests that complexation may have occurred as bicyclo-chlorins. The $\alpha$ position of the hydroxyl group on mesochlorophyllone favors the formation of $\beta$VO
BiCAP, If this is correct, this suggests that the formation of vanadyl complexes can occur with chlorins, during early diagenesis.

The formation of metal complexes involves bonding with the N atoms of the tetrapyrrole center and may have associated N-isotopic fractionations. We observe significant differences in the $\delta^{15}N$ of the three most abundant BiCAPs. Vanadyl BiCAPs are systematically $^{15}N$-depleted by an average of $2.5\%e +/- 1.5\%e$ relative to FBs, and Zn BiCAPs are equivalent to FB BiCAPs within error ($^{15}N$-enriched by $+0.1\%e$) but are variable ($+/- 1.5\%e$). The $\delta^{13}C$ values of the Zn, VO and FB BiCAPs are equivalent, suggesting that the three compounds share a common chlorophyll source, in agreement with structural data. A single chlorophyll source requires that the differences in $\delta^{15}N$ values between the VO, Zn and FB BiCAPs are the result of fractionation during the formation of metal complexes.

Application of the ‘+5%e rule’ results in very different values for primary biomass through OAE II and raises the question of whether we can use the +5%e rule for porphyrin $\delta^{15}N$ records. Despite the complexities, the covariance of bulk and BiCAP $\delta^{15}N$ through OAE II suggests that the observed variability in the bulk $\delta^{15}N$ record represents primary variability in the N-cycle.

4-6 References


Hodgson G. W. and Peake E. (1961) Metal Chlorin Complexes in Recent Sediments Ass
Initial Precursors to Petroleum Porphyrin Pigments. Nature 191, 766-&.
Huseby B. and Ocampo R. (1997) Evidence for porphyrins bound, via ester bonds, to the
Messel oil shale kerogen by selective chemical degradation experiments.
isotope fractionation during microbial reduction of iron: The importance of
adsorption. Geology 32, 205-208.
Cenomanian-Turonian oceanic anoxic event II. Geochemistry Geophysics
Geosystems 8, Q03002.
Unexpected occurrence and significance of zinc alkyl porphyrins in Cenomanian-
Reconstruction of the biogeochemistry and ecology of photoautotrophs based on
the nitrogen and carbon isotopic compositions of vanadyl porphyrins from
Miocene siliceous sediments. Biogeoosciences 5, 797-816.
and purification of sedimentary porphyrins by high-performance liquid
chromatography for compound-specific isotopic analysis. Journal of
Chromatography a 1138, 73-83.
Kashiyama Y., Ogawa N. O., Kuroda J., Shiro M., Nomoto S., Tada R., Kitazato H. and
Ohkouchi N. (2008) Diazotrophic cyanobacteria as the major photoautotrophs
during mid-Cretaceous oceanic anoxic events: Nitrogen and carbon isotopic
Keely B. J. (2006) Geochemistry of Chlorophylls. In Chlorophylls and
Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications (ed.
Bernhard Grimm, Robert J. Porra,Wolfhart Rudiger and Hugo Scheer). Springer,
pp. 531-565.
Kupper H., Kupper F. and Spiller M. (1996) Environmental relevance of heavy metal-
substituted chlorophylls using the example of water plants. J. Exp. Bot. 47, 259-
266.
Lewan M. D. (1984) Factors Controlling The Proportionality Of Vanadium To Nickel In
Acta 46, 2547-2560.
Morse J. W. and Luther G. W. (1999) Chemical influences on trace metal-sulfide
importance of diazotrophic cyanobacteria as primary producers during Cretaceous


Figure 4-1. The Treibs Scheme as adapted from Keely (2006). The traditional view of Treibs Scheme depicted on the right, showing formation of DPEP and metallo DPEP from chlorophyll \( a \). The left side of the diagram depicts the structural evolution of the bicyclo macrocycle to BiCAP and metallo-BiCAP. The three structures, mesochlorophyllone, BiCAP and metallo-BiCAP are all present in the Demerara Rise black shales.
<table>
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<th>BiCAP</th>
<th>Hexane:Acetone</th>
<th>Flow Rate</th>
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<tr>
<td>FB</td>
<td>93:7</td>
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<tr>
<td>VO</td>
<td>94:6</td>
<td>2ml*min⁻¹</td>
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Figure 4-2. Purification of the free base BiCAP. Panel A is the reversed phase chromatogram from which the free base BiCAP is collected. Panel B is the LC-MS chromatogram of the interval collected, with a coeluting porphyrin at m/z 475. Panel C is the UV/Vis chromatogram of the normal phase purification step, demonstrating the isolation of the BiCAP peak and the accompanying LC-MS chromatogram in panel D.
Figure 4-3. Purification of the Zn BiCAP. Panel A is the reversed phase chromatogram from which the free base BiCAP is collected. Panel B is the LC-MS chromatogram of the interval collected, with several coeluting porphyrins. Panel C is the UV/Vis chromatogram of the normal phase purification step, demonstrating the isolation of the Zn BiCAP peak and the accompanying LC-MS chromatogram in panel D.
Figure 4-4. Purification of the VO BiCAP. Panel A is the reversed phase chromatogram from which the VO BiCAP is collected. Panel B is the LC-MS chromatogram of the interval collected, with coeluting porphyrins at m/z 540. Panel C is the UV/Vis chromatogram of the normal phase purification step, demonstrating the isolation of the VO BiCAP peaks and the accompanying LC-MS chromatogram in panel D.
Figure 4-5. Nano EA system diagram adapted from Polissar et al., (2009). The autosampler has been retrofitted with a vacuum purge that effectively removes trace atmospheric gases trapped in sample capsules and lowers the N-background. A bleed valve splits flow, maintaining flow from the autosampler, which was found to be a source of increasing blank values through a multi-sample run. The Cu-reduction furnace is a narrow-bore, custom-made quartz furnace that reduces trapping times. The water trap has been replaced with a pyrex tube with a locking nut/Teflon ferrule setup that is a significant improvement in procedural N blank relative to the stock EA water trap. Sample gas is trapped in a silica trapping column in liquid-N; flow is diverted to a low-flow regime and the trap is heated and sample gas is focused further in a carbon PLOT column before introduction to the isotope ratio mass spectrometer.
Figure 4-6. Abundances of the Zn, FB and VO BiCAPs on a TOC normalized basis. The gray bar marks the depth range (mbsf, meters below sea floor) of bulk sediment metal depletion (see Figure 7).
Figure 4-7. Running average sedimentary metal concentrations from ODP Site 1258 adapted from Hetzel et al., 2008. Metal concentrations drop to those of average marine shale during the heart of OAE II and are over an order of magnitude greater in the overlying and underlying sediments.
Figure 4-8. Stable isotope data for bulk (N and C) and porphyrin for Zn, FB, and VO N from ODP Site 1261 through OAE II. Scale is in meters below sea floor (MBSF). Error on porphyrin $\delta^{15}$N measurements is conservatively estimated at +/- 0.5‰.
Figure 4-9. Cross plot of bulk and BiCAP δ¹⁵N values.
Figure 4-10. Stable carbon isotope data from bulk sediments and co-occurring BiCAPs. Whole chlorophylls typically approximate bulk organic matter in $^{13}$C abundance. The offset between bulk $\delta^{13}$C and porphyrin $\delta^{13}$C is expected and is the result of the loss of the esterifying alcohol, which is biosynthesized using different precursors than tetrapyrroles (Ohkouchi et al., 2008). Error on porphyrin $\delta^{13}$C measurements is conservatively estimated at +/- 1‰.
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Figure 4-11. Eh/pH stability zones for the formation of Ni, Zn and VO porphyrins, adapted from Lewan (2004). The presence of sulfide limits formation of Ni and Zn porphyrins through the formation of insoluble sulfide minerals.
Figure 4-12. Porphyrin data from the Livello Bonarelli, Italy from the CT boundary black shales from Kashiyama et al., (2008). Here they reconstruct the primary N and C isotopic composition of phototrophic biomass from Ni-DPEP using 5‰ addition to the porphyrin $\delta^{15}$N values. They also subtract 2‰ from $\delta^{13}$C values to account for the loss of the $^{13}$C-depleted esterifying alcohol.
Table 3. Zn Metallation Experimental Data
(accepted value for OEP= -12.8‰)

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<th>Zn $\delta^{15}$N</th>
<th>FB $\delta^{15}$N</th>
<th>$\Delta^{15}$N$_{fb-Zn}$</th>
<th>$\delta^{15}$N mass balance</th>
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Figure 4-13. Schematic describing the predicted isotopic fractionations during the formation of Zn and VO metalloporphyrins in a closed system. Branch points have potential fractionations ($\varepsilon$) that leave the residual FB pool isotopically enriched in accordance with Zn-OEP experiments (Table 4 and Figure 14). The numbers in the top left corner of the boxes refer to two scenarios: 1. sequential formation of Zn porphyrins from the free base pool, followed by VO porphyrins formation from the remaining free base pool; 2. Formation of Zn porphyrins from the free base pool and formation of VO porphyrins from the Zn pool by transmetallation. Here we are assuming that in each metallation step the $^{15}$N-depleted porphyrins favor the product. Scenario 1 would result in Zn complexes that have the lowest $\delta^{15}$N values with the unreacted residual FB porphyrins being the most $^{15}$N-enriched pool. Scenario 2 has Zn porphyrins forming from the original FB pool as in Scenario 1, but VO complexes are formed from by transmetallation reactions. In Scenario 2, the transmetallation reactions would drive the isotopic composition of the Zn porphyrins higher resulting in VO porphyrins that are $^{15}$N-depleted and Zn and FB porphyrins that are $^{15}$N-enriched.
Figure 4-14. Rayleigh fractionation model for Zn-OEP experiments. $\varepsilon_{P/E} (-3.9)$ was calculated by plotting the $\delta^{15}$N of the Zn-OEP against $[f/(1-f)]/\ln(f)$. 

$y = -16.786 \times 3.903x \quad R^2 = 0.99995$
Figure 4-15. A range of possible scenarios to that may result in N-isotopic effects.
Figure 4-16. The N isotopic composition of VO and FB BiCAPs (solid lines) and bulk sediments. Addition of 5‰ to the VO and FB BiCAPs (dashed lines) result in very different ‘primary’ $\delta^{15}$N values.
Chapter 5: Global expansion of N₂-fixation supported primary productivity during mid-Cretaceous Oceanic Anoxic Event II

Abstract
In the modern ocean, spatial coupling between N₂-fixation and denitrification is predicted by models and geochemical proxy data (Deutsch et al., 2007) but is difficult to observe directly. The widespread expansion of anoxic water masses during Oceanic Anoxic Events (OAEs) in the Mesozoic (Schlanger et al., 1987) provides an ancient test case for a modern biogeochemical problem. Here we demonstrate that during the Cretaceous, Cenomanian-Turonian (CT) OAE II (93.5 Ma) widespread marine anoxia resulted in an extraordinary expansion of biological N₂-fixation. New compound-specific δ¹⁵N data from chlorophyll a-derived geoporphyrins, a global δ¹⁵N dataset and the results of a N-cycle isotope box model indicate that N₂-fixation-enhanced primary productivity fueled organic matter burial during OAE II on a global scale. Geoporphyrin δ¹⁵N data from Demerara Rise confirm the validity of low δ¹⁵N values from OAE II sediments where δ¹⁵N values are almost exclusively below 0‰ and consistent with a N₂-fixation source. Additionally, the geoporphyrin δ¹⁵N data suggests that the bulk δ¹⁵N values underestimate the magnitude of the δ¹⁵N excursion by ~1‰. Simple box model results demonstrate that a doubling of P-flux sufficiently stimulates N₂-fixation to drive a 3‰ negative shift in the δ¹⁵N of dissolved inorganic nitrogen (DIN) which is directly observed in the sedimentary δ¹⁵N record. These data indicate that the δ¹⁵N-depleted signal of N₂-fixation lowered the δ¹⁵N DIN and was transferred to other primary producers communities. These results support a tight coupling between denitrification and N₂-fixation, fueled by enhanced recycling of phosphorus under anoxic conditions (Van Cappellen and Ingall, 1994). The interplay between anoxia, denitrification and phosphorus release present an interesting geochemical situation where N₂-fixation, a process that occurs under nutrient limitation, was a necessary component for high carbon burial rates during OAEs and other intervals of widespread marine anoxia under greenhouse conditions.

5-1 Introduction
Many ancient organic matter-rich black shales deposited under episodically anoxic or euxinic water-columns have δ¹⁵N signatures near 0‰ that are best explained by diverse primary producer communities utilizing DIN produced supplied by N₂-fixing cyanobacteria (Kashiyama et al., 2008; Kuypers et al., 2004; Junium and Arthur, 2008; Dumitrescu et al., 2006; Levman and von Bitter, 2002; Cao et al., 2009; Fulton, 2009). δ¹⁵N evidence for N₂-fixation in ancient black shales presents an intriguing problem because modern environments associated with high rates of water column denitrification do not appear to have significant diazotrophic communities and do not have low δ¹⁵N values. The δ¹⁵N record of ancient black shales supports a strong spatial and temporal
link between N$_2$-fixation and denitrification that is predicted in the modern ocean (Deutsch et al., 2007). It also suggests that of the major nutrients P and N, that P is the ultimate limiting nutrient for marine productivity (cf. Tyrell, 1998) in the Cretaceous ocean. Here we present compound specific $\delta^{15}$N data, a globally distributed bulk $\delta^{15}$N dataset and modeling results that support a global expansion of N$_2$-fixation-fueled productivity during the Cenomanian-Turonian OAE II.

5-2 Oceanic Anoxic Event II

OAE II is characterized by quasi-global deposition of black shales (Schlanger et al., 1987), enhanced marine productivity (Kuypers et al., 2002; Forster et al., 2008), and expansion of water-column anoxia and perturbation to the balance of the carbon cycle (Arthur et al., 1988) under greenhouse conditions (Forster et al., 2008; Forster et al., 2007) over a period of ~560 ka (Sageman et al., 2006). Black shale deposition, occurred in many basins globally (Figure 1) but was greatly enhanced in the near-equatorial region of the incipient North and South Atlantic Basins where organic matter accumulation rates are ~10x greater other CT black shale localities (Forster et al., 2008). Biomarker evidence demonstrates significant shifts in marine water-column microbial and phytoplankton ecology (Kashiyama et al., 2008), including communities of phototrophic sulfide oxidizing bacteria, (Forster et al., 2008; Kuypers et al., 2002; van Bentum et al., 2009) and calcareous nannoplankton that thrive in eutrophic conditions (Hardas and Mutterlose, 2007). The observed changes in water-column ecology are associated with a warming of bottom waters and a reduction of thermal stratification (Friedrich et al., 2008; MacLeod et al., 2008) that allowed for upward movement of a sulfidic, P-rich chemocline (e.g. Kump et al., 2006).

The mechanism for OAE II is a topic of active debate and invokes diverse evidence that indicate a probable increase in Carribbean large igneous province (LIP) volcanism in the period immediately preceding OAE II (Adams et al., 2010; Barklay et al., 2010) and at the initiation of the OAE (Turgeon and Creaser, 2008; MacLeod et al., 2008; Kuroda et al., 2008). Rapid decreases in $^{187}$Os/$^{188}$Os isotopic ratios (Turgeon and Creaser, 2008) and Pb-isotopic anomalies (Kuroda et al., 2008) immediately prior to the rise in $\delta^{13}$C values and continuing through the first half of OAE II suggest that LIP emplacement and associated feedbacks (e.g. warming, enhanced weathering) may have
been a trigger for the spread of anoxia in the latest Cenomanian. Further evidence for volcanic influences on water mass chemistry and changes intermediate-water circulation is suggested by a rapid rise in εNd values at Demerara Rise (MacLeod et al., 2008). Sulfur isotope data suggest an increase in the flux of volcanic sulfate in the ~600 ka prior to OAE II (Adams et al. 2010), and is associated with increases of atmospheric CO2 on the basis of stomatal idices (Barklay et al., 2010).

Transient volcanic events and the long-term greenhouse climate of the Cretaceous would have supported enhanced weathering rates under elevated atmospheric CO2 providing a higher P-flux from rivers and allowing for a larger deep water P-reservoir (Kump et al., 2000). Circulation changes coupled with an elevated P-flux to surface waters appear to have provided, in part, the necessary conditions to support enhanced productivity and black shale deposition. Primary producer communities may have also benefited from addition of volcanogenic trace metals such as Zn, Co and Cu which are significantly enriched in some CT sediments (Snow et al., 2005; Brumsack, 2006; Hetzel et al., 2009).

