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Compound specific amino acid $\delta^{15}N$ in marine sediments: A new approach for studies of the marine nitrogen cycle

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Abstract

The nitrogen (N) isotopic composition ($\delta^{15}N$) of bulk sedimentary N ($\delta^{15}N_{bulk}$) is a common tool for studying past biogeochemical cycling in the paleoceanographic record. Empirical evidence suggests that natural fluctuations in the $\delta^{15}N$ of surface nutrient N are reflected in the $\delta^{15}N$ of exported planktonic biomass and in sedimentary $\delta^{15}N_{bulk}$. However, $\delta^{15}N_{bulk}$ is an analysis of total combustible sedimentary N, and therefore also includes mixtures of N sources and/or selective removal or preservation of N-containing compounds. Compound-specific nitrogen isotope analyses of individual amino acids ($\delta^{15}N_{AA}$) are novel measurements with the potential to decouple $\delta^{15}N$ changes in nutrient N from trophic effects, two main processes that can influence $\delta^{15}N_{bulk}$ records. As a proof of concept study to examine how $\delta^{15}N_{AA}$ can be applied in marine sedimentary systems, we compare the $\delta^{15}N_{AA}$ signatures of surface and sinking POM sources with shallow surface sediments from the Santa Barbara Basin, a sub-oxic depositional environmental that exhibits excellent preservation of sedimentary organic matter. Our results demonstrate that $\delta^{15}N_{AA}$ signatures of both planktonic biomass and sinking POM are well preserved in such surface sediments. However, we also observed an unexpected inverse correlation between $\delta^{15}N$ value of phenylalanine ($\delta^{15}N_{Phe}$; the best AA proxy for N isotopic value at the base of the food web) and calculated trophic position. We used a simple N isotope mass balance model to confirm that over long time scales, $\delta^{15}N_{Phe}$ values should in fact be directly dependent on shifts in ecosystem trophic position. While this result may appear incongruent with current applications of $\delta^{15}N_{AA}$ in food webs, it is consistent with expectations that paleoarchives will integrate N dynamics over much longer timescales. We therefore propose that for paleoceanographic applications, key $\delta^{15}N_{AA}$ parameters are ecosystem trophic position, which determines relative partitioning of ^{15}N into source AA versus trophic AA pools, and the integrated $\delta^{15}N_{AA}$ of all common protein AA ($\delta^{15}N_{THAA}$), which serves as a proxy for the $\delta^{15}N$ of nutrient N. Together, we suggest that these can provide a coupled picture of regime shifts in planktonic ecosystem structure, $\delta^{15}N$ at the base of food webs, and possibly additional information about nutrient dynamics.

1. INTRODUCTION

Paleoceanographic studies of the nitrogen (N) cycle have employed the stable N isotopic composition of total N ($\delta^{15}N_{bulk}$) in marine sediments as a proxy for $\delta^{15}N$ of the sinking flux of organic matter (OM) (Altabet et al., 1999).

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By extension, past $\delta^{15}N_{bulk}$ fluctuations have often been interpreted as a change in photoautotrophic utilization of the surface dissolved inorganic nitrogen (DIN) reservoir. However, in HNLC regions (Altabet and Francois, 1994) and in regions that exhibit complete utilization DIN sources, the $\delta^{15}N_{bulk}$ is often taken to reflect the $\delta^{15}N$ value of sub-euphotic zone DIN (Altabet et al., 1999; Liu et al., 2008).

However, mixtures of sedimentary N sources, as well as pre- and post- depositional $\delta^{15}N$ alteration, can confound accurate paleoceanographic interpretations of marine sedimentary $\delta^{15}N_{bulk}$ records. First, $\delta^{15}N_{bulk}$ records are susceptible to time varying changes in the contribution of marine and terrestrial N or inorganic and organic N (e.g., Kienast et al., 2005). Further, alteration of bulk organic matter (e.g., selective removal or addition of specific N containing compounds) can variably alter the sedimentary $\delta^{15}N_{bulk}$ in open-ocean sedimentary environments during early diagenesis (Robinson et al., 2012), analogous to similar changes in $\delta^{15}N_{bulk}$ widely documented for suspended particulate organic matter (POM) in the water column (Altabet, 1988, 1996; Hannides et al., 2013). Such changes are thought to be microbially mediated (Macko and Estep, 1984), although exact mechanisms are not well understood, and $\delta^{15}N$ changes associated with sedimentary organic matter (SOM) degradation have also been shown to variable (Lehmann et al., 2002). Various approaches have attempted to circumvent these issues, with the goal of developing proxies that might directly reflect $\delta^{15}N_{bulk}$ of exported surface production. These include measuring microfossil-bound organic N (Robinson et al., 2004; Ren et al., 2009), or specific $\delta^{15}N$ values of well-preserved biomarkers such as chlorins and porphyrins (Sachs and Repeta, 1999; Higgins et al., 2010). However, such approaches typically directly reflect the $\delta^{15}N$ value for only a very minor fraction of total sedimentary organic N (SON).

Compound-specific nitrogen isotope analyses of individual amino acids ($\delta^{15}N_{AA}$) are novel measurements that have the potential to simultaneously provide information regarding the $\delta^{15}N$ of sub-euphotic zone DIN and trophic transfer, potentially circumventing other issues that may directly impact $\delta^{15}N_{bulk}$ measurements, such as $\delta^{15}N$ alteration in deep, remote, organic-poor depositional settings (e.g., Altabet, 1988) or in coastal settings that are susceptible to contamination by terrestrial N sources (e.g., Kienast et al., 2005). Early work by McClelland and Montoya (2002) and McClelland et al. (2003) showed that two groups of protein AA are differentiated based on their N isotopic behavior during trophic transfer; $\delta^{15}N$ of *source* AA (Src) group remain essentially unaltered, while $\delta^{15}N$ of *trophic* AA (Tr) become isotopically enriched with each trophic step. Recent work has corroborated the differential enrichment of these groups in multiple organisms (Chikaraishi et al., 2007), and in many recent studies the offsets in $\delta^{15}N$ between characteristic Src (e.g., phenylalanine) and Tr (e.g., glutamic acid) AA have been used to estimate the trophic position (TP) of individual organisms (e.g., Chikaraishi et al., 2007, 2009; Popp et al., 2007; Hannides et al., 2009) and of suspended and sinking POM (e.g., McCarthy et al., 2007). Further, diagnostic changes in $\delta^{15}N_{AA}$ patterns also accompany bacterial degradation and AA resynthesis (McCarthy et al., 2007; Calleja et al., 2013). Because the large majority of particulate organic N (ON) is composed of amino acids, this suggests that $\delta^{15}N_{AA}$ parameters may be able to directly reveal the extent of microbial alteration to the major ON constituent of sediments, and at the same time provide a molecular-level view of potential effects of degradation on $\delta^{15}N_{bulk}$ values.

$\delta^{15}N_{AA}$ measurements may have great potential in paleo biogeochemical reconstructions of the marine N cycle. $\delta^{15}N_{AA}$ measurements of oceanic sinking particles indicate that $\delta^{15}N$ values of Src AA from surface planktonic sources are preserved in deep ocean sediment traps, and also that planktonic trophic structure information is maintained in diagnostic $\delta^{15}N$ offsets between the Tr and Src AA group (McCarthy et al., 2007). Further, analyses of deep-sea proteinaceous coral skeletons have confirmed that specific $\delta^{15}N_{AA}$ proxies can directly record $\delta^{15}N$ values of surface nitrate and long term records of change in planktonic trophic structure in some regions (Sherwood et al., 2011, 2014). Together, early work has suggested that $\delta^{15}N_{AA}$ measurements in paleoarchives may be able to simultaneously monitor baseline excursions in surface nutrient dynamics, record the mean planktonic ecosystem structure, and constrain the degree to which microbial degradation has shifted $\delta^{15}N_{bulk}$ values. Carstens et al. (2013) demonstrated that lacustrine sedimentary $\delta^{15}N_{AA}$ patterns are similar to those observed for plankton in several alpine lakes where $\delta^{15}N_{AA}$ patterns and specific $\delta^{15}N_{AA}$–based indices (e.g., ΣV and trophic or "transformation" level) appear well preserved relative to sinking POM. However, to our knowledge there are no previously published reports examining $\delta^{15}N_{AA}$ in the marine sedimentary record. $\delta^{15}N_{AA}$ measurements in sediments offer major opportunities, as well as potential challenges. Marine sedimentary measurements of $\delta^{15}N_{AA}$–based indices would greatly extend the time horizon beyond the relatively short time periods recorded by deep-sea proteinaceous corals (Sherwood et al., 2011, 2014), allowing for a new way to examine N cycle changes over millennial time scales, and potentially longer. However, sedimentary N is a complicated matrix, and much more susceptible to post depositional alteration. Therefore, the first step for $\delta^{15}N_{AA}$ applications is to evaluate preservation of $\delta^{15}N_{AA}$ signatures, as well as existing $\delta^{15}N_{AA}$ parameters in a well-characterized sedimentary system.

We present here a first investigation of marine sedimentary $\delta^{15}N_{AA}$ patterns, coupling $\delta^{15}N_{AA}$ measurements from shallow marine sediments with both sediment trap and mixed plankton tow data from the overlying water column. Santa Barbara Basin (SBB) was selected as a study site because it is a well-characterized region of high primary production and sedimentation rates, with suboxic bottom waters and thus excellent sedimentary organic matter (SOM) preservation. The main objectives of this study were first to examine the extent to which $\delta^{15}N_{AA}$ of fresh marine algal biomass and sinking POM is reflected in SOM in a high accumulation, low oxygen depositional setting. To do this, we analyzed selected water column samples to confirm the expected universal $\delta^{15}N_{AA}$ patterns for fresh algal-based sources, and then compared these with $\delta^{15}N_{AA}$

patterns preserved in sediments. Second, we assessed paleobiomarker potential for existing $\delta^{15}N_{AA}$ parameters in a marine sedimentary system. Specifically, our goal was to evaluate the potential for $\delta^{15}N$ of Src AA as a potential paleoproxy for relative changes in DIN $\delta^{15}N$, not influenced by sources of terrestrial particulate organic and inorganic N in coastal settings like SBB. Also we examined $\delta^{15}N_{AA}$-based estimates of trophic position, TP (e.g., Chikaraishi et al., 2009), as a new tool to reconstruct planktonic ecosystem changes, as reflected in primary production. Finally, these AA-based parameters in the SBB surface sedimentary record were evaluated in the context of regional ecosystem data.

Overall, our goal in choosing the SBB system was to evaluate current assumptions regarding CSI-AA parameters in a setting with strong OM preservation, reducing the potential that diagenetic change could not complicate basic interpretations. Our results indicate excellent preservation of $\delta^{15}N_{AA}$ patterns in SOM. However our results, together with a $\delta^{15}N$-based isotope mass balance model, also strongly suggest that a new paradigm will be required for applications of $\delta^{15}N_{AA}$ in paleoarchives, in which the constraints of long-term N mass balance create interdependence of common CSI-AA proxies.

