Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter

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Abstract

Bulk nitrogen (N) isotope signatures have long been used to investigate organic N source and food web structure in aquatic ecosystems. This paper explores the use of compound-specific $\delta^{15}N$ patterns of amino acids ($\delta^{15}N$-AA) as a new tool to examine source and processing history in non-living marine organic matter. We measured $\delta^{15}N$-AA distributions in plankton tows, sinking particulate organic matter (POM), and ultrafiltered dissolved organic matter (UDOM) in the central Pacific Ocean. $\delta^{15}N$-AA patterns in eukaryotic algae and mixed plankton tows closely resemble those previously reported in culture. $\delta^{15}N$ differences between individual amino acids (AA) strongly suggest that the sharply divergent $\delta^{15}N$ enrichment for different AA with trophic transfer, as first reported by [McClelland, J.W. and Montoya, J.P. (2002) Trophic relationships and the nitrogen isotopic composition of amino acids. *Ecology* **83**, 2173–2180], is a general phenomenon. In addition, differences in $\delta^{15}N$ of individual AA indicative of trophic transfers are clearly preserved in sinking POM, along with additional changes that may indicate subsequent microbial reworking after incorporation into particles.

We propose two internally normalized $\delta^{15}N$ proxies that track heterotrophic processes in detrital organic matter. Both are based on isotopic signatures in multiple AA, chosen to minimize potential problems associated with any single compound in degraded materials. A trophic level indicator (ΔTr) is derived from the $\delta^{15}N$ difference between selected groups of AA based on their relative enrichment with trophic transfer. We propose that a corresponding measure of the variance within a subgroup of AA (designated ΣV) may indicate total AA resynthesis, and be strongly tied to heterotrophic microbial reworking in detrital materials. Together, we hypothesize that ΔTr and ΣV define a two dimensional trophic "space", which may simultaneously express relative extent of eukaryotic and bacterial heterotrophic processing.

In the equatorial Pacific, ΔTr indicates an average of 1.5–2 trophic transfers between phytoplankton and sinking POM at all depths and locations. The ΣV parameter suggests that substantial variation may exist in bacterial heterotrophic processing between differing regions and time periods. In dissolved material $\delta^{15}N$-AA patterns appear unrelated to those in POM. In contrast to POM, $\delta^{15}N$-AA signatures in UDOM show no clear changes with depth, and suggest that dissolved AA preserved throughout the oceanic water column have undergone few, if any, trophic transfers. Together these data suggest a sharp divide between processing histories, and possibly sources, of particulate vs. dissolved AA.
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1. INTRODUCTION

Stable N isotope ratios ($\delta^{15}N$) have been widely used in ecosystem research for over three decades. Because of the

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consistent increase in $^{15}N/^{14}N$ ratios from food source to consumer, bulk sample $\delta^{15}N$ measurements have become central to studies of marine food webs as an indictor of trophic position, as well as source and processing of organic materials (e.g., Peterson and Fry, 1987; Lajtha and Michener, 1994). The effects of multiple sources and fractionating processes, however, can make interpreting bulk $\delta^{15}N$ measurements challenging. In living organisms multiple food sources can be difficult to resolve, even with coupled multi-isotope analyses (Peterson, 1999). In detrital organic matter these challenges are further compounded because the molecular identity of only a portion of total organic N is usually known, multiple N sources may exist, and diagenetic alteration has often occurred (Benner et al., 1987).

Compound-specific isotopic measurements can be used to address some of the challenges of complex samples by making $\delta^{15}N$ measurements directly on specific molecules or compound classes. Because AA represent most organic N in both planktonic organisms and sinking particles (e.g., Cowie and Hedges, 1992), the advent of gas chromatography linked to isotope ratio mass spectrometry (GC-IRMS) for rapidly measuring $\delta^{15}N$ values of individual AA (Macko et al., 1997) now makes it possible to directly examine the major organic N component in biota, as well as much detrital organic material. In addition, the fundamental biochemical underpinning for overall $\delta^{15}N$ shifts can be examined by observing changes in $\delta^{15}N$ patterns of common protein AA (Macko et al., 1987).

Recent work by McClelland and co-authors (2002, 2003) were the first published studies showing how $\delta^{15}N$ values of individual AA ($\delta^{15}N$-AA) change with trophic transfer. McClelland and Montoya (2002) showed that regular increases in bulk $\delta^{15}N$ commonly observed with each trophic transfer masks a large disparity in the relative enrichment of individual AA. Some, such as aspartic acid (Asp) and glutamic acid (Glu), display very large $\delta^{15}N$ enrichments with each trophic step, while others such as phenylalanine (Phe), serine (Ser), and threonine (Thr) have $\delta^{15}N$ values which remain essentially unchanged. Based on these observations the authors suggested that $\delta^{15}N$ values of specific non-enriching AA might provide a unique tag for source $\delta^{15}N$ values at the base of the food chain, and the difference in $\delta^{15}N$ between these source AA and those that enrich strongly with each trophic transfer would represent an internally normalized indicator of trophic history, unaffected by unknown mixture components (McClelland and Montoya, 2002; McClelland et al., 2003).

Most organic N in the ocean water column is not in living organisms, but exists as detrital nitrogenous material in DOM and POM. POM is the major source of both remineralized N and carbon to the ocean's interior and supports sedimentary biological communities. Dissolved organic N (DON) is by far the largest reservoir of organic N in the water column, and plays a key role in N cycling in oceanic regions. While some DON cycles rapidly, a large amount survives on time scales long enough to be advected out of surface waters. Its biological inaccessibility acts as an important control on upper ocean biological cycles, and its advection and slow remineralization at depth represents

an additional N "pump", fundamental to closing upper ocean budgets (Jackson and Williams, 1985; Williams and le, 1995). In detrital organic N pools, AA are by far the main component that can be identified at the molecular level (e.g., McCarthy et al., 1997; Kaiser and Benner, 2000; Bronk, 2002). Understanding the specific sources and processing histories of AA may thus prove central to elucidating the processes controlling cycling and long-term preservation of the ocean's DON pool. Molecular-level AA analysis has long been a powerful tool toward this end (e.g., Lee and Cronin, 1984; Cowie and Hedges, 1992; Dauwe et al., 1998, 1999); however because AA molar ratios from diverse sources tend to be similar (e.g., Cowie and Hedges, 1992; McCarthy et al., 1996), in many oceanic settings there is a limit to information available from traditional approaches.

If the $\delta^{15}N$-AA trophic signatures identified by McClelland and Montoya are also preserved in detrital materials, $\delta^{15}N$-AA may offer a novel approach to examine both source and subsequent transformation of the ocean's major non-living N reservoirs. However, in detrital materials microbial processes are key, both as the primary pathway for remineralization (e.g., Williams, 2000) as well as sources of DOM (McCarthy et al., 1998; Ogawa et al., 2001). In contrast to the clear $\delta^{15}N$ shifts typically associated with zooplankton feeding, overall $^{15}N/^{14}N$ fractionation patterns in microbial food webs are less predictable. Flagellates and ciliates excrete NH_4^+ and DON depleted in ^{14}N relative to their bacterial prey (Hoch et al., 1996), and thus become enriched in ^{15}N in the same manner as larger zooplankton. However, because of the ability of bacteria to rapidly take up ambient dissolved N, the $\Delta\delta^{15}N$ between microzooplankton and bacteria has been found to depend heavily on the degree of coupling between these microorganisms, and may differ between ocean regions or time periods (Hoch et al., 1996). In addition, because bacteria can use a variety of N sources, a generalized ^{15}N shift between bacterial biomass and organic substrate may not occur. Overall, in contrast to trophic transfers in zooplankton, bulk $\delta^{15}N$ shifts due to microbial process are probably smaller and more variable, and in bacterial biomass may often be undetectable.

Any approach using $\delta^{15}N$-AA signatures in detrital materials must also take potential bacterial changes of these patterns into account. Recent work on $\delta^{13}N$ signatures of individual AA has suggested that bacterial and protist heterotrophy often results in strong fractionation in only a few AA (Ziegler and Fogel, 2003; McCarthy et al., 2004) that are tied directly to specific microbial biochemical pathways (Scott et al., 2006). If $\delta^{15}N$ shifts in only selected AA also occur, $\delta^{15}N$-AA patterns might largely preserve trophic transfer information, while at the same time providing a tracer for both microbial sources and alteration to DON and PON not observable in bulk N isotope measurements. Individual stable isotopic AA signatures might thus better reflect both heterotrophic and autotrophic sources than either bulk $\delta^{15}N$ or traditional AA Mol% compositional analyses.

In this paper, we examine $\delta^{15}N$-AA signatures in sinking particles, high molecular weight DOM, and selected plank-

ton samples from the central Pacific ocean. Our goals are to first examine whether δ^{15}N-AA patterns and enrichment behaviors identified by McClelland and Montoya (2002) are observed in detrital materials from a very different oceanic environment, and then to investigate the behavior of δ^{15}N-AA parameters in light of possible microbial alteration in the water column.

2. MATERIALS AND METHODS

2.1. Sampling

All samples were collected in the central Equatorial Pacific Ocean in association with the US Joint Global Ocean Flux (US-JGOFS) study in 1992. Plankton and sediment trap samples are sub-fractions of the JGOFS sample set, collected in February or August–September, 1992 between 12°N and 12°S along 140°W. Dissolved organic samples were collected aboard the R.V. John Vickers during the same time period (February–March, 1992) while occupying a JGOFS station.

Rhizosolenia sp. is a diatom of the order Bacillariophyceae, collected from an enormous bloom along a ~1000 km long front ("The line in the sea", Yoder et al., 1994) during the US JGOFS EqPac Survey II cruise in August 1992, at approximately 2°N, 140°W. Samples were collected from buoyant 1–2 m deep surface patches sampled directly from deck (Lee et al., 2000). Net plankton (26–850 μm), containing a mixture of zooplankton and phytoplankton, were collected at 2°N, 140°W during Survey II cruise (February 1992). The sample was collected at 100 m depth as an oblique tow with a 26 μm net, and later passed through a 850 μm sieve, as described previously (Lee et al., 2000).

Sinking POM samples were collected at the base of the mixed layer, 1000 m below the surface, and 1000 m above the bottom (Lee et al., 2000). POM exiting the photic zone was collected using floating rotating-sphere sediment traps on either side of the equator (1°S and 1°N, 135°W) at 105 m during Survey I. Deep trap samples (1000 and 3650 m) were taken using rotating sphere traps from year-long (February 1992–March 1993) moored deployments at 5°N (Honjo et al., 1995). All samples were poisoned with mercuric chloride, and processed as described previously (Wakeham et al., 1997a; Lee et al., 2000).