The restricted nature of the incipient Atlantic Basin likely supported an estuarine style of overturning circulation that enhanced nutrient trapping in the near-equatorial region, a process that is linked to the development euxinic conditions (Meyer and Kump, 2008; Meyer et al., 2008). Long-term records of organic-rich deposition on the northeast coast of South America (Erbacher et al., 2004) and in the Tarfaya Basin (Kolonic et al., 2005) suggest that regional conditions were indeed conducive to anoxia. The initiation of anoxic conditions resulted in enhanced recycling of P through Van Cappellan and Ingall-type feedbacks, as demonstrated in the resulting in the C/P relationships in CT black shales (Ingall and Jahnke, 1997; Mort et al., 2007; Nederbragt et al., 2004). This style of P-cycling has been linked to the deposition of black shales and the maintenance of water column anoxia and euxinia during the CT and other time periods (Arthur and Sageman, 2005; Meyer and Kump, 2008).

The addition of P from weathering, enhanced recycling of P through redox feedbacks and the loss of DIN through anaerobic microbial metabolism during OAE II would have resulted in a significant decrease in the marine N/P ratio below the Redfield Ratio. Similar processes are observed in the nutrient trapping system of the modern Black
Sea (Fuchsman et al., 2008), where N/P ratios of deep waters are significantly lower than Redfield averages. Over the time scale of OAE II, a global decrease in N/P would have been conducive to higher globally integrated rates of N₂-fixation, processes contingent on an elevated P-flux to surface waters where available DIN from deep waters is absent or depleted before P is fully consumed. This model is in contrast with conceptual models that invoke stratification and stagnation to promote anoxia. Rather, advection of phosphorus, and micro-nutrients such as Fe (Falkowski et al., 1998) to the surface from deep-waters stimulates N₂-fixation thus allowing for the elevated organic matter flux that is the necessary condition for long-term anoxia.

The predicted isotopic response to an expansion of N₂-fixation would be expressed in a global decrease in the average δ¹⁵N of DIN. Similar behavior is observed at the termination of the Northern Hemisphere glaciation; denitrification expands, resulting in an initial rise in δ¹⁵N values followed by an expansion in N₂-fixation responding to the net loss of fixed-N and imbalance in N and P reservoirs. The modern N-cycle displays significant spatial heterogeneity both in the concentrations and isotopic composition of DIN; the presented data and model do not require the global presence of diazotrophic communities during OAE II, merely that they are the primary source of DIN and control the ¹⁵N-abundance.

5-3 Methods and Materials
5-3-1 Bulk Analyses

Powdered samples were treated at room temperature for 24 hours with buffered acetic acid (pH 4) to remove carbonate minerals. Isotopic analyses for nitrogen and carbon 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 and He-bleed system. Data are reported using delta notation relative to atmospheric N₂ for nitrogen and the Vienna Pee Dee Belemnite International Standard (V-PDB) for carbon. Reference gases were calibrated relative to standards IAEA N1.
(0.4%) for nitrogen and ANU sucrose for carbon in combination with in-house, Devonian black shale and Peru mud isotopic standards for nitrogen and carbon. 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. Carbon isotope precision is ±0.1%.

**5-3-2 Compound Identification**

Reverse phase HPLC analysis of total acetone extracts was conducted at York University, Department of Chemistry using a Waters system (Milford, MA, USA) comprising of a 717 autosampler, 600 MS system controller and 966 photodiode array (PDA) detector. The system was controlled, and data recorded and processed using Waters Millenium 2010 software. All solvents were degassed by sparging with helium or by vacuum degassing. Separations were achieved using two Waters Spherisorb ODS2 3 μm columns (4.6 x 150 mm i.d.) in series. Aliquots of acetone extracts were analysed using a quaternary gradient elution program comprising acetonitrile, methanol, water and ethyl acetate over 85 min with a flow rate of 0.7 ml min⁻¹ (Airs et al., 2001). Determination of complexing metal was achieved by examination of online UV/vis-PDA spectra, which are diagnostic of metal type.

**LC–MSⁿ analysis** was performed using a Finnigan LCQ system comprising a Thermo Separations AS3000 autosampler, P4000 gradient pump, UV2000 UV/Vis detector and a Finnigan MAT LCQ ion trap mass spectrometer equipped with an atmospheric pressure chemical ionisation (APCI) source. Concentrated formic acid was infused into the eluent following chromatographic separation at the rate of 7 μl min⁻¹ immediately prior to introduction into the LC-MS source to prevent metallation of free-base porphyrins and chlorins within the source (cf. Airs and Keely, 2000). The interface conditions were as follows: vaporiser 450°C; capillary 150°C; discharge current 50 μA; sheath gas flow 40 (arbitrary units); auxiliary gas flow 10 (arbitrary units), collision energy 40%. Structural determinations were based on multi-stage mass spectra and comparison to spectra of authentic standards where possible.

**5-3-3 Porphyrin Purification for Isotopic analysis**

Preparation of porphyrins for isotopic analysis was adapted from 2-dimensional (reversed/normal phase) HPLC methods developed by Sachs and Repeta, (1999) and are
similar to those detailed in Kashiyama et al., (2007). Porphyrin fractions were collected from analytical reverse phase effluent (Method B of Airs et al., 2001) and dried under N₂ stream and stored at -20°C until normal phase purification. The isolated reversed phase BiCAP porphyrin aliquot is diluted in a small volume of 1:2 DCM:Hexane, typically 40ul, but adjusted based on porphyrin concentration of individual samples. Small volumes (10 μl) of the highly concentrated reversed phase fraction are injected to maintain the baseline resolution necessary for effective tetrapyrrole purification (cf. Kashiyama et al., 2007). Normal phase purification is achieved with 2, 250mm, 5 μm, 4.6mm ID Agilent Sil HPLC columns linked in series under isocratic elution at 2 ml*min⁻¹ (Figure 4-4) (Table 4-1).

5-3-4 Porphyrin δ¹⁵N

Isotopic analyses of porphyrins were conducted using a modified elemental analysis, isotope ratio mass spectrometer (EA-IRMS) system that employs a cryotrapping/capillary-column focusing method that increases the proportion of analyte gas sampled by the IRMS, and effectively increases sample peak height. Details of this method, the analytical system and its capabilities are detailed in Polissar et al., (2009) (Figure 4-5). All data are reported using standard, delta notation and calibrated within individual runs to octaethylporphyrin (Frontier Scientific), amino acids (methionine and alanine) house standards and IAEA N1, N2 and ANU-Sucrose.

Recent analytical improvements have resulted in a reduction in the size of procedural N-blank from ~80 to 20 nanomoles. Bypassing of the stock Costech-EA He regulator with He flow regulated directly from the He tank resulted in the largest decrease in the procedural blank. This allows for the use of stock EA oxidation furnaces and quartz inserts. The addition of inserts allows for the use of smooth-walled tin boats which are sonically cleaned in dichloromethane and methanol. This produces a precision of +/-1.0‰ for as little as 5 nanomoles of N and better than +/-0.5‰ for samples of 10 nanomoles N and greater for single samples, quantities that are easily isolated using analytical HPLC given sufficient porphyrin concentrations in samples. The drawback associated with use of smooth-walled tin cups, as opposed to roasted silver boats, is an increase in the size and variability of the procedural-C blank. However, the high C peaks largely reduces the influence of the C blank on the isotopic composition of samples and
standards. Precision is reduced from the system described in Polissar et al., (2009) to +/- 1.0‰ for single samples of 100 nanomoles. Though this is largely overcome through multiple analyses and the use of Keeling style plots (Keeling, 1958; Polissar et al., 2009), we are conservatively estimating the error for multiple analyses at +/- 1‰ for porphyrin δ¹³C measurements.

5-4 Results

5-4-1 Bulk δ¹⁵N Data

Here we present new bulk δ¹⁵N from Cenomanian-Turonian boundary sections from Rock Canyon, Colorado, DSDP Site 603b, Wunstorf, Germany, Oued Bahloul, Tunisia, the Danish Central Graben and and higher resolution data from ODP Site 1261 than has been reported previously (Junium and Arthur, 2007). Bulk δ¹⁵N data from OAE II sections are exclusively ¹⁵N-depleted (Figures 5-1 and 5-2) and range from +1‰ to -3‰ (Figure 5-3). The complete and well-characterized Cenomanian-Turonian boundary sections of Demerara Rise (Erbacher et al., 2005; Junium and Arthur, 2008), DSDP Site 603b, Rock Canyon, Colorado (Sageman et al., 2006) and DSDP Site 603b all show general decreases in δ¹⁵N values at the onset, or through the duration of OAE II. Notable increases in δ¹⁵N values are observed immediately prior to the rise in δ¹³C values at Demerara Rise and DSDP Site 603b; a rise in δ¹⁵N is also observed at Oued Bahloul, concomitant with the rise in δ¹³C, followed by a decrease in δ¹⁵N.

5-4-2 Porphyrin δ¹⁵N

4-3-2 BiCAP δ¹⁵N and δ¹³C

δ¹⁵Nporphyrin values are ¹⁵N-depleted compared to δ¹⁵Nbulk (Figure 5-2). N-isotope effects during the biosynthesis of chlorophyll a result in an offset between δ¹⁵Nbiomass and δ¹⁵Nchlorin (Sachs et al., 2000) and accounts, in part, for the observed differences between δ¹⁵Nbulk and δ¹⁵Nporphyrin. The Δ¹⁵Nbiomass-chlorin in modern algae averages +5‰, as determined by cultures and collected algae; this value has been confirmed in modern sediments between bulk sediments and sedimentary chlorins (Sachs and Repeta, 1999). Variability in Δ¹⁵Nbiomass-chlorin values exist between different strains of algae (Sachs and Repeta, 1999) and cyanobacteria (e.g. Beaumont et al., 2006) and differences in the dominant phototrophic community may contribute to differences in the Δ¹⁵Nbiomass-chlorin-
The $\Delta^{15}N_{\text{bulk-porphyrin}}$ values in the Demerara Rise sediments are consistent for the FB BiCAPs but offset between the different compounds (Figure 4-9).

**5-5 Discussion: The nitrogen isotopic record of OAE II organic matter**

Multiple pools of nitrogen exist in sediments that have the potential to mask primary marine productivity signals and can result in bulk $\delta^{15}N$ data that are ambiguous or misleading. Compound-specific nitrogen isotope methods that utilize chlorophyll-derived geoporphyrins for $\delta^{15}N$ analyses have the benefit of linking $\delta^{15}N$ directly to a photosynthetic source, allowing for more robust interpretations of $\delta^{15}N$ values (Sachs and Repeta, 1999). The chlorophyll $a$-derived BiCAP (Junium et al., 2008; Keely et al., 1994) $\delta^{15}N$ record through OAE II at ODP Site 1261 Demerara Rise, supports the variability in bulk nitrogen isotopic data (Figure 5-2a), and suggests that the bulk $\delta^{15}N$ record is muted and underestimates the magnitude of the $\delta^{15}N$ excursion as recorded by primary producers. However, the porphyrin $\delta^{15}N$ data affirm the validity of the $^{15}N$-depletion in bulk sediments and the variability in $\delta^{15}N$ values at Demerara Rise and suggest the same for other OAE II black shales where organic matter sources are demonstrably marine.

Covariance between $\delta^{15}N_{\text{bulk}}$ and $\delta^{15}N_{\text{porphyrin}}$ demonstrates that biomass from chlorophyll $a$-producing organisms is the most important source of N in these systems. Abundant remains of calcareous nannoplankton (Erbacher et al., 2004) and 2α-methylhopanes (Kuypers et al., 2004) produced primarily by cyanobacteria (Summons et al., 1999) suggest that these two classes of phototrophs constituted the balance of the chlorophyll $a$ production and preserved porphyrins at Demerara Rise (Junium et al., 2008). Allochthonous N sources from terrestrial organic matter or clay-bound N and organic N from phototrophic sulfide oxidizers and other organisms are therefore not significant sources of sedimentary N. Additionally, and most importantly, the BiCAP data indicate that the $\delta^{15}N$ of DIN decreased in response to OAE II at Demerara Rise.

Low $\delta^{15}N$ values during OAE II are ubiquitous and found in a wide range of depositional environments over a much of the CT-ocean (Figure 5-1). These data do not directly support the presence of diazotrophs in the proximal water column for all of the sites analyzed, although in some locations active diazotrophic communities were certainly present and prominent (Kashiyama et al., 2008; Kuypers et al., 2004). Rather, we hypothesize that the $^{15}N$-depletion reflects the cumulative influence of $\text{N}_2$-fixation.
globally, and the decreasing influence of water-column denitrification on the $^{15}$N-abundance of the global DIN reservoir. This situation allows for the local variability in the N-cycle that we observe in δ$^{15}$N records, and explains widespread $^{15}$N depletion without requiring diazotrophs to be the only sources of organic-N to sediments or present in all environments.

To explore this hypothesis, we present a one-box ocean, N-cycle, isotope mass balance model that incorporates the salient features of the nitrogen and phosphorus cycles and the feedbacks associated with anoxia (Lenton and Watson, 2000; Van Cappellen and Ingall, 1996). The model is driven by doubling of riverine phosphorus for 600ka, in agreement with proposed increases in volcanism (Turgeon and Creaser, 2008) and weathering of volcanics at the onset of OAE II. The excess P results in a 5X expansion in the extent of water column anoxia and an 8X increase in primary productivity. An 8X increase in primary productivity is in general agreement with higher organic carbon mass accumulation rates during the OAE II interval (Forster et al., 2008). The increase in P availability stimulates a doubling in N$_2$-fixation and a 3.5‰ decrease in the $^{15}$N of the global DIN reservoir (Figure 5-3).

Decreasing δ$^{15}$N values with the initial rise in δ$^{13}$C or shortly after, are evident at Demerara Rise, DSDP Site 603b (Figure 5-2), ODP Site 1138 (Meyers et al., 2008), the Levant Platform (Sepulveda et al., 2009) and Oued Bahloul (Figure 5-1) and can be explained by a decrease in the δ$^{15}$N of DIN related to N$_2$-fixation. Rock Canyon, CO (Figure 5-1), DSDP Site 367 (Kuypers et al., 2004) and Gubbio, Italy (Jenkyns et al., 2007) also show small decreases or minima in δ$^{15}$N values during the OAE II envelope, but the presence of a well-defined excursion similar to that observed at Demerara Rise or DSDP Site 603b is absent. A secular, synchronous δ$^{15}$N record at all sites is not an expectation despite the well-defined negative δ$^{15}$N excursion observed at Demerara Rise and DSDP Site 603b, and predicted by our model. Sub-euphotic zone nitrate, the reservoir that is ultimately utilized at the surface by phototrophs, ranges in $^{15}$N-abundance by ~8‰ in the modern ocean, but deep-water values converge on single value (+5‰) (Sigman et al., 2009). The range of δ$^{15}$N values for OAE II sediments and organic matter are consistent with the range of variability observed in modern δ$^{15}$N of sub-euphotic zone nitrate. However, our data suggest that the δ$^{15}$N of the DIN reservoir is
significantly lower than that of the modern ocean and more reflective of a N₂-fixation source, with limited influence from incomplete denitrification.

It could be envisioned that the expansion of anoxia at the outset of OAE II would have been accompanied by a temporary increase in δ¹⁵N from more widespread denitrification. At Demerara Rise and DSDP Site 603b δ¹⁵N values rose prior to OAE II and may reflect a temporary increase in the influence of water-column denitrification on the local δ¹⁵N of DIN. However, rising δ¹⁵N values are not characteristic at most sites (Jenkyns et al., 2007; Kuypers et al., 2004) and may not be a necessary component of developing anoxia if water-column denitrification was counteracted rapidly by increasing N₂-fixation, or if the primary change in local nutrient availability was driven initially by an increase in phosphorus availability (e.g. Mort et al., 2007), as is depicted in the model (Figure 5-4). The minima in δ¹⁵N values observed at Demerara Rise, Rock Canyon and elsewhere (Jenkyns et al., 2007; Kuypers et al., 2004; Meyers et al., 2009) are lower than what is expected for δ¹⁵N values for diazotroph biomass and may reflect local recycling of NH₄⁺ from the chemocline into the photic zone (Junium and Arthur, 2007).

