Impact of water mass mixing on the biogeochemistry and microbiology of the Northeast Atlantic Deep Water

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1. Introduction

[2] The formation of cold dense waters in the Labrador and Greenland-Iceland-Norwegian Sea and the large-scale southward transport of North Atlantic Deep Water (NADW) drives the thermohaline circulation of the world’s oceans, which plays a decisive role in the regulation of the Earth’s climate [Bryden et al., 2005]. Accompanied with the formation of the NADW at a rate of about 17 Sverdrup [Smethie et al., 1993], the export of relatively fresh dissolved organic matter (DOM) from the surface layer into the mesopelagic and bathypelagic realms [Carlson et al., 2010]. Other than particulate organic material (POM) that is capable of sinking, the vertical transport of DOM mainly depends on convective overturning and mixing of water masses. Thus, considering the constrained direct input of DOM from the surface to the deep waters, any increase or decrease of metabolically utilisable substrates for prokaryotes in the deeper strata of the water column is largely due to the solubilization of DOM and mineralization of the available and steadily aging DOM during the evolution of the NADW in the global conveyor belt [Nagata et al., 2000].

[3] In general, the mineralization of DOM derived from POM is mediated by the abundant prokaryotes. The modern view of the microbial community comprising an active component [Herndl et al., 2005; Reinthaler et al., 2006] contrasts with the previous idea of slow-growing prokaryotes in the dark ocean [Jannasch and Taylor, 1984]. Although some authors indicated a highly reduced potential for heterotrophic productivity due to pressure effects and the highly refractory nature of the DOM pool in the dark ocean when compared to the surface layer [Bauer et al., 1992; Turley, 1993; Tamburini et al., 2013], on longer time scales, prokaryotes determine to a large extent the distribution and stoichiometry of the inorganic material in the dark ocean [Nagata et al., 2010].

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The decrease in dissolved organic carbon (DOC) and oxygen concentrations in the individual deep water masses as they age in the thermohaline circulation is an important indicator for heterotrophic metabolic activity of prokaryotes [Bendtsen et al., 2002]. Comparing the DOC decrease with the decrease in oxygen concentrations indicated that the contribution of DOC to dark ocean respiration is only about 10–20% [Aristegui et al., 2002; Carlson et al., 2010]. Most of the DOC in the dark ocean is refractory (> 90%) which is reflected by its radiocarbon age of > 4000 years [Williams and Druffel, 1987; Bauer et al., 1992]. However, the DOC may be differentiated into four pools in the dark ocean: a pool of semirefractory DOC with a lifetime of 1.5 years, a pool of semirefractory DOC with a lifetime of 20 years, a pool of refractory DOC with a lifetime of 14,000 years, and an ultrarefractory pool with a lifetime of 40,000 years [Hansell, 2013]. Consequently, it is the semirefractory and refractory DOC components that may eventually be assimilated by prokaryotes.

Acknowledging the deficiencies in methodology and parameters influenced by different time and spatial scales, several recent reports point to major discrepancies between the available organic matter and the apparent metabolic requirements of the deep ocean prokaryotic community [Reinthaler et al., 2006; Steinberg et al., 2008; Burd et al., 2010; Reinthaler et al., 2010]. These studies show that the carbon demand of prokaryotes is orders of magnitude higher than the export primary production. The conversion of POM to DOM via extracellular ectoenzymes is the main mechanism for prokaryotes to obtain assimilable substrates. Thus, part of the DOM in the dark ocean must result from the cleavage of sinking organic matter particles and aggregates apart from the DOC injected into the dark ocean during water mass formation and convective overturn at lower latitudes [Baltar et al., 2009]. Hansell et al. [2009] estimated that ~80% of organic matter transported from the surface to the deep ocean is in the form of POM with the remainder being DOM.

In this respect, analyzing the stoichiometry of the major biogenic elements in the oceans is a useful tool to assess the mineralization of organic matter in the framework of the biological pump [Anderson and Sarmiento, 1994]. While Anderson and Sarmiento [1994] considered that mineralization ratios are essentially constant with depth and basin, suggesting that large, fast-sinking phytoplankton-derived material of Redfieldian elemental composition is exported from the surface ocean and consumed in all the depth horizons, other authors concluded that there are remarkable changes in the nutrient mineralization ratios in the deep waters of the different ocean basins [Li and Peng, 2002] or different depths within the same basin [Shaffer et al., 1999]. However, the estimation of nutrient regeneration ratios from dissolved nutrient concentrations can be distorted by the method used to eliminate the effect of the conservative mixing of water masses with different initial nutrient concentrations [Schneider et al., 2005]. Direct measurements of microbial activity by prokaryotes in the dark ocean, however, are not implemented in models on DOM and/or POM mineralization, one reason being the lack of data on the conversion efficiency from particulate to dissolved organic matter by microbes.

Here we study the deep pelagic realm of the NE Atlantic basin from 60°N to the equator following the core of Northeast Atlantic Deep Water (NEADW) (Figure 1). This core is identified as a deep vertical salinity maximum at about $\sigma_z = 41.42$ or 2700 dbar [van Aken, 2000] in between the overlying Lower Deep Water (LDW) and underlying Labrador Sea Water (LSW) and Iceland-Scotland overflow water (ISOW). The linear trend in the potential temperature-salinity relationship for the NEADW [Mantyla, 1994] derives from the sluggish circulation below 2500 dbar in the NE Atlantic basin, although some coherent patterns are discernable as an eastward current over the Mid-Atlantic Ridge (MAR) at the Charlie-Gibbs Fracture Zone (CGFZ), a southward slope current near the MAR and the continental slope of Africa [Paillet and Mercier, 1997] over a general cyclonic deep and abyssal circulation [Reid, 1994].

