Characterization of particulate organic matter cycling during a summer North Atlantic phytoplankton bloom using amino acid C and N stable isotopes

A.J.M. Sabadel\textsuperscript{a,b,c}, N. Van Oostende\textsuperscript{d}, B.B. Ward\textsuperscript{d}, E.M. S. Woodward\textsuperscript{c}, R. Van Hale\textsuperscript{e}, R.D. Frew\textsuperscript{a}

\textsuperscript{a} Department of Chemistry, University of Otago, PO Box 56, Dunedin, New Zealand
\textsuperscript{b} Department of Marine Science, University of Otago, 310 Castle Street, Dunedin, New Zealand
\textsuperscript{c} Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, UK
\textsuperscript{d} Department of Geosciences, Princeton University, Princeton, NJ, USA

1. Introduction

Pelagic food webs are the central driver of carbon (C) and nitrogen (N) cycling in the ocean, of which particulate organic matter (POM) is a key component. However, determining the source and fate of fixed C and N in marine food webs usually requires a suite of techniques such as the elemental and isotopic composition of POM, or flow cytometry and microscopic analyses of the planktonic community, or even better, a combination thereof (Fawcett et al., 2011; Painter et al., 2014).

The temperate North Atlantic Ocean is characterized by nutrient-deficient surface waters in the summer (Moore et al., 2008; Nieldottir et al., 2009). Vernal thermal stratification of the water column reduces the effective mixing of nutrient-rich deeper water into the euphotic zone, limiting the supply of nitrate and other nutrients, such as phosphorus, to the surface and thus limiting new production by phytoplankton (Dugdale and Goering 1967). Contributions of reactive N species by dinitrogen (N\textsubscript{2}) fixation and atmospheric deposition to fuel plankton (Dugdale and Goering 1967). Contributions of reactive N species by dinitrogen (N\textsubscript{2}) fixation and atmospheric deposition to fuel plankton (Dugdale and Goering 1967).

δ\textsubscript{13}C\text subscriptsAAA approach has not been widely applied to open ocean POM and the available data of phytoplankton or bacteria-specific δ\textsubscript{13}C\text subscriptsAAA and δ\textsubscript{15}N\text subscriptsAAA values are mostly derived from laboratory cultures (Calleja et al., 2013; McCarthy et al., 2013), or from the Pacific Ocean (Hannides et al., 2013; 2009; McCarthy et al., 2007; McMahon et al., 2013; McMahon et al., 2015a; Yamaguchi and McCarthy, 2018). Moreover, few field studies have investigated the lower food web in the North Altantic (Sherwood et al., 2011), and most have focused on multicellular zooplankton, the second trophic link of the marine food web (Hannides et al., 2013; McClelland et al., 2003; Mompeán et al., 2013; 2016). The rationale behind CSIA-AAA as a biogeochemical tool lies in the different fractionation behavior of certain AAs as they are synthesized by different species and move through a food web. Briefly, different taxonomic groups, e.g. phytoplankton and bacteria, utilize specific metabolic pathways that leave behind a C isotopic imprint in POM that allows distinguishing between the two groups of organisms (Larsen et al., 2009). But, not enough is known about the isotopic changes in the δ\textsubscript{13}C\text subscriptsAAA of sinking POM. Additionally, AAs that are central to metabolic N cycling (e.g. alanine, aspartic acid, glutamic acid, isoleucine, leucine, proline, valine; McClelland and Montoya (2002), Popp et al. (2007)) are strongly 1\textsuperscript{5}N-enriched relative to their diet during trophic transfer. As such, they are collectively known as the “trophic” AAs (Popp et al., 2007). In contrast, several other AAs (mostly notably phenylalanine; Phe) undergo little or no 1\textsuperscript{5}N trophic enrichment and therefore provide a direct proxy for the δ\textsubscript{15}N value at the base of food webs. These are
commonly known as “source” AAs. Finally, a third type of AA: threonine (Thr), has been named “metabolic” AA as it shows 15N depletion relative to a species’ food source (Germain et al., 2013; McMahon et al., 2015b). Overall, based on a good understanding of the various AA metabolic pathways (McMahon and McCarthy, 2016; O’Connell, 2017), it is possible to create CSIA-AA-based proxies to decipher the origin and fate of organic matter. Nonetheless, there are known limitations to these proxies. For instance, even though the minor fractionation of δ15N values during trophic transfer can be used to assess the inorganic N utilized to build AAs at the base of a food web, only few studies have looked at the effect of the source and δ15N value of inorganic N on the resulting δ15N (McClelland et al., 2003; Mompéan et al., 2016). Most of the available POM δ15N values in the literature are not presented with, and related to, the δ15N of the main inorganic N fueling the local food web. Moreover, while the difference between a trophic and a source AA can help estimate the relative position of a given species within a food web, there is growing evidence that the actual AA-based trophic position (TP) equation (Chikaraishi et al., 2009) needs to be adjusted to the specific system under study (McMahon et al., 2015c), thus removing its original universality and straightforwardness. It thus becomes critical to select the appropriate AA to obtain an accurate TP assessment, depending on the studied system. Additionally, it is not known why the N isotopic fractionation of Thr, the metabolic AA, behaves in the opposite manner to trophic AAs and depletes rather than enriches with increasing trophic position. Finally, heterotrophic bacteria will assimilate and transform (recycle) POM AA, again leaving an isotopic imprint on the remaining POM (i.e. the remaining AAs will become 15N-enriched). The degree of microbial alteration, based on the δ15N values of six key trophic AAs, can be assessed by the proxy 2V, described for the first time by McCarthy et al. (2007). However, other studies have since shown that bacterial reworking does not always fractionate AAs in a similar fashion (Steffan et al., 2015; Yamaguchi and McCarthy, 2018). To the best of our knowledge, there is currently no information regarding the validity of this proxy during a phytoplankton bloom event, a key phenomenon during which most pelagic POM is produced.

The aim of the present study is to investigate the above listed limitations and characterize POM cycling in late stage blooms using CSIA-AA and complementary conventional approaches, such as flow cytometry and nutrient analyses, in a relatively well-known environment: the temperate North Atlantic open ocean. We report the investigation using CSIA-AA to determine the origin and cycling of POM, for the first time during a late summer bloom at two biologically similar stations. These two stations provide a test case for the application of the CSIA-AA proxies for organic matter cycling in an open ocean setting. We determine i) the biotic origin of N and C in suspended POM, ii) the trophic status of POM and discuss an alternative way to assess TP, iii) the degree of heterotrophic resynthesis in suspended POM.