Alternatively, high Fe availability has been shown to result in δ¹⁵N values as low as -4‰ for diazotroph biomass (Zerkle et al., 2008) and proximity to relatively high concentrations of Fe from the chemocline (Lewis and Landing, 1991) could impact N-fractionation in diazotrophic organisms. The variable characteristics of the δ¹⁵N records at different localities underscores the influence of local water-column conditions and the trend to more ¹⁵N-depleted values during OAE II supports a global drop in the δ¹⁵N of DIN.

5-6 Conclusions

Low δ¹⁵N values are common for many locations through the mid-Cretaceous (Rau et al., 1987; Junium and Arthur, 2007; Kuypers et al., 2004; Meyers et al., 2009; Dumitrescu et al., 2006) suggesting that N₂-fixation was a more important source of DIN for carbon-fixation than in the modern ocean. Increased seafloor area by as much as 10% under high eustatic sea level (Arthur et al., 1987) would have allowed for higher rates benthic denitrification, greenhouse climate would have resulted in lower O₂ solubility and higher rates of denitrification. Coupled with higher weathering rates, elevated P-fluxes and continental configurations that were conducive to estuarine styles of circulation, it is
possible that the mid-Cretaceous ocean was predisposed to higher overall rates of N2-fixation and is reflected in the $\delta^{15}N$ record.

Our findings also suggest that higher rates of N2-fixation are required components of widespread anoxia and intervals of enhanced carbon burial such as Oceanic Anoxic Events. Loss of fixed-N from anoxic water-columns exerts a negative feedback on productivity that is rapidly overcome by enhanced P-recycling stimulating N2-fixation. The link between higher CO$_2$ and expansion of anoxic water bodies during OAE II also has implications for future climates. A substantial increase in the area of oxygen-depleted waters is predicted as a direct result of CO$_2$-induced warming (Keeling et al., 2010). The record of the N-cycle response to climatic events such as OAE II suggests that a warming future Earth has the potential to greatly alter the balance of the marine N and C cycles.

5-7 References


Junium, C. K., and M. A. Arthur. 2007. Nitrogen cycling during the cretaceous, Cenomanian-Turonian oceanic anoxic event II. *Geochemistry Geophysics Geosystems* 8,


Kuypers, M. M. M., R. D. Pancost, I. A. Nijenhuis, and J. S. S. Damste. 2002. Enhanced productivity led to increased organic carbon burial in the euxinic North Atlantic basin during the late Cenomanian oceanic anoxic event. *Paleoceanography* 17, no. 4:.


Figure 5-1. Plate tectonic reconstruction of Cenomanian-Turonian time (93.5 Ma) adapted from data provided by ODSN.com. Gray area represents the approximate aerial extent of organic matter rich sediments of OAE II age. The dark gray area marks an area that is characterized by elevated OM accumulation rates during OAE II and very thick sequences of black shales. Numbered squares correspond to data table, raw data from Rock Canyon, CO, DSDP 603b, Oued Bahloul, Tunisia, Danish Central Graben, and Wünstorf, Germany are available in the supplementary material.
Figure 5-2. $\delta^{15}N_{\text{bulk}}$ and $\delta^{13}C_{\text{org}}$ records from ODP Site 1261a and DSDP Site 603b, exact locations are detailed in Figure 1. The OAE II interval is delineated by the gray bar on the basis of $\delta^{13}C_{\text{org}}$ data (Sageman et al., 2006). Y axes correspond to meters below sea floor (MBSF). The molecular structure in panel A is bicycloalkano-porphyrin (BiCAP) and is distinguished from other cycloalkano porphyrins by the 7-membered ring. Compound specific $\delta^{15}N$ data from the BiCAPs are offset from $\delta^{15}N_{\text{bulk}}$, a trend that is observed in modern marine phototrophs. Whole cell biomass of marine algae is typically $^{15}N$-enriched relative to chlorophyll $\delta^{15}N$ with the average $\Delta^{15}N_{\text{chl-biomass}}$ being -5‰, with values ranging from -3 to -10‰ (Sachs et al., 1999).
Figure 5-3. Stable isotope data from Wunstorf, Germany, the Danish Central Graben and Oued Bahloul, Tunisia. The gray shaded regions delineate OAE II on the basis of $\delta^{13}$C data and referenced to the CT stratotype $\delta^{13}$C of Rock Canyon, CO (Sageman et al., 2006).
Figure 5-4. Box model results of a doubling of P-flux for 600 ka; the results are a 2.6x increase in N$_2$-fixation, a 2.3x increase in denitrification, and an 8x increase in C-burial. The isotopic response is a 2.75‰ drop in $\delta^{15}$N of DIN, in agreement with observations.
Chapter 6: Controls on bulk and compound specific $\delta^{15}\text{N}$ and pigment distributions in surface sediments of the Peru Margin

Abstract

Lateral and downslope transport of organic matter by bottom currents on the Peru Margin results in a decrease in bulk OM quality in surface sediments through the oxygen minimum zone (OMZ). Indicators of bulk organic matter quality (pyrolysis hydrogen index, pyrolysis S2 and C/N) demonstrate the most significant degradation between 150 and 400 m water depth. Concentrations of the three most abundant chlorophyll derivatives (chlorophyllone, pheophytin and pyropheophytin) decrease from 750 to 150 nanomoles*g organic carbon$^{-1}$ from 150 to 400 meters water depth. The abundances of the chlorins relative to each other do not change significantly with depth, supporting a common source from shelf sediments for the chlorins and indicating that they have similar reactivities. $\delta^{15}\text{N}_{\text{bulk}}$ values decrease by 3‰ from the inner shelf to the upper slope (1000m); co-occurring $\delta^{15}\text{N}_{\text{chlorin}}$ values show no decrease in $^{15}\text{N}$ abundance downslope. We attribute the decreasing $\delta^{15}\text{N}_{\text{bulk}}$ values to degradation of a $^{15}\text{N}$-enriched fraction such as proteins during downslope transport in a low-oxygen setting. The low variability of $\delta^{15}\text{N}_{\text{chlorin}}$ values supports a single, shelf source for the chlorins, and demonstrates that despite significant reduction in chlorin concentrations downslope, their primary $\delta^{15}\text{N}_{\text{chlorin}}$ values remain unaltered. These data demonstrate that in active sedimentary environments, such as the Eastern Tropical Pacific, transport of organic matter can significantly alter geochemical parameters used for paleoceanographic reconstructions.

6-1 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., 1995 Kienast et al., 2002) and are used as an analog for deposition of ancient organic matter-rich sediments (e.g. Algeo et al., 2008). Many of these studies rely upon nitrogen isotopes and concentrations of chlorophyll derivatives (chlorins) (Liu et
and are integral to our understanding of the impacts of rapid climate change on ocean ventilation and paleoproductivity.

Arthur et al., (1998) demonstrated that organic matter of the Peru Margin less well preserved than would be expected despite low dissolved oxygen (<5 μmol/kg) within the water column and impinging upon much of the sea bottom. This is attributed to advection of low concentrations of dissolved oxygen and nitrate, activity of low-oxygen tolerant organisms, and particularly, resuspension and lateral transport of organic matter by strong bottom currents at the shelf break. Nephloid transport of organic matter is a process that has been recognized in other localities such as the Benguela Upwelling system of offshore Namibia (Inthorn et al., 2006; Mollenhauer et al., 2008) and elsewhere (e.g. Mollenhauer et al., 2006; Ohkouchi et al., 2002). These processes effectively extend the residence time of sediments at the sediment water interface and exposure time to oxidant (dissolved oxygen, nitrate and sulfate), thereby allowing for progressive degradation that negatively impacts the fidelity of paleoceanographic proxies.

The preservation of primary δ¹⁵N signals in ancient sediments has been an issue of discussion and concern for studies utilizing δ¹⁵N data (e.g. Altabet et al., 1999; Milder et al., 1998; Sachs et al., 1999; Junium and Arthur, 2007). ¹⁵N-enrichment of sinking organic matter in oxic water-columns have been described (Altabet and Francois, 1994; Fruedenthal et al., 2001) however, in low-oxygen settings, water-column diagenetic alteration is generally assumed to have a negligible effect on δ¹⁵N values. No studies have focused on how nitrogen isotope values vary under the range the conditions that are present in core-top sediments down-slope in a modern upwelling zone. The background of previous research on Peru Margin sediments provides an excellent locality to determine the influence of lateral and downslope transport on nitrogen isotopes and the preservation of chlorophyll a-derived chlorins used for compound specific δ¹⁵N studies in recent (Sachs et al., 1999) and ancient sediments (Kashiyama et al., 2008).

Here we present δ¹⁵N and δ¹³Corg from bulk sediments and photosynthetic pigments, and chlorin concentrations from core top sediments of the Peru Margin from two depth transects that intersect the oxygen minimum zone (OMZ). Our goal is to document the impact of lateral transport on the preservation of δ¹⁵N and chlorophyll derivatives. We then discuss the implications for bulk nitrogen isotope and pigment
paleo-data in modern and recent upwelling zones.

6-2 Materials and Methods

6-2-1 Material Collection and Preparation.

Samples were recovered with submersible executed push cores and ship-deployed box cores on two transects at 12º and 13.5º south latitude during December-January of 1991 and 1992. Retrieved box cores and push cores were sub-sampled and refrigerated or frozen shipboard. Samples were freeze-dried, homogenized and archived in The Pennsylvania State University, Department of Geosciences, Sedimentary Geology Lab until analysis. Sampling was focused on the inner-shelf (~100m) through the upper-slope to 1070 m. From the inner-shelf to the upper-most slope the water column is largely anoxic and the oxygen minimum zone impinges upon the bottom (Figure 1). Below ~600 m in both transects oxygen concentrations rise, and reach ~50 μmol/kg by 1000 m.

Sediment characteristics are generally controlled by the presence of bottom currents and the degree of phosphogenesis (Arthur et al., 1998; Arthur and Dean, in review). Inner-slope settings are dominated by mud surfaces with common Thioploca microbial mats (Hogslund 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 ranges from mud to phosphorite crusts and glauconite sands.

6-2-2 Bulk Geochemical Parameters

Freeze dried samples were treated at room temperature for 24 hours with buffered acetic acid (pH 4) to remove carbonate minerals. Isotopic analyses for nitrogen and carbon 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 1020ºC 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 and the Vienna Pee Dee Belemnite International Standard (V-PDB) for carbon. Reference gases were calibrated relative to standards IAEA N1 (0.4‰) for
nitrogen and ANU sucrose for carbon in combination with in-house, Devonian black shale and Peru mud isotopic standards for nitrogen and carbon. 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. Carbon isotope precision is ±0.1‰. Carbon and nitrogen percentages on the decarbonated fraction were produced on a Costech Elemental analyzer in conjunction with isotopic measurements. Carbon and nitrogen isotopic peak heights were calibrated to acetanilide (Costech) and Devonian black shale and Peru Mud standard of known elemental composition with a standard error of ±0.1wt% for carbon and ±0.1 wt% for nitrogen. C/N values are reported as atomic ratios. RockEval 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). The principle pyrolysis data discussed are Hydrogen Indices (HI) and S2. HI is mg of hydrocarbon per gram of organic carbon and S2 is mg of hydrocarbon per gram of sediment. HI values are the most commonly reported bulk pyrolysis data, but S2 values are useful in that they are independent of TOC.

6-2-3 Pigments

Freeze-dried sediments were sonically extracted in acetone to clarity under low-light and low temperature conditions. 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 i.d x 150mm; 3 μm stationary phase) linked in series with a Phenomenex, 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 (APCI) source. Chlorin quantification was achieved via on-line UV-Vis absorbance data (665μm). 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.

6-2-4 Compound-specific δ¹⁵N and δ¹³C analyses of pigments

Six samples, frozen since the time of sampling, were selected for compound-specific isotope analyses of the three most abundant pigments (chlorophyllone, pheophytin and pyropheophytin) from 130 to 989m water-depth (Figure 2). The employed 2D-HPLC purification has been shown to be a very effective way to purify pigments from isotopic analysis (Sachs and Repeta, 2000) and base line resolution between peaks in the normal phase purification step (detailed below) is necessary to assure compound purity (Kashiyama et al., 2007).

Samples were fraction collected from quaternary gradient reversed phase effluent (Figure 2) and dried under N₂ stream and frozen until further purification. Normal phase purification for pheophytin and pyropheophytin were adapted from Sachs et al.,(2000) and utilize an isocratic solvent composition of 8% acetone in hexane at 2ml*min⁻¹ on 2, 25cm Agilent 5µm Sil analytical columns linked in series. Concentrated samples were dissolved in 8% acetone in hexane and injected in 20-40ul 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. The isocratic solvent composition and gradient will need 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 was achieved with baseline resolution using a 20% acetone in hexane solution on a 15cm Restek 5µm amino column under isocratic conditions (Figure 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 the C/N ratio. Samples for which the C/N ratios were not consistent with pure chlorins were not considered (cf. Sachs and Repeta, 2000).
Isotopic analyses of porphyrins were conducted using a modified elemental analysis, isotope ratio mass spectrometer (EA-IRMS) system that employs a cryo-trapping/capillary-column focusing method that increases the proportion of analyte gas sampled by the IRMS, and effectively increases sample peak height. Details of this method, the analytical system and its capabilities are detailed in Polissar et al., (2009) (Figure 5). All data are reported using standard, delta notation and calibrated within individual runs to octaethylporphyrin (Frontier Scientific), amino acids (methionine and alanine) house standards and IAEA N1, N2 and ANU-Sucrose.

Recent analytical improvements have resulted in a reduction in the size of procedural N-blank from ~80 to 20 nanomoles. Bypassing of the stock Costech-EA He regulator with He flow regulated directly from the He tank resulted in the largest decrease in the procedural blank. This allows for the use of stock EA oxidation furnaces and quartz inserts. The addition of inserts allow use of smooth-walled tin boats which are sonically cleaned in dichloromethane and methanol. This produces a precision of +/-1.0‰ for as little as 5 nanomoles of N and better than +/-0.5‰ for samples of 10 nanomoles N and greater for single samples, quantities that are easily isolated using analytical HPLC given sufficient porphyrin concentrations in samples. The drawback associated with use of smooth-walled tin cups, as opposed to roasted silver boats, is an increase in the size and variability of the procedural-C blank. However, the high C peaks largely reduces the influence of the C blank on the isotopic composition of samples and standards. Precision is reduced from the system described in Polissar et al., (2009) to +/-1.0‰ for single samples of 100 nanomoles. Though this is largely overcome through multiple analyses and the use of Keeling style plots (Keeling, 1958; Polissar et al., 2009), we are conservatively estimating the error for mutiple analyses at +/- 1‰ for porphyrin δ13C measurements.

6-3 Results

6-3-1 TOC, HI and S2

Total organic carbon (TOC), Rock-Eval pyrolysis and δ13C data are originally reported in Arthur et al., (1998). Here we present merged data from the two transects in combination with new bulk and pigment δ15N and chlorin abundances to provide better data coverage over the range of depths sampled. Arthur et al., (1998) demonstrated that
similar patterns in the distribution of TOC and sediment type exist between the two transects. Additionally, average HI (382 and 393), and S2 vs. TOC slopes suggest that OM sources are very similar. For these reasons we combine the two datasets, which foster analysis of down-slope nitrogen and pigment trends. TOC and HI values are highest on anoxic shelf and decrease across the shelf-break into the anoxic slope environment (Figure 4). Outlier TOC values from the inner-shelf are associated with *Thioploca* microbial mats. Elevated TOC and HI values below the OMZ may represent redeposited material from up-slope (Figure 4). Rock-Eval S2 data (hydrocarbons per gram dry weigh released during kerogen cracking) are useful for comparing 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 S2, rather than HI for comparison to other geochemical parameters. S2 values range significantly over the upper 400 m, but below 200 m S2 values drop rapidly to values below 5. S2 outliers found below 400 m are associated with TOC-rich samples that were potentially recently redeposited from upslope.