Samples of high molecular weight DOM were isolated by tangential flow ultrafiltration (UF) from four depths (2–4000 m) at 12°S, 135°W, after 0.1 μm pre-filtration to remove particles and bacteria (Benner et al., 1997). Briefly, UF isolates 20–35% of the total DOM pool based primarily on molecular size, without chemical modification. Ultrafiltered DOM (UDOM) samples in this study were isolated using 1000 Da Amicon spiral-wound polysulfone membranes, as described previously (e.g., Benner et al., 1992; McCarthy et al., 1996; Benner et al., 1997). Many UDOM chemical properties, including C/N ratios, stable carbon isotopic ratios, radiocarbon 'ages', overall amino-acid content and AA D/L ratios, are generally similar to those for total DOM, and are clearly distinct from those of either marine biomass or sinking particles, making UF a widely used tool to study seawater DOM (e.g., Benner et al., 1992, 1997; McCarthy et al., 1996; Guo et al., 1996; Aluwihare et al., 1997). At the same time some UDOM properties are also shifted relative to lower molecular weight DOM, supporting the idea that HMW DOM is typically less degraded, in accordance with size-reactivity continuum ideas (Amon and Benner, 1994). For example, radiocarbon measurements indicate that UDOM is younger than total DOM (Loh et al., 2004).

2.2. Amino acid hydrolysis and isotopic analyses

Individual AA isotopic analyses on UDOM, POM, and biota samples were made after acid hydrolysis (6 N HCl, 100°, 20 h) using isopropyl-TFA derivatives based on the method of Silfer (Silfer et al., 1991). UDOM samples were typically hydrolyzed in duplicate, while trap and plankton samples were hydrolyzed singly. After derivatization samples were analyzed on a Varian gas chromatograph coupled to a Finnegan Delta-Plus isotope ratio mass spectrometer (GC-IRMS). Samples were chromatographed using a 50 m, 0.32 ID Hewlett Packard Ultra-1 column. δ^{15}N values were typically measured for alanine (Ala), aspartic acid + asparagine (Asp), glutamic acid + glutamine (Glu), leucine (Leu), isoleucine (Ile), proline (Pro), valine (Val), glycine (Gly), lysine (Lys), serine (Ser), phenylalanine (Phe), threonine (Thr), and tyrosine (Tyr). For plankton tows and ocean particles, derivatives were injected 2–3 times. Reproducibility for POM samples was typically less than 1‰ and never exceeded 2‰. While individual standard deviations can not be calculated for all samples, the average of mean deviations for all POM sample replicates was 0.66‰.

For UDOM the more complex chromatograms typical of DOM samples (McCarthy et al., 2004) made resolution more difficult. UDOM samples had to be highly concentrated to obtain adequate peak sizes of less abundant components; however this decreased resolution, increased problems with interfering peaks, and in some cases led to co-elution of target AA. As a result, Tyr and Phe were not resolved in UDOM; (Ala + Gly) and (Ser + Thr) co-eluted and were measured as combined peaks; and for all AA an increase in analytical variation was observed. For each UDOM derivative 3–6 replicate injections were made, and typical reproducibility was ~3‰, but ranged as high as 5‰. This increase in analytical variation relative to biota and POM samples mirrored what has been observed with compound-specific stable carbon AA patterns (δ^{13}C-AA; McCarthy et al., 2004).

2.3. δ^{15}N-AA parameters

ΔTr (proxy for trophic transfers) is based on the offset ($\Delta\delta^{15}$N) between the mean δ^{15}N values of two groups of AA: the first group is composed of five "trophic" AA (Tr-AA; those which enrich with each trophic transfer) Asp, Glu, Leu, Val, and Pro. The second group are two of the "source"-AA (Src-AA; those which do not enrich with trophic transfer), Phe and Thr. The rationale for choosing these specific AA is discussed below (Section

3.1). The ΔTr value (representing a specific number of trophic transfers) is calculated by defining the average offset between these same AA in eukaryotic phytoplankton as ΔTr = 0, and then using the corresponding $\Delta\delta^{15}N$ for a single trophic transfer, as reported in culture experiments (food-consumer = 4.67; McClelland and Montoya, 2002). Thus:

$$\Delta Tr = [\Delta\delta^{15}N_{sample} - \Delta\delta^{15}N_{phytoplankton}]/4.67$$

where $\Delta\delta^{15}N$ = AVG $\delta^{15}N$ (Asp, Glu, Leu, Val, Pro) − AVG $\delta^{15}N$ (Phe and Thr). Taking the literature value of 4.67 as a constant and using the reproducibility for POM and Biota sample analysis described above (±0.66‰), the propagated average error for ΔTr would be ±0.13‰.

The ΣV parameter (proxy for total heterotrophic resynthesis) is defined as the average deviation (e.g., Bevington and Robinson, 2003) in the $\delta^{15}N$ values of the Tr-AA Ala, Asp, Glu, Ile, Leu, and Pro. Thus:

$$\Sigma V = 1/n \ \Sigma \ Abs(\chi_{AA})$$

where χ (deviation) of each Tr-AA = $[\delta^{15}N_{AA} - AVG \ \delta^{15}N$ (Ala, Asp, Glu, Ile, Leu, and Pro)], and n = the total number of Tr-AA used in the calculation. Again, reasons for specific AA choices are discussed below (Section 4). Using the reproducibility for POM and biota analysis (±0.66‰), the propagated standard analytical error for the ΣV calculation is ±0.29‰.

For UDOM samples, ΔTr_{UDOM} and V_{UDOM} were calculated as above; however because fewer individual AA were resolved in this matrix the AA included were slightly different. First, only AA resolved in every sample were included in the UDOM-specific parameters. Ile or Leu were not quantifiable in some samples due to co-elution. V_{UDOM} was thus calculated in an identical manner as ΣV, but based on Asp, Glu, Pro, Val, and the combined peak Gly + Ala. Using the ±3‰ reproducibility typical for UDOM, propagated error for V_{UDOM} is ±1.5‰. For the ΔTr_{UDOM} the Tr-AA used were Asp, Glu, Pro, and Val. For the Src-AA (Ser + Thr) was used, measured as a single combined peak. Based on culture data (McClelland and Montoya, 2002), this pair should provide a solid Src-AA reference comparable to the Phe + Thr pair used for plankton and POM samples. Error propagation yields a standard analytical error for ΔTr_{UDOM} of ±0.5‰ corresponding to the typical reproducibility of ±3‰ for UDOM samples.

2.4. DI index

The degradation index (DI) proposed by Dauwe et al. (1998, 1999) was calculated for POM samples using molar percentage (Mol%) AA compositions published previously for these same samples (Lee et al., 2000). DI was calculated using the formula originally proposed (Dauwe et al., 1999):

$$DI = \Sigma \ [var_i - AVGvar_i/STDvar_i] * Fact.Coef._i$$

where DI is the degradation index, Var is the Mol% of the individual AA, AVG$_{var}$, and STD$_{var}$ are the mean Mol% and standard deviation of each AA, respectively, and

Fact.Coef.$_i$ is the factor coefficient from Table 1 in Dauwe et al. (1999).

2.5. Statistical tests

Statistical tests discussed in the text (unpaired t-test, ANOVA, linear correlation) were conducted with either the INSTAT (GraphPad software, Inc.) or SYSTAT statistics packages. With small data sets many common statistical tests are problematic. The assumption of normality underlying parametric tests may not be met, while the data set here (n = 5 to 10) is too small to reliably test for normality. Non-parametric statistical tests could alternately be used; however with very small sample sets the inherent limitations of non-parametric approaches are exaggerated, and such tests become limited in their ability to detect real differences (e.g., Taylor, 1987; Bevington and Robinson, 2003). Because common parametric tests such as t-test and ANOVA are considered relatively robust for distributions that are not strictly Gaussian (e.g., Taylor, 1987), we have chosen to apply parametric tests, bearing in mind the caveats above. Unless otherwise stated, all tests results refer to 95% confidence intervals.

3. ASSUMPTIONS AND TERMINOLOGY

3.1. Amino acid groupings

As indicated above, AA in plankton have been found to fall into two groups with respect to the behavior of individual $\delta^{15}N$ values within food webs (McClelland and Montoya, 2002): one group (Tr-AA; Asp, Glu, Ala, Ile, Leu, Val, and Pro) have been found to be strongly enriched with trophic transfer, while a second group (Src-AA; Gly, Ser, Thr, Phe, Tyr, and Lys) have $\delta^{15}N$ values which remain largely unchanged with each trophic shift. These same groupings also largely agree with those found for AA $\delta^{15}N$ changes in higher animals, including mammals (Hare et al., 1991) and tuna (Popp et al., In press). It should be noted that while "essential" vs. "non-essential" AA designations are useful in higher animals, there is little overlap between Tr-AA and Src-AA groups and essential vs. non-essential AA. This may not be surprising, since the essential vs. non-essential designations describe carbon flow through biochemical systems, in particular the ability to synthesize-specific carbon skeletons, but are not directly linked to N cycling. $\delta^{15}N$-AA values should instead reflect the degree of transamination experienced by the total pool of a given AA. Popp and coauthors (In Press) have suggested that the "essential" vs. "non-essential" categories are not directly relevant to $\delta^{15}N$-AA enrichment, and instead have used the terms "source" and "trophic" (Popp et al., In press). We have used these same designations here. It would also be possible to group individual AA based on their biosynthetic families (e.g., Hayes, 2001), and attempt to interpret changes in individual AA $\delta^{15}N$ values based on biochemical pathways. However, we have chosen to treat our data within the existing observation-based framework, in part because the extensive metabolic diversity of microbes, coupled with their ability to salvage, resynthesize,

or even alter synthetic pathways (Lengeler et al., 1999; Scott et al., 2006) could make assumptions about specific biosynthetic pathways tenuous.

The Tr-AA group consists of aliphatic and acidic side-chain AA (Asp, Glu, Ala, Ile, Leu, and Val) as well as Pro, observed to have $\Delta\delta^{15}$N of 4–7‰ with each trophic transfer. Each of these AA has one N (excepting the associated forms Gln and Asn), which is obtained directly from Glu via transamination (Rawn, 1983). The Tr–AA group as a whole also displays a very similar range of values in phytoplankton (McClelland and Montoya, 2002). The "enriching" behavior of this group with trophic transfer is presumably related to active transamination, suggesting that these AA are major sources for the light N that is excreted by macro and microzooplankton (Checkley and Miller, 1989; Hoch et al., 1996). The Src-AA group, observed by McClelland and Montoya to remain relatively constant with each trophic transfer ($\Delta\delta^{15}$N: -1‰ to $+1.5$‰) includes Gly as well as most of the more chemically complex side chain AA (Ser, Thr, Phe, Tyr, and Lys). The N in the Src-AA group derives from more diverse enzymatic pathways (Rawn, 1983). The Src-AA group also are universally depleted in δ^{15}N relative to the Tr-AA group in both algae and zooplankton, and values within this group show a substantially more diverse δ^{15}N distribution (McClelland and Montoya, 2002).