2. Materials and methods

2.1. Sampling stations and physical parameters

Two stations were sampled during the EN532 cruise on board the R/V Endeavor, in the North Atlantic Ocean: PS1 (54 N, 20.40 W) and PS2 (50 N, 20 W) were 450 km apart (Fig. 1) and sampled within a day of each other. Sampling occurred during the early morning, between 05:00 and 08:00 UTC, on the 7th (PS1) and the 8th of September 2013 (PS2). Physical parameters of the water column, e.g., temperature, salinity and light intensity, were measured during CTD casts. The depth of the mixed layer (ML) was derived using a difference in density threshold of 0.045 kg m−3 from the water density value at 10 m depth (de Boyer Montégut et al., 2004). For more details on ML and the hydrography of these stations, see Van Oostende et al. (2017).

2.2. Nutrient concentrations, chlorophyll a and microbial community structure

Water column nutrient concentrations were determined at sea by segmented-flow colorimetric auto-analyzer techniques for all the nutrients including NO3− and NO2−. NH4+ analysis was by a nano-molar analytical system (detection limit ~5 nmol L−1) using pH differential gas diffusion across a Teflon membrane, followed by fluorescence analysis. Seawater (50 mL) was collected in acid clean HDPE bottles from Niskin bottles mounted on a CTD. Details of nutrient methods can be found in Woodward and Rees (2001). Clean sampling protocols were employed according to the GO-SHIP nutrient repeat hydrography manual (Hydes et al., 2010). KANSO (Japan) certified nutrient reference materials were measured in each batch for quality control. The accuracy of the autoanalyzer batches was within 1% and 4% (1 standard deviation; SD), with reference to the KANSO reference materials.

The bloom location was initially tracked using the surface chlorophyll distribution obtained from quasi-real-time NASA Aqua MODIS level 3 remote sensing data (www.oceancolor.org - NASA Goddard Space Flight Center, 2013). Flow cytometric analysis was used to determine cell concentrations of picophytoplankton, nanophytoplankton, Synechococcus and heterotrophic bacteria (Van Oostende et al. (2017)). The detection limit for flow cytometric populations was 173 cells mL−1 with reproducibility of about 10% near the detection limit or < 5% for concentrations > 1000 cells mL−1. Chlorophyll a (Chl-a) concentration, a proxy for phytoplankton biomass, was determined fluorometrically on acetone-extracted filter samples according to Holm-Hansen and Riemann (1978) (Van Oostende et al., 2016). Measurements of size-fractionated Chl-a concentration were performed on samples of up to 4 L sequentially filtered through a 20 μm pore-size polycarbonate filter, a 2 μm pore-size polycarbonate filter, and a 0.3 μm pore-size glass fiber filter (GF-75; Sterlitech; 47 mm diameter). Filtrations were performed in the dark under low vacuum (~< 200 mbar). Flow cytometry, Chl-a and nutrient data can be accessed at http://www.bco-dmo.org/dataset/651784/data. CTD: 563684, Chl-a: 651784, nutrients: 651816, FCM: 651890, 15N PON and 15N NO3−: 652025 are the codes for each respective dataset.

2.3. Bulk and amino acids stable isotope sampling, preparation and measurements

A total of 22 samples was collected spanning the surface water to 200 m depth (20 from the CTD rosette - Cast 29 for PS1 and Cast 30 for PS2 - and 2 from the surface inflow system). Seawater (10 L per depth) was collected in 10 L HDPE containers. Samples were immediately filtered through a 20 μm pore-size polycarbonate filter, a 2 μm pore-size polycarbonate filter, and a 0.3 μm pore-size glass fiber filter (GF-75; Sterlitech; 47 mm diameter). Filtrations were performed in the dark under low vacuum (~< 200 mbar). Flow cytometry, Chl-a and nutrient data can be accessed at http://www.bco-dmo.org/dataset/651784/data. CTD: 563684, Chl-a: 651784, nutrients: 651816, FCM: 651890, 15N PON and 15N NO3−: 652025 are the codes for each respective dataset.

Marine Chemistry 214 (2019) 103670
diamine tetra acetic acid and international isotope reference materials USGS40 and USGS41). Analytical reproducibility for replicates couldn’t be calculated because bulk samples were unique.

Amino acids were extracted by hydrolyzing whole dried filters with 2 mL 6 M HCl at 110 °C for 24 h in a N₂ atmosphere. An internal standard, norleucine (Nle, 50 μl of 1 mg/mL solution), was added to monitor the wet chemistry and AA stable isotope values. Solutes were then dried under a gentle flow of N₂ at 60 °C, dissolved in 0.1 M HCl, purified on a column packed with Dowex 50 ×-W8 resin and subsequently converted into N-Acetylisopropyl (NAIP) ester derivatives following the protocol described in Sabadel et al. (2016), modified from (Styring et al., 2012). Details of the derivatisation procedure can be found in the Supplement.

\[ \delta^{13}C_{AA} \] and \[ \delta^{15}N_{AA} \] were measured by gas chromatography/combustion/isotope ratio mass spectrometry (GC-IRMS), using a Thermo Trace gas chromatograph, the GC combustion III interface, and a Delta plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific). 200 nl aliquots of derivatised AA were injected onto the inlet at 270 °C in splitless mode, carried by helium at 1.4 mL min⁻¹ and separated on a VF-35 ms column (30 m, 0.32 mm ID and a 1.0 μm film thickness). The GC program and other set parameters for the GC-IRMS can be found in the Supplement, Table S1. Samples were analysed in triplicate along with amino acid standards of known isotopic composition (measured by EA-IRMS) and bracketing each triplicate measurement. Each run contained no > 6 samples. Raw \[ \delta^{13}C_{AA} \] was individually corrected relative to the amino acid \[ \delta^{13}C_{AA} \] of the standards to account for the added C and kinetic fractionation introduced during the derivatisation procedure (see Supplement section 1). The \[ \delta \] values were reported following the conventional method of expressing \[ \delta \] at natural abundance, in per mil (‰), relative to an international standard: Vienna PeeDee Belemnite (VPDB) for \[ \delta^{13}C_{AA} \] and atmospheric N₂ for \[ \delta^{15}N_{AA} \].