2. MATERIALS AND METHODS

2.1. Study site

All samples were collected within the Santa Barbara Basin (SBB), a borderland basin on the California Margin, bounded to the north by the California coastline and to the south by the Northern Channel Islands. The SBB is a superb location for high-resolution paleoceanographic records in part because of high rates of marine primary production and rapid accumulation rates dominated by marine OM (~0.5 cm/year), although episodic precipitation events during fall and winter storms also can contribute terrestrial input (Thunell et al., 1995). High sedimentation rates and limited flushing of SBB below its sill depth (475 m), combined with a regionally intense oxygen minimum zone, produce a suboxic depositional environment, which results in annually varved sediments. These attributes have made the SBB the focus of numerous high-resolution Holocene paleoclimate studies (Barron et al., 2010 and references therein).

2.2. Water column and sedimentary OM samples

The mixed plankton tow (>35 μm) from 30 m and a 40 cm multicore from 588 m were collected in SBB (34°14′ N, 120°02′ W) in June 2008. The multicore was immediately sectioned into 1 cm intervals after core recovery, and samples were then stored frozen until freeze-dried for further processing. The sediment trap sample is a one-year nitrogen-flux weighted composite of twelve individual one-month collections from April 1999 to March 2000 in the SBB (34°14′ N, 120°02′ W) at a water depth of 450 m. Roland et al. (2008) provides a thorough description of sample preservation and the sediment-trap time-series methodology for these samples.

2.3. Age model: unsupported ^{210}Pb

The age model for the multicore was constructed using ^{210}Pb dating. Between 2 and 4 g of dried, disaggregated sediment was sealed in counting vials and stored for at least 21 days to allow for the in-growth of ^{222}Ra and ^{214}Pb to approximate equilibrium values. Samples were counted for 2–5 d using a Princeton Gamma-Tech Ge well detector (Princeton, NJ), detecting the 46.3 keV ^{210}Pb peak. Detector efficiencies were determined by counting standards filled to the same vial height as the samples using EPA standard pitchblend ore. Supported ^{210}Pb activities were determined from the total ^{210}Pb activity at the base of the core, whereas excess ^{210}Pb was calculated by subtracting the supported ^{210}Pb from the total ^{210}Pb activity. Sediment ages were inferred using the constant rate of supply (CRS) model (Appleby and Oldfield, 1978) down to 32 cm, and an average linear sedimentation rate (0.18 cm/year) from 20–30 cm was applied for intervals below 32 cm.

2.4. Bulk stable isotopic analysis

All isotopic analysis were performed at the UCSC Light Stable Isotope Facility (http://es.ucsc.edu/~silab) using a Carlo Erba CHNO-S 1108 interfaced with a Finnigan Conflo II to a Thermo-Finnigan Delta Plus XP isotope ratio mass spectrometer. Bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values were measured on lyophilized and homogenized material, pelletized in tin capsules. The $\delta^{13}C$ analyses of organic carbon ($\delta^{13}C_{OC}$) was measured on samples pretreated with 10% HCl to remove carbonates (Hedges and Stern, 1984), followed by several rinses with *Milli-Q* grade water, and then oven-dried overnight at 50° C. Measurements were performed in duplicate. Acetanilide from Indiana University (http://mypage.iu.edu/~aschimme/compounds.html) was used as a laboratory standard, with a typical analytical precision of 0.2‰ (±1 s.d.), for calibration of both $\delta^{15}N$ and $\delta^{13}C$. Bulk isotope values are reported in standard per mil (‰) notation, relative to VPDB and air for $\delta^{13}C$ and $\delta^{15}N$ respectively.

2.5. Amino acid hydrolysis, purification and derivatization for CSIA

Approximately 6 mg of lyophilized mixed plankton tow (~0.5 to 1 mg OC) ~180 mg lyophilized of sediment trap material (~7 mg OC) or ~500 mg of sediment (~11 mg OC) were used for CSIA-AA. Individual amino acids were liberated using standard acid hydrolysis conditions (110 °C, 20 h; e.g., Lee and Cronin, 1982), followed by purification with cation-exchange chromatography (Fabian et al., 1991; Veuger et al., 2005; Takano et al., 2010), and finally conversion to trifluoroacetyl/isopropyl ester (TFAA) derivatives, as detailed in previously published procedures (Silfer et al., 1991; McCarthy et al., 2007, 2013; Calleja et al., 2013). The AA mole percent ($mol\%_{AA}$) compositions were quantified independently on the same TFAA derivatives using a GC–MS (Agilent 7890 GC coupled to a 5975 MSD). The N isotopic composition of individual AA ($\delta^{15}N_{AA}$) was determined by gas chromatography/

continuous flow isotope ratio mass spectrometry (GC-C-IRMS) using previously described chromatographic conditions (Sherwood et al., 2014) and standard corrections (McCarthy et al., 2013). Under our analytical conditions, we were typically able to determine the $\delta^{15}N$ values for 13 AA: alanine (Ala), glycine (Gly), serine (Ser), valine (Val), threonine (Thr), and leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), tyrosine (Tyr), lysine (Lys), glutamine + glutamic acid (Glx) and asparagine + aspartic acid + (Asx). We note that during acid hydrolysis, glutamine (Gln) is quantitatively converted to glutamic acid (Glu), and asparagine (Asn) to aspartic acid (Asp), thus isotopic results for these combined AA are by convention reported as Glx and Asx, respectively. More detailed explanations of both CSI-AA protocols and Mol% calculations can be found in the Electronic Annex.

2.6. $\delta^{15}N_{AA}$ nomenclature, groupings, and parameter definitions

Protein AA are divided into two main groupings, based on current literature conventions (e.g., Popp et al., 2007; McCarthy et al., 2007). The *trophic AA* (*Tr AA*; Ala, Asx, Glx, Ile, Leu, Pro, Val) are those generally exhibiting more enriched $\delta^{15}N$ values, du to assumed larger ^{15}N enrichment with trophic transfer. The *source AA* (*Src AA*; Gly, Lys, Phe, Ser, Tyr) are those with typically lower $\delta^{15}N$ values, based on the assumption of little to no $\delta^{15}N$ change with trophic transfer. We note that Thr was originally grouped with source AA (McClelland and Montoya, 2002; Chikaraishi et al., 2009), however has now been shown to in fact have "inverse" N isotopic fractionation with trophic transfer that is unique among commonly measured AA (Germain et al., 2013). Following the convention of previous authors (e.g., McCarthy et al., 2013) $\delta^{15}N_{AA}$ data are organized for SBB samples by Tr and Src AA groupings, and then in alphabetical order.

The $\delta^{15}N$ value of total hydrolysable amino acids ($\delta^{15}N_{THAA}$) is employed as a proxy for total proteinaceous $\delta^{15}N$ value. $\delta^{15}N_{THAA}$ is calculated as the mole percent weighted sum of $\delta^{15}N_{AA}$ values following McCarthy et al. (2013):

$$\delta^{15}N_{THAA} = \Sigma\left(\delta^{15}N_{AA} \cdot \text{mol}\%_{AA}\right) \quad (1)$$

where $\delta^{15}N_{AA}$ is the $\delta^{15}N$ value of individual AA determined by GC-IRMS and $\text{mol}\%_{AA}$ is the molar contribution of each AA quantified with GC–MS.

Individual $\delta^{15}N_{AA}$ values were also normalized to $\delta^{15}N_{THAA}$ (see Eq. (1)). The result, $\Delta\delta^{15}N_{AA-THAA}$, offers an internally consistent way to compare $\delta^{15}N_{AA}$ patterns between samples by removing the influence of potential variations in inorganic N source isotopic value (McCarthy et al., 2013), for example in this region variation in the $\delta^{15}N$ of sub-euphotic zone DIN through time.

Trophic positions were calculated from $\delta^{15}N_{AA}$ values of Glx and Phe, following the most widely applied current formulation from Chikaraishi et al. (2009). This approach is based on a calibrated difference in $\delta^{15}N_{Glx}$ and $\delta^{15}N_{Phe}$ defined as:

$$TP = \frac{\left(\delta^{15}N_{Glx} - \delta^{15}N_{Phe} - 3.4‰\right)}{7.6‰} + 1 \quad (2)$$

where 3.4‰ is the empirical difference between $\delta^{15}N_{Glx}$ and $\delta^{15}N_{Phe}$ in aquatic marine autotrophs and 7.6‰ is the N isotopic trophic enrichment factor (TEF) of $\delta^{15}N_{Glx}$ relative to $\delta^{15}N_{Phe}$ per trophic transfer.

Analogous measures of relative trophic position were calculated from the difference in the mean $\delta^{15}N$ of Tr AA ($\delta^{15}N_{Tr}$) and Src AA ($\delta^{15}N_{Src}$) groups, $\delta^{15}N_{Tr-Src}$ after Sherwood et al. (2011).

2.7. Diagenetic indicators

Three independent metrics were used to assess early diagenetic alteration, based on AA changes in proteinaceous SOM. The degradation index (DI) (Dauwe and Middelburg, 1998; Dauwe et al., 1999) is based on multivariate analysis of protein amino acid composition, and was calculated after Dauwe et al. (1999):

$$DI = \sum_i \left[\frac{var_i - AVG_i}{STD_i}\right] \times \text{fac. coef}_i \quad (3)$$

where var_i is the mole percentage of AA i in our data set and the AVG_i and STD_i are the mean and standard deviation of AA i in the reference data set used by Dauwe et al. (1999), and fac. $coef_i$ is the factor coefficient for AA i based on the first principal component factor from Table 1 in Dauwe et al. (1999). The reactivity index (RI) (Jennerjahn and Ittekkot, 1997) is an independent quantitative metric proposed for the "quality" of OM, based on the ratio of aromatic AA (Tyr and Phe) to two non-protein AA (β-ala and γ-aba),

$$RI = \frac{\text{Tyr} + \text{Phe}}{\beta\text{ala} + \gamma\text{aba}}. \quad (4)$$

Finally, the ΣV parameter is a proxy for total heterotrophic AA resynthesis, originally proposed by McCarthy et al. (2007), and is based on the average deviation of individual $\delta^{15}N$ values of Tr AA (Ala, Val, Leu, Ile, Pro, Asx, Glx) from the $\delta^{15}N_{Tr}$ (the mean $\delta^{15}N$ of Tr AA). The ΣV value essentially expresses relative "scatter" around the mean in the $\delta^{15}N_{AA}$ *pattern*, and so is by definition internally normalized, eliminating the influence of inter-sample variation in bulk $\delta^{15}N$ values. We calculated ΣV:

$$\Sigma V = \frac{1}{n}\Sigma|\chi_i| \quad (5)$$

where χ_i, is the offset in $\delta^{15}N$ of each AA i from the average $= [\delta^{15}N_i - AVG\delta^{15}N_i]$, and n is the number of AA used in the calculation.