3.2. Heterotrophic processing

The main focus of this paper is interpreting δ^{15}N values of AA in terms of the heterotrophic processing histories of POM and DOM, with particular emphasis on the possibility of distinguishing between bacterial and eukaryotic processes. However, in addition to "heterotrophy", a large number of related terms (e.g., degradation, decomposition, reworking, alteration, cycling, diagenesis, etc.) are commonly used to discuss similar, and often overlapping, aspects of the biological transformation of organic matter. This makes it important to clarify the specific terms we use in relation to δ^{15}N-AA patterns we will discuss.

Heterotrophic reworking of proteinaceous material encompasses a range of processes promoted by heterotrophic organisms (e.g., bacteria, protists, zooplankton, etc.) including hydrolysis, uptake and de novo synthesis, salvage AA incorporation into new protein, as well as strict catabolism. Heterotrophically "processed" material thus represents a mixture of new biosynthate as well as remnant macromolecular material that has never been hydrolyzed. In turn, protein in new biosynthate also represents a mixture of resynthesized and salvaged AA.

We are concerned here primarily with observed changes in δ^{15}N-AA *pattern*, i.e., instances in which substantial change in δ^{15}N values of only *selected* AA are observed against a backdrop of other δ^{15}N-AA values that remain relatively unaltered. Because AA broad molar distributions of ocean plankton sources are generally similar (e.g., Lee and Cronin, 1984; Cowie and Hedges, 1992; McCarthy et al., 1996), it is unlikely that mixtures of sources with different bulk δ^{15}N values would usually result in only *selected*

δ^{15}N-AA changes. Salvage AA uptake similarly should not alter original δ^{15}N values of AA incorporated into new protein. Thus an underlying assumption we make is that substantial δ^{15}N-AA shifts in only selected AA indicates primarily de novo *heterotrophic* AA resynthesis: i.e., that at least the N of an assimilated AA has been replaced via a fractionating metabolic pathway. For us to observe these changes in particulate or dissolved material, new heterotrophic biosynthate must also have become incorporated as a significant part of the organic matter mixture. Heterotrophy in which strict catabolism dominates, and new heterotrophic biomass does not become a significant part of detrital organic matter, would likely leave δ^{15}N-AA signatures of original unhydrolyzed material largely unchanged. Thus, any δ^{15}N-AA shifts observed suggest that heterotrophy has occurred, but also indicates that new heterotrophic biosynthate constitutes an important part of material measured.

In what follows, we use "heterotrophy" as a broad term to encompass the combined processes associated with bacterial growth or animal feeding. We use "resynthesis" as a shorthand term to indicate heterotrophic synthesis of AA, i.e., AA incorporated into new *heterotrophic* biomass in which at least one N has been replaced via an enzymatic pathway (the broader term "de novo synthesis" could also include AA synthesis occurring in autotrophs from mineral carbon and N sources). "Salvage incorporation" is used to indicate uptake and incorporation of AA with both the carbon skeleton and all N atoms unchanged. "Remnant material" refers to macromolecular proteinaceous material (or biomass) that has never been hydrolyzed, and thus has survived heterotrophy with its primary AA structure and original isotopic signatures largely intact. In addition, we use the term "trophic" specifically in the context of resynthesis or salvage incorporation during one transfer of material between a consumer (protozoan or zooplankton) and its prey. We do not use the term "trophic" in relation to bacterial heterotrophy.

Finally, a note concerning archaea. Archaea are increasingly recognized as major components of oceanic microbial assemblages (e.g., Karner et al., 2001; Ingalls et al., 2006a). It is likely that the effects of AA resynthesis by heterotrophic archaea would not be distinguishable from resynthesis by bacteria based on the parameters in this paper. Limited available data on compound-specific isotope patterns in archaea suggest that, as with bacteria, archaeal heterotrophy acts to increase the "scatter" of AA patterns (Scott et al., 2006). However, because very little archaea-specific data exists, we have limited the following discussion to bacterial heterotrophy, bearing in mind that archaea might also contribute to changes we observe.

4. RESULTS

4.1. Plankton samples

Values for plankton and POC are given in Table 1. The *Rhizosolenia* sp. sample provides an unusual opportunity to compare δ^{15}N-AA values from essentially a natural algal monoculture with results from lab cultures (McClelland

and Montoya, 2002). As noted previously (*sampling*, Section 2.1), this sample was collected at ~2.5°N during August, in contrast to the other samples that were collected during February. Because our goal is not to examine absolute $\delta^{15}N$ values, but rather δ^{15}N-AA *patterns*, this offset in sampling time should not be important. This inference is supported by the nearly identical patterns in the natural *Rhizosolenia* sp. vs. cultured alga (from McClelland and Montoya, 2002) discussed below. To directly compare the *Rhizosolenia* sp. δ^{15}N-AA pattern with both natural and cultured samples having differing N sources, distributions were normalized by difference to Phe = 0 in Fig. 1, chosen because Phe was the benchmark AA identified as least changed with trophic level by McClelland and Montoya (2002).

The δ^{15}N-AA distribution from *Rhizosolenia* sp. was very similar to the δ^{15}N-AA pattern previously observed in the eukaryotic algae *Tetraselmis suecica* (Fig. 1). The Tr-AA group values in both algae are similar to Glu, and most Src-AA values are slightly depleted. The main exceptions are Gly in the Src-AA group, and Pro in the Tr-AA group, both of which appear substantially depleted in *Rhizosolenia* relative to *T. suecica*. These diatoms can have complex life histories, sometimes harboring symbiotic N-fixing bacteria and undergoing vertical migration (e.g., Villareal and Carpenter, 1989; Villareal et al., 1997). It is not known whether these factors influence the differences in δ^{15}N-AA pattern between natural *Rhizosolenia* sp. and cultured *T. suecica*. Of specific importance for the following discussion is the comparison between the Tr-AA and Src-AA groups, because it is the increase in this offset that we use as a proxy for trophic transfers. The offset between Tr-AA vs. Src-AA groups (Table 1) used in the ΔTr calcu-

lation are indistinguishable within analytical error (3.5‰ and 3.7‰ for *T. suecica* vs. *Rhizosolenia* sp., respectively; vs. propagated error for Avg (Tr-AA) – Avg (Thr,Phe) = ±0.6‰). A *t*-test indicates no significant difference between the two means.

Relative to the algae, the mixed plankton tow (26–850 μm) shows substantial $\delta^{15}N$ enrichments in the Tr-AA group (Fig. 1b). Every Tr-AA is enriched in ^{15}N relative to Phe, as well as relative to the Src-AA group as a whole. As in the pure phytoplankton, the individual $\delta^{15}N$ values in the Tr-AA group continue to fall within a narrow range near Glu. The Src-AA show somewhat greater variation in the mixed tow relative to the phytoplankton. Lys and Tyr were depleted relative to Phe (~5‰), while Gly and Ser were enriched (2–3‰). However, the shifts of all these AA relative to Phe are also similar to shifts previously observed in the Src-AA group in zooplankton consumers (McClelland and Montoya, 2002). Overall, the mean value for (Tr-AA – Src-AA) was 10.7 ± 0.6‰ (Table 1), vs. the corresponding value of 3.7 ± 0.6‰ for phytoplankton discussed above. A *t*-test indicates these offsets are significantly different.

4.2. Sinking particles

δ^{15}N-AA distributions in sinking POM at 2°N (collected at the same site and time as the plankton tow; Table 1, Fig. 2a and b), are similar to the mixed tow in showing large and consistent offsets between Tr-AA and Src-AA in every sample. The mean $\delta^{15}N$ of (Tr-AA – Src-AA) range from 10.2 to 12.9 (Table 1). Assuming normal statistics, one way ANOVA indicates no significant difference between any of these values. As with the mixed tow, the same

Table 1
δ^{15}N-AA Values and parameters for plankton and sinking POM

		$\delta^{15}N$ Plankton (‰)		$\delta^{15}N$ Sinking POM (‰)			
		Rhizo. 2.5°N	Tow 2°N	105 m 2°N	105 m 1°S	1000 m 5°N	3600 m 5°N
	ΔTr	0.0	1.5	1.9	1.4	1.8*	1.9
	ΣV	1.0	1.5	3.4	2.9	1.4	1.7
	DI	nd	−0.07	−0.90	0.37	−2.3	−2.7
	TrAA -SrcAA	*3.7*	*10.7*	*12.8*	*10.2*	*12.9**	*12.7*
Trophic	Ala	6.3	15.8	8.6	8.3	15.7	17.8
	Asp	5.4	11.6	14.1	11.7	11.4	12.6
	Glu	5.7	15.3	21.0	16.4	15.5	15.7
	Ile	5.6	14.9	9.5	13.4	15.2	11.6
	Leu	4.3	13.8	17.6	15.4	14.6	13.9
	Pro	2.6	11.7	14.2	7.9	12.9	13.5
	Val	10.6	15.2	12.9	8.1	16.2	17.6
Source	Gly	−3.5	7.7	nd	nd	8.5	9.8
	Lys	nd	nd	12.0	4.5	nd	nd
	Ser	0.6	8.3	nd	nd	7.3	6.2
	Thr	**1.5**	**0.4**	**−0.6**	**−2.2**	**1.2**	**−1.7**
	Tyr	2.4	4.9	nd	nd	6.3	8.0
	Phe	**2.6**	**5.3**	**7.0**	**5.6**	**10.0**	**5.5**

AA abbreviations are as defined in text (Section 2.2). Underlined AA are those used in the ΔTr calculation; TrAA-SrcAA is the mean value of the Src-AA used in ΔTr subtracted from the mean value of Tr-AA (Section 2.4). Asterisk (∗) at values for the 5°N, 1000 m sample indicates that the value for Phe was excluded from parameters for this sample, as described in the text. DI values are calculated from AA Mol% data reported in Lee et al. (2000).

test indicates that all these values are significantly higher than the algal mean offsets above.