The NEADW is a body of water defined by mixing of four water types (WT), each one characterized by a unique combination of thermohaline and chemical property values. When these properties are taken in the source region of the WT, it is called a source water type (SWT). The four WT that contribute to NEADW are Labrador Sea Water (LSW), Iceland-Scotland overflow water (ISOW), Mediterranean Water (MW), and Lower Deep Water (LDW) [van Aken, 2000]. LSW is a proper SWT but ISOW, MW, and LDW are WT. The property values of the ISOW were taken at the sills between Iceland and Scotland, where it forms by entrainment of the Norwegian overflow. MW was defined at about 1000 m in the Gulf of Cadiz after the intense mixing of the eastern North Atlantic central water with the Mediterranean overflow water that spills at the Strait of Gibraltar. The properties of the LDW were taken at the entry of the Vema Channel at 11°N of the Mid-Atlantic Ridge (see Table S1 in the supporting information).
[9] If NEADW would be a distinct water mass transported through the deep NE Atlantic with negligible mixing, then the $O_2:C:N:P$ stoichiometry of the mineralization of biogenic materials could be assessed by simply comparing the dissolved oxygen and nutrient distributions. However, the previously described WT contribute to the salinity maximum of the NEADW [van Aken, 2000]. We hypothesized that the mixing WT does not only mask the latitudinal and longitudinal patterns of variability of nutrient mineralization but also prokaryotic activity that is intrinsic to the pure NEADW. We used a multiparameter water mass modeling approach that is based on predefined WT and thus allows calculating the proportions of the different WT influencing the NEADW. The ultimate goal was to explore the link between prokaryotic biomass and activity and nutrient mineralization and stoichiometry along the NEADW core in the NE Atlantic basin that is corrected for water mass mixing and hence yielding a better approximation of microbe-mediated biogeochemical cycling in the NEADW.

2. Methods

2.1. Sampling Methodology

[10] Four cruises were conducted in the eastern basin of the North Atlantic spanning a transect from 62°N to 5°S. The cruises were carried out in the fall of the years 2002 (60°N–25°N), 2005, 2006, and 2007 (25°N–5°S) (Figure 1) resulting in a total number of 113 stations relevant for the current analysis. The stations sampled in the subtropical Atlantic at the different cruises were only partly overlapping. At 5 depth levels, discrete seawater samples for physicochemical and biological parameters were taken from 10 L Niskin bottles mounted on a Seabird conductivity-temperature-depth rosette (for details of sampled depth levels, see Table S2). Water masses were identified on board using downcast records of conductivity (salinity) and temperature [van Aken, 2000]. Initial readings of the conductivity, temperature, pressure, and oxygen (SBE 43, Seabird Electronics) were calibrated and corrected for water mass changes into duplicate 8 mL precombusted amber glass ampoules. From samples that were transferred directly from the Niskin bottle (60°N–25°N), 2005, 2006, and 2007 (25°N–5°S) (Figure 1) resulting in a total number of 113 stations relevant for the current analysis. The stations sampled in the subtropical Atlantic at the different cruises were only partly overlapping. At 5 depth levels, discrete seawater samples for physicochemical and biological parameters were taken from 10 L Niskin bottles mounted on a Seabird conductivity-temperature-depth rosette (for details of sampled depth levels, see Table S2). Water masses were identified on board using downcast records of conductivity (salinity) and temperature [van Aken, 2000]. Initial readings of the conductivity, temperature, pressure, and oxygen (SBE 43, Seabird Electronics) were calibrated and passed World Ocean Circulation Experiment requirements [WOCE operations manual, 1994]. The derived parameters of potential temperature ($θ$) and apparent oxygen utilization (AOU) were calculated using the algorithms implemented in the software package Ocean Data View 4.4.2 [Schlitzer, 2002].

2.2. Nutrient Measurements

[11] Nutrient measurements followed standard segmented flow analysis with Joint Global Ocean Flux Study recommendations [Gordon et al., 1993]. The concentrations of NH$_4$, NO$_2$, NO$_3$, PO$_4$, and SiO$_4$ were determined on a continuous flow autoanalyzer (Technicon TRAACS 800) immediately after collecting the samples and gentle filtration through 0.2 μm Acrodisc filters. Total dissolved nitrogen and total dissolved phosphorus were analyzed following the persulfate oxidation method as described in Kramer et al. [2005] (for details and detection limits, see Supporting Information Material and Methods).

2.3. DOC Measurements

[12] DOC was measured as unfiltered total organic carbon from samples that were transferred directly from the Niskin bottles into duplicate 8 mL precombusted amber glass ampoules. The ampoules were heat sealed after acidification to pH < 2 with phosphoric acid and stored frozen at −20°C until analysis back in the lab. DOC analysis was performed using the high-temperature combustion method on a Shimadzu TOC-5000A. Quadruplicate sample injections compared to a three-point standard curve, prepared with potassium hydrogen phthalate, were used to calculate DOC concentrations. The instrument’s performance and the validity of the calibration were determined using reference material of the Hansell consensus reference materials program (44–46 μmol L$^{-1}$ for the reference samples; n = 3 and 1–2 μmol L$^{-1}$ for low carbon water; n = 3). The average analytical precision of the instrument was < 3%.