**6-3-2 C/N Ratios**

In sediments where the organic matter is predominantly of marine origin, variability in C/N ratios may be used as an indicator of differential preservation of C and N pools (Junium and Arthur, 2007; Freudenthal et al., 2002). C/N ratios of phytoplankton biomass range from 4 to as high as 10 and degradation under most conditions results in rising C/N ratios of the residual biomass. If degrading phototrophic biomass is preserved as heterotrophic bacterial organic matter, C/N ratios can rise less significantly or decrease (Lehmann et al., 2001). Additional factors such as sorption of ammonium from degrading organic matter onto clays in sediments can result in C/N ratios that appear to indicate fresh, unaltered organic matter when in fact organic matter preservation is very poor (e.g. Peters, 1978). Thus, the sediment composition, geochemical conditions and the primary C/N of phototrophic biomass can strongly govern C/N values preserved in sediments, and care is required when interpreting C/N data. In the Peru Margin, sedimentary C/N ratios gradually increase offshore with greater water depth. Shelf C/N values average 11, and at the shelf break reach values as high as 21 at 400 m and remain elevated through the anoxic region of the upper-slope (Figure 5). At the oxic/anoxic transition at ~550 m C/N values drop to values below 15.
6-3-3 Bulk $\delta^{15}$N and $\delta^{13}$C

Inner-shelf and upper-slope $\delta^{15}$N values range between +6.5 and +10.9‰. Shelf values average +8‰, drop slightly from the shelf to the upper-most slope and return to values of ~+8‰ at 350 m. $\delta^{15}$N values drop to less than +6‰ below 700 m (Figure 5). $\delta^{13}$C values range from -19.6 to -21.8 with values gently decreasing 1‰ from the shelf break through to 1100 m (Figure 3). The observed $\delta^{15}$N and $\delta^{13}$C values are typical of surface sediments and particulates from upwelling zones (Pancost et al., 1997; Altabet et al., 1999).

6-3-4 Geochemical Parameter Relationships

6-3-4-1 S2 Data

S2 data reveal relationships with C/N, $\delta^{13}$C$_{org}$ and $\delta^{15}$N (Figure 6). Where S2 values are greater than 20, C/N ratios range narrowly between 12 and 9; for S2 values below 20, C/N values range from 10 to 30 and trend lower as S2 decreases. Similar trends are observed in $\delta^{13}$C$_{org}$ where variability for shelf samples is limited and values are $^{13}$C-enriched; and $\delta^{13}$C$_{org}$ decreases when S2 drops below 20. $\delta^{15}$N values have a slightly more complex trajectory; values become slightly enriched as S2 values drop from 60 to 30 but decrease as S2 values drop below 20.

6-3-4-2 $\delta^{15}$N Data

$\delta^{15}$N values display relationships with %N, C/N, $\delta^{13}$C$_{org}$, and HI, in addition to S2. The trend between %N and $\delta^{15}$N is very similar in appearance to the relationship between S2 and $\delta^{15}$N (Figure 6). $\delta^{15}$N values increase slightly as %N drops from 3% to 1% and decrease below 1% N. When % N is less than 0.7% N, $\delta^{15}$N values drop below 6.7‰; a similar trend is also observed for HI, where most $\delta^{15}$N values that are lower than 6.7‰ have corresponding HI values that are below 225. A positive relationship between $\delta^{15}$N and $\delta^{13}$C$_{org}$ is also observed. A general trend of increasing C/N with increasing $\delta^{15}$N is observed; however, the relationship is not especially strong (Figure 7). Relationships between C/N and $\delta^{15}$N have been observed in other organic matter-rich settings such as Cretaceous black shales (Junium et al., 2007) or Mediterranean Sapropels (Milder et al., 1999). However, in Cretaceous examples, elevated C/N values correspond to better organic matter preservation, indicated by a positive relationship between HI and C/N.
6-3-5 Chlorins

6-3-5-1 Compound Identification.

HPLC-UV/Vis and LC-MS 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 (Figures 2 and 8). All chlorins here are identified on the basis of strong absorbance at 655nm and elution order. Structures were confirmed using LC-MS$^n$ spectra compared to published results (Airs et al., 2001). Two closely eluting peaks at 11 and 12 minutes (Figure 2, peaks 1 and 2) display full mass spectra that are dominated by single ions at m/z 533 which are consistent with protonated masses [M/H$^+$] of chlorophyllone epimers (Harris et al., 1995; Mawson and Keely, 2007). 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. Two small peaks eluting at 43 and 44 minutes (Figure 2, peaks 3 and 4) have full mass spectra that are dominated by a single ion at m/z 887 and an MS$^2$ spectra indicating loss of the esterifying alcohol phytol, and are consistent with protonated masses [M/H$^+$] of hydroxychlorophyll $a$ epimers (Walker et al., 2002).

Peak 5 (Figure 2) has a full mass spectra displaying a single ion at m/z 871 and a MS$^2$ spectra consistent with the loss of phytol and is consistent with protonated masses [M/H$^+$] of pheophytin $a$. Peak 7 (Figure 2) has a full mass spectra at m/z 814 and an MS$^2$ spectra indicating the loss of phytol and is consistent with protonated masses [M/H$^+$] of pyropheophytin $a$. No Mg-chlorophylls, phaeoporphide or non-chlorophyll $a$ tetra pyrroles, 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, however since the goals of our study are focused upon the factors controlling the preservation of most abundant tetra pyrroles, these other compounds will not be discussed.

6-3-5-2 Chlorin Abundances

Chlorophyllone is the most abundant chlorin in all samples with an average chlorophyllone/(pheophytin + pyropheophytin) ratio of 3.7 +/-1.5. The most abundant pigment concentrations are present at the most shallow, inner slope sites (Figure 8). Pigment concentrations decrease with increasing water-depth, with a sharp decrease in
pigment concentrations at the slope break. The chlorophyllone/(pheophytin + pyropheophytin) ratios do not reveal relationships with other geochemical parameters that are significant for the number of samples analyzed.

6-3-6 Chlorin $\delta^{15}$N and $\delta^{13}$C

$\delta^{15}$N$_{chlorin}$ values for chlorophyllone, pheophytin and pyropheophytin average 0.2‰, range between -1 and +1.6‰ (Figure 9). They display a strong correspondence between the different structures and show no trend with increasing water depth and distance offshore (Figure 9). The similarity between the pheophytins and chlorophyllone $\delta^{15}$N$_{chlorin}$ supports a chlorophyll $\alpha$ source for chlorophyllone. The offset between $\delta^{15}$N$_{bulk}$ and $\delta^{15}$N$_{chlorin}$ is typical of chlorophyll derivatives and is the result of biochemical fractionation during chlorophyll biosynthesis (Sachs et al., 2000). The canonical value for $\Delta^{15}$N$_{biomass-chlorin}$ in modern algae is +5‰, a value that has also been observed between bulk sediments and chlorin (Sachs et al., 1999; Bidigare et al., 1991). The $\Delta^{15}$N$_{bulk-chlorin}$ for Peru Margin sediments averages +7‰ and is within the range of observed $\Delta^{15}$N$_{bulk-chlorin}$ values (Sachs et al., 2000). $\delta^{13}$C$_{chlorin}$ values decrease generally with depth but there is significant scatter in the data (Figure 10). $^{13}$C-enrichment of chlorophyllone relative to pheophytin and pyropheophytin is expected and results from the loss of $^{13}$C-depleted phytol.

6-4 Discussion

6-4-1 Bulk and Chlorin $\delta^{15}$N and $\delta^{13}$C

Bulk sedimentary $\delta^{15}$N data in upwelling zones such as those of the Eastern Pacific (e.g. Ganeshram et al., 2002) and Arabian Sea (Altabet, 1995) are $^{15}$N-enriched relative to the average $^{15}$N-abundance of marine nitrate (~+5‰), and there is a strong correspondence between sub-euphotic zone $\delta^{15}$N$_{nitrate}$ and the $\delta^{15}$N of underlying sediments (Thunnel et al., 2004). These data demonstrate that sedimentary $\delta^{15}$N accurately records the $^{15}$N abundance of dissolved inorganic nitrogen (DIN) and that the enriched $\delta^{15}$N values in the sediments of upwelling zones reflect N-isotope effects imparted during water-column nitrate reduction (e.g. Mariotti et al., 1981). The importance of other anaerobic metabolisms that result in net loss of fixed-N species, such as anaerobic oxidation of ammonium, certainly play a significant role in the isotopic
evolution of DIN within upwelling zones since it is apparent that they play a very significant role in N-loss in OMZs (e.g. Kuypers et al., 2005).

These observations allow for interpretation of down-core $\delta^{15}N$ records in similar environments with respect to the balance of the N-cycle and links to the global climate over the last 4 Ma (Ganeshram et al., 2000; Altabet et al., 1999; Altabet et al., 2004). The fact that the observed trends are replicated regionally and globally provides further evidence that the observed data reflect primary signals and supports their use to quantify changes in the N-cycle in deterministic models (e.g. Meissner et al., 2005). Modeling efforts provide important benchmarks for understanding the glacial to interglacial transitions, and provide important test cases for understanding future change (e.g. Schmittner et al., 2009). If considered on their own, the decreasing bulk $\delta^{15}N$ values we observe in surface sediments could be the result of primary changes in surface water nutrient conditions despite evidence for decreasing organic matter quality downslope (Arthur et al., 1998). A decrease in water-column denitrification or nitrogen fixation offshore (cf. Deutsch et al., 2007) could result in $^{15}N$-depletion of NO$_3^-$ outboard of the shelf, thus accounting for the decreasing bulk $\delta^{15}N$ data. However, $\delta^{15}N_{chlorin}$ data suggest that phototrophic biomass preserved in these Peru Margin surface sediments does not vary significantly in $^{15}N$-abundance offshore (Figure 9). This fact suggests that bulk $\delta^{15}N$ measurements in surface sediments from the Peru Margin are not recording primary, phototrophic signals and thus may not be used directly for constraining N-cycle models. It is possible that bulk organic matter is at least partially sourced from the overlying water and pigments are derived from shelf material with a consistent $\delta^{15}N$. The correlation of decreasing bulk $\delta^{15}N$ with decreasing bulk $\delta^{13}C$ (Figure 7) suggests that the variability in both parameters reflects the decrease in productivity and reduced nitrate utilization offshore.

On the basis of S2 and HI values (Arthur et al., 1998) and C/N ratios, OM quality decreases significantly downslope, and over the same interval bulk $\delta^{15}N$ values decrease by 3‰ (Figures 4,5). Our suggestion that $\delta^{15}N$ variability in high-productivity, low-[O$_2$] environments is the result of diagenesis is contrary to the accepted paradigm that change in $\delta^{15}N$ values is negligible, and that diagenetic reactions would result in $^{15}N$-enrichment. Indeed, decreasing $\delta^{15}N$ values are not typical of canonical diagenetic reactions. Early
diagenetic $^{15}$N-enrichment is well described in sinking particles and sediments under oxidizing conditions during microbial degradation (Altabet and Francois et al., 1994; Fruedenthal et al., 2001; Gaye-Haake et al., 2005). However, under anoxic conditions $^{15}$N-depleted ammonium is released and fixed on and within clays and can result in $^{15}$N-depletion of bulk N (Lehmann et al., 2001).

An additional possibility is selective degradation of more reactive pools of organic matter under the anoxic conditions present in the OMZ such as $^{15}$N-enriched amino acid-N (Figure 4). Degradation of amino acid nitrogen has been shown to be the most important source of inorganic nitrogen in the water column and sediments (Burdige and Martens, 1988; Pantoja and Lee, 2003), and in OMZ environments proteins and amino acids are targeted by heterotrophic denitrifying bacteria as a carbon source (van Mooy et al., 2002). Percentages of N present as amino acids ($\%T_{AA}N$) in surface sediments of upwelling zones can be as high as 70% of the total sedimentary N (Pantoja and Lee, 2003). 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 (Figure 11). Amino acid nitrogen is typically $^{15}$N-enriched relative to bulk biomass (Macko et al., 1986) and selective degradation or protein-N has the potential to impart diagenetic $^{15}$N-depletion on organic-N in the water column and sediments.

While the overall trajectory of $\delta^{15}$N values trends toward $^{15}$N-depletion with greater depth, higher values are observed from 200 to 400m. The rapid drop in S2 at the shelf break is matched by an increase of 1.5‰ in $\delta^{15}$N and 10 in C/N before $\delta^{15}$N values drop sharply by 2‰ (Figures 4 and 5). The rise in $\delta^{15}$N is associated directly with a sharp decrease in the S2 values suggests $^{15}$N-enrichment takes place during diagenesis. However, the lower slope data indicate a complete removal of this pool of $^{15}$N-enriched organic matter, presumably protein and amino acids, leaves the residual OM $^{15}$N-depleted. The source of the $^{15}$N-depletion is also likely to be ammonium sorbed to mineral surfaces. Degradation of organic N releases the $^{15}$N-depleted ammonium which fills available sites on mineral sources. As N-degradation continues the ammonium becomes progressively more $^{15}$N-enriched but is not retained because mineral surfaces are have reached their sorptive capacity (Freudenthal et al., 2001). If a majority of the N
in degraded sediments remains as ammonium-N, the $\delta^{15}$N values are expected be lower than primary organic-N (Rau et al., 1987).

The concept of oxygen exposure time (Hedges and Keil, 1995; Hartnett et al., 1998; Hedges et al., 1999) has been utilized in many studies to describe the progressive degradation of organic matter in oxidizing marine setting with time (e.g. Demaison and Moore, 1980). In environments where molecular oxygen is not present, or is present in very low concentrations, oxidant can be plentiful in the form of MnO$_2$, FeOOH, NO$_3^-$ and SO$_4^{2-}$ (Aller, 1993; Froelich et al., 1979) Heterotrophic microbial degradation of organic matter using MnO$_2$, FeOOH, NO$_3^-$ and SO$_4^{2-}$ as electron acceptors is very efficient and can result in significant degradation of organic matter in anoxic environments until the buildup of water column or pore water sulfide (cf. Canfield, 1989; Canfield, 1994).

Below the sediment-water interface in marine sediments, sulfate concentrations deplete rapidly, effectively decreasing the efficiency of microbial degradation by limiting microbial metabolisms to less efficient, fermentative pathways. Preservation of organic matter is therefore enhanced by limiting the oxidant exposure time not simply molecular-O$_2$.

Lateral transport of sediment near the sediment-water-interface has been detailed in many marine environments (Arthur et al., 1998; Ganeshram et al., 1999; Inthorn et al., 2006) and explains the presence of surface sediments with anomalous age distributions owing to resuspension and transport of particles (Ohkouchi et al., 2002; Mollenhauer et al., 2006). Arthur et al., (1998) demonstrated that organic matter from Peru Margin surface sediments is more poorly preserved than would be expected despite the low-oxygen conditions ($<5 \mu$mol/kg). This is attributed to benthic currents that advect low concentrations of dissolved oxygen and high concentrations of nitrate, activity of benthic organisms, and lateral transport of organic matter by strong bottom currents up to 30 cm/s that initiate at the shelf break. These processes extend residence time or organic matter at or above the sediment water interface and progressively degrade organic matter. Additional factors such as winnowing and hydrodynamic sorting of size fractions and types of organic matter during transport could increase heterogeneity could further impact downslope trends (Bergamaschi et al., 1997). Regardless of the relative role of sorting or exposure time, it is clear that organic matter quality decreases significantly at
the shelf break due to extended exposure to oxidant, primarily NO$_3^-$, despite anoxic conditions in the heart of the Peru Margin OMZ. The uniformity of $\delta^{15}$N$_{chlorin}$ data suggest that the analyzed chlorins share a common source from shelf sediments and were transported to depth by lateral currents driving net transport down-slope.

Overprints on bulk $\delta^{15}$N values are also encountered where the addition of chemotrophic microbial biomass is significant. Two $^{15}$N-enriched samples from the inner-shelf stand out in our dataset (Figure 5); they are relatively TOC-poor but have very high S2 and HI values, indicating high hydrocarbon concentrations and good preservation of organic matter. These two samples are associated with significant contributions from *Thioploca* biomass; visible as white microbial filaments in uncrushed samples. *Thioploca* oxidize sulfide with nitrate and are known to accumulate high concentrations of nitrate within their cells (Fossing et al., 1995). The elevated $\delta^{15}$N values are caused by fractionation during reduction of nitrate by *Thioploca* communities. *Thioploca* form dense mats over large areas of the Peru and Chile oxygen minimum zone at around 200-400 m water depth (Hogland et al., 2009) and if their biomass is indeed a significant contributor to ancient organic matter, their signature is capable of altering primary $\delta^{15}$N signals, as we observe.