3. RESULTS AND DISCUSSION

3.1. $\delta^{15}N_{AA}$ patterns in water column vs. sedimentary organic matter

To explore whether $\delta^{15}N_{AA}$ in sediments reflects expected patterns for exported primary production, we compared $\delta^{15}N_{AA}$ patterns in SOM with a local mixed

plankton tow, and also a one-year flux-weighted composite of monthly SBB sediment trap samples (Fig. 1). The $\delta^{15}N$ distribution of Tr AA and Src AA values in all samples had similar patterns (Fig. 1a, Table EA-1). Mixed plankton tow and sediment trap $\delta^{15}N$ values of Tr AA ranged from 10.4‰ to 17.1‰ (mean: 13.9 ± 2.2‰) and 10.0–15.5‰ (mean: 13.0 ± 2.0‰), respectively. The $\delta^{15}N$ values of Src AA for the tow and trap ranged from 6.1‰ to 12.1‰ (mean: 9.2 ± 2.3‰) and 3.9–10.2‰ (mean: 7.1 ± 2.3‰), respectively (Table EA-2). The general $\delta^{15}N$ offsets of Tr and Src AA in all of our sample types (plankton, POM, and SOM) are consistent with expectations mixed planktonic source material. This is further supported by the $\delta^{15}N_{AA}$ based TP estimates, which ranged from 0.6 to 1.6 in all samples (see Section 3.3.2), very similar to the range in trophic positions in POM from the Pacific (1.0 to 2.4; McCarthy et al., 2007), consistent with a mixture of primary and secondary production. The general $\delta^{15}N_{AA}$pattern evident in all three sample types (Fig. 1b) is also consistent with expected $\delta^{15}N_{AA}$ patterns from autotrophs, as first observed by Macko et al. (1987). Other authors (c.f., McClelland and Montoya, 2002; McClelland et al., 2003; Chikaraishi et al., 2007, 2009; McCarthy et al., 2007, 2013) have since confirmed that $\delta^{15}N_{AA}$ patterns in both natural and cultured photoautotrophs are largely universal, regardless of taxonomic group or N source. The $\delta^{15}N_{AA}$patterns preserved in SOM here are therefore very similar not only to algae, but also to those in ocean sinking POM previously measured in the equatorial Pacific (McCarthy et al., 2007, 2013). We note that based on the essentially "universal" $\delta^{15}N_{AA}$ patterns expected in fresh marine algal sources (e.g., McCarthy et al., 2013), these findings for our selected water column samples serve primarily to verify the expected AA isotope patterns in the main sources to SBB sediments.

There are, however, also several finer scale differences in $\delta^{15}N$ values of specific AA between the tow and trap samples evident in Fig. 1a. Trap $\delta^{15}N_{AA}$ values of Ala, Glx, Gly, Phe, Ser and Thr are all significantly different from plankton tow values (Student's *t*-test, $n \geqslant 3$, $p < 0.05$), with each of these AA depleted by 1.7–6.9‰ (Table EA-1). These differences cannot be attributed to AA molar compositional changes, which remain similar across samples (see Section 3.2). It is more likely differences are related to the different time scales represented by the plankton tow versus the yearly-integrated trap sample. As noted above, the general photoautotrophic *pattern* is very similar, however specific $\delta^{15}N$ values would be expected to vary seasonally. Further, different algae species can also express different $\delta^{15}N_{AA}$ fractionations for some specific AA, particularly for Gly and Ser, both within and among taxa (c.f., Chikaraishi et al., 2009).

Because the plankton tow in this study represents a single time point, we hypothesize that the $\Delta\delta^{15}N_{tow-trap}$ for specific AA may reflect a difference in the relative mix of planktonic sources for a single sampling versus the annual integration represented by the composite trap sample. The AA Thr, as noted above, is unique, not falling within either Source or Trophic AA groups in terms of its $\delta^{15}N$ values observed in ecosystems. Thr typically shows an inverse

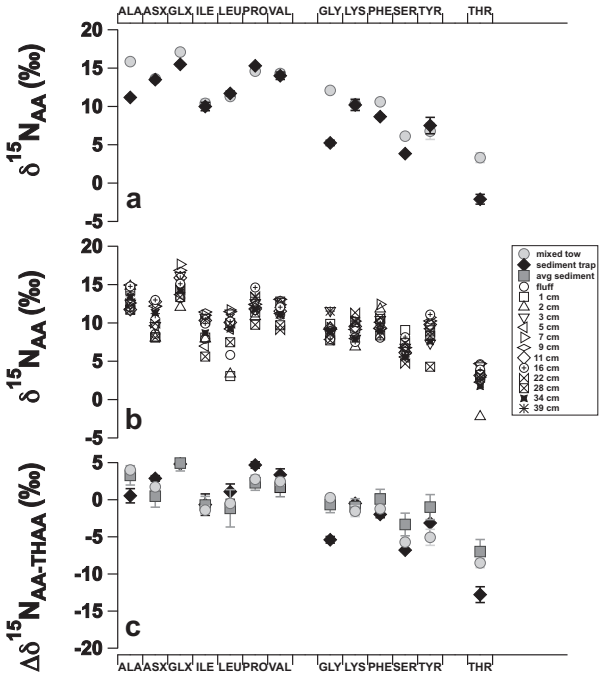

Fig. 1. $\delta^{15}N_{AA}$ values in water column (plankton tows vs. sinking POM) vs. sedimentary OM from SBB. As noted in text, AA are arranged by "Source" (Ala to Val), "Trophic" (Gly to Tyr), and "Metabolic" (Thr) groupings now common in the literature. (a) Water column OM: a single mixed tow (>30 μm, 30 m) (circles) and 1-year N flux-weighted sediment trap composite (diamonds); (b) values from 13 sediment intervals of 40 cm multicore; and (c) normalized comparison of all three sample types. $\delta^{15}N_{THAA}$ values are normalized to total hydrolysable amino acids (THAA; $\Delta\delta^{15}N_{AA-THAA}$) to allow direct comparison of relative $\delta^{15}N_{AA}$ patterns. Vertical bars in (a) represent mixed tow and trap sample standard deviations of replicate $\delta^{15}N$ analyses ($n \geqslant 3$). In (b) specific analytical errors are not shown for clarity, however standard deviation for individual AA measurements averaged 1.1‰ (see Electronic Annex Table 1). In (c) vertical bars indicate propagated analytical errors from replicate analysis of individual $\delta^{15}N_{AA}$ and $\delta^{15}N_{THAA}$ for the mixed tow and trap sample, and represent the average propagated analytical error for all sediment samples.

isotopic effect with trophic transfer, and so may have potential utility as an indicator of metabolic differences (Germain et al., 2013). We hypothesize that the apparent offset in Thr $\delta^{15}N$ values between trap and tow samples could reflect temporal changes in planktonic community structure, however because the systematics of Thr $\delta^{15}N$ values are very poorly understood, changes are difficult to interpret. Overall, however, the similarity in the broader $\delta^{15}N_{AA}$ pattern for the trap versus tow samples (see also discussion of normalized data below) suggests that water column OM sources to sedimentary ON in the SBB have similar $\delta^{15}N_{AA}$ patterns, as would be expected. This is consistent with relatively little change in $\delta^{15}N_{AA}$ patterns previously observed in sinking POM (McCarthy et al., 2007) and in oligotrophic and eutrophic lacustrine sedimentary environments (Carstens et al., 2013). Together, this implies that $\delta^{15}N_{AA}$ patterns in both trap and tow are likely representative of long-term POM sources to SBB SOM.

The $\delta^{15}N_{AA}$ patterns in SOM of multicore intervals (fluff layer to 39 cm; Fig. 1b) also generally resemble those of the mixed tow and sediment trap. Average $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ for all multicore intervals range from 3.1‰ to 17.6‰ (mean: 11.4 ± 2.6‰) and 4.7–12.4‰ (mean: 8.8 ± 1.7‰), respectively (see Table EA-2). The mean $\delta^{15}N_{Tr}$ in the mixed tow, sediment trap and average sediment intervals are 13.2‰, 12.5‰, and 11.4‰, respectively. In each multicore interval, $\delta^{15}N_{Glx}$ is the most enriched "trophic" AA and $\delta^{15}N_{Thr}$ the most depleted AA, as in both water column sample types. Overall, $\delta^{15}N_{AA}$ compositions of averaged multicore intervals are significantly correlated to both sediment trap (Pearson's $r = 0.86$, $p \ll 0.01$, $n = 13$) and mixed tow (Pearson's $r = 0.95$, $p \ll 0.01$, $n = 13$) $\delta^{15}N_{AA}$ signatures.

Normalizing $\delta^{15}N_{AA}$ to $\delta^{15}N$ of total hydrolysable proteinaceous material ($\Delta\delta^{15}N_{AA-THAA}$, see Section 2.6) offers an internally consistent way to specifically compare $\delta^{15}N_{AA}$ *patterns*, by removing potential variation in the $\delta^{15}N$ of baseline inorganic N sources through time. The striking uniformity in $\Delta\delta^{15}N_{AA-THAA}$ patterns of the mixed tow, sediment trap and averaged sediment core data (Fig. 1c) confirms similar offsets between $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ in all three sample types, and in fact even extends to the level of individual AA variations within each group. The similarity of both water column and sedimentary $\Delta\delta^{15}N_{AA-THAA}$ patterns again is consistent with the conclusion that $\delta^{15}N_{AA}$ compositions in SOM in SBB are a well-preserved archive of primary production $\delta^{15}N_{AA}$ patterns. This result is also consistent with conclusions from a recent study in alpine lacustrine environments (Carstens et al., 2013). Together this suggests that at least in similar suboxic depositional regimes, $\delta^{15}N_{AA}$ derived proxies are likely faithfully preserved, and so have substantial potential as paleoceanographic tools.

3.2. AA content and indicators of diagenetic alteration

The degree of diagenetic alteration of accumulated sedimentary OM provides the fundamental context for understanding downcore trends in $\delta^{15}N_{AA}$, including potential paleoenvironmental interpretations discussed below (Section 3.3). Because AA are one of the most labile of compound classes (Cowie and Hedges, 1994; McCarthy and Bronk, 2008), a number of different AA-based proxies for relative degradation state have been developed. These include proxies based on relative AA yields (e.g., THAA), others related to relative changes in molar% of individual AA (the DI and RI indices), and a newly defined compound-specific isotopic metric proposed to specifically indicate microbial AA resynthesis (ΣV). Because these proxies and $\delta^{15}N_{AA}$ data all derive from the same pool of hydrolysable AA, we would expect that the behavior of diagenetic proxies should directly indicate the relative alteration of the OM pool, and ultimately be consistent with $\delta^{15}N_{AA}$ changes we observe.

The contribution of THAA normalized to organic carbon (OC) content of the mixed tow and sediment trap composite is 180 and 48 mg THAA (per 100 mg OC), respectively (following the reporting convention of Cowie and Hedges, 1992) (Fig. 2a). This represents a 73% loss of THAA between fresh plankton and sinking trap material, and is consistent with the large decrease in exported POM relative to surface plankton OM, and also rapid degradation with depth in the upper water column (Cowie and Hedges, 1992, 1994; Cowie et al., 1995; Wakeham et al., 1997; Lee et al., 2000; Hedges et al., 2001). Rapid remineralization of THAA in sinking POM in the upper water column has been attributed to multiple factors, including animal heterotrophy in the euphotic zone, loss of protein-rich intracellular material through autotrophic cell lysis (Cowie and Hedges, 1992, 1994; Cowie et al., 1995; Wakeham et al., 1997; Lee et al., 2000; Hedges et al., 2001).