2.4. Prokaryotic Abundance

[13] Counts of prokaryotic abundance (PA) broadly followed the protocol of Gasol et al. [1999]. For each sample, 1 mL of unfiltered seawater sample was fixed with 37% of 0.2 μm filtered formaldehyde (2% final concentration), incubated for 10 min at room temperature in the dark, and stored frozen in liquid nitrogen. Prior to the analysis, samples were thawed and stained with 10 μL of SYBR Green I (Molecular Probes) of a 1:200 dilution of the stock solution and incubated in the dark for 15 min. Prokaryotic cells were enumerated with an on-board FACSCalibur flow cytometer (BD Biosciences) using the excitation of the argon laser line at 488 nm and scatterplots of right angle light scatter versus green fluorescence measured at 530 nm. Counts were calibration with fluorescent microspheres (Molecular Probes) of 1 μm diameter added to all samples. Data were acquired in log mode until 10,000 events were registered. High nucleic acid (HNA) prokaryotes were distinguished from low nucleic acid (LNA) cells in the side scatter versus green fluorescence plot where HNA populations show higher fluorescence compared to LNA cells (see Figure S1). Prokaryotic carbon biomass (PB) was calculated assuming a carbon content of 10 fg C cell$^{-1}$ [Ducklow et al., 2000].

2.5. Prokaryotic Heterotrophic Production

[14] Prokaryotic heterotrophic production (PHP) of the unfiltered seawater was measured by $^3$H-leucine incorporation (specific activity: 595.7 × 10$^{10}$ Bq mmol$^{-1}$; final concentration 5–10 nmol L$^{-1}$) and followed the method described in Reintzhaler et al. [2010]. Three 10–40 mL samples and three blanks were incubated in the dark. The blanks were fixed with concentrated 0.2 μm filtered formaldehyde (4% final concentration, v/v) 10 min prior to adding the tracer. After incubating the samples and the blanks at in situ temperature for 4–48 h, depending on the expected activity, the samples were fixed with formaldehyde (4% final concentration), filtered onto 0.2 μm polycarbonate filters using nitrocellulose supporting filters, and rinsed twice with 5 mL ice cold 5% trichloroacetic acid for 5 min. Thereafter, the filters were transferred to 20 mL scintillation vials and the filters were allowed to dry. Subsequently, 8 mL of scintillation cocktail (Filter Count, Perkin-Elmer) was added and after 18 h, the radioactivity counted in a liquid scintillation counter. Leucine incorporated into prokaryotic biomass was converted to carbon production using the theoretical conversion factor of 1.55 kg C mol$^{-1}$ leucine assuming no isotope dilution [Simon and Azam, 1989].

2.6. Multiparameter Water Mass Analysis

[15] Briefly, the proportions of the four water types (MW, LSW, ISOW, and LDW) contributing to the NEADW were
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Figure 2. Observed latitudinal gradients of physicochemical parameters in the NEADW core. (a) Salinity, (b) potential temperature (°C), and (c) silicate (SiO₄; μmol kg⁻¹) along the transect from 70°N to 10°S. Numbers in the inset indicate average ± standard deviation over the transect.

objectively quantified by solving a set of linear mixing equations of potential temperature (θ), salinity (S), and silicate (SiO₄) conservation with the constraint that the contributions of all WT must be positive and sum up to 100% [Brea et al., 2004] (for details, see supporting information Material and Methods).

[16] The θ, S, and SiO₄ of the water types used in the analysis (see Table S1) is based upon previous studies in the area [van Aken, 2000; Alvarez et al., 2004; Alvarez-Salgado et al., 2013]. Furthermore, we assume that these tracers behave conservatively in the deep NE Atlantic basin. The robustness of the estimation of the water mass proportions was assessed using a perturbation test according to Lawson and Hanson [1974] (for details, see supporting information Material and Methods).

[17] After calculating the WT proportions contributing to each sample, the concentration of any nonconservative parameter, N, was modeled by calculating the parameters that better fit the equation:

\[ N_j = \sum_i x_{ij} N_i \quad j = 1 \text{ to } 113 \text{ samples} \quad (1) \]

Where \( N_j \) is the measured concentration of \( N \) in sample \( j \), \( N_i \), the adjustable parameters, are the expected concentrations of \( N \) in WT \( i \). Note that whereas the water type values of conservative parameters (θ, S, and SiO₄) retain the conditions in the area where they were defined, the water type values of nonconservative parameters retain the variability due to both: (i) the conditions in the area where they were defined (i.e., the initial preformed concentrations) and (ii) the mineralization of biogenic materials from the area of definition to the center of mass of each water type in the study area [Perez et al., 1993; Alvarez-Salgado et al., 2013]. A system of 113 linear mixing equations (one per sample) with four unknowns, \( N_i \) (one per WT) was solved by minimizing the residuals of these equations in a least squares sense. The higher the correlation coefficient (\( r^2 \)) and the lower the standard deviation of the residuals (SD) of the least squares analysis, the larger the impact of WT mixing on the distribution of parameter \( N \). Local biogeochemical variability, i.e., biogeochemical differences between samples with the same WT composition, is contained in the residuals of equation (1), which represent the proportion of \( N \) that cannot be modeled just by WT mixing.

[18] In summary, the relationship between any pair of nonconservative parameters (\( N_1 \), \( N_2 \)), either geochemical or microbial, depends upon (i) conservative mixing of WT with contrasting \( N_i \) values and (ii) nonconservative biogeochemical processes that occur during that mixing, both at the basin scale (from the area where they were defined to the center of mass of each WT in the study area) and the local scale (different processes or intensities of the same processes in samples of the same WT composition). Thus, the following equation allows modeling the relationship between \( N_1 \) and \( N_2 \):  

\[ N_{1j} - \sum_i x_{ij} N_{1i} = \beta (N_{2j} - \sum_i x_{ij} N_{2i}) \quad \text{or} \]

\[ N_{1j} = \sum_i x_{ij} (N_{1i} - \beta N_{2i}) + \beta N_{2j} \quad j = 1 \text{ to } 113 \text{ samples} \quad (2) \]