In contrast to the 105 m trap samples, δ^{15}N-AA patterns in both year-long deep trap samples at 5°N are more similar to each other. The specific δ^{15}N values of most individual AA are similar at both depths (Table 1, Fig. 2c), and the average δ^{15}N of the Tr-AA group (12.9 ± 0.6 vs. 12.7 ± 0.6) were within analytical error, and not significantly different via t-test. The 5°N site is well outside the variable equatorial upwelling zone, and these similarities suggest that both mid and deep-water traps at 5°N had similar year-averaged sources of proteinaceous material. A few AA δ^{15}N values, however, are clearly shifted between the two depths. In the Tr-AA group, Ala is enriched by 2‰ at 3650 m vs. 1000 m, while Ile is depleted by 3.6‰. Both these AA are among those most commonly observed to show strong δ^{13}N shifts with bacterial heterotrophy (Fogel and Tuross, 1999; Keil and Fogel, 2001; McCarthy et al., 2004). In the Src-AA group, both Thr and Phe in the deep trap samples are depleted relative to values at 1000 m. Together, this suggests that additional heterotrophic (likely bacterial) resynthesis has taken place between mid-water and the deep ocean, consistent with other characteristics of these sinking particles (Wakeham et al., 1997b; Lee et al., 2000). Phe shows the largest shift of any AA

($\Delta\delta^{15}$N of 4.5‰) between mid and deep water. However, as discussed below, we believe the elevated Phe value at 1000 m (+10‰) is likely in error.

4.2.1. ΔTr in sinking POM

The calculated ΔTr values for POM at all depths fall into a similar range, indicating that exported POM in this region has undergone approximately 2 trophic transfers (Table 1; Fig. 3). There are also smaller ΔTr differences between POM samples from different sites. ΔTr indicates an offset of approximately 0.5 average trophic transfers between the mixed tow and 105 m at 2°N (ΔTr = 1.4 vs.

Fig. 2. δ^{15}N-AA Signatures of Sinking POM vs. Plankton. In each panel the δ^{15}N-AA pattern for the pure alga (*Rhizosolenia* sp.) is included for reference. All δ^{15}N values are those directly measured, except those for *Rhizosolenia* sp. which have been adjusted by difference such that Phe = average Phe value for other samples compared (as in Fig. 1 this is done so that δ^{15}N-AA *patterns* can be directly contrasted). AA abbreviations are as defined in the text. (a) δ^{15}N-AA patterns for contemporaneous mixed tow vs. POM exiting the photic zone (105 m) at 2°N. (b) δ^{15}N-AA patterns for 2°N vs. 1°S 105 m traps. (c) δ^{15}N-AA patterns for samples from 1000 to 3650 m year-long trap deployments at 5°N.
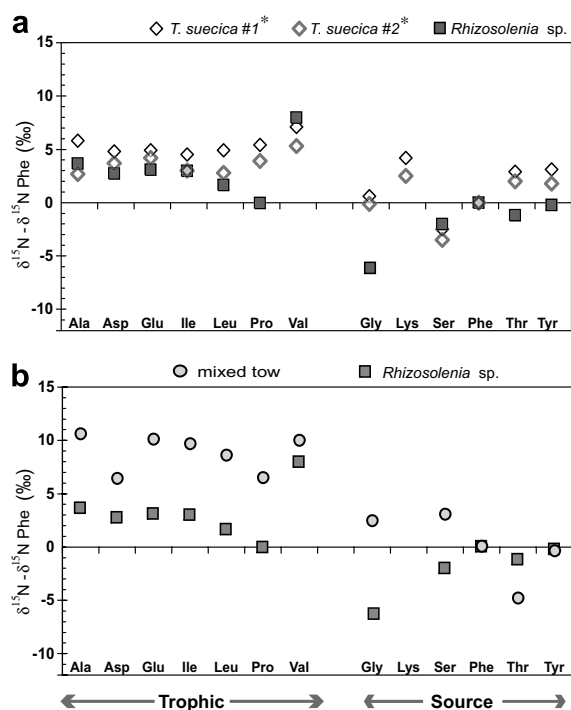

Fig. 1. δ^{15}N-AA Signatures of Phytoplankton and Zooplankton. To allow direct comparison of internal variation in δ^{15}N values between samples having different N sources (e.g., natural vs. cultured, or plankton from different regions), data are normalized to the value of Phe by subtraction (such that Phe = 0). AA abbreviations are as defined in the text. (a) δ^{15}N-AA patterns in green alga *Tetraselmis suecica* vs. a natural diatom (*Rhizosolenia* sp.) bloom. *T. suecica* data from McClelland and Montoya (2002). (b) δ^{15}N-AA patterns in mixed tow (26–850 μm = zooplankton + phytoplankton) vs. *Rhizosolenia* sp. bloom.

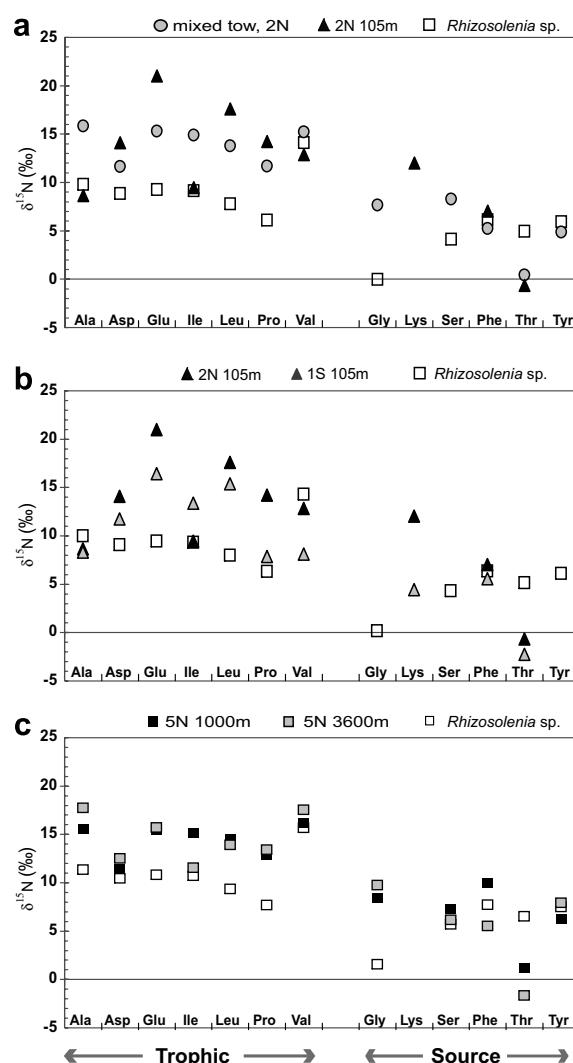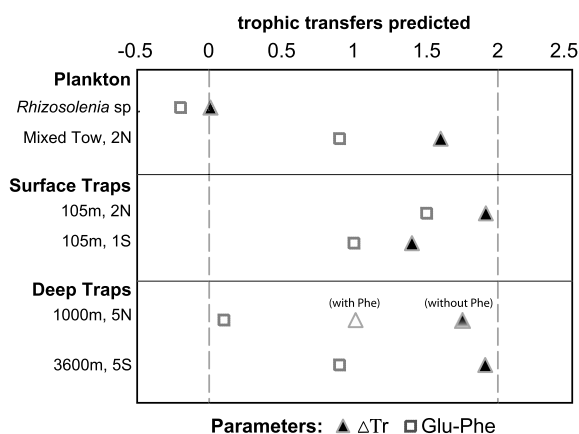Fig. 3. Predicted Trophic Transfers (ΔTr) for Plankton and Sinking POM. Number of overall trophic transfers predicted by ΔTr for plankton and POM sources, compared with those indicated by the simpler (Glu-Phe) pairing. Note that because the 1000 m trap sample appears to have an unreliable Phe value (as addressed in the text), ΔTr recalculated without Phe is also plotted for the 1000 m sample. AA abbreviations are as defined in text.

1.9), as well as the same offset between the north and south 105 m trap samples. Differences of this magnitude ($\sim$0.5 ΔTr) are about 5× larger than the propagated standard error for ΔTr ($\pm$ 0.1 see Section 2). If one uses propagated analytical error as standard error for analysis of variance, one-way ANOVA of ΔTr values from all mixed tow and POM samples (but excluding the *Rhizosolenia* bloom sample) yields a *P*-value of 0.002, indicating a high degree of overall significance in differences between individual ΔTr values. The Tukey–Kramer multiple comparisons post-test also indicates that an offset of 0.5 ΔTr between individual samples is significant at 95% confidence. However, since for each individual environmental sample type or location we have one sample, the environmental variation is untested. Using a one-sample *t*-test on all ΔTr data (i.e., treating the data as replicate cases of the same variable) yields a 95% confidence interval of 1.4 to 1.9, and thus would predict no statistically significant variation from the mean in any samples except the *Rhizosolenia* bloom (ΔTr = 0.0). Without additional samples from each environment, it is not possible to determine relative magnitude of environmental vs. analytical variation.

4.2.2. ΣV in sinking POM

Further alterations in POM δ^{15}N-AA distributions are also observed within the Tr-AA group (ΣV, see Section 2) relative to plankton samples. In the 105 m trap samples, both the overall δ^{15}N range and ΣV in the Tr-AA group are higher than plankton (Table 1). δ^{15}N of Glu is no longer representative of other Tr-AA, but instead Gly is the most enriched AA, and both Ile and Ala are generally depleted. The elevation in ΣV relative to plankton is present in all the trap samples, however the 105 m trap samples from 2°N and 1°S show larger changes relative to 5°N deep traps. There are also differences between the 1°S and 2°N locations, with both total range and ΣV at 2°N 105 m trap greater relative to the 1°S 105 m trap sample (Table 1; Fig. 2b and c). In the Tr-AA group Glu, Pro, and Val are

all depleted at 1°S relative to 2°N, and Ile is elevated strongly. In the Src-AA group Lys is elevated in the 2°N sample. Nevertheless, Phe δ^{15}N values in both samples are very similar to each other, as well as to δ^{15}N of Phe in the 2°N tow (Fig. 2a, Table 1).