In surface sediments OC-normalized THAA content further decreased in the top 3 cm from a mean of 40 ± 7 to 10 ± 3 mg THAA (per 100 mg OC) below 3 cm (Fig. 2a), representing an additional 75% loss of sedimentary THAA. This loss of THAA during sedimentary burial, and the percent of OC comprised by THAA-C that can be recovered

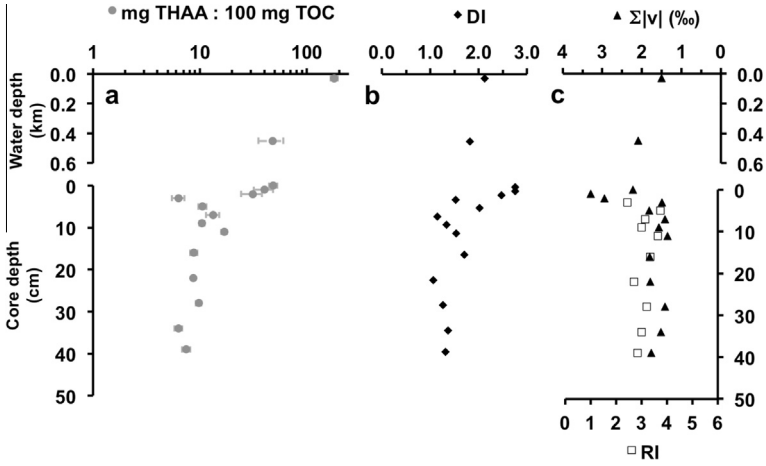

Fig. 2. Amino-acid diagenetic indicators in water column and sediments. Water column samples are shown on upper axis, and multicore sediment values are shown on lower axis for (a) total hydrolyzable amino acids (THAA), expressed as mg THAA per 100 mg OC (after Cowie and Hedges, 1994); (b) the degradation index (DI) (e.g., Dauwe et al., 1999); (c) the reactivity index (RI) (e.g., Jennerjahn and Ittekkot, 1997) and ΣV (McCarthy et al., 2007; as defined in *methods*).

with the hydrolysis techniques applied here, are well within ranges observed in sediments from multiple oceanic regions (2–30%; e.g., Moore et al., 2012 and references therein). The overall loss of THAA in SBB sediments is similar to observations in other coastal sediments (Burdige and Martens, 1988; Cowie and Hedges, 1994; Cowie et al., 1995), and the relative magnitude of THAA lost in SBB *surface* sediments is remarkably consistent with similar quantitative comparisons of THAA loss. For example, in observations from Dabob Bay, WA ~80% of the measured amino acid flux through midwater depths was lost and apparently remineralized at the sediment–water interface (Cowie and Hedges, 1992).

However, despite the overall 94% decrease in OC-normalized THAA from the mixed tow to SOM intervals (deeper than 3 cm), the AA mol% composition in all water column and sediment samples are the same within error (Fig. 3). This implies that minimal changes in the AA composition of OM have occurred between water column and sedimentary samples. This further implies that, at least on the bulk compositional level, the primary processes underlying large THAA losses are *removal and remineralization*, not degradation or diagenetic alteration of overall AA composition. Put another way, the relative mol% composition data suggests that despite the remineralization of >90% of AA, removal of AA was not selective, and the OM which has survived burial is molecularly similar to water column sources and therefore "fresh" or "unaltered". A caveat to this interpretation is that photosynthetic and heterotrophic sources of proteinaceous material can have similar bulk AA compositions (e.g., Cowie et al., 1992). However, in coastal, high-productivity and low oxygen environments such as SBB, sinking POM are expected to be the dominant source of SOM. Prior CSI-AA observations in both shallow and deep sediment traps (McCarthy et al., 2007; Hannides et al., 2013), was well as solid state NMR-based explorations of sinking POM composition observations (Hedges et al., 2001) strongly support the idea that remnant algal material dominates sinking flux to the sediments. Finally, the minimal contribution of non-protein AA in all samples from this study (<1.5 mol% of THAA), lends further support the idea that heterotrophic or bacterial sources are not major components of proteinaceous material in sinking POM.

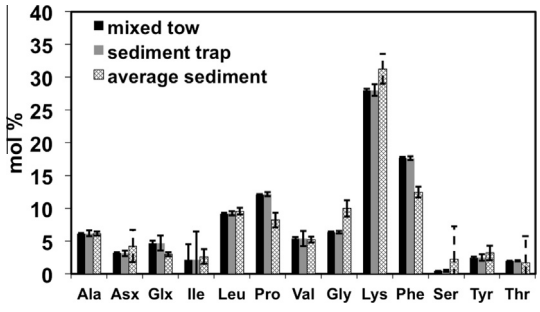

Fig. 3. Average amino acid compositions for plankton, sediment trap, and sediment samples. For plankton, error bars indicate standard deviation of replicate analyses for a single tow; for traps, error bars indicate standard deviation of replicate analyses for a single 1 year flux-weighted composite sample; for sediments error bars represent standard deviation of all multicore intervals analyzed ($n = 13$).

Despite the fact that AA mole compositions of various marine sources are all generally similar (Cowie et al., 1992), fine scale AA mole compositional changes can provide a more sensitive metric to assess OM alteration. Several widely used degradation parameters have been shown to provide more quantitative and sensitive assessments of OM preservation compared to qualitative $mol\%_{AA}$ comparisons. The DI is a multivariate metric commonly used to assess OM preservation, and is based on the relative changes in multiple AA compositions with degradation (see Section 2.7; Dauwe and Middelburg, 1998; Dauwe et al., 1999). The exact suite of AA we used to calculate DI differs slightly from those proposed by Dauwe et al. (1999), since Methionine, Arginine, and Histidine were either not abundant or are not detected in our GC-based protocol. For this reason, the exact DI values calculated here are not directly comparable to those reported in the original papers. However, variable AA groupings are now commonly used to calculate DI, and it is *relative shifts* in DI values (using the same AA suite) that are most diagnostic (e.g., McCarthy et al., 2007).

The DI values for all sample types measured here are positive, ranging from 1.1 to 2.8 (Fig. 2b). Specifically, the mixed tow and sediment trap composite have DI values of 2.1 and 1.8, respectively; the top three cm of SOM have DI values between 2.5 and 2.8, declining to an average of 1.4 ± 0.3 with a weak downcore trend (Pearson's $r = -0.02$). Downcore DI values were also significantly correlated to OC-normalized THAA (Pearson's $r = 0.89$, $p = 0.0021$). Lower DI values indicate a higher degree of OM alteration, with typical plankton DI values greater than 1, sinking POM often in the range −1 to 1, and marine sediments generally less than 0. Overall, DI values in average SOM samples of 1.4 are similar to water column values of ~2.0, which suggests that compositional changes relative to sinking POM with planktonic biomass are minimal. This is consistent with algal biomass as the primary component of sinking POM preserved in SOM of high productivity, rapid accumulation in coastal suboxic depositional settings as present in SBB.

RI is an independent quantitative metric proposed for the "quality" of OM, based on the ratio of aromatic AA (Tyr and Phe) to two main non-protein AA (β-ala and γ-aba), see Section 2.7 (Jennerjahn and Ittekkot, 1997). The basis for RI is that aromatic AA (Tyr and Phe) are concentrated in cell plasma, and thus are sensitive indicators of cell lysis and/or decay processes (Mobius et al., 2011), while the non-protein AA β-ala and γ-aba are microbial degradation products. Consistent with this expectation, β-ala and γ-aba were only quantified in downcore sediments intervals ($\geqslant 3$ cm). Combined, these non-protein AA make up less than 1.5 mol%, with no significant downcore trend, similar to observations by Cowie and Hedges (1994) in WA margin coastal sediments. As originally defined, RI values near 0 indicative extensive OM degradation, while living marine plankton have RI values between 4 and 6 (Jennerjahn and Ittekkot, 1997). Our multicore intervals below 3 cm

exhibited RI values between 2.5 and 3.8, without any significant downcore trend (Fig. 3c), also consistent with DI, which also indicates excellent preservation of remaining algal sourced OM.

Finally, we also explored a new parameter based specifically on relative changes in CSI-AA values. The ΣV index is based on the average deviation of a subset of AA ($\delta^{15}N_{Tr}$ values; see Section 2.7) and has been proposed to indicate total heterotrophic *resynthesis* of proteinaceous material (e.g., McCarthy et al., 2007, 2013). The ΣV tracks separate processes compared to the indicators discussed above, as it fundamentally indicates whether the AA pool has been isotopically altered by microbial uptake and resynthesis, irrespective of any appreciable change having occurred in AA mol% composition. As such, this parameter is perhaps most germane to the preservation of source information in $\delta^{15}N_{AA}$ isotopic patterns. Water column POM samples have ΣV values ranging from 1.5‰ to 2.1‰ for the plankton tow and sediment trap composite respectively (Fig. 3c). These values are consistent with increased heterotrophic contributions to biomass in sinking POM (McCarthy et al., 2007). Most downcore ΣV values are also similar to water column sources, ranging from 1.3‰ to 1.8‰. The only slightly higher ΣV values observed (2.2–3.3‰) are in fact at the sediment–water interface. It would seem unexpected that surficial sediments would be more microbially altered than the layers lying below. One possible explanation is provided by Prokopenko et al. (2006) and references therein, noting that thick mats of *Beggiatoa* spp. in this region are not buried, but occur commonly at the surface to upper few cm of sediments in the anoxic SBB. Although visual evidence of these mats were not noted, it is possible that the elevated surficial ΣV values might indicate some incidental direct sampling of microbial mat biomass at this location.

Taken together, however, all diagenetic parameters tell a similar story of well-preserved proteinaceous material. In spite of the marked decline in recoverable THAA in the SBB sample suite, all molecular-level AA proxies (DI, RI, ΣV) consistently indicate very minimal AA alteration in these SBB SOM samples relative to surface and sinking POM. This strongly supports the hypothesis that SOM $\delta^{15}N_{AA}$ data, at least in suboxic to anoxic and high OM depositional environments like SBB, and likely faithfully reflect planktonic source $\delta^{15}N_{AA}$ patterns, and so have strong potential for ecological and environmental reconstruction.