Where \( N_{1j} \) and \( N_{2j} \) are the concentrations of \( N_1 \) and \( N_2 \) in sample \( j \). \( N_{1i} \) and \( N_{2i} \) are the adjustable WT concentrations of \( N_1 \) and \( N_2 \), respectively. \( \beta \) is the adjustable coefficient of the relationship between parameters \( N_1 \) and \( N_2 \), which is independent of the mixing and models the local-scale mineralization. Again, a system of 113 linear mixing equations (one per sample) was solved with 5 unknowns, in this case four parameters (one per WT) and \( \beta \). As for the case of equation (1), the goodness of this linear physical-biogeochemical parametric model was tested using the correlation coefficient (\( r^2 \)) and the standard error of the residuals of the least squares analysis. Furthermore, to assess the robustness of the estimated \( \beta \), we performed a perturbation test of equation (2) following the previously referred method of Lawson and Hanson [1974] in which the values of \( N_1 \), \( N_2 \), and \( x_{ij} \) were modified, introducing normally distributed random numbers within the uncertainties of their respective estimates: the analytical errors in the case of \( N_1 \) and \( N_2 \) and the standard deviation of the results of the 100 perturbation experiments previously conducted to obtain the water mass proportions in the case of \( x_{ij} \).

2.7. Conversion of O₂:N:P Molar Ratios Into Biochemical Marine Phytoplankton Composition

[19] Assuming that changes in the O₂:C:N:P stoichiometry of the oceans are due to variations in the proportions of
**Figure 3.** Proportions of water types contributing to the NEADW core as calculated using multiparameter water mass analysis. The percent contributions of (a) Mediterranean Water (MW), (b) Labrador Sea Water (LSW), (c) Iceland-Scotland Overflow Water (ISOW), and (d) Lower Deep Water (LDW) along the transect from 70°N to 10°S are indicated by dots. Error bars indicate standard deviations from the perturbation analysis. Numbers in the inset indicate average ± standard deviation over the transect.

**Figure 4.** Observed latitudinal gradients of chemical parameters in the NEADW core. (a) Nitrate (NO₃; µmol kg⁻¹), (b) apparent oxygen utilization (AOU; µmol kg⁻¹), (c) total organic carbon (DOC; µmol kg⁻¹), and (d) dissolved organic nitrogen (DON; µmol kg⁻¹) along the transect from 70°N to 10°S. Numbers in the inset indicate average ± standard deviation over the transect.
3. Results

3.1. Latitudinal Gradients of Physicochemical Parameters and SWT Proportions in the NEADW Core

[20] The NEADW core was identified by a conspicuous relative vertical salinity maximum at about 2700 m depth at each of our 113 stations. The highest salinity (34.98) and lowest potential temperature (1.76°C) at the northern end of the study area pointed to the major contribution of Iceland-Scotland Overflow Water (ISOW) to NEADW (Figures 2a and 2b). In fact, the contribution of the ISOW (mean ± sd: 18.4 ± 19.1%) decreased exponentially toward the equator from ~96% at >60°N to 5% in the subtropical North Atlantic (Figure 3c).

[21] A relative salinity minimum of ~34.93 was encountered near the entrance of the Charlie-Gibbs Fracture Zone (CGFZ; 48°–52°N), a major conduit of the LSW originating in the western basin of the Atlantic (Figure 2a). At the CGFZ, the contribution of the LSW (mean ± sd: 40.9 ± 9.3%) peaked with proportions of around 62% and was rather stable toward the south with contributions of ~40% to the NEADW core (Figure 3c).

[22] At the latitudinal range of the Strait of Gibraltar (34.5°–25.5°N), the highest influence of the MW of 5.2% led to an increase in NEADW salinity to 34.96 (Figures 2a and 2b). Overall, the contribution of the MW was low and on average 3.3 ± 0.6% (mean ± sd).

[23] South of 30°N, the influence of LDW (Figure 3d) led to a decrease in the salinity of the NEADW (Figure 2a) and an increase in silicate concentrations (Figure 2c). The LDW contribution (mean ± sd: 37.4 ± 19.9%) increased from 3% in the northern North Atlantic to ~60% near the equatorial Atlantic (Figure 3d).

[24] NO₃ (Figure 4a) and PO₄ concentrations (not shown) ranged from 15.2–22.1 μmol N kg⁻¹ and 1.0–1.6 μmol P kg⁻¹, respectively. Both increased almost linearly southward toward 25°N, where they reached highest and essentially constant concentrations between 25°N and 5°S. Dissolved inorganic nitrogen mainly consisted of NO₃ (>99%), whereas NO₂ did not exhibit any latitudinal trend (data not shown). Coinciding with the increasing influence of LDW, higher silicate concentrations were detected toward the south of our study region, increasing monotonically from 9.6 μmol Si kg⁻¹ at the northern end of the transect to 37.9 μmol Si kg⁻¹ at the equator (Figure 2c).

[25] Similar to the distribution of inorganic nutrients, the AOU increased from 35.9 μmol O₂ kg⁻¹ at the northernmost station to a maximum of 90.1 ± 16.1 μmol O₂ kg⁻¹ from ~25°N southward (Figure 4b). DOC concentrations were in the range of 39–62 μmol C kg⁻¹ (mean ± sd: 48 ± 5 μmol C kg⁻¹) and decreased significantly (p < 0.0001, n = 91) toward the equator (Figure 4c).

[26] Dissolved organic nitrogen (DON) decreased linearly toward the south from 5.1–1.8 μmol N kg⁻¹ (mean ± sd: 3.5 ± 0.8 μmol N kg⁻¹) (Figure 4d). DON comprised between 7 and 30% of the total dissolved N pool with decreasing proportions toward the equator. DOP exhibited a large scatter along the transect ranging from 0.04–0.18 μmol P kg⁻¹ (mean ± sd: 0.10 ± 0.03 μmol P kg⁻¹; n = 72) representing 6.5% of the total dissolved P pool (data not shown).