Overall, there is a general increase in ΣV values from algae, through mixed plankton and into detrital POM (Fig. 4). ΣV data in Fig. 4 was categorized into four groups for statistical analysis. Group 1(autotrophic plankton) included the *Rhizosolenia* sp. sample and the two *T. suecica* samples from McClelland and Montoya (2002). Group 2 (heterotrophic and mixed plankton) included the mixed plankton tow and two *B. plicatilis* samples (McClelland and Montoya, 2002). Group 3 (surface POC) included the two 105 m trap samples) and group 4 (deep POC) included both 5°N deep trap samples. One way ANOVA yields a *P*-value of 0.0006, indicating a high degree of significance in the differences of ΣV between selected groups. Applying a post-test for linear correlation (e.g., Altman, 2006) to the same data indicates a highly significant linear trend ($P = 0.0018$), with an r^2 value of 0.30 (Fig. 4). Because this data is categorical, the high degree of significance to the linear trend test is what is important. While a significant linear correlation would be predicted across samples which generally increase in resynthesis, an r^2 close to 1.0 would not be expected, since the categories into which data is grouped for this test would not likely have a simple monotonic relationship. In fact, testing for significance of the residual non-linear variation in the ANOVA results also yields a very low *P*-value ($P = 0.0008$), indicating that after accounting for the linear trend the remaining variation is also significant. The two equatorial surface trap (105 m) samples are the most important in this latter result; if these two samples
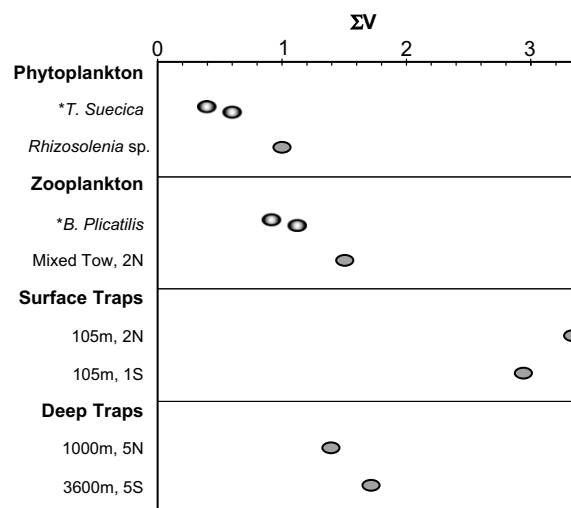

Fig. 4. ΣV for Plankton and Sinking POM. Average deviation in the Tr-AA group (ΣV) for cultured plankton, central Pacific plankton and sinking POM. Samples are grouped into four categories: autotrophic plankton, heterotrophic plankton, surface and deep sediment trap samples. ANOVA post-test for linear regression indicates a highly significant linear trend of increasing ΣV between sample categories. Cultured plankton data from McClelland and Montoya (2002) are indicated by open center symbols.

Table 2
Comparison of trophic transfers predicted by selected Tr-AA and Src-AA

Tophic	Source	Plankton		Sinking POM			
		Rhizo. 2.5°N	Tow 2°N	105 m 2°N	105 m 1°S	1000 m 5°N	3600 m 5°N
*Glu	*Phe	−0.2	0.9	1.5	1.0	0.1	0.9
Asp	Phe	−0.4	0.5	0.7	0.4	−0.7	0.6
Glu	Thr	0.3	1.6	2.7	2.3	1.8	2.1
Asp	Thr	0.4	1.6	2.2	2.1	1.4	2.1
Asp, Glu	Phe, Thr	0.0	1.2	1.8	1.5	0.8	1.5
A, G & Leu	Phe, Thr	0.0	1.4	2.1	1.8	1.0	1.7
A, G & Leu, Val	Phe, Thr	0.2	1.5	1.9	1.5	1.0	1.9
**A, G& Leu, Val, Pro	**Phe, Thr	0.0	1.5	1.9	1.4	1.0	1.9

*(Glu-Phe) indicates the trophic indicator proposed for living biota by McClelland and Montoya (2002). Double asterisk (**) indicates combination chosen for ΔTr parameter in this work, as described in the text. *Rhizo.* = *Rhizosolenia* sp. sample. "Tow" = mixed 26–850 μm plankton tow at 2°N. To save space, A, Asp; and G, Glu in lower rows. Other parameters and AA abbreviations are as defined in the text.

are removed, a significant linear trend remains and the r^2 value increases ($P = 0.02$; $r^2 = 0.67$), but the test for variation remaining after accounting for the linear trend is no longer significant ($P = 0.52$).

4.2.3. DI in sinking POM

DI values in sediment trap materials ranged from +0.37 (1°S, 105 m trap sample) to −2.7 (5°N, 3600 m trap sample). The mixed plankton tow DI value was −0.07. AA Mol% data was not available for the *Rhizosolenia* sp. sample, or the UDOM samples. In sinking particles DI generally varied as would be expected, with higher DI values in surface trap samples and plankton, and progressively more negative values in mid-water (1000 m) and the deep (3600 m) samples. However, a strong offset was also observed between the two upper water (105 m) trap sample values. DI indicated the 2°N sample (−0.90) was more degraded than the 1°S (+0.37) sample.

4.3. Dissolved organic matter

While fewer AA could be quantified in the UDOM matrix (see Section 2), most members of the Tr-AA group were well

Table 3
δ^{15} N-AA Values and Parameters for UDOM Isolates

		UDOM δ^{15}N-AA(‰)			
		2 m	100 m	375 m	4000 m
	ΔTr_{UDOM}	−0.7	−0.2	−0.8	0.3
	V_{UDOM}	0.45	0.67	0.19	0.94
	Avg-TrAA	*7.9*	*8.2*	*5.9*	*9.6*
Trophic	Ala + Gly	8.2	9.5	5.5	8.5
	Asp	8.8	12.6	4.9	12.0
	Glu	4.7	6.3	7.2	10.3
	lle	nd	17.9	nd	nd
	Leu	nd	11.6	5.7	4.9
	Pro	8.2	2.9	6.7	7.3
	Val	9.5	9.9	5.0	9.9
	Ser + Thr	6.2	6.2	6.2	4.9

AA and parameter abbreviations are as defined in the text. Underlined AA are those used in the ΔTr_{UDOM} calculation. Avg-TrAA is the mean value of Tr-AA used for the ΔTr_{UDOM} calculation (Section 2.4).

resolved, providing a good basis for examining trophic transfers and potential bacterial resynthesis. Unlike POM samples, the distributions of Tr-AA in UDOM show no consistent differences with depth. The $\delta^{15}N$ values of individual Tr-AA are more variable than for POM or plankton; however, this variability generally falls within the higher analytical variability typical of the UDOM matrix (~3–5‰, see Section 2). Tr-AA values are similar to Src-AA values (Ser + Thr) in all samples (Table 3; Fig. 6), in contrast to the large offsets in Tr-AA vs. Src-AA observed in POM samples. The Tr-AA value at 375 m (oxygen minimum) appears lower than the others, however it is within propagated analytical error (Fig. 6), and is not significantly different via one way ANOVA ($P = 0.29$). The fact that the 4000 m mean Tr-AA value falls directly in line with surface waters supports the inference that the lower 375 m value is in fact due to analytical variation. In addition, the measured (i.e., non-normalized) $\delta^{15}N$ values of Tr-AA from all depths fall close to values measured in *Rhizosolenia* sp. (5–10‰), and none are in the elevated range found in the mixed tow or sediment trap samples (15–20‰). Asp might be one exception, with enriched $\delta^{15}N$ in the deep ocean sample, but a depleted value in the oxygen minimum zone (375 m). However, as neither Glu nor any other Tr-AA follows this pattern, it is difficult to ascribe much significance to change in a single AA.

The ΔTr_{UDOM} values are similar; unlike POM they are also generally negative, with most falling in the region of 0 to −1 (Table 3). In addition, there is no consistent increase in the average deviations of UDOM Tr-AA patterns with depth, as reflected in V_{UDOM} (Table 3). V_{UDOM} at 100 m is elevated relative to the other depths, while surface, oxygen minimum zone, and deep ocean values are all similar (Fig. 7). However, ANOVA indicates that none of the ΔTr values ($P = 0.40$) or V_{UDOM} values ($P = 0.97$) can be shown to be significantly different from the others.

5. DISCUSSION

Our results indicate that trophic behavior of Tr-AA and Src-AA groups predicted by McClelland and Montoya's culture experiments are consistent with patterns observed in the central Pacific POM and plankton. The similarity between published $\delta^{15}N$-AA patterns and those from natural

Rhizosolenia supports the idea that δ^{15}N-AA patterns are similar in algae. In detrital POM, both the sense and magnitude of these selective AA enrichments are consistent with previous observations for zooplankton (McClelland and Montoya, 2002). Together, these comparisons suggest that diagnostic δ^{15}N-AA patterns are well preserved in detrital organic matter.

5.1. Record of trophic transfers preserved in detrital organic matter

Based on observations in culture, McClelland and Montoya (2002) suggested that δ^{15}N (Glu-Phe) as the most sensitive recorder of trophic history in living plankton. To examine the degree to which variation in specific AA might affect such estimates in detrital material, we selected specific groups of Tr-AA and Src-AA and compared the resulting predictions for trophic transfers in central Pacific POM. The AA groups compared were chosen based on (1) predicted sensitivity to trophic transfer in plankton, (2) relative abundance and analytical variability, avoiding minor or highly variable AA, and (3) apparent susceptibility to resynthesis during heterotrophy, based on both literature observations and changes in our POM samples. In the Tr-AA group we focused on the acidic AA Asp and Glu, and the neutral AA Val, Leu, and Pro. We avoided Ile and Ala, since these are commonly resynthesized during bacterial heterotrophy (e.g., Keil and Fogel, 2001; McCarthy et al., 2004). In the Src-AA group we included only Phe and Thr. These were the only two Src-AA in our data set that fit the above criteria and were also measured in every sample. Gly was also excluded, because changes in both relative abundance as well as carbon isotopic value have been widely observed during organic matter degradation (e.g., Dauwe et al., 1999; Yamashita and Tanoue, 2003; McCarthy et al., 2004).

5.1.1. Comparison of multi-amino acid proxies

AA groups examined gave similar estimates for living plankton trophic level (Table 2): near 0 for *Rhizosolenia* sp., and 1–1.5 for the mixed plankton tow. This similarity, in particular the close relation with the expected value for phytoplankton, reinforces the close correspondence between algal δ^{15}N-AA patterns in culture and those in natural plankton. The sediment trap sample values were more variable, however most groups of AA yielded similar estimates, ranging from 1 to 2 trophic transfers. This broad agreement suggests that despite likely resynthesis in some AA, δ^{15}N-AA trophic level information from plankton sources are well preserved in sinking POM.

However, some AA groups, in particular combinations using a small subset of AA, did give consistently different estimates. For example, [Glu-Phe] vs. [Asp-Phe] gave estimates that are a factor of two different for almost all samples (Table 2). This is somewhat surprising, as Glu and Asp are both abundant, chemically similar, and each strongly enriched with trophic level. Phe is similarly abundant and was the most stable benchmark Src-AA in published culture experiments (McClelland and Montoya, 2002). Acidic AA are known to sorb to inorganic particles,

and this might affect their behavior. Also, carbonates are enriched in Asp and Glu, so that as these mineral dissolve in sinking particles, compounds of a different origin may be released. Such differences suggest caution in interpreting internal parameters such as these using only a few AA in non-living organic matter.