3.3. Paleo-environmental and -oceanographic implications

3.3.1. $\delta^{15}N_{bulk}$ and $\delta^{15}N_{THAA}$ as complementary proxies for sedimentary N studies

Because planktonic $\delta^{15}N_{AA}$ patterns appear well preserved in SBB SOM, we expect that $\delta^{15}N$ value of total proteinaceous OM ($\delta^{15}N_{THAA}$; see Eq. (1) in Section 2.6) is also well preserved. For paleoceanographic applications, sedimentary $\delta^{15}N_{THAA}$ values provide new kinds of information beyond what can be gleaned from $\delta^{15}N_{bulk}$ measurements alone. First, in part because of generally high AA liability, terrestrial AA in riverine sediment sources are rapidly replaced with marine-derived AA (e.g., Keil and Fogel, 2001), therefore SOM THAA likely represents almost uniquely marine sources (Keil and Fogel, 2001). Since $\delta^{15}N_{bulk}$ in continental margin sediments are often influenced by temporally varying amounts of terrestrial N (N_{terr}), $\delta^{15}N_{THAA}$ therefore likely provides a better measure than $\delta^{15}N_{bulk}$ of changes in the $\delta^{15}N$ of marine N_{org}. Second, because $\delta^{15}N_{THAA}$ derives from a dominant compound class of particulate organic N, $\delta^{15}N_{THAA}$ eliminates potential confounding influences of other N-bearing compound classes (e.g., chlorophylls, nucleic acids, carbohydrates), each with their own unique $\delta^{15}N$ signatures (Sachs and Repeta, 1999; Werner and Schmidt, 2002; Higgins et al., 2010).

$\delta^{15}N_{THAA}$ and $\delta^{15}N_{bulk}$ were therefore directly compared in each sample of our three sample types. The values of $\delta^{15}N_{THAA}$ in the mixed tow (11.8 ± 0.7‰), sediment trap composite (10.6 ± 0.7‰) and average multicore intervals (9.9 ± 0.7‰) are all *enriched* relative to $\delta^{15}N_{bulk}$ (by +4.6‰, +3.9‰ and +2.9‰, respectively; Fig. 4, Table 1). We note that while the water column samples are not extensive in this study, there nevertheless seems to be a progressive trend in the offset between the $\delta^{15}N$ value of the total hydrolysable proteinaceous pool, $\delta^{15}N_{THAA}$, and the $\delta^{15}N$ value of the total N in our samples, $\delta^{15}N_{bulk}$. For brevity, we define this offset as $\Delta\delta^{15}N_{THAA-bulk}$. The $\Delta\delta^{15}N_{THAA-bulk}$ is greatest in the freshest samples, and then appears to narrow progressively from sediment trap samples into buried sediments.

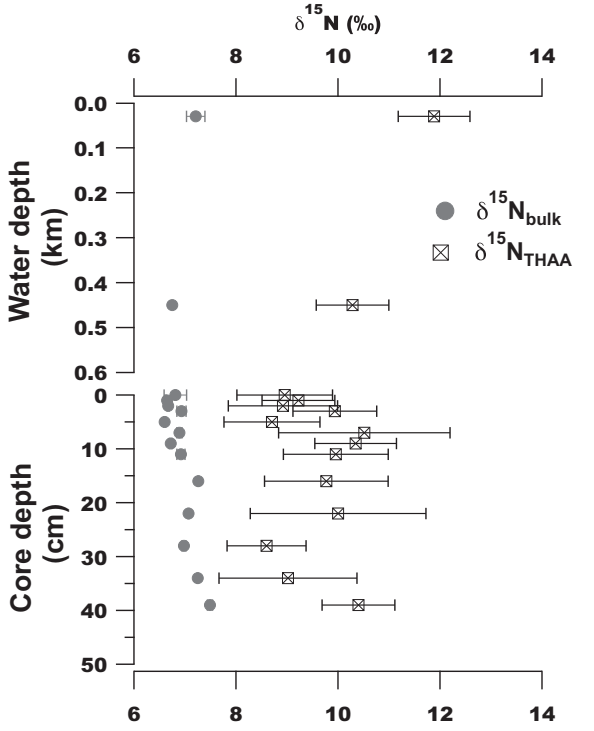

Fig. 4. $\delta^{15}N_{bulk}$ and $\delta^{15}N_{THAA}$ in water column and sedimentary samples. Top axis: plankton tow and sinking POM, bottom axis: multicore sediment intervals. As defined in text (*methods*), $\delta^{15}N_{bulk}$ represents $\delta^{15}N$ measurement on total N in sample, while $\delta^{15}N_{THAA}$ is a proxy for $\delta^{15}N$ value of the total hydrolyzable amino acid pool.

Table 1
$\Delta\delta^{15}N_{THAA-bulk}$ for published $\delta^{15}N_{AA}$ data sets, including regression statistics for $\delta^{15}N_{Bulk}$ vs. $\delta^{15}N_{THAA}$. $\delta^{15}N_{THAA}$ and $\Delta\delta^{15}N_{THAA-bulk}$ parameters are as defined in text.

Sample type	n	$\Delta\delta^{15}N_{THAA-bulk}$ (‰)	Slope	y-int	r^2	p	Reference
Autotrophs, bacteria	6	3.5 ± 0.3	1.0	3.5	1.0	1.2E−06	Macko et al. (1987)
Bacteria	2	2.3 ± 0.9	1.3	3.3	1.0	0.24	Pan et al. (2007)
Autotrophs, bacteria	7	2.5 ± 3.0	0.9	1.7	0.9	0.09	McCarthy et al. (2013)
Mixed tow	1	4.6					This study
Sediment trap	1	3.9					This study
Sediment	13	2.9 ± 0.6	0.9	3.4	0.1	1.5E−09	This study

There are several possibilities for the uniform enrichment of $\delta^{15}N_{THAA}$ relative to total N (e.g., *positive* values of $\Delta\delta^{15}N_{THAA-bulk}$) in all samples. One might be the admixture of terrestrial material. The $\delta^{15}N$ of N_{terr} can vary, but typically has an approximate $\delta^{15}N$ range of approximately 0–3‰ (e.g., Sigman and Casciotti, 2001), depleted primarily due to anthropogenic fixed N introduced as synthetic fertilizers. Therefore, the $\Delta\delta^{15}N_{THAA-bulk}$ offset might be hypothesized to be due to a mixture of isotopically distinct N_{terr}. Direct estimates of $\delta^{15}N_{terr}$ values for our study region support lower values for $\delta^{15}N_{terr}$. For example, Sweeney and Kaplan (1980) estimated $\delta^{15}N_{terr}$ to be 2.8‰ from terrestrial runoff at the eastern end of the Southern California Bight southeast of the SBB, and recent measurements of $\delta^{15}N_{bulk}$ of POM reported values of 2.9 ± 1.5‰ ($n = 5$) in 8 coastal streams along the Santa Barbara Channel (Page et al., 2008). Therefore, neither the inter-sample comparison, nor the uniformly positive $\Delta\delta^{15}N_{THAA-bulk}$ support terrestrial N as the explanation. Further, the generally similar $\delta^{15}N_{bulk}$ values between the mixed tow (6.8‰), sediment trap composite (7.2‰) and all multicore intervals (6.6–7.5‰; mean = 6.9 ± 0.3‰; Fig. 4) also do not support N_{terr} inputs, which would by definition be essentially absent in fresh plankton tow, and should increase (not decrease) in trap and sediment samples. We also note that the $\delta^{15}N_{bulk}$ values of tow, sediment and sediment trap samples are all consistent with previous results of suspended POM of 6.8 ± 0.8‰ (Page et al., 2008), sinking POM of (Thunell, 1998) and SOM (Prokopenko et al., 2006) from SBB. Finally, the fact that plankton tow sample (presumably devoid of N_{terr}) exhibits the *greatest* $\Delta\delta^{15}N_{THAA-bulk}$ offset, while sediments on average had the *smallest* offset, is also contradictory to what would be expected from an increasing contribution of ^{15}N-depleted N_{terr} in traps and sediments.

To further test this inference, we estimated the N_{terr} versus N_{mar} contribution to total N in all samples based on simple stable isotopic end-member mixing. First, stable carbon isotope values, $\delta^{13}C_{org}$, were used to estimate the terrigenous OC contribution, OC_{terr}. Applying terrestrial end-member, $\delta^{13}C_{terr}$ of −28.0‰ (Thunell, 1998), and a marine end-member, $\delta^{13}C_{mar}$ of −21.0‰ (this study and Roland et al., 2008), yields an OC_{terr} contribution of 8–19%. This range is also consistent with OC_{terr} estimates by Prokopenko et al. (2006) for SBB sediments, and also the relatively small OC_{terr} contribution to SBB sediment trap material based on compound-class $\delta^{13}C$ and $\Delta^{14}C$ values (Roland et al., 2008). A similar bulk isotope approach yields a N_{terr} contribution of 15–21%, based on $\delta^{15}N_{bulk}$ end-member values for $\delta^{15}N_{mar}$ of 8.0‰ from subsurface nitrate $\delta^{15}N$ (Sigman et al., 2003) and for $\delta^{15}N_{terr}$ of 2.9 ± 0.8‰ from riverine suspended POM (Page et al., 2008).

We can then use these estimates of N_{terr} to consider the potential influence of terrestrial input on the $\Delta\delta^{15}N_{THAA-bulk}$. The *highest* N_{terr} contribution estimate (21%) would correspond to a change in $\delta^{15}N_{bulk}$ of ~1‰. This could therefore explain less than a third of the observed $\Delta\delta^{15}N_{THAA-bulk}$ offsets. We note that it is not possible from $\delta^{15}N_{AA}$data alone to assess the effect of a given contribution of N_{terr} on the $\delta^{15}N_{THAA}$ values, because this would require knowing what fraction of AA are ultimately derived from terrestrial sources. However, as noted above, prior literature would suggest that terrestrial derived AA contributions in these samples is likely very small. An additional approach to assess the same question would be to consider the implication of possible terrestrial derived AA on AA-based trophic position (TP) estimates of SBB samples. Because characteristic AA offsets in primary producers are very different in terrestrial systems (Chikaraishi et al., 2011), AA-based TP estimates would likely be altered in samples with any substantial terrestrial AA contributions. TP estimates from these sediments are discussed in more detail below (Section 3.3), however the fact that TP estimates for all sample types closely match expectations for purely marine particles (~1.5) (McCarthy et al., 2007), together with excellent preservation noted by degradation indices above, further supports the conclusion that N_{terr} is not a major contributor to $\delta^{15}N_{THAA}$.