The impact of OM degradation on bulk $\delta^{13}$C is less clear than $\delta^{15}$N. Decreasing $\delta^{13}$C of particulate organic carbon and core-top sediments downslope (Figures 5 and 12) could be the result of primary changes in phytoplankton $\varepsilon_p$ or community ecology in the overlying water column (Pancost et al., 1997; Pancost et al., 1999). The $\delta^{13}$C values of phytol and POC decrease slightly offshore (Pancost et al., 1999) suggesting that changes in the $^{13}$C composition of OM in overlying surface waters may have control on the $\delta^{13}$C of sediments (Figure 12), but there is significant scatter in the data. Slope sediments are likely to be an admixture of material derived from rainout and reworked shelf organic matter, but the $\delta^{15}$N$_{chlorin}$ values suggest that most of the chlorins are derived predominantly from the shelf. $\delta^{13}$C$_{chlorin}$ values display scatter that is similar to phytol and POC (Figure 10). Sediment bulk $\delta^{13}$C values follow a similar trend to bulk $\delta^{15}$N in that they decrease significantly (1‰) for corresponding S2 values that are below 20. This suggests that OM degradation can also impact bulk $\delta^{13}$C values for surface sediments through selective degradation or hydrodynamic sorting during transport.
6-4-2 Chlorin Distribution and Downslope Trends

In all samples, chlorophyllone is present as two enantiomers (Figure 2, Peaks 1 and 2), and is the most abundant chlorin, with pyropheophytin and pheophytin following in abundance (Figure 8). Bi-cyclo-chlorins, including chlorophyllone are commonly found in water column particulates, (e.g. Walker et al., 2004), core-top sediments (Chillier et al., 1993; Harris et al., 1995; Ocampo et al., 2000) and occasionally in more ancient sediments (Mawson et al., 2007; Junium, Chapter 2), and are the probable precursors of bicyclo-alknoporphyrins (BiCAPs). Their occurrence in modern settings appears to be associated directly with zooplankton herbivory (Goericke et al., 2000; Watanabe et al., 1993; Walker and Keely, 2004) suggesting that the chemical conditions necessary for cyclization are probably favorable within the guts of zooplankton. Fecal pellets and marine snow are the primary initial delivery mechanisms for organic matter to the sediment water interface in the Peru Margin (Arthur et al., 1998; Arthur and Dean, in review) and most upwelling zones (Staresnic et al., 1983). The high ratio of chlorophyllone to pheophytin and pyropheophytin (3.7 +/- 1.5) in Peru surface sediments suggests that at least 50-90% of phototrophic biomass in sediments was consumed by herbivores and delivered as fecal material or directly as the bodies of herbivorous organisms. Goericke et al., (2000) suggested that chlorophyllone is formed following the degradation of 132-175cyclopheophorbide a-enol during solvent extraction. Indeed, 132-175cyclopheophorbide a-enol has been shown to be present in the extracts of sediments from the Peru Margin (cf. Ocampo et al., 1999) but at relatively low concentrations compared to the other chlorins. Additionally, the abundances of the two chlorophyllone peaks in Peru Margin sediments are dominated by the 132(S) isomer which is a result of enzymatic processes associated with herbivory, not an operational artifact (Aydin et al., 2003).

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 allow for greater abundances of the more functionalized pheophytins and pyropheophytins than chlorophyllone (Figure 8). This suggests that the transformation of chlorophyll a to chlorophyllone, and the relative abundances of chlorophyllone, pheophytin and pyropheophytin are controlled in the upper, oxic region.
of the water-column, and that the reactivity of the three most abundant chlorins in the geochemical conditions present in the sampled interval are similar for those three structures.

The decrease in chlorin concentration at 200m appears to be the result of degradation associated with resuspension and extended residence time of organic matter at or above the sediment water interface. These data are consistent with S2 values that decrease by a factor of 6 at depths between 200 and 400m (Figure 8). It is surprising that the 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. At low concentrations of dissolved oxygen (< 5 μM), 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 in poorly, chlorophyllone remained in low concentrations (Figure 8). 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 expedited delivery of fecal pellets delivery to sediments. The large decrease in chlorin concentration is not directly associated with a large drop in productivity. Application of the Chlorin Index (CI) to the geologic record has been useful for characterizing changes in surface water phototrophic productivity (e.g. Higginson et al., 2003). However, in sediments where there are large changes in preservation downcore, particularly associated with variability in current activity (e.g. Ganeshram et al., 1999), application of the CI may be inappropriate.

The maximum concentration of chlorins in Peru Margin shelf sediments (660 nmol*gTOC⁻¹) is lower than the average porphyrin concentrations from mid-Cretaceous (~94 Ma) black shales of the Demerara Rise (1285 nmol*gTOC⁻¹) (Junium, Chapter 4). We hypothesize that the primary difference between the two locations is related directly to oxidant exposure time. Both water columns are characterized by oxygen deprivation but the mid-Cretaceous deep water over Demerara Rise was episodically euxinic. However, the main control on tetrapyrrole concentration is not within the water column, but at the sediment-water interface. The Cretaceous sediments on Demerara Rise are
laminated, indicating a sedimentary environment that was sufficiently euxinic to limit bioturbation by eukaryotes and there is little evidence for strong bottom current. Without resuspension and reintroduction of organic matter into the water-column where oxidants such as sulfate and nitrate are plentiful, preservation potential of tetrapyrroles is clearly greater, even considering the long time-scale of post burial diagenesis for Cretaceous strata.

6-4-3 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) (Figure 13). 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 drop of ~120 m 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 decreases in $\delta^{15}N$ of the Mexican outer-shelf could be the result of OM degradation and not decreasing denitrification (Ganeshram et al., 2002; Figure 13). Compound-specific $\delta^{15}N$ analysis provides a method for testing this hypothesis in future studies.

6-5 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 transect down the Peru Margin demonstrate that bulk sedimentary $\delta^{15}N$ values from surface sediments are altered and the concentration of chlorophyll derivatives decreases due to lateral transport of sediment and degradation of
OM within the Peru Margin OMZ. Lateral transport increases the residence time of OM at or above the sediment water interface, allowing for significant degradation by organisms despite reducing conditions. Compound-specific δ¹⁵N analyses of chlorophyll derivatives demonstrate that the ¹⁵N-abundance of phototrophic N in surface sediments does not decrease offshore as is observed in bulk organic N. These data suggest that OM is sourced from the shelf and transported downslope, and that bulk δ¹⁵N values are altered during transport and reworking and are not reliable proxies for the state of the water-column N-cycle outboard of the shelf. Alteration of primary δ¹⁵N signals proceeds through selective degradation of ¹⁵N-enriched proteins leaving bulk sediments ¹⁵N-depleted; this process is clearly enhanced by bottom currents and reworking on the upper slope indicated by a significant drop in OM quality at the shelf break. The kinetics of degredative reactions typically result in isotopic enrichment however our data suggest that selective removal of isotopically enriched OM fractions can result in the observed decrease in δ¹⁵N and δ¹³C values with depth. Additional factors such as addition of abundant microbial biomass, (e.g. Thioploca), further complicate interpretation of bulk δ¹⁵N 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 is a powerful approach to support bulk δ¹⁵N analyses.

6-6 References


Figure 6-1. Site location of 1991-1992 Peru Margin Cruise.
Figure 6-2. HPLC UV/Vis chromatogram (665 μM) of a typical acetone extract. Sample 3354 3 from 183m water depth (15.3 % TOC).
Figure 6-3. 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. (Figure 7). Early eluting compounds are primarily carotenoids that coelute with chlorophyllone. Panel B is the integrated LC-MS chromatogram 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.
Figure 6-4. Bulk geochemical parameters from Puru Margin surface sediments (Arthur et al., 1998). Data are plotted onto the Y axis as water depth and are projected as a function distance from shore (not to scale). The depths of samples matches the depth in first panel. Circles represent the 12°S location and triangles are from the 13.5°S location.
Figure 6-5. Bulk geochemical parameters from Puru Margin surface sediments. Data are plotted onto the Y axis as water depth and are projected as a function of distance from shore (not to scale). The depths of samples match the depth in the first panel. Circles represent the 12°S location and triangles are from the 13.5°S location.
Figure 6-6. Scatter plots of bulk geochemical data with respect to bulk S2. Circles represent the 12°S location and squares are from the 13.5°S location.
Figure 6-7. Scatter plots of bulk geochemical data with respect to bulk $\delta^{15}$N. Circles represent the 12°S location and squares are from the 13.5°S location.
Figure 6-8. Abundance data from the three most abundant chlorins and S2 data from Peru Margin surface sediments. S2 values and chlorin abundances drop significantly from 200 to 400 m. Water at the sediment water interface is anoxic from 70 m to ~600 m. Red circles are from the 12°S location and blue circles are from the 13.5°S location (S2 values only).
Figure 6-9. Compound specific $\delta^{15}$N data from 6 Peru Margin surface sediment samples.
Figure 6-10. Compound specific $\delta^{13}$C data from 6 Peru Margin surface sediment samples.
Figure 6-11. Percentage of N as amino acids ($\%T_{aa}N$) from Peru Margin surface sediments from (Lomstein et al., 2009)
Figure 6-12. Particulate organic carbon $\delta^{13}$C (red dots) and phytol $\delta^{13}$C from water column filter samples off the Peru Margin (Pancost et al., 1997 and 1999).
Figure 6-13. Data presented in Ganeshram et al., (1999) (HI) and Ganeshram et al (2002) ($\delta^{18}$O, $C_{org}$ and $\delta^{15}$N) and adapted for this presentation. The core was taken from 425m depth from the N.W. Mexican Margin, south of the Baja Peninsula at 23.6°N. Gray bars are used here to mark laminated intervals and to correlate HI data with $\delta^{15}$N as HI data were plotted with time on the Y-axis rather than depth in a separate publication.
Chapter 7: Biogeochemical controls on black shale deposition in the Neoproterozoic Kwagunt Formation, Chuar Group, Grand Canyon, USA.

Abstract

Significant effort has been given to understanding the sedimentary and geochemical processes control the genesis of the organic matter rich sediments known as black shales. The current paradigms are based primarily on Phanerozoic strata and our knowledge of the factors controlling black shale genesis in the Precambrian is limited. A wide range of The black shales of the Neoproterozoic, Kwagunt Formation, Chuar Group, Grand Canyon, USA were deposited in the time prior to the first Neoproterozoic, Snowball Earth Episode (770-742 Ma). These sediments provide an excellent example from which to study the processes that control the genesis of black shales in this important time period. Organic carbon in the Awatubi Member and much of the lower Walcott Member is associated with sedimentary structures that are consistent with production and of organic matter in situ by benthic microbial mat communities. The co-occurrence of mat structures with high abundances of Sphaerocongregus microfossils and low $\delta^{15}N$ values (2-3‰) is consistent with a significant proportion of biomass having been produced by mat forming, diazotrophic cyanobacteria. Base level rise in the Walcott Member enhanced nutrient flux from the Neoproterozoic ocean allowing for the development of euxinia and deposition of black shales. $\delta^{15}N$ values increased sharply and decrease gradually at lower values through the black shale interval. Euxinic conditions during black shale deposition are inferred on the basis of gammacerane indices, total thiophene concentrations and C/N data. The $^{15}N$-enrichment can be attributed to episodic oxygenation and overturning allow for partial denitrification, a process that may be expected in the relatively shallow Chuar Basin. $\delta^{15}N$ values decrease from +4.5‰ to below +3‰ through the lower Walcott black shale interval, signaling a transition to an nitrogen fixation dominated regime, and similar to observe in Phanerozoic black shales. Decreasing base level in the upper Walcott limits nutrient exchange with the Neoproterozoic Ocean; here riverine P-flux supported nitrogen fixation and primary productivity, but at lower rates than in the black shale intervals.

7-1. Introduction

The Neoproterozoic (800-542 Ma) contains some of the most significant and confounding biogeochemical events in Earth’s history. Large variations in the carbon isotope record, global-scale, low-latitude glaciations (Schragg et al., 2002; Halverson et al., 2005; Fike et al., 2006) (Figure 1), and the rise of metazoan lineages (e.g. Valentine 2002; Knoll and Carroll, 1999; Love et al., 2009) have been associated with the termination of large-scale marine euxinia and a putative rise atmospheric $O_2$ concentrations from ~10 to 90% of present atmospheric levels (Canfield, 1998; Canfield 2005; Canfield et al., 2008) (Figure 2). The burial of organic carbon is an important
modulator of a CO₂ driven climate and atmospheric O₂; characterizing the sedimentary and biogeochemical factors that control the deposition of organic matter rich sediments will help identify the potential links that exist between carbon burial and the major climate transitions of the Neoproterozoic.

The black shales of the mid-Neoproterozoic, Kwagunt Formation, Chuar Group were deposited during the time preceding to the Sturtian Glaciation and provide important constraints on the nature of the earth system prior to the first Snowball Earth episode (Dehler et al., 2005; Nagy et al., 2009). Here we present a broad dataset that integrates a refined mudstone stratigraphy, elemental ratios, organic biomarkers and nitrogen isotopic data that expand our understanding of biogeochemical processes controlling deposition of the Kwagunt Formation and will help in understanding of the processes governing the genesis of black shales in the Neoproterozoic. These data also represent the first detailed attempt at reconstructing the nitrogen cycle from isotopic data in Neoproterozoic strata.

The carbon isotopic record of the Neoproterozoic displays numerous, large positive excursions that suggest elevated fractional burial rates of organic carbon (Figure 1) (e.g. Karlstrom et al., 2000; Knoll and Kaufmann, 1994; Halverson et al., 2005). Indeed, high carbon burial rates aided by euxinic deep waters and elevated primary productivity may have supported a reduction in atmospheric CO₂ concentrations to levels that allowed low-latitude glaciations (Schrag et al., 2002). Recent evidence suggests that the Walcott member of the Kwagunt Formation was deposited under an anoxic and potentially euxinic water column and eutrophic conditions (Canfield et al., 2008; Nagy et al., 2009).

Phanerozoic trends demonstrate that in anoxic basins nitrogen-fixing organisms proliferate (Rau et al., 1988; Levman and von Bittern, 1999; Kuypers et al., 2004; Junium and Arthur, 2007; van Capellen and Ingall, 1996; Meyer et al., 2008; Haug et al., 1998). The loss of inorganic nitrogen species resulting from suboxic metabolic activity (e.g. Deutsch et al., 2007) and the regeneration of P from OM and authigenic phases (van Cappellen and Ingall, 1994) supports a lower N/P on basinal scales (e.g. Fuchsman et al., 2008) creating conditions favorable for N₂-fixation. If our understanding of links between nitrogen fixation and anoxia and euxinia for the Phanerozoic are correct, nitrogen fixation
may be an important factor in the deposition of prominent Neoproterozoic black shales, a hypothesis we aim to test with the Kwagunt Formation black shales.

7-2. Geologic Setting

The Chuar Group comprises a 1600 meter succession of supracrustal sediments that were deposited in an intracratonic rift basin on the north side of Laurentia at near equatorial latitudes (Karlstrom et al., 2000) (Figure 3). Chuar deposition spans ~28 Ma +/- 6Ma (770-742 Ma), is synchronous with the late-stage break-up of the supercontinent Rodina and correlates in time with similar, supracrustal sediments which are often overlain by glacial sediments of putative Sturtian age (Dehler et al., 2005).

The Kwagunt Formation is the uppermost formation within the Chuar Group and is composed of ~500m of silty carbonaceous claystones with interbedded siltstones, sandstones and dolomites (Dehler et al., 2001; 2005) (Figure, 4). Kwagunt formation deposition is believed to have occurred under relatively shallow, episodically emergent, but marine influenced conditions (Dehler et al., 2001). Interbedded sandstones, siltstones and mudstones contain asymmetric and symmetric ripple structures, subaerial exposure surfaces, and tidally influenced sedimentary structures indicate that for the majority, but not all of deposition, water depth in the Chuar basin did not exceed storm wave base, and it is more likely that water depth was only 10s of meters (Karlstrom et al., 2000). Cosmopolitan acritarch assemblages also support at least a surface connection with the open ocean (Nagy et al., 2009).

Of particular interest is the juxtaposition of dolomite beds and black shales in the Walcott Member of the Kwagunt Formation. The dolomites contain pseudomorphs of evaporite minerals and probable exposure surfaces (Summons et al., 1988; Dehler et al., 2001) that suggest deposition in a near-tidal environment. The interbedded organic matter-rich black shales, therefore, may have been deposited under relatively shallow, potentially hypersaline conditions (Dehler et al., 2001; Summons et al., 1988). Dehler et al.,(2005) suggest that a combination of drier climate, on the basis of decreasing chemical index of alteration (CIA) data and kaolinite percentages coupled with low-amplitude, eustatic sea-level changes contribute the deposition dolomite beds in the Walcott Member, and the facies relationship between the dolomites and black shales suggests relatively shallow conditions predominated Walcott Member deposition.
7-3. Materials and Methods

7-3-1. Sample Preparation

Samples were collected from trenched outcrops by Mobil Exploration at Nankoweap Butte (Figure 5) during a field excursion in 1988. Samples were stored in cotton sample bags and the largest individual pieces were selected for powdering for geochemical analyses. Selected samples for biomarker analyses were cleaned of weathered surfaces and processed in the ExxonMobil Upstream Research Company Petroleum Geochemistry Lab (PGL) for biomarker analyses. Samples for bulk isotopic analysis were powdered in a vanadium carbide ball mill and treated with 1N HCl at room temperature for 24h for the removal of any trace carbonates, washed with deionized water (4X) and freeze-dried.