Eleven amino acids from each sample were measured with no peak co-elutions, listed below. In order of elution: alanine (Ala), glycine (Gly), valine (Val), leucine (Leu), isoleucine (Ile), Threonine (Thr), serine (Ser), proline (Pro), asparagine + aspartic acid (Asx), glutamate + glutamic acid (Glx) and phenylalanine (Phe) (see Supplement, Fig. S2, for a typical chromatogram). Note that during the hydrolysis step, asparagine is converted to aspartic acid (hence the notation Asx) and glutamate is converted to glutamic acid (hence the notation Glx). The \[ \delta^{13}C_{AA} \] values were obtained from all depths from surface to 200 m depth, while the reported \[ \delta^{15}N_{AA} \] values are from the surface to 100 m at PS2, and to 60 m at PS1 because \[ \delta^{15}N_{AA} \] was not quantifiable for deeper samples due to their low nitrogen content. Precision (1SD) of corrected \[ \delta^{13}C_{AA} \] ranged from 0 to 1.0‰ with a mean of 0.3‰, while the precision for \[ \delta^{15}N_{AA} \] ranged from 0.1 to 1.0‰ with a mean of 0.5‰.

2.4. Trophic position and diagenetic proxy

Calculation of the TP proxy provides an estimation of the trophic status of an organism based on the known isotopic fractionation of N in some specific AAs with trophic transfer. TP was calculated based on the algal-based Eq. (1) from Chikaraishi et al. (2009):

\[ \text{TP} = \frac{\delta^{15}N_{AA} - \delta^{15}N_{N_2}}{\Delta\delta^{15}N_{AA}} \]

Fig. 1. Composite remote sensing image of chlorophyll a concentration in region of the two stations during their sampling (composite of merged, weighted average CHL1 L3 product at 1/24° resolution from 6th to the 13th of September 2013) (GlobColour, data accessed on 6 September 2016). PS1 (54°00′N, 20°40′W) and PS2 (50°00′N, 20°00′W).
Where $\beta$ represents the isotopic difference between Glx and Phe in the primary producers: $\delta^{15}\text{N}_{\text{Glx}} - \delta^{15}\text{N}_{\text{Phe}} = 3.4\%o$ for aquatic cyanobacteria and algae (Chikaraishi et al., 2009). The trophic enrichment factor (TEF) value of 7.6%o is commonly used in aquatic food web studies for low TP (McMahon and McCarthy, 2016; Ohkouchi et al., 2017). The TEF is the average $^{15}$N enrichment in one or more trophic AAs relative to source AAs per TP and is key to estimate the TP accurately. Recent studies have highlighted the issues with the use of a fixed TEF across a food web and more specifically at higher TPs (Bradley et al., 2011). Indeed, as pointed out by the meta-analysis of Nielsen et al. (2015), TEF decreases with higher TP. Change in diet with higher trophic position species was found to be the main cause of this TEF decrease (McMahon and McCarthy, 2016). However, and as demonstrated by Monpéan et al. (2016), a TEF of 7.6‰ yields reasonable estimates for lower TP organisms retrieved in suspended POM, and much of the early work to determine the TEF has been conducted on low TP species (Chikaraishi et al., 2009; McClelland and Montoya, 2002). Uncertainties associated with the trophic position calculation using Eq. (1) were calculated by the propagation of errors following Dale et al. (2011), using $\sigma_\beta = 0.9\%o$ and $\sigma_{\text{TEF}} = 1.1\%o$ (Chikaraishi et al., 2009); details can be found the Supplement.

The $\Sigma$ parameter is a complementary POM degradation proxy (McCarthy et al., 2007). It is based on the assumption that de novo heterotrophic AA resynthesis preferentially cleaves $C^{1-14}N$ bonds of selected AAs, thus producing $^{15}$N-enriched AA and a more scattered $\delta^{15}N_{\text{AA}}$ pattern in POM. The equation used to calculate the $\Sigma$ parameter shows the average degree of variation around a mean of six trophic AAs. The use of this parameter eliminates inter-sample variation and only focuses on changes in the $\delta^{15}N_{\text{AA}}$ patterns. Thus, other bacterial processes, such as extracellular protein hydrolysis, or during animal-like $\delta^{15}N_{\text{AA}}$ fractionation of POM AA, where all AAs involved in the $\Sigma$ parameter fractionate equally (Hannides et al., 2013; Yamaguchi and McCarthy, 2018) and their $\delta^{15}N_{\text{AA}}$ pattern shifting identically compared to the substrate (Ohkouchi et al., 2017), the $\Sigma$ parameter value should remain relatively low and stable. $\Sigma$ is calculated for each POM sample as follow:

$$\Sigma V = \frac{1}{n} \sum_{i} \text{Abs}(\chi_i)$$

With $\chi_i$, the deviation in $\delta^{15}N$ of each AA from the average, $\chi_i = [\delta^{15}N_{\text{AA}} - \text{Avg.}\delta^{15}N_{\text{AA}}]$ and $n$, the total number of AAs used in the equation. Note that this parameter can be calculated for an exclusive group of AAs (e.g. trophic or source (McCarthy et al., 2007)) or for all AAs at once (Calleja et al., 2013). Here, only six trophic AAs are used: Ala, Asx, Glx, Ile, Leu, and Pro.

### 2.5. Data treatment and statistical analyses

All data were expressed as the arithmetic mean of triplicate measurements ± 1SD. Differences between stations, depth layers and AA stable isotope signatures were tested using $t$-tests and deemed significant at the $p < 0.05$ level. When the independent variables were categorical (e.g. differences between the two stations), unequal variance $t$-tests were used to detect differences among categories. When the variables could be paired (e.g. data within one station), a two-tailed paired $t$-test was used. The degree of linear association between two variables was evaluated using Pearson’s correlation coefficient $r$.

To assess the algal or heterotrophic bacterial origin of organic matter based on $\delta^{15}C_{\text{AA}}$ signatures, we focused on the isotopic composition of Leu and Ile, which have been identified to be good discriminators between these two groups (Larsen et al., 2009). The theory behind this strong $^{13}C_{\text{AA}}$ discrimination lies within the fact that bacteria (marine or terrestrial), synthesize certain AAs differently than plants or phytoplankton (including chlorophytes, chrysophytes, cyanobacteria, diatoms, and haptophytes). We compiled $\delta^{15}C_{\text{AA}}$ values from various datasets, including a validation set of ocean microalgae and bacterial cultures (Larsen et al., 2015), a validation set of forest bacteria (Larsen et al., 2009) as it was used by Hannides et al. (2013) and a marine POM dataset from Hannides et al. (2013). We standardized the assembled dataset (for each AA: $\text{Norm}(\delta^{15}C_{\text{AA}}) = (\delta^{15}C_{\text{AA}} - \text{mean}(\delta^{15}C_{\text{AA}})) / \text{SD}(\delta^{15}C_{\text{AA}})$), and plotted the resulting values of $\text{Norm}(\delta^{15}C_{\text{AA}})$ vs. $\text{Norm}(\delta^{13}C_{\text{AA}})$ from the library in a bi-variate scatter plot. We then compared the $\delta^{15}C_{\text{AA}}$ of POM values from this study against this library by adding the standardized POM data and graphically assessed to which group they belonged: algal, bacterial or a mix of both.