Instead, we hypothesize that the observed magnitude of $\Delta\delta^{15}N_{THAA-bulk}$ in SBB samples is more likely linked to variation in the biochemical composition of marine OM. Estimates of $\Delta\delta^{15}N_{THAA-bulk}$ with existing $\delta^{15}N_{AA}$ and $\delta^{15}N_{bulk}$ data from laboratory cultures and natural samples, for both autotrophs and bacteria (McCarthy et al., 2007,2013; Pan et al., 2007), strongly support this hypothesis (see Electronic Annex for explanation of $\Delta\delta^{15}N_{THAA-bulk}$ estimates). Average $\Delta\delta^{15}N_{THAA-bulk}$ values in autotrophic and bacterial biomass from published datasets (Table EA-1) range from +2.3‰ to +3.5‰, similar to the range observed in all SBB sample types, +2.9‰ to +4.6‰. Further, linear regressions of $\delta^{15}N_{bulk}$ versus $\delta^{15}N_{THAA}$ of these data (Table 1, Fig. EA-1) offers an approximation of the mean $\Delta\delta^{15}N_{THAA-bulk}$ in cultured and natural biomass. The y-intercept of these regressions predict a range of $\Delta\delta^{15}N_{THAA-bulk}$ from +1.7‰ to 3.5‰ derived from culture studies for which CSI-AA data exists. This offset in pure cells *must* be related to offsets in the

average $\delta^{15}N$ values between protein and other nitrogenous compound classes (e.g., nucleic acids, carbohydrates, chlorophylls, lipids) each with unique ranges of biosynthetic N fractionation (Hayes, 2001; Werner and Schmidt, 2002). While $\delta^{15}N_{THAA}$ data are not yet extensive, this literature comparison (Table 1, Fig. EA-1) strongly suggests that while $\Delta\delta^{15}N_{THAA-bulk}$ values in fresh biomass fall in a generally similar range, there could also be variations between specific groups of algae microorganisms.

Overall, however, the culture data suggests that $\Delta\delta^{15}N_{THAA-bulk}$ in both tow, trap, and sediments are mostly related to a relative mixture of nitrogenous organic compound classes, as opposed to terrigenous or other inputs. That $\Delta\delta^{15}N_{THAA-bulk}$ values in all sample types are similar to ranges in pure cultures (Table 1, Fig. EA-1) supports this conclusion, and also strongly supports the inference of excellent N_{org} preservation discussed above. We hypothesize that the differences in the magnitude of $\Delta\delta^{15}N_{THAA-bulk}$ between the mixed tow and trap samples could be associated with planktonic community composition, especially considering different time scales of the specific samples that were available for this study (i.e., annual integration for traps versus a single sampling for the mixed plankton tow). Alternatively, it is also possible that preferential loss of different nitrogenous compound classes during water column remineralization and sediment burial could explain the apparent trend in decreasing $\Delta\delta^{15}N_{THAA-bulk}$; indeed, the fact that this offset is lowest in sediments is also consistent with extensive NMR data suggesting that proteinaceous material dominates sedimentary N_{org} preserved in recently deposited sediments (Knicker et al., 1996, 2002; Nguyen and Harvey, 1998; Knicker and Hatcher, 1997, 2001).

Overall, we therefore hypothesize that sedimentary $\delta^{15}N_{THAA}$ will therefore reflect $\delta^{15}N$ of integrated *proteinaceous* material exported from the surface ocean. If a consistent average relationship exists between $\delta^{15}N_{THAA}$ and $\delta^{15}N_{bulk}$ in primary production (as has been indicated both by our comparison here, and also in earlier literature; e.g., McCarthy et al., 2013) then an appropriate calibration factor should be able to reconstruct $\delta^{15}N$ values of bulk exported primary production from $\delta^{15}N_{THAA}$ measured in sediments. Characterizing the range in $\Delta\delta^{15}N_{THAA-bulk}$ values of fresh marine OM and comparing $\Delta\delta^{15}N_{THAA-bulk}$ of sinking and sedimentary OM in various depositional settings will therefore be important for developing $\delta^{15}N_{THAA}$ as a potential paleoproxy. In any environmental application, we also suggest that ΣV values will be useful to evaluate if microbial resynthesis may have altered initial relationships.

3.3.2. Interrelationship of source and trophic AA values in sedimentary archives: dependence of individual values of $\delta^{15}N_{AA}$ on trophic position

As noted above, the most important applications of $\delta^{15}N$ CSI-AA to date have been based on the ability to decouple primary production $\delta^{15}N$ signatures from the effects of trophic transfer, and so simultaneously indicate baseline $\delta^{15}N$ values together with precise values for trophic position (TP; *methods*). Among the source AA, $\delta^{15}N_{Phe}$ in particular has been shown to be the best proxy for integrated $\delta^{15}N$ values at the base of the marine food webs. This conclusion has come primarily from measurements in individual marine organisms, both in culturing and feedings studies (e.g., Chikaraishi et al., 2009; Germain et al., 2013), and in environmental studies of specific organisms or mixed plankton samples (e.g., Schmidt et al., 2004; McCarthy et al., 2007; Popp et al., 2007; Chikaraishi et al., 2009; Hannides et al., 2009, 2013). Recently, the same conclusion has also been strongly supported by paleoceanographic work with deep-sea corals (Sherwood et al., 2011, 2014). Together, past work therefore suggests that $\delta^{15}N_{Phe}$ in marine sediments could represent a direct proxy for integrated $\delta^{15}N$ value of baseline nitrogen sources. Sedimentary $\delta^{15}N_{Phe}$ values in multicore intervals are ~10‰ at the core top, declining to ~8‰ in the upper half (<20 cm), and increasing again toward 10‰ at the bottom of the core (Fig. 5a). The range of sedimentary $\delta^{15}N_{Phe}$ values, 8.1–12.4‰ (mean = 10.0‰) span $\delta^{15}N_{Phe}$ in the mixed tow (10.6‰) and sediment trap composite (8.6‰) samples (Fig. 5a, Table EA-1). If sedimentary $\delta^{15}N_{Phe}$ values directly track baseline values, as hypothesized, then these trends might indicate regional excursions in the $\delta^{15}N$ of exported primary production.

The TP record shows a similar pattern of change over most of the downcore profile, except the deepest 3 intervals (Fig. 5b). Most TP values in all three sample types fall in the range of 1–1.5, consistent with prior sinking POM results (McCarthy et al., 2007); the overall average TP of sediment intervals (1.2 ± 0.2) is slightly lower than the mixed plankton tow and sediment trap composite samples (both 1.4 TP, within error). The TP values of these sample types should represent the average TP of a mixture of marine OM sources in the (McCarthy et al., 2007). However, what is most striking in the data is that TP values appear to mirror $\delta^{15}N_{Phe}$ throughout most of the core (0 to ~30 cm). In the upper depths (with the best resolution), this inverse relationship is strongest, with TP values progressively increasing from ~1.0 at the core top, to ~1.5 at 16 cm depth, coincident with a *decrease* in $\delta^{15}N_{Phe}$. However, in the deepest few samples ($\geqslant$28 cm; 3 out of 15 samples) this relationship is not observed, with TP and $\delta^{15}N_{Phe}$ values trending in the same direction (both increasing with depth). Overall, however, variations of TP and $\delta^{15}N_{Phe}$ over both water column and multicore samples exhibit a significant inverse correlation between TP and $\delta^{15}N_{Phe}$ (Pearson's $r = -0.56$, $p = 0.029$, $n = 15$). Assuming the $\delta^{15}N_{THAA}$ is well preserved (as the data discussed above clearly indicate) this relationship suggests *interdependence* exists between $\delta^{15}N_{Phe}$ and TP values. Such a conclusion would run directly counter to current $\delta^{15}N_{AA}$ theory and common applications in animals, which assumes these qualities are fundamentally independent.

The most likely explanation for this apparent contradiction may lie in the long time scales of sedimentary accumulation, as opposed to more discrete temporal $\delta^{15}N_{AA}$ measurements in organisms. Over long time scales, $\delta^{15}N$ values in a system must be constrained by N isotopic and mass balance. We hypothesize that this constraint should in fact create an intrinsic interdependence between TP (i.e., related to the isotopic separation between source and trophic AA groups) and the $\delta^{15}N$ values of all AA. To test

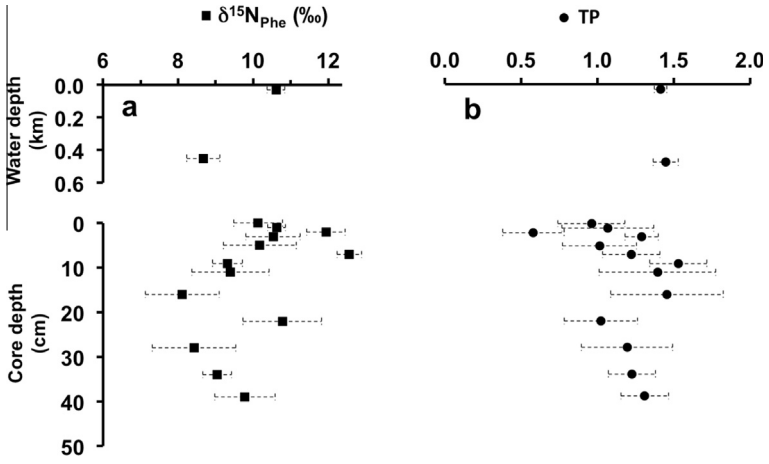

Fig. 5. Comparison of $\delta^{15}N_{Phe}$ and trophic position (TP) in water column POM and multicore sediment samples. An inverse relationship of TP vs. $\delta^{15}N_{Phe}$ is significant at the 95% confidence interval (Pearson's $r = -0.56$, $p = 0.029$, $n = 15$). This suggests interdependence between $\delta^{15}N_{Phe}$ values and planktonic ecosystem TP over timescales of sedimentary accumulation.

this idea we constructed a simple isotope mass balance model to examine TP dependence on $\delta^{15}N$ values of source AA ($\delta^{15}N_{Src}$), and trophic AA ($\delta^{15}N_{Tr}$).

Fig. 6 is a schematic of the main assumptions underlying the isotope mass balance model, showing the partitioning of $\delta^{15}N$ between trophic and source AA groups, as well as trophic enrichment factors (TEF) during trophic transfer. Our model assumes a steady state isotope mass balance for a fixed $\delta^{15}N$ composition of the proteinaceous pool, $\delta^{15}N_{THAA}$, and also a constant ratio of trophic AA, f_{Tr}, and source AA, f_{Src} (e.g., mole fraction of each AA group) which comprise the total THAA pool, f_{THAA} (Eq. (6)):

$$f_{THAA} \cdot \delta^{15}N_{THAA} = f_{src} \cdot \delta^{15}N_{Src} + f_{Tr} \cdot \delta^{15}N_{Tr} \quad (6)$$

The $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ values in Eq. (6), as well as the average $\delta^{15}N$ offset *between* the two groups, $\beta_{Tr/Src}$, are determined by three factors: (1) the baseline $\delta^{15}N$ of primary production and fractionation for both AA groups, trophic, α_{Tr}, and source, α_{Src}, relative to $\delta^{15}N_{THAA}$, (2) trophic enrichment factors (TEF) for trophic AA, TEF_{Tr}, and source AA, TEF_{Src}, groups, and (3) the value of TP (Fig. 6, Eq. (7)):

$$\delta^{15}N_{Tr} - \delta^{15}N_{Src} = \alpha_{Src} + (TEF_{Src} \cdot TP) + \alpha_{Tr} + (TEF_{Tr} \cdot TP) \quad (7)$$

Combining Eqs. (6) and (7):

$$\delta^{15}N_{Src} = \frac{f_{THAA} \cdot \delta^{15}N_{THAA} - f_{Tr}[\alpha_{Src} + (TEF_{Src} \cdot TP) + \alpha_{Tr} + (TEF_{Tr} \cdot TP)]}{f_{Src} + f_{Ttr}} \quad (8)$$

A specific $\delta^{15}N_{Phe}$ value can than be calculated with the following:

$$f_{Src} \cdot \delta^{15}N_{Src} = \sum_{i=1}^{n} f_{Src_i} \cdot (\delta^{15}N_{Phe} + \varepsilon_{Src_i\text{-}Phe}) \quad (9)$$

where, f_{Src_i}, is the mole fraction of each source AA, and $\varepsilon_{Src_i\text{-}Phe}$ is the offset between the $\delta^{15}N$ of each source AA and $\delta^{15}N_{Phe}$. See Table EA-3 for values of model parameters.