7-3-2. Bulk Geochemical Analyses

Hydrogen Index, TOC and major oxide analyses were performed at PGL. Isotopic analyses and weight percent data for nitrogen and carbon 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 and He-bleed system. Data are reported using delta notation relative to atmospheric N₂ for nitrogen and the Vienna Pee Dee Belemnite International Standard (V-PDB) for carbon. Reference gases were calibrated relative to standards IAEA N1 (0.4%) for nitrogen and ANU sucrose for carbon in combination with in-house, Devonian black shale and Peru mud isotopic standards for nitrogen and carbon. 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. Carbon isotope precision is ±0.1%. To ensure proper combustion of moderately thermally mature samples, like the Chuar sediments, extra precautions must be taken. All samples were run with the ‘macro’ oxygen loop at 1.2 bars pressure. The efficiency of combustion was confirmed through the use of a thermally mature Devonian black shale house standard.
7-3-3. Organic Extract $\delta^{15}$N

Selected samples were sonically extracted 3 times for 5 minutes in 4:1 DCM:Methanol, and evaporated to dryness and stored until analysis. Samples were diluted in 250ul DCM and 10 to 40ul was added to smooth-walled tin capsules and allowed to dry at room temperature prior to isotopic analysis. N-isotopic measurement were performed using a cryotrapping/focusing method detailed in Polissar et al., (2009). The high C/N values of organic extracts necessitates the removal of CO$_2$ from the sample gas. Even at high He dilution, the quantity of sample required for N analyses overwhelms the IRMS source. Removal of CO$_2$ was achieved with the addition of an Ascarite trap upstream of the water trap. Addition of the Ascarite trap does not impact the precision estimates of Polissar et al., (2009) which was determined, conservatively, to be +/- 0.5‰ by multiple sample analyses and octaethylporphyrin and methionine house standards.

7-3-4. Biomarker Analyses

Saturate biomarker and on-line pyrolysis gas-chromatography tandem mass-spectrometry (Py-GCMS) analyses were performed in the ExxonMobil Upstream Research Company Petroleum Geochemistry Lab. Prepared sample powders were Soxlet extracted using 4:1 DCM/Methanol. Separations of saturate, aromatic and polar fractions were achieved using preparative high-performance liquid-chromatography using standard techniques. Saturate and aromatic fractions were analyzed and quantified using gas chromatography tandem mass spectrometry GC MS/MS in metastable reaction monitoring mode.

7-4. Results

7-4-1. Mudstone Facies Descriptions (Figure 6)

*Facies 1:* (Awatubi Member, Kwagunt Formation) consists of gray to brown silt-bearing clay-rich, mudstones, with prominent dark gray to black carbonaceous laminations. Total organic carbon percentages range from 0.1 to 0.7% and average 0.5%. Laminations range in thickness from 1-3 mm to ~ 0.1mm. Individual mm-scale laminae are often packages of sub-mm scale laminations. The laminations show a large relative range in form from plane parallel to wavy and fenestral with dessication features, roll-up structures and are often laterally discontinuous on a cm-scale. Closer examination of apparently plane-parallel laminations shows that they are wavy, variable in thickness and laterally
discontinuous on the sub-mm scale. Bedding surfaces are irregularly wavy and can have “pustulose” and wrinkly fabrics. The Acritarch *Chuaria circularis* is commonly found on bedding surfaces (Figure 7).

**Facies 2**: (Walcott Member, Kwagunt Formation) consists of dark gray silt-bearing clay-rich mudstones and clay-bearing, silt-rich mudstones with black carbonaceous laminations. Total organic carbon percentages range from 0.1 to 2.9% with an average of 1.4%. Laminations are typically less than 1 mm in thickness and appear to be plane-parallel but are often composed of numerous of sub-mm scale arcuate, discontinuous carbonaceous threads and lenses. Inclined and pseudo-cross-laminations are also found.

**Facies 3**: (Walcott Member, Kwagunt Formation) consists of very dark gray to black silt-bearing clay-rich mudstones and clay-bearing, silt-rich mudstones with black carbonaceous laminations. Weathered surfaces are often yellowed, presumably from the oxidation of pyrite. Total organic carbon percentages range from 0.1 to 2.9% with an average of 1.4%. Laminations are typically less than 1 mm in thickness and appear to be plane-parallel but are often composed of numerous of sub-mm scale arcuate, discontinuous carbonaceous threads and lenses. Inclined laminations of a similar affinity are also found.

**Facies 4**: (Walcott Member, Kwagunt Formation) consists of sub-mm-scale laminated to massive black clay dominated mudstones. Total organic carbon percentages range from 4.9 to 11.8%. Laminations are discontinuous or anastomose with wavy carbonaceous lenses.

### 7-4-2. Mudstone Stratigraphy

The Awatubi Member comprises much of the lower half of the Kwagunt Formation and is dominated by relatively organic matter-poor claystones deposited under shallow to periodically emergent conditions (Dehler et al., 2001). Fine-scale sedimentary structures within the mudstones display features that are of unambiguous microbial mat origin (Figure 6); apparently plane-parallel laminations are, in fact, wavy, with variable thickness and are laterally discontinuous. Bedding surfaces are irregularly wavy and can have "pustulose" or wrinkled fabrics and occasional roll-up structures that appear to have occurred during desiccation or remobilization of microbial mats by currents (cf. Schieber, 2004). Inclined laminae sets in mudstones are ‘false-cross-lamination’
resulting from the layered growth of microbial mats over undulatory bedding (Shieber et al., 2004). A majority of the organic carbon observed in thin section and hand samples is associated with microbial mats (Figure 6).

The Walcott Member comprises the upper half of the Kwagunt formation and is composed primarily gray to black, siltstones and claystones with interbedded dolomites and thin sandstones (cf. Dehler et al., 2001 for detailed data on sandstones and dolomites). The mudstones of the Walcott Member are significantly more organic carbon-rich than the underlying Awatubi Member; %TOC values increase gradually from 0.4 to 2.5% for the bottom 100 meters of the Walcott Member and increase significantly to greater than 8% TOC in the middle Walcott Member and remain organic carbon-enriched in the mudstones upsection (Figure 7). Microbial mat features remain common in the Walcott Member, however organic matter is not exclusively associated with mat features in contrast to what is observed in the Awatubi Member (Figure 6).

Throughout the Kwagunt Formation we observe a direct relationship between %TOC and %silt (Figure 8), except in association with black mudstones that are significantly enriched in TOC within the Walcott Member. Likewise, a direct relationship between Si/Al and %TOC is observed for the Walcott and Awatubi members with the exception of the most organic rich intervals (Figure 10). This may support the positive relationship of %Silt (predominantly quartz) with %TOC for all but Facies 4. The silt could be derived from either fluvial inputs or eolian sources. Aluminum content versus %TOC displays a similar, but reversed trend.

7-4-3. Bulk Geochemical Data

The relatively TOC-poor (average TOC 0.5%) Awatubi Member is characterized by very wide range of $\delta^{13}$C$_{org}$ values (-31.7 to -13.2‰) but are more typically $^{13}$C-enriched with an average of -19.1‰ (Figure 7). These $\delta^{13}$C values are in agreement with previously published data from the Chuar (Dehler et al., 2005) and with the wide range of $\delta^{13}$C values that are typical of the Neoproterozoic (Kaufman and Knoll, 1995; Halverson et al., 2005; Figure 1) but direct comparison of $\delta^{13}$C$_{org}$ values to the carbonate record may not be appropriate. Nitrogen isotope values range from +2.5 to +5.7‰ but are, on average, moderately $^{15}$N-enriched with an average $\delta^{15}$N value of +4.5‰ (Figure 9). C/N
ratios rise at the base of the Awatubi Member to values as high as 25 and decrease upsection to below 10 (Figure 9).

Overall TOC-enrichment and two prominent TOC-rich black shale intervals differentiate the Walcott Member from the Awatubi Member; TOC contents average 3.0% for the Walcott Member and are as high as 10.9% (Figure 7). From the base of the Walcott member δ¹³C_{org} values rise from a local minimum of -26.9‰ to a maximum of -24.3‰ within the lower of the two Walcott TOC-rich black shales (Figure 7 and 11). Following deposition of two prominent dolomite beds (Dehler et al., 2005) δ¹³C_{org} values drop to a Walcott Member minima of -28.3‰ and then rise to -27.2‰. The nitrogen isotope record for the lower Walcott Member maintains values of 2-3‰ until initiation of the lower Walcott TOC-rich black shale. The spike in TOC-enrichment is followed by an increase in δ¹⁵N values that remain above +4‰ for 9 meters and drop to below +3‰ as TOC remains elevated. The upper TOC-rich black shale has the lowest δ¹⁵N values for the entire Kwagunt Formation with values dropping as low as +1.7‰ with ¹⁵N abundances returning to near Walcott Member averages as TOC decreases at the top of the section (Figure 9). C/N ratios range from 2.3 to 32.7 with the highest C/N ratios typically corresponding to the most TOC-rich intervals a trend that is commonly observed in other TOC-rich sequences from Phanerozoic black shales (Figure 9) (e.g. Junium and Arthur et al., 2007).

7-4-4. Organic Extract δ¹⁵N

Analyses of whole organic extracts are used to determine if observed trends in bulk δ¹⁵N through the deposition of the Walcott Member were representative of the organic N fraction. The composition of nitrogenous compounds present in the organic extracts is unknown, however various classes aromatic nitrogen may be present and includes carbazoles, indoles, and pyrroles (porphyrins and maleimides). Analyses revealed no detectable porphyrins, however it is possible that they are present in quantities that are below detection limits. The absolute values of δ¹⁵N_{extract} are lower than δ¹⁵N_{bulk}; this is an expected result and suggests that the organic-N fraction is derived from chlorophyll derivatives. δ¹⁵N values for chlorophylls and porphyrins are ¹⁵N-depleted relative to total algal biomass and bulk sediment δ¹⁵N (Bidigare et al., 1991; Sachs et al., 1999). The modern calibration of Δ¹⁵N_{biomass-pigment} is ~5‰ (Sachs et al.,
The observed range of values for $\Delta^{15}N_{\text{biomass-pigment}}$ and $\Delta^{15}N_{\text{bulk-pigment}}$ can range from 10 to -10‰ (Chicarelli et al., 1993; Beaumont et al., 1999; Fulton, 2010; Junium, Chapter 4), but in most cases the values range from 2-6‰ (i.e. chlorophyll derivatives are $^{15}N$-depleted relative to biomass or bulk sediments by 2-6‰). The values for the Chuar sediments range from 7.0 to 1.6‰ and average 3.5 (n=17), well within the range of chlorophyll derivatives. The positive excursion observed in $\delta^{15}N_{\text{bulk}}$ in the middle Walcott Member is also present in $\delta^{15}N_{\text{extract}}$ (Figure 11), but the range of values is significantly larger; $\delta^{15}N_{\text{extract}}$ values rise and fall by from background values of ~-2‰ to +3‰ and return to below -2‰ as TOC values return to background values of ~1%. The $\delta^{15}N_{\text{bulk}}$ signal may not reflect the full magnitude of the $\delta^{15}N$ variability during black shale deposition. $\delta^{15}N_{\text{extract}}$ data also validate the $\delta^{15}N_{\text{bulk}}$ bulk values in the TOC-poor intervals of the Walcott Member from 200-135 m and above 110 m.

7-4-5. $C_{\text{org}}/S_{\text{total}}$ Data

In modern, non-euxinic settings sulfur is incorporated into sediments through the formation of pyrite during pore-water sulfate reduction. The quantity of sulfur is limited by the relatively small pool of available $SO_4^{2-}$ and downward diffusion of $SO_4^{2-}$ from the overlying water column (Raiswell and Berner, 1985). Euxinic water columns, such as the Black Sea deep waters foster the formation of pyrite in the water column (e.g. Wilkin et al., 1998) and early sulfurization of organic matter (e.g. Sinninghe-Damsté et al., 1989). These processes can result in sedimentary sulfur concentrations that are significantly higher than can be achieved by pore water reduction of $SO_4^{2-}$ alone. This is manifested as anomalously low C/S ratios, below 3 for modern marine environments. $C_{\text{org}}/S_{\text{total}}$ data are not definitive of euxinic environments but are useful as indirect proxies of water column euxinia in the past. Low sulfate environments such as those found in fresh waters and for intervals in the past when sulfate concentrations were less than modern (e.g. Hurtgen et al., 2006), the $C_{\text{org}}/S_{\text{total}}$ can be depressed, despite strong evidence for euxinic conditions, such as the presence diagnostic biomarkers for phototrophic sulfide oxidizing bacteria (e.g. Sinninghe-Damsté and Köster, 1998). For the Kwagunt Formation, total sulfur percentages range from .01 to 1.68% and display a $C_{\text{org}}/S_{\text{total}}$ averages over 10 (figure 12).
A wide range of biomarker ratios and RockEval data indicate moderate thermal maturity within the early to peak oil generation phase (Figure 13) and agree with data presented by Summons et al., (1988). The diversity and distribution of biomarkers, namely gammacerane (Rullkötter, et al., 1984), which degrades at higher thermal maturity suggests that observed distributions in biomarker ratios reflect environmental variability rather than overprints of thermal maturity (Summons et al., 1988). However, consideration of thermal maturity is also important in the consideration of bulk isotopic parameters, particularly $\delta^{15}N$. Moderate thermal maturity may cause small, positive shift, in the isotopic composition of bulk nitrogen, however significant $^{15}N$ enrichments on the order of 1-2‰ are not anticipated until thermal metamorphism equivalent to greenschist facies or 350°C (Jia, 2006). We therefore consider after extraction and analysis that thermal influences on bulk and extract $\delta^{15}N$ data to be minimal.

Our best efforts (GC-MS, LC-MS, Py-GC-MS) have not yielded diagnostic biomarkers such as porphyrins and carotenoids, including those produced by phototrophic sulfur bacteria, that are known to form sulfur cross-links. One consequence of moderate thermal maturity and oil generation is the cracking of carbon sulfur links that form during early diagenesis (Koopmans et al., 1998) and the potential loss and migration of the compounds that are most susceptible to these types of reactions.

**7-4-7. Biomarker and Pyrolysis GC-MS data**

The Kwagunt Formation is well-known for unusual saturated hydrocarbon biomarker distributions. Summons et al., (1988) detail high proportions of gammacerane and C27 Steranes. Gammacerane is derived from the pentacyclic compound tetrahymanol; it is produced in place of sterols by ciliates that graze primarily in chemocline (Harvey and McManus, 1991), and by some purple non-sulfur bacteria (Kleeman et al., 1990). Tetrahymanol is a common component of sediments (Venkatesan, 1987) but in modern settings has only been found in the water column of the Black Sea (Wakeham et al., 2007). Gammacerane indices (gammacerane/C30:17α,21β-hopane) are elevated for all samples analyzed but vary significantly through the Walcott Member; values range from 0.07 to 0.53 and are highest within the main region of TOC-enrichment (Figure 19). Gammacerane indices in notable Phanerozoic sediments from the Permian of Meishan (Cao et al., 2009) and OAE II of Jordan (Sepulveda et al., 2009) are
as high as 0.15 and 0.6 respectively. Hopane/sterane ratios, relative indicators of bacterial versus eukaryotic production (reference) remain above unity for much of the Walcott Member. Sterane distributions reveal a very unusual predominance of C27 steranes through the Walcott Member and are similar to those reported by Summons et al., (1988) for the Chuar and Granham et al., (1986a) for Oman oils. C27 steranes are produced primarily by the modern eukaryotic red algae (*Rhodophyta*) and the predominance of the C27 form suggests a relatively uniform eukaryotic algal community under predominantly marine conditions (Zhang et al., 1996). Curie Point Pyrolysis GC-MS thiophene data (total thiophenes) range significantly through the Walcott Member (from 0.5 to 5.9 mg*gOC⁻¹); values enriched by 3-6X during the deposition of the lower black shale compared to average background values.