3.2. Latitudinal Gradients of Prokaryotic Biomass and Heterotrophic Production in the NEADW Core

[27] Prokaryotic biomass (PB) decreased exponentially toward the south from 164 to 10 nmol C L⁻¹ and was rather stable south of 36°N with an average biomass of 19 ± 6 nmol C L⁻¹ (overall mean ± sd: 35 ± 32 nmol C L⁻¹, n = 107; Figure 5a). The fraction of HNA cells decreased by roughly 30% from the north toward the Azores region (~36°N) and
subtropical Atlantic toward 5°S, PHP was variable with an increasing inorganic nutrient concentrations from North to South (Figure 3).

Overall, the contribution of HNA cells to the total prokaryotic abundance was rather stable with an average of 61 ± 7% (n = 107; Figure 5b). Prokaryotic heterotrophic production (PHP) revealed a similar pattern as PB and decreased from the north to 36°N from 1.46 to 0.01 nmol C L⁻¹ d⁻¹. In the subtropical Atlantic toward 5°S, PHP was variable with an average of 0.03 ± 0.01 nmol C L⁻¹ d⁻¹ (Figure 5c). The increasing inorganic nutrient concentrations from North to South coincided with decreasing PB and PHP. Prokaryotic growth rates decreased southward and was 0.020 ± 0.008 d⁻¹ north of 36°N (turnover time: 93 ± 66 d) and 0.002 ± 0.001 d⁻¹ under the subtropical gyre south of 36°N (turnover time: 775 ± 325 d; data not shown).

4. Discussion

4.1. Choice of Model

There are several parametric inverse models that can be used to analyze the mineralization of organic matter in the dark ocean. Most approaches rely on the assumption that mixing occurs strictly on isopycnal surfaces [Takahashi et al., 1985; Hansell and Carlson, 2001; Li and Peng, 2002]. We have used a multiparameter inverse model that accounts for both, diapycnal and isopycnal mixing, as a relatively simple approach to separate the contribution of the different water types that mix to form a water mass. Other than defining the initial WT properties, no further a priori assumptions on the sources and sinks of constituents are needed for the data analysis, and thus, the method lends itself to be extended to biological data. A potential source of bias in the multiparameter inverse model is its sensitivity to the definition of the end-members needed for the mixing analysis. By carefully selecting the end-member properties, the analysis has been shown to compare well to more complex models of ocean carbon cycling [Schneider et al., 2005]. By means of a perturbation test, we have also shown that the variability of θ, S, and SiO₄ in the areas where the four water types that contribute to the NEADW were defined did not substantially affect the results of the model; the average error of the estimation of the WT proportions was generally low and ~1% for the MW and LDW and ~6% for the ISOW and LSW (Figure 3).

### Table 1. Least Squares Regression Results of the Mixing-Model Applied Along the NEADW Core

| Parameter | Unit | LSW | ISOW | LDW | r²c | SDd | n° | p f | Var (%)b |
|-----------|------|-----|------|-----|-----|-----|----|-----|--------|
| AOU       | μmol kg⁻¹ | 15 ± 4 | 26 ± 3 | 118 ± 3 | 0.95 | 4.8 | 113 | < 0.001 | 5       |
| PO₄      | μmol kg⁻¹ | 1.00 ± 0.03 | 0.95 ± 0.02 | 1.64 ± 0.02 | 0.93 | 0.45 | 113 | < 0.001 | 7       |
| NO₃      | μmol kg⁻¹ | 15.1 ± 0.4 | 13.8 ± 0.3 | 24.4 ± 0.3 | 0.95 | 0.49 | 113 | < 0.001 | 5       |
| DON      | μmol kg⁻¹ | 0.14 ± 0.03 | 0.08 ± 0.02 | 0.12 ± 0.03 | 0.18 | 0.04 | 82  | < 0.225 | –       |
| DOC      | μmol kg⁻¹ | 3.8 ± 0.5 | 6.3 ± 0.3 | 2.6 ± 0.3 | 0.63 | 0.5  | 100 | < 0.001 | 37      |
| PAh       | cells mL⁻¹ | 60 ± 4  | 53 ± 3  | 45 ± 3  | 0.30 | 4.0  | 91  | < 0.001 | 70      |
| HNAh     | cells mL⁻¹ | 0.0 ± 0.2 | 2.0 ± 0.1 | 0.3 ± 0.1 | 0.79 | 0.18 | 104 | < 0.001 | 21      |
| PHP      | nmol C L⁻¹ d⁻¹ | 0.7 ± 0.3 | 2.0 ± 0.2 | 0.0 ± 0.2 | 0.64 | 0.31 | 91  | < 0.001 | 36      |

*From equation (1). Values ± SE represent coefficients of chemical and biological parameters (N) of each SWT.

*Abbreviations of parameters and water masses see text.

*Coefficient of determination, indicates the total variability of the parameter accounted for by SWT mixing.

*Standard deviation of the residuals.

*Percentage of the variance accounted for by biogeochemical processes (Var = 1 – r² × 100) for each variable along the NEADW core.

*Prokaryotic abundance × 10⁷.

4.2. Impact of Conservative Mixing Versus Nonconservative Biogeochemical Processes on Dissolved Oxygen and Inorganic Nutrients

Based on the proportions of the four water types forming the NEADW (Figure 3), our model explained 93–95% of the total variability of dissolved oxygen and inorganic nutrient concentrations (see r² in Table 1), suggesting that conservative mixing and basin-scale mineralization are the main factors explaining the variability of these parameters along the NEADW core. Although the variability not explained by equation (1) is only 5–7% (see Var% in Table 1), the standard deviation of the residuals of the mixing model of these variables (see SD in Table 1) is about an order of magnitude larger than the corresponding analytical errors (see section 2). Therefore, the impact of local-scale nonconservative biogeochemical processes is still considerable. The distribution of the residuals allows obtaining further insights into mechanisms causing this variability.