Change in the $\delta^{15}C_{\text{AA}}$ values with depth has previously been reported (Hannides et al., 2013; McCarthy et al., 2004) and can be associated with bacterial activity and/or change in POM composition as particles sink through the water column. Differences in $\delta^{15}C_{\text{AA}}$ values between the euphotic zone (depth ≤ 55 m) and the twilight zone (depth ≥ 100 m), were assessed by calculating the $\delta^{15}C_{\text{AA}}$ offset of the averaged $\delta^{15}C_{\text{AA}}$ values of each zone: $\Delta_{\text{euphotic-twilight}} = \delta^{15}C_{\text{AA-euphotic}} - \delta^{15}C_{\text{AA-twilight}}$.

To graphically represent relationships among other AAs and compare them to the existing literature (Ohkouchi et al., 2017), the $\delta^{15}N_{\text{AA}}$ data were normalized to $\delta^{15}N_{\text{Glx}}$ within sample (for each AA: $\text{Norm}(\delta^{15}N_{\text{AA}}) = \delta^{15}N_{\text{AA}} - \delta^{15}N_{\text{Glx}}$). Data were plotted, and statistical analyses were performed using R (version R2015b).

### 3. Results

#### 3.1. Hydrography, nutrient concentrations and microbial community structure

In order to contextualize the cycling of POM assessed by CSIA-AA at our open ocean sites we first describe the hydrography, nutrient concentrations and microbial community structure of the two stations. Of the two North Atlantic stations, PS2 represented a later bloom stage, based on surface nutrient depletion, biomass composition, and nutrient uptake rates (Peng et al., 2018) (Fig. 2). The recent mixing history of the water column was reflected in the higher concentration of surface NO$\text{3}^-$ and the greater depth of the ML at PS1 (50 m), compared to PS2 (35 m, Fig. 2). The euphotic zone was defined as the depth at which light intensity is attenuated to 1% of its surface value (~55 m at both stations). The distributions of NO$\text{3}^-$, NO$\text{2}^-$ and NH$\text{4}^+$ concentrations with depth were very similar between the two stations (Fig. 2). Average upper ML concentration of NO$\text{3}^-$ was ~2 μmol L$^{-1}$ at PS1 while NO$\text{3}^-$ was depleted at PS2 (~0.5 μmol L$^{-1}$). The nitrate was located at 45 m for PS1 and 40 m for PS2, below which the average concentration of NO$\text{3}^-$ increased to similar values at 100 m at PS1 and PS2 (9.6 μmol L$^{-1}$ and 11.1 μmol L$^{-1}$, respectively (Fig. 2a). NO$\text{2}^-$ concentration peaked at 50 m at PS1 to reach 0.485 μmol L$^{-1}$, and 55 m at PS2 to reach 0.560 μmol L$^{-1}$. NO$\text{2}^-$ concentration was at or below the detection limit in the upper ML at PS2 but reached 0.190 μmol L$^{-1}$ at PS1. NH$\text{4}^+$ showed a peak concentration of 0.400 μmol L$^{-1}$ at 45 m at both stations, just above the nitrate maximum, and decreased to very low concentrations (~0.025 μmol L$^{-1}$) below the ML.

The two stations were sampled during a summer phytoplankton bloom as indicated by remote sensing (Fig. 1) and in situ Chl-α concentration measurements (Van Oostende et al., 2017). Mean Chl-α concentrations integrated over the depth of the ML were similar at PS1 (0.81 µg chl L$^{-1}$) and PS2 (1.12 µg chl L$^{-1}$). Total Chl-α at both stations was strongly dominated by pico- and nanophytoplankton, whereas the microphytoplanktonic size fraction made up < 4% of the total Chl-α. PS1 had 30–50% more autotrophic nano-eukaryotes (2–20 μm cell...
diameter) and 50% more heterotrophic eukaryotes (e.g., heterotrophic flagellates) in the top 50 m of the water column than PS2 (Fig. 3a). PS2, on the other hand, had ~25% more autotrophic pico-eukaryotes (<2μm cell diameter) and about double the abundance of *Synechococcus* cells, and between 60 and 90% higher cell abundance of heterotrophic bacteria than at PS1 in the top 40 m. The higher relative abundance of bacterial cells is indicative of the more advanced stage of the bloom at PS2 (Fig. 3b–d, Buchan et al. (2014)).
δ−11.1‰ for PS1 and from −36.4 to −9.3‰ for PS2 in the whole sinking out of the euphotic zone. The average offset across all AAs was increased between the euphotic zone and the twilight zone, as POM is displayed with red squares. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Carbon and nitrogen elemental and stable isotope composition of particulate organic matter

The C/N ratio of elemental composition of POM averaged 7 ± 1 (range 6–10) for PS1 and 7 ± 1 (range 6–8) for PS2 (Table S1), typical of marine phytoplankton, which usually have a C/N ratio of 4–10 (Meyers, 1997). The average δ13CBulk isotopic composition in the upper 60 m was very similar at both stations (Fig. 4, FigS6) (δ13CBulk: PS1–23.9 ± 0.5‰ and PS2–23.6 ± 0.5‰, unequal variance t-test: t = 2.20, p > 0.05), and δ13CBulk variability with depth was not significant (PS1 ANOVA, F = 0.98, p > 0.05; PS2 ANOVA, F = 1.06, p > 0.05). In contrast to δ13CBulk, the average δ15N Bulk at PS1 (5.6 ± 0.6‰) was lower than at PS2 (7.1 ± 0.4‰, Figs. 4, 5, 6, unequal variance t-test: t = 2.45, p < 0.05). However, no significant differences in δ15N Bulk with depth within each station were found (ANOVA, Fdeg of freed = 0.90, p > 0.05 for PS1 and ANOVA, Fdeg of freed = 0.94, p > 0.05 for PS2).