### 7.5. Discussion

#### 7.5-1. The Neoproterozoic Diagenetic Environment

The efficiency with which carbon is remineralized is controlled by the availability of electron acceptors. In the modern ocean, the most efficient means for degradation of organic carbon is with molecular oxygen, and is largely a function of the Earth’s well-oxidized atmosphere. The secondary effect of a large atmospheric oxygen reservoir is the presence of significant quantities of oxidized dissolved cations and metal oxides (MnO, FeOOH, NO₃⁻, SO₄²⁻; Froelich et al., 1978). For example, the oxidative capacity of sulfate (28 mM as sulfate) is significantly larger than the concentration oxygen in seawater (avg 150 μM) and provides a very efficient pathway for organic matter degradation in sediments (Canfield, 1989), and in some water column environments such as the Black Sea.

There is considerable evidence that the size of the Earth’s sulfate reservoir is tied to the concentrations of atmospheric O₂. Prior to the rise of oxygen at the Archean-Proterozoic transition; sulfate concentrations may have been as low at 200 μM (Habicht et al., 2002) and likely remained low for much of the Proterozoic as concentrations of atmospheric O₂ increased slowly (Canfield et al., 2005; Hurtgen et al., 2004). In pore waters, sulfate would be quickly and OM degradation would be limited less efficient fermentative pathways (Lovely and Klug, 1986). Under low sulfate and oxygen concentrations that are believed to be characteristic of the Neoproterozoic (Canfield et al.,
degradation of organic matter in the water column and sediments would have been limited, increasing the burial efficiency of organic matter that reaches the sediment water interface.

**7-5-2. The Role of Microbial Mats**

Layered benthic microbial communities create a highly efficient path for burial of organic carbon. Intimate association of organic matter with sedimentary substrates minimizes remobilization and facilitates passing organic matter through the sulfate reduction window (e.g. Canfield et al., 1989), maximizing preservation potential. In contemporary environments microbial mat communities are subject to consumption by grazers and irrigation by burrowers, limiting organic matter preservation. The preserved remnants of microbial mats are very common in the Proterozoic siliciclastic and carbonate records (Schieber, 1998; Schieber, 1999; Logan et al., 1999). Lower concentrations of oxidant (Canfield et al., 2008; Hurtgen et al., 2004) and a lack of burrowing organisms during the Neoproterozoic would have limited degradation by aerobic heterotrophic bacteria and sulfate reducers. Under these conditions, preservation of microbial mat-produced carbon would have been very efficient.

The association of organic carbon with microbial mats in the Awatubi Member and the relatively TOC-poor intervals of the Walcott Member (>3.0%) suggests that a significant proportion of the ‘background’ organic production preserved in the Kwagunt Formation was produced *in situ* by shallow water benthic microbial communities. Nagy et al., (2009) detail high abundances of the microfossil *Sphaerocongregus* from the mid-Awatubi Member through the base of the Walcott Member. *Sphaerocongregus* display a range of morphologies that are similar to modern *Pleurocapsa* cyanobacteria (Moorman, 1974; Knoll et al., 1981) which live in a range of environments and salinities, are capable of nitrogen fixation and live epiphytically (Waterbury and Stanier, 1978). The co-occurrence of mat structures with high abundances of *Sphaerocongregus* provides additional evidence that they are related to *Pleurocapsa* and are mat-forming cyanobacteria. Targeted biomarker analyses and identification of diagnostic compounds such as 2α-methylhopanes or scytonemin in the microbial mat structures of the Walcott Member and their modern analogues may provide further evidence for a link between *Pleurocapsids* and *Sphaerocongregus*. Nitrogen isotope values for much of the
Sphaerocongregus interval (140-200m) of the Walcott Member range from 2-3‰ (Figures 9 and 11). While δ¹⁵N values of 2-3‰ are not definitive evidence for nitrogen fixation (e.g. Junium and Arthur, 2007), they are consistent with a significant amount of the organic matter having been produced by nitrogen fixing cyanobacteria such as Pleurocapsa.

Thin-section photomicrograph evidence (Figure 6) demonstrates that organic matter in the TOC-poor intervals of the Kwagunt Formation was produced largely by benthic microbial communities. Microbial mat production may have been an extremely important mechanism for carbon burial on the Precambrian Earth, and particularly during Neoproterozoic. Thick sequences of supracrustal, siliciclastics deposited in rift basins have been recognized from the mid-Neoproterozoic associated with the breakup of Rodinia (Karlstrom et al., 2000). Microbial mat communities would have capitalized on the expanse of shallow, epicontinental seas, promoting carbon burial in regions that are episodically emergent and not conducive to highly productive, pelagic photosynthetic communities. In fact, the mat facies from the Neoproterozoic Centralian Superbasin are significantly more TOC-rich (0.7%) than the non-mat facies (0.1%) (Logan et al., 1999), a trend that underscores the potential importance of microbial mats in facilitating the burial of organic carbon on the Precambrian Earth in the absence of large pelagic, algal communities and burrowing meiofauna.

7-5-3. Geologic controls on Walcott Member Black Shale Deposition

The organic carbon content of sediments is essentially a function of the delivery and preservation of organic matter to sediments, and the rate of sedimentary dilution. Within the Kwagunt Formation it is clear that both processes were significant in the observed variability in %TOC (Figure 8). A positive relationship between %TOC with Si/Al (Figure 10) and covariance in %Silt and %TOC for non-black shale facies (1-3) (Figure 8) suggest that the factors controlling the addition of quartz silt are enhancing production and burial organic carbon. It is reasonable to consider that a fluvial control on silt flux during episodically wetter intervals would have provided excess riverine phosphorus, stimulating phytoplankton growth and fresh substrate for new microbial mat communities, a behavior that is observed in the development of false cross-lamination where newly deposited silt is colonized and stabilized by mat communities (Schieber,
2004). If the additional silt is of eolian origin, seeding of P and Fe from the surfaces of silt grains may provide nutrients sufficient to stimulate organic production.

Reduced siliciclastic dilution was an important mechanism in the deposition of the most TOC-rich intervals of the Walcott Member. Reduction in fluvial discharge or eolian input could also result in a decreased silt flux, however chemical index of alteration (CIA) data do not point toward discernable changes in weathering intensity in the Walcott Member (Dehler et al., 2005) that may have been associated with significant changes in regional climate. Therefore it appears that increased weathering intensity in sediment source area is not responsible for the black shale deposition and decreases in silt content. The observed reduction in silt content (Figures 8 and 10) could have been the result of deepening base level and trapping of silt shoreward, driven by modest sea level change of tectonic or glacioeustatic origin (Dehler et al., 2005). The duration of deposition within the Chuar Basin places some constraints on sedimentation rates for the Walcott Member which are useful for understanding the mechanisms controlling Walcott Member black shale deposition. A simple linear sedimentation rate on the basis of Chuar Group thickness (1600m) and the estimated duration of deposition (28 Ma, +/- 6Ma ) (Dehler et al, 2005) yields sedimentation rate 5.7cm*ky⁻¹ and ~ a 230ka duration for the 13 meter black shale in the lower Walcott Member. Certainly, this is a very rough estimate for the duration of the lower Walcott black shale, but the duration is within the range of time that may be expected for sea-level variation of a glacioeustatic origin or typical of Oceanic Anoxic Event duration during the Phanerozoic (e.g. Sageman et al., 2006).

The relationship between Si/Al₂O₃ and Al₂O₃ and %TOC for the black shale facies (4) falls well off the dominant trends that characterize a majority of the Kwagunt Formation (Figures 8 and 10) suggesting that reduced sedimentary dilution is not the only factor responsible for TOC-enrichment in the Kwagunt Formation and black shale deposition requires increased delivery of carbon to sediments. The visible organic matter in Walcott black shales is disseminated and massive rather than found in discreet mat laminae (Figure 6) indicating a water column source and delivery to sediments primarily via rain-out. Maxima in gammacerane indices support development of a chemocline conducive to purple non-sulfur bacterial production within the water column. High
gammacerane indices have been linked to hypersaline conditions (ten Haven et al., 1988; Brassel et al., 1987) but are probably more reflective of redox-stratified environments (Sinninghe-Damsté et al., 1991) which are often found in hypersaline basins (e.g. Hofmann et al., 1993; Hollander et al., 1993). Facies association of the black shales with evaporite-bearing dolomites suggests that salinity stratification may have been an important in the time leading up to the dolomite deposition. Elevated gammacerane indices through the Walcott Member are also consistent with high abundances of vase-shaped microfossils (Nagy et al., 2009). Vase-shaped microfossils are believed to have been produced by testate amoebae, simple protozoans which are known to graze in the chemocline and may be, in part, responsible for enhancing the organic flux to sediments through the production of fecal pellets.

Indeed, sea-level rise or sill breaching could have enhanced exchange with an anoxic, nutrient-rich Neoproterozoic ocean, stimulating productivity. If riverine discharge to the Chuar Basin was sufficient, an estuarine-style of overturning circulation could have been a key facet in maintaining the redox stratified and eutrophic conditions inferred by Fe speciation, microfossils (Nady et al., 2009), gammacerane indices and thiophene concentrations (Figure 9). Estuarine circulation has been postulated to be an important factor in the development of euxinic conditions because it enhances nutrient trapping in deep waters but maintains delivery of trapped nutrients to surface waters (Meyer and Kump, 2008). Similar conditions are observed in the modern Black Sea (Arthur and Sageman, 2006), Framvaren Fjord (Velinsky and Fogel, 1999) and inferred for many ancient occurrences of euxinia such as during the Late Permian (Demaison and Moore, 1980; Meyer and Kump, 2008). Thus, the deposition of the Walcott Member black shales appears to have been associated with the development of redox stratification and basinal anoxia resulting from an estuarine style of circulation that supported elevated primary productivity.

7-5-4. The role of Euxinia

The role of water-column anoxia and euxinia as the primary contributing factor in the widespread deposition of organic matter-rich sediments has been a point of contention (e.g. Calvert et al, 1996). However, a new paradigm seems to be emerging that focuses on a positive coupling between enhanced productivity and sedimentary evidence of
euxinia. In this model, anoxia and euxinia reduce the burial efficiency of phosphorus (e.g. Van Capellan and Ingall, 1994; Mort et al., 2007; Athur and Sageman, 2006) resulting in elevated deep-water phosphate concentrations associated with estuarine styles of circulation (Meyer and Kump, 2008). Under the same conditions, fixed nitrogen species are lost from the water column via suboxic microbial metabolisms (e.g. denitrification, anaerobic ammonium oxidation) creating a water column that is replete with phosphate but depleted in nutrient nitrogen. Nitrogen fixing organisms, primarily cyanobacteria, utilize the excess phosphate allowing for carbon fixation and burial to continue despite macronutrient limitation.

The deep-water euxinia that characterized the Mesoproterozoic (Canfield et al., 1998) may to have continued into the mid-Neoproterozoic (Canfield et al., 2008). On the basis of reactive Fe speciation (Canfield et al., 2008; Nagy et al., 2009), the Walcott Member water column during black shale deposition might have been euxinic. During the deposition of the lower Walcott Member black shale, a more substantial role for sulfurization of organic matter is evident in substantially elevated concentrations of total thiophenes (Figure 9). Reaction of reduced sulfide species with the unsaturated bonds of lipids, pigments and carbohydrates during early diagenesis favors the incorporation of sulfur as thiophenes, a process that is enhanced under high sulfide conditions (Sinninghe-Damsté et al., 1990; Sinninghe-Damsté et al., 1989; Sinninghe-Damsté et al., 1998). Carbon-sulfur relationships for the Kwagunt Formation do not directly support euxinia (Figure 12), but this could be the result of low sulfate concentrations, a situation that was at times characteristic of the Neoproterozoic Ocean (e.g. Hurtgen et al, 2006).

Additionally, if the exchange of the Chuar Basin water column with the open ocean was restricted and fresh water inputs were sufficient, sulfate concentrations could be depressed, resulting in C-S relationships that are not indicative of water-column euxinia (Raiswell and Berner, 1985).

One of the curious characteristics of many Phanerozoic black shales composed of marine organic matter are C_{org}/N_{total} ratios > 20 (Rau et al., 1988; Junium and Arthur, 2007). Elevated in C/N ratios are anticipated with the loss reactive amino N from organic matter (e.g. Lehmann et al., 2002) so long as all of the inorganic N is not scavenged by mineral surfaces (e.g. Peters et al., 1978). However complete loss of only amino N should
not result in C/N ratios that are much higher than 20 (Junium et al., 2007). This suggests that pathways exist that enhance the relative preservation of reactive C-rich compounds during black shale deposition. Sulfurization processes, which act to limit degradation of organic carbon under euxinic conditions may be reflected in elevated C/N ratios. Prominent intervals of widespread water-column euxinia in the past, such as the late Devonian and mid-Cretaceous are marked by C/N ratios >20 (Junium et al., 2007) and similar values in the Walcott Member (Figure 9) appear to be the result of more strongly euxinic conditions on the basis of thiophene data and gammacerane indices.

7-5-5. Evolution of the Walcott Member Nitrogen Cycle

Chemocline rise occurred rapidly at the onset of OAE II at Demerara Rise; green sulfur bacterial biomarker concentrations increase substantially (van Bentum et al., 2009) and are matched by 2‰ drop in $\delta^{15}$N values in response to the expansion of nitrogen fixation (Junium and Arthur, 2007; Chapter 5). If the Chuar Basin fit the euxinic black shale model as informed by Cretaceous data, it would be expected that the $\delta^{15}$N would indicate a nitrogen-fixation source for DIN during black shale deposition. Rather, $^{15}$N-enrichment characterizes a majority of the lower Walcott black shale. $\delta^{15}$N values are more in agreement with those of modern deep-sea nitrate $\delta^{15}$N (Altabet et al., 1999) and indicative of a DIN pool that been subject to partial denitrification forcing consideration of other possibilities. Foremost, the depositional setting of the Chuar Basin is directly comparable to the open-ocean, slope environment of many of the Phanerozoic black shale examples on which the nitrogen fixation-euxinia model is based. The Chuar Basin was relatively shallow, potentially restricted from exchange with the open ocean and therefore may have been more sensitive to small-scale environmental variability.

Basin deepening and a more significant connection with the Neoproterozoic ocean likely supplied DIN in some form to the Chuar basin, but there are currently no constraints on the mid-Neoproterozoic open ocean nitrogen cycle, the $\delta^{15}$N of DIN or the dominant DIN species (NH$_4^+$ or NO$_3^-$). A largely euxinic mid-Neoproterozoic ocean (Canfield et al., 2008) could have allowed advection of nutrient rich, anoxic waters during deepening of the Chuar Basin. If this is correct, it is the key component to the development of euxinia, eutrophication and black shale deposition.
Elevated gammacerane indices and high abundances of vase shaped microfossils led Nagy et al. (2009) to surmise that a significant proportion of the organic matter within the Walcott Member black shales may have been delivered by testate ameobae (probable bactivorou ciliates) grazing within the chemocline. Indeed, the $\delta^{15}N$ of NH$_4^+$ can be significantly $^{15}N$-enriched in the upper reaches of a sulfidic chemocline, however sulfide oxidizer biomass is typically $^{15}N$-depleted, in some cases by up to -20‰ due to high NH$_4^+$ concentrations (e.g. Fayetteville Green Lake; Fulton, 2009); biomass produced and excreted by vase shaped microfossils should reflect the $^{15}N$-depletion. Additionally, the abundance of biomarkers for red algae (C27 steranes), hopane/sterane ratios near unity and $\delta^{13}C$ values suggests that OM was composed largely of marine algal and cyanobacterial biomass, and not of chemocline origin.

Recent study of the Holocene Black Sea (Fulton, 2009) demonstrates a correlation between $^{15}N$-enrichment of organic matter with molecular evidence for photic-zone euxinia immediately following the initiation of sapropel deposition. A similar association between $^{15}N$-enrichment and photic-zone euxinia is also observed from the Permian of China (Cao et al., 2009) and in Toarcian black shales (van Breugel et al., 2006; Jenkyns et al., 2000). The $^{15}N$-enrichment in the Black Sea during intervals of photic zone euxinia was the result of a strengthening of halostratification via increased freshwater flux, and delivery of $^{15}N$-enriched riverine NO$_3^-$ driving productivity and chemocline rise (Fulton, 2009). Elevated $\delta^{15}N$ values in Permian of Meishan are attributed to a normal marine nitrogen cycle with a primary producers utilizing $^{15}N$-enriched nitrate in a basin that was subject to episodic photic zone euxinia (Cao et al., 2009). A similar model may explain the nitrogen cycle during Chuar black shale deposition; the strongest indicators for euxinia are accompanied by $^{15}N$-enrichment (Figures 9 and 11).