The WT values of AOU (N_i in equation (1)) in Table 1 are significantly larger than 0. Therefore, the mixing model includes the mineralization of these water types from the areas where they were defined to the center of mass of the study site. LSW, the WT that is closest to its formation area, has the lowest AOU_i, while the high AOU_i of LDW indicates that the area where it was defined, in the Vema Channel at 11°N (see Table S1), is furthest away from the study area and basin-scale mineralization has occurred. For the inorganic nutrients, the molar N:P ratio of these water types is 15 ± 1 (Table 1), very similar to the canonical Redfield ratio of 16.

The WT values of the MW are not included here because the average proportion of this WT in the study area is so small (~4%; Figure 3a, see Table S1) that the estimated WT values have unacceptably high errors.

4.3. Impact of Conservative Mixing Versus Nonconservative Biogeochemical Processes on Organic Carbon and Nutrients

For the organic carbon and nutrients, the total variability explained by the mixing model is much lower, ranging from 18–63% (Table 1), indicating a higher influence of local metabolic activity on the variability of dissolved organic
4.4. Impact of Conservative Mixing Versus Nonconservative Biogeochemical Processes on Microbial Activity

Between 64–79% of the total variability in prokaryotic abundance, including HNA cells and PHP, was explained by conservative mixing (Table 1). Therefore, a notably large fraction of the variability, ranging from 21–36%, was explained by the local biological activity (see Var% in Table 1). This might indicate that a resident prokaryotic community exists that is typical for the NEADW, as has been shown by Agogué et al. [2010]. Similar to the nutrient analyses, the fraction of variability in prokaryotic abundance, HNA and PHP that is not accounted for by equation (1), is interpreted as the variability due to nonconservative biogeochemical processes. Analytical and sampling errors might also contribute to this residual variability. However, if the variability would be exclusively due to analytical and sampling errors, then the values of β would not be statistically significant and would produce unrealistic values.

4.5. Variability of Nutrient Ratios

It is commonly assumed that sinking particulate organic matter (POM) fuels heterotrophic activity in the dark ocean [Aristegui et al., 2009] and that the particles are solubilized by prokaryotes before the organic matter is utilized for biomass production, respiration, and energy acquisition [Baltar et al., 2009]. There is evidence that an abundant particle-attached microbial community exists in the deep ocean [Moeseneder et al., 2001; Lauro and Bartlett, 2008] providing substrate for pelagic microbes [Karl et al., 1988] and leading to a preferential removal of N- and P-containing organic compounds [Schneider, 2003]. However, recent studies suggest that surface derived organic matter might not be enough to fuel the metabolism of heterotrophic prokaryotes in the dark ocean [Reinthaler et al., 2006; Baltar et al., 2009].

When using equation (2) and applying $N_1 = \text{AOU}$ and $N_2 = \text{PO}_4$ (or $\text{NO}_3$), then β yields the stoichiometric coefficient of dissolved oxygen consumption to inorganic nitrogen (or phosphorus) production. Considering the values and the standard errors of these β coefficients (Table 2), the modeled $O_2$:N:P ratios independent of the mixing within the NEADW core of $126(\pm11)$:$13.0$ ($\pm0.7$):$1$ are in good agreement with earlier studies from similar depths [Anderson and Sarmiento, 1994]. From the β coefficients, we calculated the contribution of the different biomolecules to the oxygen consumed in the NEADW. Following the approach of Fraga et al. [1998] (see supporting information Materials and Methods), the material mineralized in the NEADW averaged over the transect was composed of $14\%$ of phosphorus compounds, $43\%$ of organisms, and $43\%$ of carbohydrates and lipids. This yields a C:N:P composition of the mineralized biogenic material in the NEADW of $87$–$96$:13:1. This suggests a preferential (but not significant) mineralization of phosphorus compounds when compared to the classical surface Redfield ratio of $106:16:1$.

Applying $N_1 = \text{AOU}$ and $N_2 = \text{DOC}$ to equation (2) and dividing β by the AOU:Corg stoichiometric molar ratio which ranges from 1.31 to 1.44, depending on the possible relative proportions of consumed carbohydrates and lipids (see section 2.7 and supporting information Materials and Methods), yields the contribution of dissolved organic matter to oxygen consumption. The importance of DOM export from the surface ocean has only recently been recognized and is estimated to contribute about $20\%$ of the total organic carbon flux to the global dark ocean [Hansell et al., 2012]. Using a high-resolution DOC data set and a different modeling approach, Carlson et al. [2010] calculated that DOC

| Table 2. Mixing-Biogeochemical Model of Chemical and Biological Parameters Taking the Proportions of the Water Masses into Account$^a$ |
|------------------|------------------|------------------|------------------|------------------|
| $N_1$ $^b$ | $N_2$ $^b$ | Unit | $\beta$ | SD $^c$ | $n$ | $\beta \pm \text{SE}^d$ | p $^e$ |
| AOU | $\text{PO}_4$ | Molar ratio | 0.98 | 3.2 | 113 | $126 \pm 11$ | < 0.0001 |
| AOU | $\text{NO}_3$ | Molar ratio | 0.98 | 2.7 | 113 | $9.7 \pm 0.6$ | < 0.0001 |
| $\text{NO}_3$ | $\text{PO}_4$ | Molar ratio | 0.99 | 0.2 | 113 | $13.0 \pm 0.7$ | < 0.0001 |
| DOC | AOU | Molar ratio | | | | ns |
| DON | AOU | Molar ratio | | | | ns |
| DOP | AOU | Molar ratio | | | | ns |
| PA | $\text{NO}_3$ | cells $\mu$mol$^{-1}$kg$^{-1}$ | 0.80 | 0.17 | 104 | $4.5 \pm 2.0^b$ | 0.0260 |
| PA | $\text{PO}_4$ | cells $\mu$mol$^{-1}$kg$^{-1}$ | 0.78 | 0.17 | 96 | $-0.3 \pm 0.2^b$ | 0.0354 |
| PA | DOP | cells $\mu$mol$^{-1}$kg$^{-1}$ | | | | ns |
| HNA | PA | Ratio | 0.99 | 0.03$^b$ | 104 | $0.82 \pm 0.02$ | < 0.0001 |
| PHP | PB | $d^{-1}$ | 0.70 | 0.29 | 90 | $0.023 \pm 0.005$ | < 0.0001 |

$^a$For relationships of water mass modeled parameters according to equation (1) and the residual parameters according to equation (2), see Figure S3 and S4.