While it is not possible from this data set to suggest which AA are generally most stable or most variable, these comparisons suggest that the broadest possible average of AA should provide the most robust estimates, being least susceptible to potential resynthesis in a few components. We thus propose the δ^{15}N average (Asp, Glu, Leu, Val, and Pro)–δ^{15}N average (Phe, Thr), which we have defined as ΔTr, as the best indicator of number of trophic transfers for detrital samples based on our analytical conditions.

5.1.2. Trophic level for sinking POM in equatorial pacific

As indicated above (Section 4) ΔTr values for POM at all depths fall into a similar range, suggesting that POM exported from the upper ocean in this region has undergone approximately 2 trophic transfers. It should be noted that the single trophic transfer "constant" used to estimate ΔTr from the δ^{15}N shifts we measured (=4.67, see Section 2) was derived from a limited number of culturing experiments (McClelland and Montoya, 2002). Additional culturing or environment-specific work might in the future refine this value, and thus could alter the exact number of trophic transfers predicted by our data. However, the ΔTr values we obtained are in good agreement with independent predictions based on export production and estimates of zooplankton digestion efficiency, which previously estimated an average value of 2–3 trophic transfers for POM at this location (Hernes et al., 2001). This agreement suggests that the McClelland and Montoya results are in fact reasonable. In addition, any future refinement to the single-trophic transfer interval would not affect the *relative* offsets predicted between different samples.

In Fig. 3 trophic transfer predictions from δ^{15}N (Glu-Phe) are included to directly compare this result with our broader ΔTr parameter. For the phytoplankton bloom both estimates agree closely. In all other samples, while relative trends are always the same, the Glu-Phe estimate is also always lower than ΔTr. Both indicate a substantial increase from pure algae to the mixed tow (1-1.5), and a further increase of ~0.5 to 1 average trophic transfers for material reaching the base of the euphotic zone in the contemporaneous 105 m trap at 2°N. The deep trap data on first examination appears unusual, in that the predicted ΔTr at 1000 m is substantially *lower* than either in the surface trap sample or the 3600 m trap sample below it. The Glu-Phe only based result in particular indicates that 1000 m POM is essentially pure phytoplankton material.

This seems highly unlikely, and suggests that the 1000 m Phe δ^{15}N may be an isolated unrepresentative value. If the 1000 m Phe value were authentic, this would represent the largest shift of any AA ($\Delta\delta^{15}$N of 4.5‰) between the mid and deep water trap samples; yet Phe is an AA which has not previously been reported to be strongly tied to microbial resynthesis. This observation also would be of particular interest, since as discussed above Phe has been found to

be the most stable AA between trophic levels in culturing studies, and thus was suggested as the best marker for original autotrophic $\delta^{15}N$ values (McClelland and Montoya, 2002). A large difference between 1000 and 3650 m could indicate that Phe is highly susceptible to resynthesis during POM degradation. While this cannot be absolutely excluded, the data set as a whole is more consistent with $\delta^{15}N$ of Phe at 1000 m as an analytical outlier. Non-normalized Phe values in both the surface traps, the plankton tow, as well as at 3650 m are all very similar ($\sim 5.5\%$). The 1000 m value alone (a year-long integrated value) is substantially enriched at 10.0‰. In addition, no offset in Phe $\delta^{15}N$ is observed between the mixed plankton tow and either surface trap sample, despite the strong variation in other AA. Finally, if Phe is removed from the calculation and ΔTr is recalculated for 1000 m using only Thr as a Src-AA, the resulting value falls directly in line with that in all other traps (Fig. 3). This analysis highlights the difficulty of relying on limited AA $\delta^{15}N$ values, especially for detrital samples, and emphasizes the need for a broadly defined parameter like ΔTr in such samples.

5.1.3. Potential sensitivity of ΔTr

In addition to the larger ΔTr changes between plankton and sinking particles, there are also smaller offsets on the order of 0.5 ΔTr. As indicated above (Section 4), differences of this magnitude are statistically significant based upon analytical error, however since we have only a single sample at each location it is not possible to directly evaluate additional effects of environmental variability. Nevertheless, an important question is whether ΔTr differences of this magnitude are likely to be meaningful, in light of comparison with independent data on these same samples.

Between the 2°N plankton tow and contemporaneous 105 m trap sample, the change in ΔTr (+0.4 trophic transfers), seems consistent with the transition from mixed plankton (zooplankton + phytoplankton) to sinking detrital material. The similar magnitude ΔTr difference observed between the northern and southern 105 m trap samples collected during the same time period is less intuitive. However, a substantial body of previous data indicates strong differences in zooplankton grazing and overall POM "freshness" between these two regions. Both 105 m trap samples were short term deployments collected during EqPac Survey 1, a strong El Niño period characterized by a sharp drop in equatorial upwelling and overall primary productivity (Murray et al., 1995). Zhang et al. (1995) reported approximately double the rates of mesozooplankton grazing on the northern vs. southern sides of the equator during this time. Hernes et al. (1996) reported that glucose made up a higher percentage of neutral aldoses in 105 m trap material at southern stations, suggesting that upper-water POM at southern stations was generally "fresher" than that in the north. Based on these observations, as well as relative abundance of specific biomarker lipids (Lee et al., 2000), it was suggested that upper ocean POM on the north side of the Equator during this time period probably passed through $\sim$ one additional trophic level (Hernes et al., 1996; Lee et al., 2000). Finally, offsets in DI values between 2°N trap samples (−0.90) vs. 1°S samples (+0.37) are also

consistent with proteinaceous materials at 2°N being more degraded. The ΔTr offset at 2°N vs. 1°S is consistent with all these trends, suggesting that ΔTr is in fact recording finer scale variation in trophic structure.

5.2. Evidence of bacterial amino acid resynthesis in δ¹⁵N-AA patterns?

A major difference we observe between the living and detrital samples is not further uni-directional enrichment of the Tr-AA group, but instead that the $\delta^{15}N$-AA distribution in the Tr-AA group is "scattered" relative to the narrow range of values characteristic of biota. The larger differences in overall scatter of $\delta^{15}N$-AA values between signatures in mixed plankton and some sediment trap samples (e.g., Fig. 2a; 1°S and 2°N 105 m samples) further suggest that this may be related to continuing bacterial resynthesis in sinking detrital material.

To compare the extent of such changes we defined ΣV as the average deviation in six Tr-AA (Section 2). While the average deviation is less commonly used than the more familiar variance (SD^2), it is equally valid for expressing degree of variation around a mean (e.g., Bevington and Robinson, 2003). Because this expression uses the absolute values of the individual deviations rather than squares, the result increases linearly (rather than exponentially) as the cumulative variation around the mean increases. ΣV is thus a more appropriate expression for our idea that increasing total variation linked to progressive resynthesis. While AA shifts due to resynthesis would likely occur in both Tr-AA and Src-AA, we focused ΣV only on the Tr-AA because the tight $\delta^{15}N$-AA distributions observed in plankton provides a clearer starting point for comparison. Analytically, we were also able to measure all the Tr-AA for our sample set.

In contrast to ΔTr values, ΣV in POM does not approach a common threshold, but exhibits a greater range which increases from pure algae to detrital material (Fig. 4). Based on previous observations with $\delta^{13}N$-AA (McCarthy et al., 2004), such changes resemble those expected from heterotrophic resynthesis. Fractionations in only specific AA, leading to a "scattering" of source AA patterns, have been repeatedly observed in the $\delta^{13}N$-AA values of organic material degraded by heterotrophic bacteria (e.g., Fogel and Tuross, 1999; Keil and Fogel, 2001; Ziegler and Fogel, 2003), as well as in $\delta^{15}N$-AA patterns (Fogel and Tuross, 1999). The statistically significant linear trend in increasing ΣV values categorized from algae, through zooplankton and into detrital materials (Section 4; Fig. 4) likely reflects the fact that autotrophs have more homogenous Tr-AA distributions than heterotrophs or detrital materials. This suggests that this trend derives from progressive AA resynthesis in both animal and microbial consumers. All detrital (trap) samples then show further ΣV elevation beyond the range seen in living plankton. We hypothesize that such increases are due primarily to bacterial heterotrophic resynthesis.

5.2.1. ΣV Comparison to other amino acid proxies

One way to examine our interpretation of changes in ΣV is to consider alternate AA proxies for degradation. Two of

these are Mol% of Gly, and the AA DI (degradation) index. Increases in Mol% of Gly are thought to result from microbial activity, increases in Gly-rich cell wall materials, or terrestrial inputs (e.g., Dauwe and Middelburg, 1998; Dauwe et al., 1999; Yamashita and Tanoue, 2003). The DI index is based on principle component analysis of often minor changes in AA Mol% (Dauwe et al., 1999; Yamashita and Tanoue, 2003; Kawasaki and Benner, 2006). Exact DI values obtained can vary with specific starting materials; for example, DI values in DOM and POM have been found to be generally different, likely based on differences in initial AA composition (Davis and Benner, 2005). Limitations of the DI method can also make a standard principal components analysis more useful for complicated samples, particularly in water column studies (Sheridan et al., 2002; Ingalls et al., 2003, 2006b). In general, however, decreasing values of DI indicate increasing degradation; fresh materials typically yield positive values, while degraded materials yield more negative ones.

In our samples the changes observed in ΣV between sample pairs in similar ocean locations correspond in all cases with the expected changes in both Mol% Gly (Lee et al., 2000) and DI for these same samples (Table 1). Specifically, both Mol% Gly and DI indicate increased degradation for the 2°N tow to 100 m trap samples, both show increased degradation in the N vs. S equatorial surface trap, and both indicate increased degradation with depth between 1000 and 3600 m at 5°N (Table 1). However, the large offset between the equatorial surface traps vs. the 5°N deep traps observed in ΣV do not correspond to a similar offset in Mol% Gly or DI.

One possible explanation for this last observation is that AA Mol% changes and isotopic changes in the sinking POM samples indicate fundamentally different aspects of degradation. Changes tracked by DI are hypothesized to be driven in large part by enrichment in (more refractory) cell wall materials (Dauwe et al., 1999). In contrast, our ΣV parameter is hypothesized to track only the *resynthesis* of AA. Thus, while DI and ΣV might generally be expected to vary together, this would not necessarily be the case: enrichment of exported POM in cell wall materials might result in

very substantial increase in DI, but this alone should result in no increase at all in ΣV. Overall, the DI and ΣV should fundamentally be independent but complimentary parameters.