3.3. The determination of POM origin from the isotopic composition of CAA and NAA

δ15C CAA values of POM individual AAs ranged from −38.2 to −11.1‰ for PS1 and from −36.4 to −9.3‰ for PS2 in the whole water column (surface to 4000 m; Supplement Table S2). δ15C CAA values increased between the euphotic zone and the twilight zone, as POM is sinking out of the euphotic zone. The average offset across all AAs was of Δtwilight-euphotic = 2.7‰ at PS1 (values ranged from Δtwilight-euphotic = 1.1‰ for Ser and Asx to Δtwilight-euphotic = 4.4‰ for Ile) and of Δtwilight-euphotic = 1.4‰ at PS2 (values ranged from Δtwilight-euphotic = 0.1‰ for Thr to Δtwilight-euphotic = 3.3‰ for Pro). The contributions of phytoplankton and heterotrophic bacterial C sources to POM were estimated using the Norm(δ13C CAA) and Norm(δ15C CAA) composition of POM as discriminants, based on previous research by Larsen et al. (2015) (further details in the discussion). Based on the relatively higher δ13C CAA compared to δ15N CAA values and the clustering of this study’s samples with the microalgae-only samples from Larsen et al. (2015) (Fig. 5), the carbon in POM was of primarily phytoplanktonic origin.

δ15N CAA of POM individual AAs ranged from −3.0 to 12.3‰, between the surface and 60 m at PS1 and from −7.0 to 17.6‰ between the surface and 100 m at PS2 (Supplement, Table S3). Overall, source AA had significantly lower δ15N values (mean δ15N Source = 2.5‰ at PS1 and 1.8‰ at PS2) than trophic AA (mean δ15N Source = 8.8‰ at PS1 and 9.6‰ at PS2). At both stations, the most 15N-depleted AA was Thr (mean δ15N Thr = 0.2‰ at PS1 from 0 to 60 m and −1.9‰ at PS2, from 0 to 100 m depth), while the most 15N-enriched was Glx (mean δ15N Glx = 10.9‰ at PS1 from 0 to 60 m and 11.6‰ at PS2, from 0 to 100 m depth). The δ15N Phe values were lower and less variable at PS1 (3.3 ± 0.6‰) than at PS2 (4.3 ± 1.7‰). Thr, the unique metabolic AA, was the most isotopically 15N-depleted AA of the measured suite (δ15N Thr = −0.1 ± 2.0‰ at PS1 and δ15N Thr = −1.9 ± 3.7‰ at PS2, Table S3). The δ15N AAs in POM each varied with depth (ANOVA, F7,10 = 3.5–72.7, p > 0.01 for all AAs at PS1 and ANOVA, F9,10 = 16.3–65.2, p > 0.01 at PS2). However, no particular trends were observed in either source or trophic AA; only the metabolic AA, Thr, became more depleted with increasing depth at both stations (Fig. 6).

Several significant relationships between the δ15N values of different AAs and variables informing about the nutrient regime and biomass composition were identified by correlation analyses. The presence of NH4+, a regenerated form of N, is indicative of an imbalance between its production and consumption rates. Although it was not possible to measure δ15N NH4+, there was a negative correlation between NH4+ concentration and the isotopic baseline AA, δ15N Phe at PS2 (Table 1). At PS1, there was no correlation between δ15N Phe and the concentration of NH4+ (Table 1). δ15N Phe was negatively correlated with δ15N Bulk at PS1 and positively correlated at PS2 (Table 1; Supplement Fig. S7). δ15N Glx was only correlated with δ15N Bulk at PS1 but was significantly correlated with δ15N Phe at both stations.

The δ15N Phe enrichment with depth could be partially explained by the variation of phytoplankton cell abundance, PON concentration and trophic position (Table 2). Indeed, a decrease in δ15N Phe had previously been associated with trophic fractionation (Germain et al., 2013; McMahon et al., 2015c). Interestingly, δ15N Thr enrichment correlated with the cell abundance of heterotrophic bacteria at PS1 but not at PS2, where the decrease in cell abundance with depth was larger (Fig. 3; Table 2).

The differences between the canonical trophic and source AAs (δ15N Glx and δ15N Phe, respectively; ΔGlx-Phe = δ15N Glx - δ15N Phe) can, by definition, give a first indication on the trophic status of POM without calculating TP. ΔGlx-Phe was higher below the ML compared to the upper mixed layer at both stations. At PS1, in the upper mixed layer: ΔGlx-Phe = 7.3 ± 0.4‰ (ranged from 6.7 to 7.7‰) and at PS2, ΔGlx-Phe = 6.6 ± 0.5‰ (ranged from 5.9 to 6.9‰). Below the mixed layer, at PS1, ΔGlx-Phe = 8.1 ± 0.4‰ (ranged from 7.8 to 8.3‰) and at PS2, ΔGlx-Phe = 9.8 ± 1.4‰ (ranged from 7.9 to 11.7‰). In Fig. 8, the relationship between δ15N Glx and δ15N Phe is plotted along TP isolines together with environmental δ15N NAA endpoints. This helps to (1) visualize POM TP and (2) identify the inorganic precursor used for the synthesis of the AA found in POM. Recall, however, that δ15N Glx and δ15N Phe must be considered relative to each other and that assessing the δ15N baseline (δ15N Phe) is crucial to interpret CSIA-AA data.

3.4. Heterotrophic contribution to POM

By definition, primary producers are expected to have a TP of 1, and deviation from this value usually indicates either the inclusion of heterotrophic material (e.g. zooplankton) and/or the presence of bacterial recycling in suspended POM. The calculated TP of POM averaged 1.5 ± 0.1 above and 1.6 ± 0.0 below the ML depth at PS1, and 1.4 ± 0.1 above and 1.8 ± 0.2 below the ML depth at PS2 (Fig. 8). Thus, TP measures suggest the inclusion of at least some heterotrophic or reworked organic matter in suspended POM. In a complementary way, the EV proxy, along with the interpretation of the Norm(δ15N AA) pattern, provides an estimate of the degree of heterotrophic degradation or resynthesis (Eq. 2, Table S3) and the processes associated with it, respectively. Typically, EV values between 1 and 2 are expected for phytoplankton biomass (McCarthy et al., 2007), while a higher value of EV suggests the presence of heterotrophic bacterial AA resynthesis.
values were similar at both stations, ranging from 0.9 to 2.0, without a clear trend with depth. The pattern of variation with depth of each \( \delta^{15}N_{\text{AA}} \) at PS1 and PS2 was significantly similar (using the mean values of each \( \delta^{15}N_{\text{AA}} \) per station: unequal variance \( t \)-test: \( t = 2.11, p = 0.76 \); Fig. 7). However, \( \delta^{15}N_{\text{AA}} \) values of each AA were more variable along depth at PS2 compared to PS1 (even when excluding >100 m samples from PS2, ANOVA, \( F_{7,63} = 3.472, p < 0.05 \) at PS1 and ANOVA, \( F_{10,90} = 13.43, p < 0.05 \) at PS2) (Fig. 7).