The model output for $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$, driven by values of TP over the range observed in our sediment samples, clearly demonstrates the dependence of both $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ on the systems TP, given the model assumptions (Fig. 7). The offset between $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ increases linearly with increasing TP, as it must. Therefore, this simple model demonstrates that *under the constraint of isotopic mass balance, values of both $\delta^{15}N_{Src}$ and $\delta^{15}N_{Tr}$ become necessarily dependent on TP*. This is because any individual $\delta^{15}N_{AA}$ value fundamentally results from the partitioning of a fixed source pool of AA (with a fixed $^{15}N/^{14}N$ ratio) between the Tr and Src AA groups.

Further, we then examined if this simple model could also reproduce measured variation in $\delta^{15}N_{Phe}$ in SBB sediments. To apply the model to examine actual SBB data, we used the measured TP of each sediment interval, and then we used the model to calculate $\delta^{15}N_{Phe}$ from Eq. (9). The mole fraction of each Src AA, f_{Src_i}, was based on the measured mol% of the SBB plankton tow (Fig. 3), and N isotopic offsets of source AA relative to Phe, $\varepsilon_{Src_i\text{-}Phe}$, were derived from averages of field and culture data for autotrophic organisms (Table EA-3) (McClelland and Montoya, 2002; McClelland et al., 2003; Chikaraishi

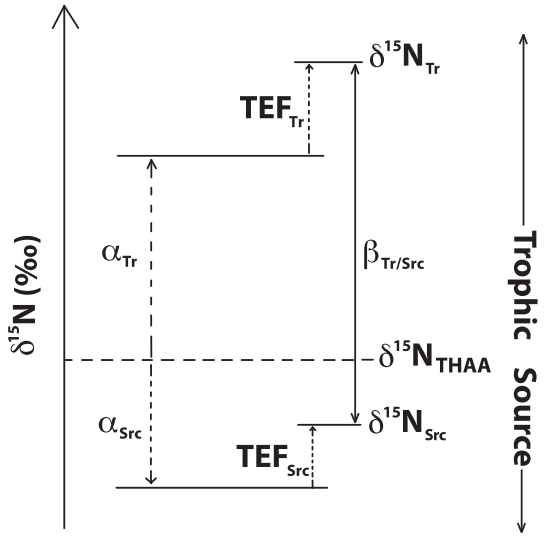

Fig. 6. Conceptual diagram showing main assumptions underlying the isotope mass balance model of amino acid $\delta^{15}N$ values and trophic position. *Y*-axis is the relative $\delta^{15}N$ value, referenced to $\delta^{15}N$ value of total AA pool ($\delta^{15}N_{THAA}$; dashed horizontal line). Trophic AA (Tr) by definition have $\delta^{15}N$ values above this line, while Source AA (Src) have $\delta^{15}N$ values below. $\delta^{15}N$ offsets of average Tr AA ($\delta^{15}N_{Tr}$) and average Src AA ($\delta^{15}N_{Src}$) values from the total AA pool (i.e., $\delta^{15}N_{THAA}$) in autotrophs are indicated by α values (see Eqs. (6)–(9), Section 3.3.2). The changing offset between $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ (or $\beta_{Tr/Src}$, connected by a solid double arrow line) then reflects the combined effects of the original offsets in primary producers, α, combined with a relative $\delta^{15}N$ change of AA groups with each progressive trophic transfer (trophic enrichment factor; TEF). At steady state, if $\delta^{15}N_{THAA}$ is assumed to be constant, than both $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ values are inherently dependent on the degree of trophic transfer (or trophic position).

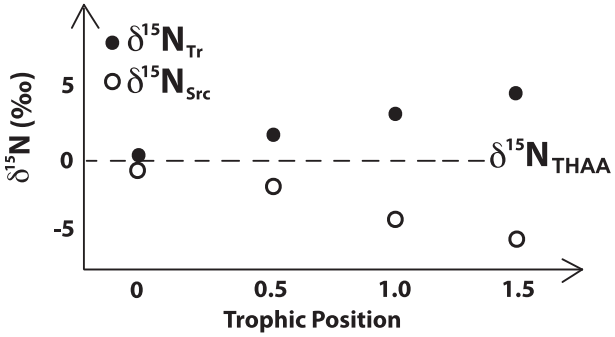

Fig. 7. Isotope mass balance model output showing $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ values as a function of trophic position. *Y*-axis indicates relative $\delta^{15}N$ values ($\delta^{15}N$ value for the total THAA pool, $\delta^{15}N_{THAA}$, is arbitrarily set at zero). Trophic positions (0.5–1.5) span total range observed in SBB sediments. The slopes of the $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ output are the average TEF values for each AA group.

et al., 2007, 2009; McCarthy et al., 2007, 2013). The magnitude of the measured SBB isotope changes, as well as overall shape of the observed $\delta^{15}N_{Phe}$ records, are clearly captured by the model output (Fig. 8). Modeled and observed sedimentary $\delta^{15}N_{Phe}$ values are significantly positively correlated (Pearson's $r = +0.58$, $p = 0.036$, $n = 13$) when $\delta^{15}N_{THAA}$ values are set to 8‰ (i.e., the approximate average of sub-euphotic zone nitrate $\delta^{15}N$ from water column data in Sigman et al., 2005).

Overall, these results strongly support our hypothesis that long-term isotope mass balance underlies the observed inverse relationship between TP and $\delta^{15}N_{Phe}$. However, our simple model also clearly cannot capture all environmental factors that could impact $\delta^{15}N_{Phe}$, and we suggest this is why exact observed $\delta^{15}N_{Phe}$ values (as opposed to the *trends*) are not identical to model output values. Some potential factors not captured by our model include time-varying changes of nutrient $\delta^{15}N$, (and also $\delta^{15}N_{THAA}$), any variation in percentage of N-utilization, the mole fraction of Tr and Src groups, variability in individual values of $\varepsilon_{Src_i\text{-}Phe}$, or differences in either α_{Tr} or α_{Src}. We note that AA preservation changes do not seem to be relevant here (see discussion above in Section 3.2), however, plankton composition changes might influence the mole fraction of Tr and Src groups, and so $\varepsilon_{Src_i\text{-}Phe}$ offsets (see Table EA-3). Further, if periods occur where basic model assumptions (e.g., complete N utilization, and a "closed system" with a constant N isotope mass balance) no longer apply, then the inverse relationship between $\delta^{15}N_{Phe}$ and TP would also no longer be predicted. It is tempting to speculate that such factors could underlie the deepest several data points in the record (below 28 cm), where $\delta^{15}N_{Phe}$ and TP appears to have a positive – not an inverse relationship (Fig. 5). However, below 28 cm, there are very few data, and interpretation of such finer details of this core is therefore beyond the scope of our current data set. Overall, what seems most striking is that a very simple mass balance model can recreate not only the observed overall interrelationship between TP and $\delta^{15}N_{Phe}$, but can also capture the relative magnitude of changes observed in a natural system.

Together, our model and the SBB data suggest that a fundamentally different paradigm may be required for CSI-AA temporal reconstructions in paleoarchives. Specifically, in contrast to past studies of samples collected in the water column, our sedimentary analyses and simple model indicate that over long time scales (given the assumption of steady state N balance), measured individual $\delta^{15}N_{AA}$ values must become directly dependent on TP change. This suggests that two key parameters, TP and $\delta^{15}N_{THAA}$ (the total proteinaceous pool $\delta^{15}N$ value), are in fact the master variables. Dependence of AA $\delta^{15}N$ value on TP change also should of course extend to each individual AA, not just to $\delta^{15}N_{Phe}$ or the average $\delta^{15}N_{Tr}$ or $\delta^{15}N_{Src}$ groups. However, the strength of the TP dependence for any specific AA in turn depends on its relative mol% and its TEF value (the very different slopes of $\delta^{15}N_{Tr}$ versus $\delta^{15}N_{Src}$ shown in Fig. 7 illustrate both these effects). Finally, however, it is also very important to note that in cases where TP in a record is *unchanging* (i.e., as in both case studies for deep sea corals so far published; Sherwood et al., 2011, 2014), then TP variation by definition has no effect, and $\delta^{15}N_{Phe}$ (or $\delta^{15}N_{Src}$) values should again directly reflect baseline $\delta^{15}N$ values. However, in cases where TP varies, then

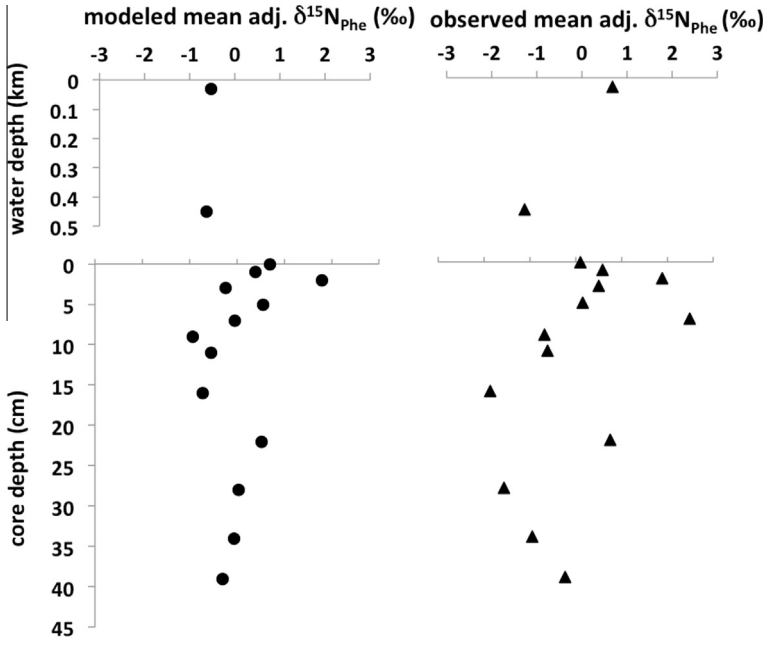

Fig. 8. Comparison of measured $\delta^{15}N_{Phe}$ values with isotope mass balance model predicted $\delta^{15}N_{Phe}$. Water column (top panels) and downcore (bottom panels) values of the mean adjusted $\delta^{15}N_{Phe}$ values from model output (left side), are compared with SBB sedimentary measurements (right). As detailed in text (Section 3.3.2), modeled $\delta^{15}N_{Phe}$ values are a function of trophic position.