$^b$Chemical and biological parameters; Abbreviations of $N_1$ and $N_2$, see text.

$^c$Adjusted coefficient of determination.

$^d$Standard deviation of the residuals of the model.

$^e$Number of data points.

$^f$Beta coefficients ($\beta$) and standard error (SE) of the multiple regression analysis based on the residuals of chemical and biological parameters ($N_i$) and the proportions of the water masses derived from the mixing model applying equation (2).

$^g$p value indicating significant regression model; ns indicates regression model not significant.

$^h$Number $\times 10^3$. 

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The SD of the estimation performed with the mixing-biogeochemical inverse models obtained with equation (2) (Table 2) is significantly better than the estimations based on the mixing model calculated using equation (1) alone (Table 1). For example, the SD of AOU decreased from 4.8 μmol O₂ kg⁻¹ with the mixing model to 2.7 (or 3.3 μmol O₂ kg⁻¹) with the mixing-biogeochemical model including NO₃ (or PO₄). However, this SD is still larger than the analytical error of the determination of AOU. In fact, an analysis of the distribution of the residuals of equation (2) (i.e., ΔN₂) along the latitude and longitude in the NE Atlantic indicates that the distribution of those residuals is not random (Figure 6). This is an indication that the coupled mixing-biogeochemical model is still insufficient to explain all the observed variability. For N₁ = AOU and N₂ = NO₃ (or PO₄), a biased distribution of Δ(N₁:N₂) shows deviations from the average AOU:N (or N:P) stoichiometric coefficients of 9.7 ± 0.6 (or 13.0 ± 0.7) obtained with equation (2) (Table 2). We interpret these deviations as an indication of changes in the biogeochemical composition of the mineralized materials with latitude and/or longitude. The Δ(AOU:PO₄) ratios (Figure 6a) are negative to up to −5 μmol O₂ mol⁻¹ P in the temperate region between 45°N and 35°N (average ± sd of Δ(AOU:PO₄) = −2.7 ± 1.6 μmol O₂ mol⁻¹ P, n = 11) suggesting the mineralization of fresher POM below the transition zone between the North Atlantic Current and the Azores Current. According to Kahru et al. [1991], higher productivity and fast sinking rates of large-size fresh POM are common in this area. In contrast, the positive Δ(AOU:PO₄) ratios near the Charlie-Gibbs Fracture Zone, between 55°N and 45°N (average ± sd of Δ(AOU:PO₄) = +1.0 ± 2.3 μmol O₂ mol⁻¹ P, n = 20) and particularly under the subtropical gyre, from 35°N to 15°N (average ± sd of Δ(AOU:PO₄) = +4.3 ± 2.4 μmol O₂ mol⁻¹ P, n = 13) point to the mineralization of relatively aged sinking material. This coincides with the inflow of LSW in the CGFZ and the low productivity in the oligotrophic surface waters of the subtropical gyre with its presumed low primary production and small slow-sinking particles into the deep sea.
narrower with predominantly lower values than the average AOU:PO₄ (average ± sd of ∆(AOU:PO₄) = −0.5 ± 2.1 mol O₂ mol⁻¹ P, n = 31), suggesting that lateral transport from the shelf region due to filaments and from the NW African eastern boundary upwelling system introduces fresh POM into the NEADW [Aristegui et al., 2003]. A similar trend is observed in the open ocean south of 15°N, i.e., in the area under the influence of the North Equatorial Current, which also transports fresh material from the African coast (average ± sd of ∆(AOU:PO₄) = −0.5 ± 1.7 mol O₂ mol⁻¹ P, n = 21) and for the residuals of the ∆(NO₃:PO₄) ratios (Figure 6b).

4.6. Prokaryotic Activity in the NEADW

[39] An increasing fraction of high nucleic acid (HNA)-containing prokaryotes from the epipelagic to the bathypelagic ocean has been reported previously [Reinthaler et al., 2006; Gasol et al., 2009]. HNA bacteria have been shown to be more active suggesting that HNA cells are metabolically more versatile than LNA cells [Gasol et al., 1999]. Bouvier et al. [2007] hypothesized that HNA- and LNA-containing bacteria might be phylogenetically different communities. Recently, the notion of distinct HNA- and LNA-containing bacterial groups has been corroborated using 16S rRNA pyrosequencing and fluorescence in situ hybridization (FISH) [Schattenhofer et al., 2011; Vila-Costa et al., 2012]. These surface ocean studies revealed that the HNA fraction is composed of mostly versatile and fast-growing bacteria such as the SAR324 and SAR406 clusters, members of the Deltaproteobacteria and Fibrobacteres. Interestingly, Agogué et al. [2010] found that members of the SAR324 and SAR406 cluster are substantially more abundant in the LSW and NEADW along a similar transect as covered in this study. FISH analysis of the prokaryotic community along a transect in the North Atlantic showed that Archaea contribute up to 20% of the picoplankton in the NEADW and constitute a metabolically active group [Teira et al., 2006; Varela et al., 2007]. With conventional flow cytometric counting using fluorescent labels to stain prokaryotes, however, it is currently not possible to separate the bacterial and archaeal contribution to the total abundance of the dark ocean prokaryotic community.