4. Discussion

4.1. Community composition and bloom status at two North Atlantic open ocean sites

The overall ML phytoplankton biomass was similar at both stations, and the low abundance of microphytoplankton is characteristic of late summer North Atlantic Ocean blooms (Dandonneau et al., 2004; Van Oostende et al., 2017). Station PS2 was in a more advanced bloom stage than PS1, as indicated by depleted surface nutrient concentrations, deeper nitracline, and the higher relative abundance of smaller planktonic groups such as pico-eukaryotes, Synechococcus, and heterotrophic bacteria. Depth profiles of ammonium and nitrite at both stations were very similar and suggest that biomass decomposition and remineralization was occurring near the bottom of the ML. The presence of maxima in the ammonium and nitrite profiles indicates spatial uncoupling between production and consumption, which may result from nitrite excretion due to light limitation of phytoplankton during nitrate assimilation (Collos, 1998; Kiefer et al., 1976; Malerba et al., 2012; Wada and Hattori, 1978), ammonium accumulation due to grazer...
excretion during a declining bloom (Johnson et al. 2007), or ammonium and nitrite oxidation below the euphotic depth (Peng et al., 2018). These results are further compared to those obtained using CSIA-AA.

4.2. δ13CAA as a biomarker of autotrophic vs heterotrophic biomass

While δ13Cbulk values between PS1 and PS2 were not significantly different, δ13CAA values can be used to identify the source of C in POM (Larsen et al., 2009, 2013, 2015) found that the isotopic values of the essential AA Leu and Ile could discriminate between the C of plant/algae vs heterotrophic bacterial origin in POM. Indeed, Leu and Ile both belong to the pyruvate family, and while it has been demonstrated that the synthetic pathways for these AAs are similar in bacteria and plants (Hagelstein et al., 1997; Keseler et al., 2005; Kohlhaw, 2003), studies have shown that the associated fractionation was however different (Blair et al., 1985; Melzer and Schmidt, 1987). Additionally, the synthesis of Ile in bacteria involves the use of acetohydroxy acid synthetase, and thus bacterially-derived Ile is isotopically distinct from plant-derived Ile (Kiel and Fogel, 2001). Thus, by plotting standardized (to z-score) δ13CLeu and δ13CIne, we were able to show the dominance of algal-derived AA as the source of C in our POM samples by comparing them to a validation dataset produced in other studies (Fig. 5). Given the dominance of autotrophic (eukaryotic) biomass (Fig. 3, Van Oostende et al., 2017) and the bloom stage at our two stations (Fig. 1), a phytoplanktonic C isotopic signature in POM was expected and so the source of C in POM’s AAs in our study appears to be primarily of algal origin as opposed to heterotrophic bacteria (Fig. 5). Results from Hannides et al. (2013), one of the few open ocean studies using the dual (C and N) AA isotopic approach on POM, but which did not include samples taken during a phytoplankton bloom, however, fell between algal and bacterial-derived δ13CAA values, indicating that the autotrophic biomass was less dominant at station ALOHA in the North Pacific Subtropical Gyre. In their study, the authors used a training set comprising “terrestrial bacteria” from Larsen et al. (2012); it was interesting to note that both forest bacteria (Larsen et al., 2009) and ocean heterotrophic bacteria (Larsen et al., 2015) fell along the same side using our dual AA approach. This indicated that, in both these datasets, heterotrophic bacteria, whether terrestrial or marine, synthesized AA with similar relative final δ13CLeu and δ13CIne values. Possible explanations for this similarity could be i) both heterotrophic bacteria groups are using identical metabolic pathways or ii) both heterotrophic bacteria groups are using different pathways that result in similar δ13CLeu and δ13CIne values. However, it is also possible that these heterotrophic bacteria are a particular subsample with similar metabolisms when it comes to Leu and Ile synthesis. More work is required to assess if this trend is observable across larger heterotrophic bacteria groups, including bacteria from systems such as hydrothermal vents, hot springs and freshwater environments.

The observed increase in δ13CAA with depth at both stations (Δtwilight-euphotic, Table S2), between the euphotic and the twilight zone, based on the ammonium and nitrite concentration depth profiles, seems to point towards isotopic fractionation caused by remineralization during organic matter decomposition of sinking particles (Siller et al., 1992), which was likely prevalent below the euphotic zone. The magnitude of change in δ13CAA was found to be similar to the magnitude of change (a few ‰) between the surface and mesopelagic depths (200–1000 m) at station ALOHA (Hannides et al., 2013). This indicates that, at least at these stations, located in different open ocean regions (North Pacific vs North Atlantic), the fate of organic C was similar, and thus it might be possible to predict the diagenetic transformation and subsequent isotopic fractionation in POM during its journey to the ocean floors. This is key information with regard to understanding the role of bacteria in the cycling of organic matter in sinking particles and in sediments (Kiel and Fogel, 2001; Larsen et al., 2015), and for the reconstruction of past climate and water column C cycling based on δ13CAA values (McMahon et al., 2015a; Schiff et al., 2014).