For the Toarcian, Permian, and Black Sea examples, $\delta^{15}N$ values are lowest, near or below 0‰, when biomakers for cyanobacteria (scytonemin, 2α-methylhopanes) are present but molecular indicators for phototrophic sulfide oxidizers are absent. A reduction in the fresh water flux to the Black Sea allowed for enhanced mixing in the upper Black Sea water column and advection of P to surface waters. Increased P availability, in turn, supported an increase in nitrogen fixation indicated by $\delta^{15}N$ values near 0‰ and the occurrence of diagnostic cyanobacterial biomarkers (sytonemin). Lower
Δ₁⁵N values in the upper half of the lower black shale and in the upper black shale are more in agreement with a nitrogen fixation source for DIN (Figure 11) and the Phanerozoic black shale, nitrogen-fixation model. However, Δ₁⁵N values are never below 0‰ as is the case in most Phanerozoic examples (Figure 1-1). Gammacerane and thiophene data suggest that the chemocline may not have been as well developed and hopane/sterane ratios suggest a shift to more bacterially dominated organic matter sources (Figure). Reactive-Fe values also decrease from maximum values of 0.76 to 0.53 suggestive of more oxidizing conditions (Nagy et al, 2009).

Application of the Black Sea hydrology model in the Walcott black shales fits our data, but is limited in that the Black Sea is a substantially deeper basin and it relies upon a significant source of ¹⁵N-enriched riverine NO₃⁻ as the source for elevated Δ₁⁵N values during intervals of photic zone euxinia. The nature and presence of an established terrestrial biota during the Neoproterozoic is a matter of debate (e.g. Knauth and Kennedy, 2009), and without a significant terrestrial biomass as a source for a riverine NO₃⁻ it would be difficult to directly link the Black Sea and Chuar records despite the correlation of ¹⁵N-enrichment and euxinia.

The nitrogen isotope data support two nutrient regimes during deposition of the Walcott Member, a N-limited, lower productivity regime (Figures 9 and 11) where N₂-fixation provides DIN for primary producers. This condition occurs under lower base-levels where exchange with the open ocean is limited and P is primarily delivered from rivers. With base level rise, increased connectivity with the Neoproterozoic ocean provides DIN and P, stimulating productivity resulting in euxinic conditions and deposition of the lower Walcott black shale (cf. Arthur and Sageman, 2006). Proximal fresh water sources may have provided sufficient fresh water to the Chuar Basin to enhance anoxia and nutrient trapping through estuarine circulation (e.g. Meyer and Kump, 2008). The ¹⁵N-enrichment that characterizes this interval may reflect the Δ¹⁵N of Neoproterozoic DIN but may also be the result of episodic oxygenation and partial denitrification, similar to processes observed in the modern Baltic Sea proper (Bianchi et al., 2000; Sohlenuis et al., 2001; Borgendahl and Westman, 2007; Fehr et al., 2008). The near equatorial latitudes of the Chuar Basin (Karlstrom et al., 2000) may have been influenced by monsoonal variability in fresh water supply, allowing for yearly
development/erosion of a chemocline. Allowing for high fluxes in the upper half of the lower Walcott black shale (Figures 9 and 11) δ\textsuperscript{15}N values drop, by ~1.5‰ but TOC, thiophenes, and C/N remain elevated; this supports a transition toward the Phanerozoic euxinia/nitrogen fixation model due to decreased water column ventilation.

7-6 Conclusions

The factors controlling carbon burial in the Neoproterozoic are illustrated by the range of processes associated with the deposition of the Kwagunt Formation sediments. Shallow epicratonic rift basins associated with the break-up of Rodinia may have been significant depocenters aiding in the burial of organic carbon and drawdown of CO\textsubscript{2} leading up to the Sturtian Glaciation, as suggested by Schrag et al. (2002). Restricted basins are often characterized by estuarine styles of circulation that promote the trapping of nutrients and are more likely to support anoxic or euxinic conditions (Meyer and Kump, 2008). These factors, combined with a smaller marine sulfate reservoir (Hurtgen et al., 2004), lower atmospheric concentrations of oxygen (Canfield et al., 2005), and a lack of burrowing organisms would have contributed to greater burial efficiency for organic matter reaching the sediment water interface.

Microbial mat communities played an integral role in the burial of organic carbon during deposition of the Kwagunt Formation. Organic carbon in the Lower Walcott Member and Awatubi Member are clearly associated with microbial mat structures, providing for consistent background TOC values averaging ~1% over 300 m of the Kwagunt Formation. Benthic carbon production provides an efficient mechanism for burial of carbon in shallow, emergent conditions that are not hospitable to significant pelagic algal populations and may have been a very important facet of Neoproterozoic carbon burial (e.g. Logan et al., 1999).

This study is a first attempt at understanding the Neoproterozoic N-cycle; the record from the Chuar Basin provides a basis from which other sequences of this time period can be assessed. The δ\textsuperscript{15}N record does not fit the Phanerozoic black shale model where δ\textsuperscript{15}N values are 0‰ or lower through black shale deposition. However, C/N ratios, thiophene concentrations, gammacerane indices and reactive Fe data of Nagy et al. (2009) suggest the presence of a euxinic watermass. Whether euxinia is purely a function of the biogeochemical system unique to the Chuar Basin or reflective of the mid-
Neoproterozoic ocean remains to be determined through further study of this time period. The N-isotopic record through Walcott Member black shale displays a strong response to transgression and increasing carbon burial rates. The $^{15}$N-enrichment that characterizes the Walcott member lower black shale may reflect the $\delta^{15}$N of Neoproterozoic DIN, but may also be the result of water mass mixing and partial denitrification in the relatively shallow Chuar Basin.

7-7. References


of breakup of Rodinia, associated change in the global carbon cycle, and ecosystem expansion by 740 Ma. Geology. 28, 619-622.


Schieber, J., 1998. Possible indicators of microbial mat deposits in shales and sandstones: examples from the Mid-Proterozoic Belt Supergroup, Montana, USA. Sedimentary Geology. 120, 105-124.


Biogeochemical Cycles Of Carbon And Phosphorus. Paleoceanography. 9, 677-692.
Figure 7-1. Secular carbon isotope curve from Halverson et al., 2005. The span of time that encompasses deposition of the Chuar Group is marked in green and is based on ash bed dates from Dehler et al., (2005).
Figure 7-2. Evolution of marine redox geochemistry and biological evolutionary events through the Neoproterozoic, adapted from Canfield et al., (2008). Water column redox conditions for the Chuar were determined on the basis of reactive Fe dat from Canfield et al., (2008).
Figure 7-3. Global plate tectonic reconstruction from the mid-Neoproterozoic adapted from Karlstrom et al., 2000. Location of Chuar Group is marked by red circle, on the north coast of Laurentia.
Figure 7-4. Stratigraphic log of the Kwagunt Formation adapted from Dehler et al., (2005).
Figure 7-5. Geologic map of the Chuar Group adapted from Dehler et al., 2005. Samples were collected from the Nankoweap Butte locality.
Figure 7-6. Stratigraphic log and bulk geochemical parameters and $\delta^{13}$C$_{org}$. Stratigraphy is adapted from Dehler et al., (2005). LBS is the lower Walcott black shale and UBS is the upper Walcott Black shale. The shaded region is expanded in figure 11.
Figure 7-7. Stratigraphic log with bulk $\delta^{15}$N, atomic C/N ratios, gammacerane indices (GI), pyrolysis-GC total thiophenes, %C27 steranes and total hopane/sterane ratios. Stratigraphy is adapted from Dehler et al., (2005). LBS is the lower Walcott black shale and UBS is the upper Walcott Black shale. Fe speciation data are from Nagy et al., (2009); values for total reactive Fe decrease from .76 to .54 through the upper half of the Walcott Member. The shaded region is expanded in figure 11.
Figure 7-8. A. Microbial mat facies from the Awatubi Member. Organic matter is found in discrete layers representing fossil mat communities. B. Well developed false-cross-lamination from the upper Walcott Member; inclined silt laminae are colonized and stabilized by microbial communities. C. “elephant skin” and pustulose textures from the Awatubi Member resulting from desiccation of mats during episodically emergent conditions. D. roll up structures from the Awatubi Member. E. Wavy bedding associated with desiccation from the Awatubi Member. F. Laminated black shale from the lower Walcott Member.
Figure 7-9. The % Silt relative to the % TOC in the corresponding sample. (Not to stratigraphic scale). The shift in mode in facies 4 indicates a prominent role for reduced siliclastic dilution in the genesis of TOC enrichment during deeper-water conditions.
Figure 7-10. Si/Al vs. %TOC. Blue data points are from the Walcott Member, yellow dots are from the Awatubi member.
Figure 7-11. Closeup of the lower Walcott black shale geochemistry. Stratigraphy corresponds to meters 100 through 200 in figure 4. Biomarker samples through this interval are labeled on the TOC plot.
Figure 7-12. Carbon and sulfur data from the Walcott Member (blue data points) and the Awatubi Member (yellow data points).
Figure 7-13. Molecular thermal maturity parameters on the basis of 9 samples from the Walcott Member.
8-1 A guide for $\delta^{15}$N studies

This section is presented as a simple guide for those wishing to perform $\delta^{15}$N studies. This is one of the important conclusions of my work but it is not directly discussed in the main text. Over the course of my dissertation I have formed what I feel are informed opinions of how $\delta^{15}$N studies are best executed based on my own work, and that of others. The simplicity of bulk $\delta^{15}$N measurements has allowed for an extraordinary quantity of data to be produced over the last decade. These data have helped develop and enrich important hypotheses about the past N-cycle (cf. the collected works of Altabet, Sigman and co-authors), but in many cases, these data have confounded (much of it certainly unpublished) as much as they have illuminated. N is a relatively labile element in the geologic environment and is subject to a range of diagenetic processes that have been shown to alter the $^{15}$N-abundance of bulk sediments (e.g. Freudenthal et al., 2001).

Bulk analyses are the starting point. First and foremost, it is important to determine whether the organic matter and N are derived from the source you are wishing to measure (i.e. derived from marine organic matter). Biomarker data, pyrolysis hydrogen indices, oxygen indices and $T_{\text{max}}$, C/N ratios, smear slides or other micrographic techniques can be used to ascertain the source of OM. This is of particular importance in depositional environments proximal to a high terrigenous flux. In such sequences it is possible that N is derived from terrestrial plant material, soil organic matter or allochthonous ammonium in association with clays. These types of analyses are also useful for determining the degree of oxidation and potential impacts of diagenesis. There is growing body of literature that will help direct these types of questions (Freudenthal et al., 2001; Lehmann et al., 2002; Junium and Arthur, 2007) Material choice is also another factor; clearly, core material is preferred, but is not always available. The work presented in Chapters 2-5 benefited greatly from the well-preserved samples recovered from Demerara Rise.

If you have established the OM source and are confident that your samples are well preserved, bulk N is a great starting point. When presented with other data such as biomarkers specific to cyanobacteria (2$\alpha$-methylhopanes, Kuypers et al., 2004;
scytonemin, Fulton, 2010), or when trends are replicated in many localities, bulk-N analyses alone can be very informative and are probably sufficient. They give a general idea of the state of the N-cycle, but they have limitations. For example, in organic matter-poor sequences, it is difficult to ascertain whether the N is present in an organic or inorganic phase. It is in these types of sediments where additional analyses, whether it is through kerogen isolation or compound specific isotope analyses, are beneficial, and may be necessary.

I have discussed at length in Chapters 4-7 how we as a community struggle to determine whether bulk $\delta^{15}$N data are meaningful. The current convention suggests that in reducing environments such as the Black Sea (Fulton, 2010) or upwelling zones (Galbraith et al., 2008 and references therein), that bulk $\delta^{15}$N values are not altered, and reflect primary processes. In modern environments the correspondence between the $\delta^{15}$N of subeuphotic zone nitrate and surface sediments in high-productivity environments suggests strongly that we are observing meaningful signals (Thunnell et al., 2004; Galbraith et al., 2008). Indeed, in most of these environments it seems as though primary N-cycle signals and variability are indeed preserved. However, in light of the results of Chapter 6, where chlorin $\delta^{15}$N values form Holocene sediments of the Peru Margin deviate from bulk $\delta^{15}$N values, it is clear that in some situations bulk $\delta^{15}$N values, even in low-oxygen settings, may be altered. These data suggest that we need to be more rigorous with our assumptions about low oxygen environments. The lesson that is best learned from the Peru Margin work is that one must assess the depositional environment and preservational conditions from which our samples are retrieved. The Peru Margin is a very dynamic environments and we must use due diligence to assess our deeper time records and place them in a clear geologic context.

In ancient sequences of the Cretaceous and Neoproterozoic I have found that $\delta^{15}$N values in black shales are supported by tetrapyrrole and bulk organic extracts, respectively. However, the data suggests that there may be more to learn about primary production from isolating photosynthetic N, be that photosynthetic pigments or phytoplankton (e.g. Sigman et al., 1999). The data I report in Chapter 4, 5 and 7 suggest that the organic nitrogen phases are capturing a larger variability than observed in bulk $\delta^{15}$N, and that we are underestimating the N-cycle response to events such as OAE II.
This is very important when considering that these data are used to calibrate deterministic models. In the absence of suitable material for compound specific isotope analyses, kerogen is an easily isolated organic phase and should be the primary target for many studies in Precambrian sediments. Additional compounds such as maleimides may be useful for bridging the gap between porphyrins and sediments where extractable yields are absent or very low.

Because of the limitations of bulk analyses, studies for which the focus is the N-cycle, more specific analyses that target organic-N phases are necessary. Chlorophyll derivatives provide a clear link between primary productivity and the N-cycle, a link that allows for a measure of certainty that is not possible with bulk analyses alone. Beyond chlorophyll derivatives, diatoms (Sigman et al., 1995), foraminifera (Ren et al., 2009) and kerogen are excellent targets and should be a part of the N-isotope toolbox where these analyses are possible.

**8-2 Future Work**

To me this work has generated many more questions than it has answered but for this section I am going to focus on just a few key areas that will comprise my future work. In the immediate future I see great opportunity in continuing and expanding the methods and knowledge I have acquired while doing my doctoral studies.

For the Cretaceous, there are portions of the ocean that have yet to be suitably sampled, the South Atlantic is an obvious target using existing materials from DSDP Site 530 or in a more long-term view, additional cruises would provide the greatest benefit and best samples.

Application of the porphyrins δ¹⁵N methods to other time periods is a clear direction. There appears to be significant links between the C N P S and Fe cycles during widespread anoxia. The framework for additional study within the Neoproterozoic is already set to begin this winter and we will focusing the links between these cycles during the deposition of black shales prior to the Sturtian glaciation and in the Ediacaran. Additional targets include the Frasnian-Famennian boundary in the Devonian and expansion of work already started in the Paleoproterozoic.
Purely molecular studies in the Cretaceous are necessary to determine the structures and stratigraphic variability of putative bacterial porphyrins found through OAE II and in the Demerara Rise sediments.

The data from the Peru Margin suggest that bulk $\delta^{15}$N records from continental margins may be altered, and utilization of down-core chlorin $\delta^{15}$N records would be a relatively simple test using easily acquired material for ODP Leg 201.

8-3 References


## Appendix 1: Data Tables

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Appendix 2

Figure A2-1. HPLC UV/Vis chromatogram of 1261a 49r1 (639.88 mbsf). The top panel is the total acetone extract. Bottom panel is the acetone extract after NiB desulfurization. These chromatograms have only very small differences following desulfurization observable only in minor peaks. The major peaks isolated for isotope analysis, the VO and FB BiCAPs show no change in abundance.
Figure A2-1. HPLC UV/Vis chromatogram of 1261a 48r3 (634.43 mbsf). This particular sample is from the middle of the OAE and has very high sulfur concentrations (up to 8 wt.% in some samples), so it was an obvious target for desulfurization. The top panel is the total acetone extract. Bottom panel is the acetone extract after NiB desulfurization. The major peaks isolated for isotope analysis FB BiCAPs show no change in abundance. There is loss of the two peaks, the 487 and 487 m/z BiCAPs. These two particular compounds have double bonds (one or two) within the 7-membered exocyclic ring. The addition of Ni-borohydride (a strong reducing agent) reduced these double bonds and in the process fostered the formation of Ni-complexed BiCAP. The exact mechanism of this process is unclear, however, it is possible that electron transfer during the reduction increases the reactivity of the porphyrin center.
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PUBLICATIONS
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Measurement of $^{13}$C and $^{15}$N isotopic composition on nanomolar quantities of C and N, Analytic Chemistry, accepted.