There are also alternate possible explanations for ΣV variations, including mixtures of sources, selective preservation, and alteration of δ^{15}N-AA signatures associated with passage through zooplankton guts. As indicated previously, because the relative molar AA distributions in biota are similar, and because the normalized δ^{15}N-AA patterns in both zooplankton and phytoplankton also appear similar (Figs. 1 and 2), it is unlikely that simply mixing plankton source material could account for substantial changes in *only* selected δ^{15}N-AA values. Selective preservation of specific sources, for example more refractory algal cell wall components (e.g., Cowie and Hedges, 1996; Hernes et al., 1996; Ingalls et al., 2003), could play a role in ΣV changes. However, examining AA distributions for specific samples suggests that this is not the most likely explanation. For example, the 2°N mixed tow and contemporaneous 2°N 105 m trap samples represent the most directly linked sample pair, and ΣV increases substantially from tow to trap. Absolute values of a number of Tr-AA (Leu, Pro, and Val) as well as the average value of the Tr-AA group, remain essentially unchanged for both these samples (Table 1), indicating that both enrichment and depletion of only a *selected* set of Tr-AA has occurred, while others have remained unaltered. Finally, it is also possible that passage through zooplankton guts, rather than microbial degradation of sinking POM in ambient seawater, is important in ΣV changes. Because zooplankton guts are abundantly colonized by bacteria (Hansen and Bech, 1996), gut bacterial activity might have an important impact on ΣV of particles leaving surface water.

5.3. ΔTr vs. ΣV: Eukaryotic vs. Bacterial heterotrophy?

If ΔTr records trophic transfers and ΣV reflects AA resynthesis during both zooplankton feeding as well as subsequent bacterial degradation, an intriguing possibility is that together these parameters could define a two dimensional heterotrophic "space," simultaneously indicating rel-
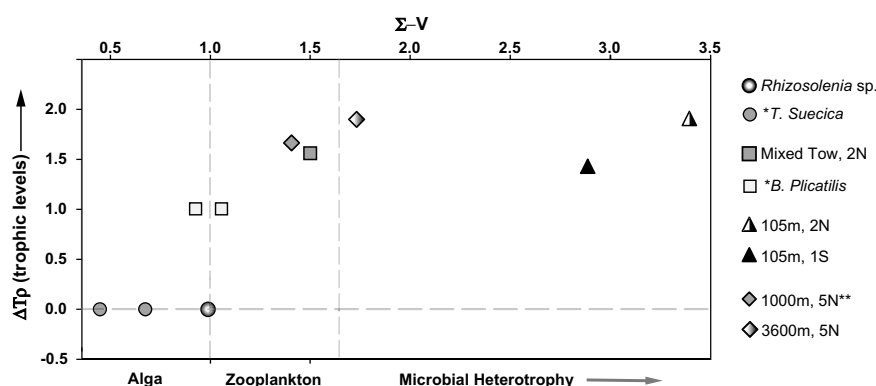

Fig. 5. ΔTr vs. ΣV for Plankton and Sinking POM. y-axis indicates predicted trophic transfers (ΔTr), while x-axis expresses proposed total resynthesis (ΣV). General regions within proposed continuum corresponding approximately to alga, zooplankton heterotrophy, and further microbial heterotrophy in detrital materials are indicated along the y-axis. Average values for cultured plankton (McClelland and Montoya, 2002) are again included for reference. As discussed in text, 1000 m trap sample ΔTr value does not include Phe. AA and parameter abbreviations are as defined in the text.
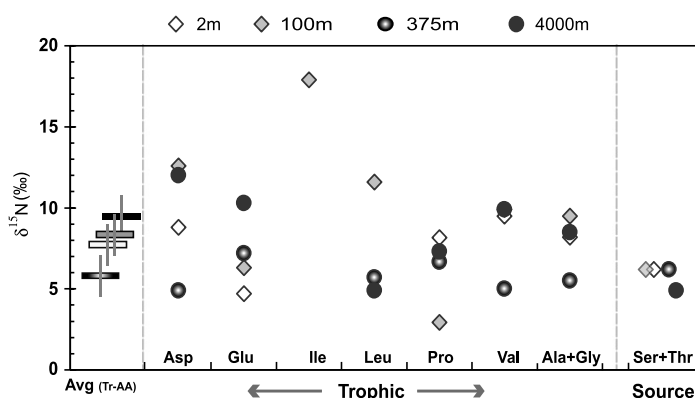
Fig. 6. $\delta^{15}N$-AA Signatures in UDOM (2–4000 m). Averages of the comparable Tr-AA for each depth (Asp, Glu, Leu, Pro, and Val) are indicated by bars at left (Avg. Tr-AA). Range bars indicate propagated standard error of the mean ($\pm 1.6‰$) corresponding to typical analytical reproducibility of $\pm 3‰$. Co-eluting AA measured as individual peaks are indicated as Ala + Gly and Ser + Thr. Other abbreviations are as defined in the text.

ative eukaryotic and bacterial heterotrophy. If the unidirectional $\delta^{15}N$ enrichment in the Tr-AA group occurs only in zooplankton consumers, ΔTr will be fundamentally tied to eukaryotic feeding. By contrast ΣV, while also changing somewhat with trophic transfer, likely continues to increase with bacterial resynthesis. Further, the homogeneous Tr-AA patterns in both plankton tows and other animal tissue samples (Hare et al., 1991; McClelland and Montoya, 2002) suggest that the region of ΣV found in eukaryotic tissue may be well defined by common enzymatic reactions. If so, ΣV increases beyond this range are likely related primarily to microbial heterotrophy.

Plotting ΣV vs. ΔTr in plankton and equatorial Pacific POM samples synthesizes the observations made previously, and suggests several distinct intervals corresponding to algal sources, zooplankton sources, and finally additional bacterial resynthesis (Fig. 5). Phytoplankton plot in

the region of ΔTr = 0 and $\Sigma V = 0$–1. Based on the similar $\delta^{15}N$-AA distributions observed for algae, this region is likely the firmest endmember. Trophic transfers increase ΔTr, and to some extent ΣV. Available data suggest that zooplankton biomass plots in a limited ΣV range of approximately 1–1.5, while the corresponding ΔTr range should be limited only by N assimilation efficiencies and total trophic levels in an ecosystem. Beyond ΣV values of ~1.5–2 we hypothesize that increasing values indicate predominantly bacterial resynthesis.

In the equatorial Pacific, relative changes in ΣV and ΔTr generally correspond in the same sense, but not magnitude, throughout the data set. This suggests that while relative eukaryotic and bacterial heterotrophy are often associated, they can also be significantly decoupled. ΔTr never exceeds ~1.5–2 in any sample, suggesting a similar trophic threshold for zooplankton processing of exported sinking POM. In
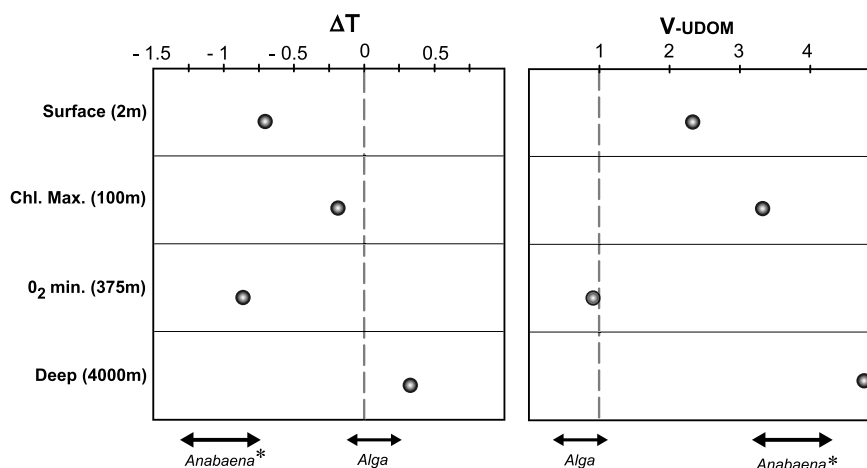

Fig. 7. ΔTr$_{UDOM}$ and V_{UDOM} for UDOM. ΔTr$_{UDOM}$ and V_{UDOM} through Pacific water column (2–4000 m). (Left panel) Predicted UDOM trophic transfers (ΔTr$_{UDOM}$). As discussed in text, there are no statistically significant differences between values. "Alga" double arrow indicates range observed in cultured alga and zooplankton (McClelland and Montoya, 2002 and this work). (Right panel) Predicted total UDOM AA resynthesis (V_{UDOM}). As discussed in text, there are also no statistically significant differences between V_{UDOM} values. In both panels dotted lines for ΔTr$_{UDOM}$ and V_{UDOM} indicate approximate values that would result from eukaryotic algal sources examined previously. (*) *Ranges in Anabaena*: arrows on the bottom indicate approximate ranges of each parameter that would result from published $\delta^{15}N$-AA values from *Anabaena* sp. cyanobacteria (Macko et al., 1987), grown on different nitrogen sources.

contrast, ΣV varies more substantially, suggesting greater variability in extent of bacterial resynthesis. If our hypothesis about ΣV is correct, these values would suggest that upper-water traps from the El Niño period have a much greater degree of microbial resynthesis than the year-integrated material north of the equator at 5°N, despite the fact that degree of zooplankton trophic transfers are similar for both. This analysis suggests that independent aspects of δ^{15}N-AA distributions may allow the examination of fundamental shifts in plankton trophic level vs. heterotrophic bacterial source and processing within ecosystems. Additional work is clearly required to test the limits of this idea.

5.4. UDOM: ΔTr vs. ΣV in dissolved material

Relative in δ^{15}N-AA patterns in dissolved material with depth (Table 3; Fig. 6) appear unrelated to those observed in sinking particles. As mentioned above (Section 4), ANOVA analysis indicates no statistically significant variation in the means of the Tr-AA δ^{15}N between depths, as well as no significant variation between either ΔTr$_{UDOM}$ or V_{UDOM} values. If interpreted in the same framework as POM samples, these observations would suggest not only that no trophic transfers have taken place, but also that once UDOM leaves the surface, little continuing bacterial resynthesis has altered its AA isotope patterns. However, significant caution is required in drawing direct parallels to patterns observed in living plankton and POM, because source pathways for DOM are both more diverse and less well known. Such pathways include solubilized products of feeding and digestion (Jumars et al., 1989), biomass and cellular contents introduced directly via viral lysis of cells (Fuhrman, 2000), as well as DOM excreted directly from living cells (Biddanda and Benner, 1997). It is not clear which pathways dominate the DOM that accumulates in the ocean, and different sources might have as yet unknown effects on δ^{15}N-AA patterns.