4.3. δ15NAA as a biomarker for the origin and contribution of different N sources to primary producers

Phytoplankton reliance on NO3− vs recycled N can be inferred from the δ15N of suspended PON using a Rayleigh distillation model, together with the concentration and δ15N of NO3− and the isotope effect of NO3− assimilation (Van Oostende et al., 2017 Fig. S9, Mariotti et al. (1982)). The interpretation of the Rayleigh model, however, can be confounded by, e.g., deviation from the open substrate pool assumption, recent export of isotopically lighter PON, or different contributions of biomass vs detritus to PON. While it is intuitive that phytoplankton at the NO3−-depleted PS2 had to rely on recycled N sources to sustain itself, it was more difficult to ascertain for phytoplankton at PS1, where NO3− was still present in the ML. However, studies conducted in parallel to this one established that during the late summer bloom, the phytoplankton community was dominated by regenerated N specialists (i.e., picocyanobacteria and picoeukaryotes) (Van Oostende et al., 2017), and demonstrated using H15NO3−, 15NO3− and 15NH4+ uptake experiments, and N15O− concentration that the phytoplankton communities at both stations were supported by recycled N (Peng et al., 2018). Further insights into the cycling of N at these two stations can be gained by using AA isotope-based biomarkers. As a source AA, Phe undergoes very little fractionation throughout trophic transfer in food webs (McClelland and Montoya, 2002; Popp et al., 2007), and its variability in δ15N values indicates a change in N source isotopic
composition at the base of zooplankton food webs. Moreover, $\delta^{15}N_{\text{Nhe}}$ has been shown to integrate and preserve the stable isotopic signature of its initial/source N representing an ideal ‘isotopic baseline’ (McMahon and McCarthy, 2016; Ohkouchi et al., 2017). In this study, POM at PS1 had lower and less variable $\delta^{15}N_{\text{Nhe}}$ values than at PS2 (3.3 ± 0.6‰ and 4.3 ± 1.7‰, respectively), which may indicate that the AA synthesis used different N precursors - with different isotopic composition - and/or that these AAs underwent different metabolic processes that affected their isotopic ratios distinctively. The latter may be due to the more advanced stage of the bloom at PS2. However, while the processes controlling the isotopic ratios of Phe seemed to extend to all N-containing molecules in POM at PS2, there was a decoupling of Phe vs the rest of the N-containing molecules in PS1’s POM, as reflected by the negative correlation between $\delta^{15}N_{\text{bulk}}$ vs $\delta^{15}N_{\text{Phe}}$. This decoupling observed at PS1 could actually be caused by the fact that Phe is not the dominant AA in POM and thus may only contribute to a small extent to $\delta^{15}N_{\text{bulk}}$. Further interpretation of these data would benefit from a Lagrangian experiment following the evolution of PON isotope values over the decay of a phytoplankton bloom.

Additionally, $\delta^{15}N_{\text{Phe}}$ values at PS1 (2.5–4.1‰) remained stable within the euphotic zone while $\delta^{15}N_{\text{NO3}}$ values ranged from 5.4–9.2‰, reflecting the depth gradient of nitrate depletion. This could not be assessed at PS2 because of the surface depletion of NO$_3^−$. A change in inorganic precursor could be the reason for a drop in $\delta^{15}N_{\text{Phe}}$ values between the ML and the euphotic depth.

The significant negative correlation of $\delta^{15}N_{\text{Phe}}$ with the concentration of NH$_4^+$ (Fig. 2), indicated that POM isotopic baseline could be decreasing due to the preferential incorporation of NH$_4^+$ by the pre-biomassing ($\delta^{15}N_{\text{Nati4+}}$, $< < \delta^{15}N_{\text{NO3}}$), as corroborated by NH$_4^+$ uptake experiments (Peng et al., 2018). There was no dominant contribution of N fixation (N-fix) in this region which could have potentially influenced the $\delta^{15}N_{\text{AA}}$ ($\delta^{15}N_{\text{Nfix}} \sim 0‰$), at these stations Peng et al. (2018).

We summarize the variations in source of inorganic N (Eglite et al., 2018; McCarthy et al., 2007; McClelland et al., 2003) and the relative trophic position they occupy within the marine food web by bracketing $\delta^{15}N_{\text{Phe}}$ values with intermediate and end-member values found in the literature (Fig. 8). We note that comparing these values, taken from different regions with different source inorganic $\delta^{15}N$ and in different bloom conditions than our study is a tricky task, but nonetheless provides a range of different bloom conditions than our study is a tricky task, but nonetheless provides a range of different N-containing molecules in POM, as reflected by the negative correlation between $\delta^{15}N_{\text{bulk}}$ vs $\delta^{15}N_{\text{Phe}}$. This decoupling observed at PS1 could actually be caused by the fact that Phe is not the dominant AA in POM and thus may only contribute to a small extent to $\delta^{15}N_{\text{bulk}}$. Further interpretation of these data would benefit from a Lagrangian experiment following the evolution of PON isotope values over the decay of a phytoplankton bloom.

4.4. Trophic positions and food web structure

The suspended POM fraction encompasses live and dead material from organisms of a range of trophic positions that make up the lower marine food web. The differences between trophic and source AAs at a given depth throughout the water column were much higher than the established isotopic difference between Glx and Phe in marine primary producers, $\beta = 3.4‰$ (Chikaraishi et al., 2009), supporting the idea that the POM at our stations was not composed entirely of primary producers. In this study, the TP of POM was either invariant throughout the euphotic zone at PS1 or was up to 50% higher below the ML at PS2 compared to the surface. While we assume that suspended POM represents the “baseline” of a local marine food web, POM contains a mixture of live and dead material from phytoplankton, bacterioplankton and other zooplankton-derived material, so its TP is not expected to be exactly 1. For instance, while primary producers would have a TP of ~1, bacterioplankton, may record a metazoan-like TP depending on what they feed on (Steffan et al., 2015), and zooplankton tissues would register a TP ≥ 2. Thus, a TP of 1.5, as we found above the ML at both stations, could be due to the presence of zooplankton (heterotrophic micro-eukaryotes or small multicellular zooplankton) in addition to phytoplankton in POM. The even higher TP below the ML, at station PS2, indicates a higher contribution of zooplankton or detrital material to POM. This would concord with the later bloom stage of PS2 which would have allowed the establishment of higher TP species grazing on the prevalent phytoplankton community.

4.5. Nitrogen isotopic values of threonine, a potential TP biomarker

Threonine is an enigmatic AA that has a behavior strictly opposite to any trophic AA: $\delta^{15}N_{\text{Thr}}$ decreases with trophic transfer, and thus, with TP (Hare et al., 1991; Styring et al., 2010). The strong negative relationship between $\delta^{15}N_{\text{Thr}}$ and TP at both stations in this study is indeed observed (Bradley et al., 2015; Mompéan et al., 2016). Batista et al. (2014) also hypothesized that the apparent offset in $\delta^{15}N_{\text{Thr}}$ values, between their trap and tow samples, could reflect temporal changes in planktonic community structure. This could be a valid explanation for the observed decrease in POM $\delta^{15}N_{\text{Thr}}$ values with depth, and a simultaneous strong correlation between water column biomass at our study sites (Table 1). As $\delta^{15}N_{\text{Thr}}$ becomes depleted when transferred up the food web, the observed depletion with depth at both stations may reflect the disappearance of phytoplankton biomass and the increasing presence of higher trophic level material, such as microzooplankton, in POM. At the time of sampling (dawn), it is possible that primary consumers, such as small flagellated zooplankton, would
have migrated down to below the ML depth, and so may be present in POM samples collected with Niskin bottles, thus raising the apparent TP of POM from 1 to ~1.6–1.8.