$\delta^{15}N_{THAA}$ should become the only direct proxy for baseline $\delta^{15}N$ values.

3.3.3. Regional interpretation of TP and $\delta^{15}N_{THAA}$ records

We have hypothesized that sedimentary profiles of $\delta^{15}N_{THAA}$, coupled with TP and $\delta^{15}N_{Tr-Src}$ (a broader proxy for TP, see Section 2.6), may be used to differentiate temporal changes in baseline $\delta^{15}N$ values from changes in ecosystem trophic structure. Our downcore records from the SBB offers a unique opportunity to evaluate these AA-based parameters as potential paleoceanographic indicators of regional physical and ecosystem changes in the Southern California Bight (SCB).

^{210}Pb-based chronology determined that the deepest sediment interval in the multicore is ca. 1870 and that the average sedimentation rate is 0.29 cm year^{-1}. This sedimentation rate is consistent with previous estimates of 0.12 to 0.5 cm year^{-1} for SBB (Barron et al., 2010). CSI-AA data were analyzed at 13 intervals throughout the sediment core at low temporal resolution, particularly prior to ca. 1980 (Fig. 9). Because of the low resolution of the record prior to ca. 1980, the lack of trends in all three CSI-AA parameters could simply be a result of aliasing of high frequency variations. However, $\delta^{15}N_{THAA}$, TP and $\delta^{15}N_{Tr-Src}$ decrease markedly after 1980. $\delta^{15}N_{THAA}$ decreases from 10.6‰ to 9.3‰, TP decreases from 1.6 to 0.6, and $\delta^{15}N_{Tr-Src}$ decreases from 5.0‰ to 2.0‰. These parameters are now briefly considered in the context of regional oceanography.

Assuming complete nitrate utilization for the system and that $\delta^{15}N_{THAA}$ reflects changes in the DIN $\delta^{15}N$ of source water to the region, then a $\delta^{15}N_{THAA}$ decrease of ca. 1.3‰ would imply a decrease in DIN $\delta^{15}N$ also of ~1.3‰ in SBB since 1980. The annual mean subsurface DIN $\delta^{15}N$ in SBB today is ~8‰ (Sigman et al., 2003, 2005). This value is likely set by the relative ratio of the dominant surface and subsurface sources throughout SBB, which include the equatorward CA Current (CC), poleward Southern CA Countercurrent (SCCC) and the poleward CA Undercurrent (CUC) (Hickey and Royer, 2001). The sub-euphotic zone nitrate $\delta^{15}N$ in the CUC (at ca. 300 m) near Baja California exhibits values up to 15–16‰, but decreases northward along the CA margin from 8% to 10‰ in the SBB (Sigman et al., 2003, 2005) to deep-ocean $\delta^{15}N$ values (ca. 6‰) off the coast of Washington (Kienast et al., 2002). One way to change the average $\delta^{15}N$ of DIN of sub-euphotic water in SBB could simply be a change in the admixture of source waters to SBB. Assuming the DIN $\delta^{15}N$ values of CUC and CC end-members have not changed since 1980, then the decreasing $\delta^{15}N_{THAA}$ values could imply a greater proportion of $\delta^{15}N$-depleted nitrate from the northerly CC relative to the $\delta^{15}N$-enriched CUC in the admixture of these currents to SBB. This is broadly consistent with documented physical oceanographic changes occurring throughout the CCS during this period, and also expected changes in the relative ratio of CCS source waters to the SBB (e.g., Palacios et al., 2004; Rykaczewski and Dunne, 2010).

Contemporaneous with the declining trend in $\delta^{15}N_{THAA}$ are declining trends in TP and $\delta^{15}N_{Tr-Src}$ parameters since ca. 1980. These trends indicate that the average TP of the export flux has decreased in the SBB over this time period. This observation also seems consistent with documented CCS-wide ecosystem changes. Specifically, declining zooplankton biomass since ca. 1950 (Lavaniegos and Ohman, 2003, 2007), as well as nutricline shoaling and increases in *Chl a* since ~1980 (Rykaczewski and Dunne, 2010), are

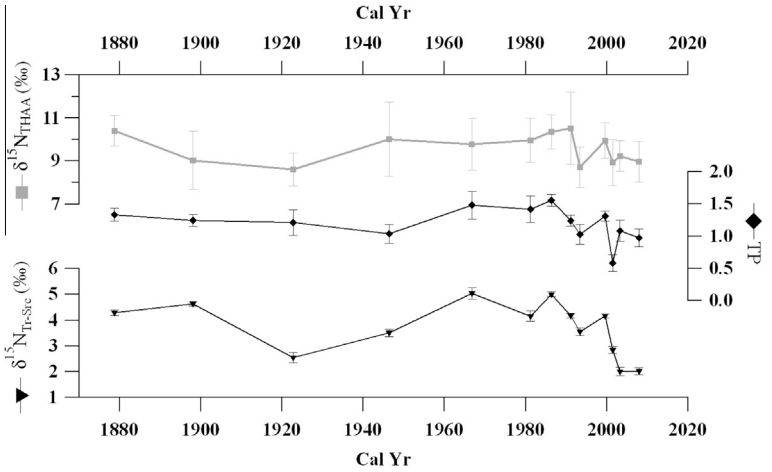

Fig. 9. The sedimentary record of CSI-AA parameters in SBB since ca. 1880. $\delta^{15}N_{THAA}$ (the $\delta^{15}N$ value of total proteinaceous material), trophic position ($TP_{Glu/Phe}$) explicitly estimated after Chikaraishi et al. (2009) and $\delta^{15}N_{Tr-Src}$ (the offset between $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$, an analogous measure of trophic enrichment) are as defined in text (*Methods*; Section 2.6). Vertical bars for $\delta^{15}N_{THAA}$, TP, and $\delta^{15}N_{Tr-Src}$ represent propagated analytical error for calculated parameters.

thought to represent a more productive and likely less complex marine food-web, having fewer trophic transfers (Rykaczewski and Checkley, 2008; Aksnes and Ohman, 2009). Such a shift would be consistent with declining average planktonic TP values, as reflected in $\delta^{15}N_{Tr-Src}$ values. This would imply that since 1980, the average TP of the export flux has decreased in the SBB.

Taken together, records of $\delta^{15}N_{THAA}$, TP and $\delta^{15}N_{Tr-Src}$ since ca. 1980 provide a first attempt to use these CSI-AA parameters as proxies of regional physical and ecosystem change. Broad trends in these new data since 1980 are consistent with general ecosystem changes throughout the CCS. Based on the observations and discussion above, we hypothesize that CSI-AA data, in organic-rich, anoxic or sub-oxic environments such as the SBB, may be a useful addition to the toolkit used to study paleoceanographic N biogeochemical cycling and ecosystem and baseline DIN changes.

4. SUMMARY AND CONCLUSIONS

Similar planktonic patterns of $\delta^{15}N_{AA}$ values were found in sedimentary organic matter (SOM) and water column POM sources from the Santa Barbara Basin. Together with molecular-level indicators of preservation (e.g., DI, RI, etc.), this supports our basic hypothesis that SOM $\delta^{15}N_{AA}$ data preserve $\delta^{15}N_{AA}$ patterns of local planktonic sources. We conclude that at least in similar environments having excellent OM preservation, $\delta^{15}N_{AA}$ derived proxies have great potential in paleoceanographic studies to track variations in the marine N cycle. Specifically, they can provide a record of both baseline $\delta^{15}N$ values and also trophic structure of exported primary production.

However, our data also suggest that a fundamentally new conceptual framework is likely required for CSI-AA proxies in paleoceanographic applications. The SBB multicore data showed an inverse relationship between TP values and individual $\delta^{15}N_{Phe}$ values through most of the record. A simple isotope mass balance model confirms that in fact an interdependence of individual $\delta^{15}N_{AA}$ values on TP is expected for any record which integrates exported production over long temporal scales, and thus is constrained by long term $\delta^{15}N$ mass balance (i.e., typical for most paleoceanographic archives). This idea represents a significant departure from current assumptions about the independence of source AA $\delta^{15}N$ values (e.g., $\delta^{15}N_{Phe}$) and TP estimates, at least as these apply in modern, short time frame, ecosystem studies. However, it is also important to note that in cases where TP is *unchanging* throughout a record, such dependence is by definition removed, and the direct linkage between source AA and baseline $\delta^{15}N$ values should again comply with utility in modern ecosystems. For example, several recent studies have demonstrated deep-sea proteinaceous coral records with essentially invariant TP (Sherwood et al., 2011, 2014; Prouty et al., 2014). When TP does not vary, the direct linkage between $\delta^{15}N_{Phe}$ (or $\delta^{15}N_{Src}$) and nitrate $\delta^{15}N$ indicated in these studies is fully consistent with our findings here.

We conclude that in sedimentary records, $\delta^{15}N_{AA}$ values will be strongly influenced by *both* changes in both TP and in $\delta^{15}N$ of the source inorganic nitrogen. This implies that in sediments changes in baseline $\delta^{15}N$ values are likely best reflected not by selected source AA (as is now assumed), but instead by $\delta^{15}N_{THAA}$ (the proxy for $\delta^{15}N$ value of total proteinaceous material). Our comparison of $\delta^{15}N_{THAA}$ in SBB water column sources and sediments also indicates that while trends in $\delta^{15}N_{THAA}$ likely track $\delta^{15}N$ of exported N_{org}, $\delta^{15}N_{THAA}$ *values* are also offset from bulk $\delta^{15}N$ values due to presence of other nitrogenous organic compounds. Examination of literature CSI-AA data from algal cultures suggests a relatively constant correction factor, whose value is also consistent with our SBB observations. This suggests that applying an offset correction to sedimentary $\delta^{15}N_{THAA}$ is necessary to reconstruct $\delta^{15}N$ of marine ON sources.

Taken together, these results suggest that in regions with complete nitrate utilization, $\delta^{15}N_{THAA}$ may represent the best new proxy for the $\delta^{15}N$ of nitrate, and (unlike $\delta^{15}N_{bulk}$) one that is independent of potentially confounding inorganic N sources. However, more work is now needed to more precisely determine the offset between $\delta^{15}N_{THAA}$ and $\delta^{15}N$ of bulk algal N_{org}, and we suggest this is one of the most important areas of future research for application of CSI-AA proxies in paleoarchives. Overall, our results imply that in paleoceanographic contexts TP together with $\delta^{15}N_{THAA}$ represent the central CSI-AA parameters, and that these must always be assessed together. We suggest that, at least in sedimentary environments where AA are well preserved, TP and $\delta^{15}N_{THAA}$ should provide a coupled picture of regime shifts in planktonic ecosystem structure, and also $\delta^{15}N$ at the base of food webs. Future work now needs to evaluate how well $\delta^{15}N_{AA}$ are preserved in other marine sedimentary environments, particularly those characterized by oxic and slow-depositional conditions. This work will be critical to assess the potential of $\delta^{15}N_{AA}$ proxies in deep, open-ocean sediments.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2014.08.002.

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