UDOM AA patterns also hint at source variation between particular and dissolved materials. If DOM were primarily derived from particulate cellular material (e.g., either via viral lysis or heterotrophic processes analogous to those in POM), both ΔTr$_{UDOM}$ and V_{UDOM} would be expected to correspond with ranges observed previously for algal sources and partially degraded POM. However, ΔTr$_{UDOM}$ and V_{UDOM} values are also less consistent with tested plankton sources or detrital POM. ΔTr values do not plot near zero (or above) as would be expected from algal data. Instead they are generally negative, with most falling in the region of 0 to -1 (Fig. 7; Table 3), while at the same time V_{UDOM} values are higher than would be expected for algal sources. The coupling of negative ΔTr with high V_{UDOM} does not fit patterns so far observed for algal (eukaryotic) sources or for sinking POM. It is possible that greater UDOM analytical variation, and the inexact correspondence of AA measured with those in reference sources, plays a role in these results. However, the uniformity of sampled eukaryotic algal δ^{15}N-AA distributions means that any sample with a similar starting distribution should yield very similar results for ΔTr and V_{UDOM}, no matter what subset of AA are chosen.

The unusual ranges of ΔTr$_{UDOM}$ and V_{UDOM} could indicate that UDOM AA from the central Pacific derive largely from sources having different δ^{15}N-AA patterns than those for sinking POM. Enantiomeric (D vs. L) AA ratios have indicated that prokaryotes are a major source of DON in the ocean (e.g., McCarthy et al., 1998; Dittmar et al., 2001; Amon et al., 2001; Jones et al., 2005), while D/L values are much lower in sinking particles (Dittmar et al., 2001). At the same time, δ^{13}C-AA patterns suggesting autotrophic source have been observed in UDOM at all depths for these same samples (McCarthy et al., 2004). Taken together, these observations have suggested that autotrophic prokaryotes (cyanobacteria) may be major sources for proteinaceous material in UDOM (McCarthy et al., 2004). While we do not have δ^{15}N-AA data for natural ocean cyanobacteria, the limited data available is generally consistent with this idea, suggesting that δ^{15}N-AA from cyanobacteria can differ from those in eukaryotic algae. Macko and coworkers (1987) reported δ^{15}N-AA patterns in a cyanobacterium (*Anabaena* sp.) that are different from those observed thus far in eukaryotic algae. If converted to our parameters, these data correspond favorably with the ΔTr$_{UDOM}$ and V_{UDOM} ranges observed in UDOM (Fig. 7). It is not clear, however, how representative these cultured organisms may be of natural cyanobacteria.

If UDOM AA were derived in large part from extracellular material, another important factor in explaining differences between POM and UDOM could be the relation of δ^{15}N-AA patterns in excreted AA vs. those in bulk biomass. It is well known that growing cells (both algae and cyannobacteria) excrete significant quantities of DOM and UDOM (e.g., Hellebust, 1974; Biddanda and Benner, 1997), and extracellular products have been hypothesized as a major source for the ocean's HMW carbohydrates (Biersmith and Benner, 1998; Aluwihare and Repeta, 1999). As indicated above, the compound-specific δ^{13}N-AA patterns measured in these same samples matches the expected autotrophic biomass δ^{13}N-AA pattern at all depths (McCarthy et al., 2004). If excreted AA were important in shaping δ^{15}N-AA distributions this would suggest that excreted AA sources differ more in their δ^{15}N isotopic patterns, vs. δ^{13}C patterns. Measurements of both δ^{15}N-AA and δ^{13}C-AA in extra-cellular DOM need to be made to directly address the possibility, and should be an important goal for future research.

Despite the uncertainties surrounding UDOM sources, a main observation remains that overall UDOM δ^{15}N-AA patterns do not appear to change in any large or systematic manner with depth. This appears to contrast with what one might expect for very old dissolved material from the deep ocean i.e., the most extreme evidence of heterotrophic reworking, as opposed to modern, relatively short-lived POM. There are at least two main ways to interpret this result. One is that since the most production and remineralization of organic matter occurs in the surface (<200 m) ocean, further heterotrophic resynthesis in the subsurface involves no trophic transfer and little heterotrophic AA resynthesis, such that its effect cannot be easily observed in δ^{15}N-AA patterns. This interpretation would also imply almost uniquely microbial heterotrophic reworking for UDOM at all depths, since any trophic transfers from the

original autotrophic AA sources would be recorded in elevated Tr-AA $\delta^{15}N$ values. A second possibility is that, as suggested by ΔTr_{UDOM} and V_{UDOM} values, autotrophic bacteria (cyanobacteria) are a major source for dissolved AA in the open ocean. This idea is also consistent with $\delta^{13}N$-AA values reported previously, which appear to preserve a largely unchanged ''autotrophic'' signature into the deep ocean (McCarthy et al., 2004). Together the current $\delta^{15}N$-AA and previously published $\delta^{13}N$-AA patterns also support the importance of rapid and long-lived protection mechanisms operating in the surface ocean for a portion of photoautotrophic biosynthate (Benner and Biddanda, 1998; Ogawa et al., 2001; McCarthy et al., 2004). Clearly, these different possibilities are not mutually exclusive, and additional work will be needed to fully interpret these early observations.

6. OVERVIEW AND CONCLUSIONS

The isotopic signatures we have observed indicate that $\delta^{15}N$-AA patterns represent a powerful tool to examine sources and heterotrophic processing for detrital organic matter. This work extends previous observations regarding the effect of zooplankton heterotrophy on $\delta^{15}N$-AA patterns, supporting the idea that only a selected group of AA become enriched in $\delta^{15}N$ with trophic transfer, while $\delta^{15}N$ values of other AA remain essentially stable (McClelland and Montoya, 2002). Our data also support the generality of both Tr-AA and Src-AA groups across different ocean regions and plankton types, as well as the degree of enrichment per trophic transfer. This suggests a firm basis for an internally normalized biochemical parameter (such as ΔTr) for trophic transfers. Importantly, these signatures appear to be preserved in detrital organic matter as well as living plankton. The agreement of previous independent trophic level estimates from the Equatorial Pacific with both our ΔTr ranges, as well as trends among samples, also suggests that ΔTr is a robust and sensitive proxy.

Our data also suggest that subsequent bacterial resynthesis may have a qualitatively different effect on $\delta^{15}N$-AA patterns, resulting in increases in variability of $\delta^{15}N$-AA values. We have proposed a measure of the variation in the Tr-AA group (ΣV) to express this aspect of $\delta^{15}N$-AA signatures. If the effects of archaeal heterotrophy on $\delta^{15}N$-AA patterns are generally similar to those in bacteria (Scott et al., 2006), then elevated ΣV may in fact be indicative of combined bacterial + archaeal AA resynthesis. Combined with the uni-directional enrichments due to trophic transfer, this suggests that these independent aspects of $\delta^{15}N$-AA distributions can distinguish between resynthesis by different groupings of heterotrophic organisms. The broader hypothesis that ΣV vs. ΔTr can simultaneously indicate relative (bacterial + archaeal) vs. eukaryotic heterotrophy is a promising topic for future research.

DOM and POM $\delta^{15}N$-AA data from the central Pacific Ocean indicates a sharp divide between sources and processing of particulate vs. dissolved proteinaceous materials. For sinking POM, our data suggest that zooplankton are the main direct source of exported proteinaceous material, having undergone 1.5–2 trophic transfers at all depths and locations examined. This indicates that, on average, biomass from primary and secondary consumers makes up the bulk of the proteinaceous material exported, and thus that consumers one trophic level above this (i.e., secondary-tertiary consumers) are the primary agents of export via fecal pellet production. It should be kept in mind that, as with all stable isotopic data, this result reflects an average of all sources, and does not rule out contributions either lower or higher on the chain of trophic transfers. Nevertheless, one corresponding implication is that direct export of primary production, or export via physical aggregation of DOM or algal biomolecules (Chin et al., 1998; Engel et al., 2004), are less important processes in our sample set. Particle aggregation would of course still be a likely initiator of sinking of zooplankton products. Physical aggregation may also be important for dissolved carbohydrates (Engel et al., 2004), but neutral sugars make up only 5–10% of particulate carbon exported to the deep ocean in this region (Hernes et al., 1996; Wakeham et al., 1997b). Because AA makes up the majority of sinking POC (as well as PON) in these waters (Wakeham et al., 1997b; Lee et al., 2000; Hedges et al., 2001), these inferences may extend also to total carbon export.

In contrast to POM, processing history and possible sources of DOM appear separate. While analytical variation is larger, there is no clear evidence of trophic transfer in UDOM at any depth. V_{UDOM} values are also similar in surface and deep ocean, suggesting that a lower degree of continuing bacterial resynthesis has altered DOM $\delta^{15}N$-AA patterns, even during long residence times in the deep ocean. These observations support ideas that major DOM AA sources are at the base of the microbial loop, perhaps via direct viral lysis, DOM excretion, or protozoan grazing. Combined with previously reported $\delta^{13}C$-AA patterns, $\delta^{15}N$-AA distributions are consistent in suggesting that cyanobacteria may be important UDOM sources.

Together, the $\delta^{15}N$-AA patterns we have observed provide not only insight into organic N sources and transformations in the water column, but suggest a novel set of tools to examine relative zooplankton vs. bacterial heterotrophic processing for organic matter. If further work reveals that bacterial vs. archaeal alteration of $\delta^{15}N$-AA follows discernible patterns, these patterns might also provide a major biochemical window into the composition and function of microbial communities. $\delta^{13}C$-AA patterns have indicated that specific AA shifts are tied to specific microorganisms or metabolic pathways (Scott et al., 2006; McCarthy et al., 2004). If the same holds for $\delta^{15}N$-AA, then specific $\delta^{15}N$ shifts might eventually be tied not only to the extent of microbial degradation in organic matter, but also to microbial community structure within specific oceanic regimes. Comparative POM $\delta^{15}N$-AA distributions might help to identify large scale regional or ecosystem trends in heterotrophic processing, which fundamentally determine carbon export and remineralization. Finally, the divergence of UDOM $\delta^{15}N$-AA signatures from those of eukaryotic algae, and similarities to known distributions in cyanobacteria, hint that $\delta^{15}N$-AA patterns might also have potential to distinguish prokaryotic vs. eukaryotic pri-

mary production. If borne out, δ^{15}N-AA might constitute not only a tool to examine heterotrophic transformations, but also a source indicator in environments where cyano-bacterial production is important. Direct investigation of δ^{15}N-AA signatures in a range of autotrophic as well as heterotrophic oceanic bacteria are needed to evaluate these possibilities.

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Associate editor: H. Rodger Harvey