[40] Several lines of evidence suggest that the fraction of HNA-containing cells are indeed the more active component in the NEADW. First, the variability of the residuals of prokaryotic abundance is essentially explained by the variability of the residuals of HNA cells: The r² of the mixing model of HNA cells increases from 0.76 to 0.99 when prokaryotic abundance is included as explanatory variable in the mixing-biogeochemistry model (compare Table 1 and Table 2), indicating that the changes in abundance occur in the HNA fraction. Concomitantly, the SD of the estimated HNA cells decreases from 0.14 × 10⁵ to 0.03 × 10⁵ cells mL⁻¹. Second, there is a positive relationship between the residuals of PHP and the residuals of PB (Table 2); the r² of the mixing model of PHP increases from 0.64 to 0.70 when PB is included as explanatory variable in the mixing-biogeochemistry model and the SD of estimated PHP decreases from 0.31 nmol C L⁻¹ d⁻¹ in the mixing model to 0.29 nmol C L⁻¹ d⁻¹ in the mixing-biogeochemical model including PB as explanatory variable (compare Table 1 and Table 2). This, in turn, indicates that any increase in PHP that is not explained by mixing must be due to a higher activity of a fraction of the cells found in the NEADW that according to the value of β when N₁ = PHP and N₂ = PB, exhibits a mean growth rate of 0.023 ± 0.005 d⁻¹ or a generation time of 43 ± 10 d (Table 2).

[41] There is uncertainty in our measurements of PHP and PB. Biases of prokaryotic activity due to pressure effects have not yet been unequivocally shown [Tamburini et al., 2013] and the conversion factor between leucine incorporation and cell abundance into units of carbon might be different for the more productive northern North Atlantic and the subtropical gyre region [Alonso-Sáez et al., 2007]. Del Giorgio et al. [2011] conducted a study comparing the relationships between long-term versus short-term incubations of PHP with calculated growth efficiencies. Their data suggest that empirically derived conversion factors from short-term incubations might be underestimates. Furthermore, a conversion factor of ~1.5 kg C mol Leu⁻¹ was required to balance the carbon budget in the Ross Sea of the Southern Ocean [Ducklow et al., 2000]. Thus, there are strong indications that our chosen theoretical conversion factor of 1.5 kg C mol Leu⁻¹ yields reasonable estimates of PHP.

[42] Figure 6c shows that ∆(PHP:PB) is not homogeneously distributed with latitude and longitude: the average ± sd of ∆(PHP:PB) is +0.004 ± 0.009 d⁻¹ (n = 41) below the subpolar-temperate eastern North Atlantic (65°N–35°N), increasing significantly to +0.019 ± 0.007 d⁻¹ (n = 7) below the subtropical gyre (35°N–15°N) and then, decreasing significantly to −0.005 ± 0.008 d⁻¹ (n = 18) within the North Equatorial Current domain south of 15°N. Finally, in the area under the influence of the Canary current eastern boundary upwelling system, values of ∆(PHP:PB) significantly lower than the average PHP:PB were also observed (−0.003 ± 0.007 d⁻¹; n = 24). This indicates that the highly active heterotrophic prokaryotes below the subtropical gyre turn over significantly faster (~24 days) than those below the subpolar-temperate North Atlantic (~37 days). However, the turnover time of heterotrophic prokaryotes below the areas influenced by the upwelling of the Canary Current (~55 days) and the North Equatorial Current (~50 days) is significantly longer than those below the subtropical gyre. Relatively short prokaryotic turnover times below the subtropical gyre region are counter-intuitive. One would expect higher growth rates or shorter turnover times where the prokaryotic substrate is supposedly fresh due to recent water mass formation or below regions where our model indicates vertical input of fresher surface-derived material. Measured PHP and PA clearly show a latitudinal trend with higher growth rates in the northern parts of the NEADW than under the North Atlantic gyre system [Reinthaler et al., 2006]. Whether these relatively high turnover rates of prokaryotes in the NEADW of the subtropical Atlantic are a genuine feature or are caused by changing conversion factors used in the calculations of PB and PHP, require further investigation.

4.7. Concluding Comments

[43] In the NEADW, most of the nutrient concentrations are influenced by the input from water masses above and below the deep North Atlantic salinity maximum indicating that mixing processes are mainly responsible for the observed latitudinal changes in the nutrient concentrations in the NEADW. The mixing-corrected mineralization ratios in the NEADW indicate a major contribution of particulate organic
matter serving as substrate for the heterotrophic prokaryotes, while the maximum contribution of dissolved organic carbon to the oxygen consumption is about 20%. In contrast to the inorganic and organic matter, biological activity behaves differently and the relatively high activity in the NEADW is less influenced by mixing than the inorganic nutrient concentrations. [44] Mixing and mineralization processes from the WT formation sites explained 64% of the variability in PHP. Considering our reported turnover times ranging from 100 to 800 days suggest that dark ocean microbial activity is representative for relatively long-time periods. Thus, the area of WT origin dictate the contrasting initial values of PB and PHP. Conversely, 36% of the variability of PHP was associated with local mineralization of sinking POM which is the substrate for an active fraction of the prokaryotic community with a turnover of just 43 ± 10 days. Thus, we were able to show that the local-scale variability of dissolved oxygen and nutrients is mainly led by microbial mineralization of sinking particles and, consequently, that both the local nutrient mineralization and local microbial activity can occur at comparable time scales. [45] The latitudinal variability of the nutrient ratios and the prokaryotic turnover suggests a dependence on the biogeochemical province crossed by the NEADW core. Hence, to decipher the mineralization and the prokaryotic activity in the dark ocean, the mixing of water masses and the surface processes in the biogeochemical provinces need to be taken into account.

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