4.6. Degree of microbial resynthesis affecting amino acids in particulate organic matter

Microbes degrade POM, including AAs, into NH$_4^+$ which can be either oxidized back to NO$_3^-$ or made directly available as a highly reduced source of N to phytoplankton, which is then transferred through the food web. While heterotrophic fractionation can also refer to fractionation caused by trophic transfer, in this section, we focus on heterotrophic bacterial processes. There are multiple processes that are likely to happen during the interaction of heterotrophic bacteria with POM AAs, these include hydrolysis, uptake and de novo synthesis, salvage AA incorporation into new protein, as well as strict catabolism (Ohkouchi et al., 2017). These different processes will affect the POM AA isotopic composition by scattering the $\delta^{15}$NAA pattern (reworking), by enriching all the remaining AAs in $^{15}$N (hydrolysis) or just the trophic AA in a metazoan-like fractionation (uptake). Based on the average degree of variation around a mean of six trophic AAs, the $\Sigma V$ parameter constitutes a proxy for this heterotrophic resynthesis (McCarthy et al., 2007). Throughout the surface water column at PS1 and PS2 (i.e., above 100 m), the $\Sigma V$ values were $< 2$, indicating minor heterotrophic POM resynthesis in the surface water column. These $\Sigma V$ values are within the range of those found for natural suspended POM in other studies and also within the range expected for fresh biomass, i.e., live phytoplankton and zooplankton (Batista et al., 2014; Hannides et al., 2013; Maki et al., 2014; McMahon and McCarthy, 2016; Mompeán et al., 2016; Sherwood et al., 2014; Sherwood et al., 2011; Yamaguchi and McCarthy, 2018).

This lack of significant imprint of bacterial resynthesis on POM does not imply that POM was not affected by other microbial processes. Indeed, the microbial community can degrade POM in two distinct ways: resynthesis, and extracellular protein hydrolysis of POM. Resynthesis is the removal of selected AAs from POM and their subsequent transformation: this creates a scattered pattern that affects the key AAs used to calculate the $\Sigma V$ parameter. On the other hand, extracellular protein hydrolysis is caused by the hydrolysis of peptide-bonds that heterotrophic microbes utilize to facilitate the assimilation of AAs. This hydrolysis affects all AA without distinction (trophic, source and metabolic) and leaves behind an enriched pool of $^{15}$N in POM (Hannides et al., 2013; Ohkouchi et al., 2017). The invariant $\Sigma N_{AA}$ isotopic pattern (Fig. 7) suggests a high contribution of fresh material (phyto- and zooplankton) to POM, while the higher variability of all Norm($\delta^{15}$NAA) in the water column at PS2 suggests the presence of heterotrophic microbial resynthesis, such as extracellular protein hydrolysis, as the active degradation process. This conclusion is comparable to the results in Hannides et al. (2013) indicating that the variation in $\delta^{15}$NAA of POM during the late summer bloom in the North Atlantic was probably mainly driven by the extracellular hydrolysis of proteins.

5. Overview and conclusions

In this study, we used AA isotopic markers as proxies, along with more conventional analyses, such as flow cytometry and nutrient concentrations, and PON and NO$_3^-$ isotopic composition, to describe in situ elemental cycling at the base of pelagic food webs during a late summer North Atlantic Ocean bloom, and to track the origin, composition and fate of organic C and N. Most of these proxies, developed in a laboratory setting, proved to be useful tools to characterize POM cycling in the water column compared to standard measures of biomass composition, especially when used in combination with each other. The bivariate scatter plot of Leu and Ile Norm($\delta^{15}$C(AA)) in the ML revealed the dominant phytoplankton-derived AA contribution to POM at both late summer bloom stations. The POM trophic position (TP ~ 1.6) derived from $\delta^{15}$NAA indicated the presence of organic matter originating from higher trophic positions, such as heterotrophic micro-eukaryotes and multicellular zooplankton in addition to the strong primary producer signal. This TP signal was closely tracked by the $^{15}$N depletion of Thr, a metabolic AA, with depth. Based on the pattern of variation in $\delta^{15}$NAA throughout the water column, hydrolysis of proteins by heterotrophic microbes was probably the main POM degradation process at work, as opposed to the resynthesis of POM represented by the $\delta^{15}$NAA-based heterotrophic resynthesis proxy ($\Sigma V$).

However, while CSIA-AA represent a useful and complementary tool to more traditional techniques and approaches, it still has some limitations and caveats that needs to be address in future research. This study also indicates research areas where improvements would yield a more refined use of CSIA-AA proxies for C and N cycling in the ocean. For example, the establishment of baseline patterns in $\delta^{15}$NAA and $\delta^{15}$N$_{\text{POM}}$ across different marine environments and ocean basins would make it possible to capture the effect of primary producer community composition, and the influence of N source (NO$_3^-$ or DON, NH$_4^+$) on their $\delta^{15}$NAA composition. The combination of this approach with in situ isotopic tracer experiments to estimate these N sources uptake rates would be a way to test this. Finally, $\delta^{15}$N$_{\text{TAM}}$ seems to have promising biomarker properties, including the possibility of assessing TP based on it’s ‘reverse-fractionation’ behavior. The development of a TP proxy using $\delta^{15}$N$_{\text{TAM}}$ values (see Fig. S8) would benefit from targeted in situ feeding experiments when possible. Overall, these research developments would augment the value of CSIA as a biogeochemical tool to track elemental cycling and trophic exchange in the variable marine environment.

Acknowledgements

The cruise was funded by National Science Foundation Grant no. OCE-1136345 to B. B. Ward. We thank Bror Jönsson for providing the MODIS data during the cruise. GlobColour data (http://globcolour.info) used in this study has been developed, validated, and distributed by ACRI-ST, France. We also thank Marta Guerra (University of Otago, New Zealand and New Zealand Whale & Dolphin Trust) for allowing us to use additional sperm whale data that she gathered during the KAI2016-06 project funded by the Ministry of Primary Industries (New Zealand).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marchem.2019.103670.

References

Chikaraishi, Yoshito, Steffan, Shawn A., Ogawa, Nanako O., Ishikawa, Naoto F., Sasaki,


