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Major contribution of autotrophy to microbial carbon cycling in the deep North Atlantic's interior

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ABSTRACT

Current estimates point to a mismatch of particulate organic carbon supply derived from the surface ocean and the microbial organic carbon demand in the meso- and bathypelagic realm. Based on recent findings that chemoautotrophic Crenarchaeota are abundant in the mesopelagic zone, we quantified dissolved inorganic carbon (DIC) fixation in the meso- and bathypelagic North Atlantic and compared it with heterotrophic microbial activity. Measuring ^{14}C-bicarbonate fixation and ^{3}H-leucine incorporation revealed that microbial DIC fixation is substantial in the mesopelagic water masses, ranging from 0.1 to 56.7 μmol C m^{-3} d^{-1}, and is within the same order of magnitude as heterotrophic microbial activity. Integrated over the dark ocean's water column, DIC fixation ranged from 1–2.5 mmol C m^{-2} d^{-1}, indicating that chemoautotrophy in the dark ocean represents a significant source of autochthonously produced 'new organic carbon' in the ocean's interior amounting to about 15–53% of the phytoplankton export production. Hence, chemoautotrophic DIC fixation in the oxygenated meso- and bathypelagic water column of the North Atlantic might substantially contribute to the organic carbon demand of the deep-water microbial food web.

1. Introduction

The dark ocean, i.e. below ca. 200 m depth, comprises about 75% of the global ocean's volume and contains more than 98% of the global dissolved inorganic carbon (DIC) pool (Gruber et al., 2004). While geochemical measurements provide major insights into the general ocean carbon cycle, our mechanistic understanding of the dark ocean's carbon cycle and the role of the microbial communities in the transformation of carbon remains rudimentary (Arístegui et al., 2009).

The dark ocean harbors around 65% of all pelagic Bacteria and Archaea (Whitman et al., 1998) and a large fraction of the global ocean's remineralization of organic matter occurs below 200 m depth (Del Giorgio and Duarte, 2002). The generally rapid attenuation with depth of sedimenting particulate organic carbon (POC) (Martin et al., 1987) resulting in low POC inputs, and the constant background of refractory DOC resisting microbial oxidation (Barber, 1968), however, led to the widespread view that microbes in the dark ocean are extremely slow growing or dormant. This view is contradicted by several recent studies showing that microbes in the dark ocean are overall metabolically active (Herndl et al., 2005; Teira et al., 2006, Reinthaler et al., 2006b). Genomic studies on deep-sea microbial communities identified novel genes and metabolic pathways that make it possible for some microbes to thrive as chemoautotrophs on inorganic substrates (Berg et al., 2007; Hallam et al., 2006).

Microbial growth in the interior of the ocean is considered to be mainly supported by the organic matter exported from the euphotic zone, with most of the exported particulate and dissolved organic matter remineralized in the mesopelagic zone (Del Giorgio and Duarte, 2002). While the notion that the microbial carbon demand exceeds the particulate organic carbon flux into the dark ocean is not new (Ducklow, 1993; Karl et al., 1988; Simon et al., 1992), recently direct evidence has been presented by comparing biogeochemical carbon flux estimates with microbial activity measurements (Baltar et al., 2009; Reinthaler et al., 2006b).

Chemoautotrophy by microbes is common in hypoxic and anoxic environments such as the redox transition zones in the Cariaco Basin, the Black Sea and parts of the central Baltic Sea where uptake of DIC is supported by reduced end-products of anaerobic decomposition (Jost et al., 2008; Karl and Knauer, 1991; Taylor et al., 2001). However, in the oxygenated water column, DIC uptake by the heterotrophic bacterioplankton is generally attributed to anaplerotic reactions in the tricarboxylic acid cycle

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(TCA) (Dijkhuizen and Harder, 1995). Measurements of CO_2 fixation by heterotrophic bacterioplankton are scarce; however, reports suggest that about 1–8% of the biomass production in heterotrophic Bacteria is derived from anaplerotic reactions (Romanenko, 1964; Sorokin, 1993). The extent of the anaplerotic reactions is largely determined by the availability of labile organic carbon whereas more complex organic carbon sources, such as those dominating the deep-ocean dissolved organic matter (DOM) pool, decrease the need for replenishing reactions in the TCA cycle (Doronia and Trotsenko, 1985). In the dark ocean, organic carbon availability is generally limiting heterotrophic bacterial activity. Hence, anaplerotic reactions likely play only a minor role in deep-sea Bacteria metabolism, although clearly more research needs to be done on this aspect.

While commonly the microbial community of the oxygenated water column of the dark ocean is considered to be heterotrophic, as shown for Archaea and Bacteria taking up amino acids (Ouverney and Fuhrman, 2000; Teira et al., 2006), DI^{14}C fixation might be more common than hitherto assumed, particularly for the abundant Crenarchaeota (Herndl et al., 2005; Hansman et al., 2009). Evidence of a major autotrophic crenarchaeal community in the deep ocean is also provided by the isotopic composition of archaeal lipids from a 600 m deep station in the Pacific indicating that DIC fixation accounts for about 80% of the archaeal carbon uptake (Ingalls et al., 2006).

We determined microbial DIC fixation rates and microbial heterotrophic production in the oxygenated meso- and bathypelagic realm of the North Atlantic along a transect from 65°N to 5°S following the eastern branch of the North Atlantic Deep Water (NADW) and additionally, following the western branch of the NADW from 65°N to 35°N. In this regional analysis, we show that dark ocean DIC fixation is substantially more important in the deep-ocean carbon cycle than hitherto appreciated and not restricted to sub- or anoxic regions of the global ocean.

2. Methods

2.1. Sampling

Seawater was sampled from well-defined water masses. A calibrated Seabird SBE9/11+ CTD was used to measure conductivity, temperature, pressure and oxygen. The CTD was fitted with 16 bottles each of 12 dm^3 volume. The water masses were identified based on their salinity and temperature characteristics during the downcast. The factory calibration of the conductivity and the oxygen sensor was checked for stability with appropriate reference samples measured on board. Salinity standards and samples were measured with a Guildline 8400B salinometer, the oxygen sensor (SBE 43) was compared to Winkler measurements (Reinthaler et al., 2006a). Both instruments were operated in a temperature-controlled container at 20 ± 1 °C. Prior to initial sampling, the Niskin bottles were soaked with 10% bleach and rinsed by deploying them to 1000 m depth.

2.2. Sampled water masses and conducted cruises

Generally, samples were taken from the main water masses encountered during 4 cruises and from the subsurface layer (SSL; ~100 m depth) (Fig. 1). During TRANSAT-1 (Sep 2002) in the eastern North Atlantic (62°N to 35°N), samples were taken from the Labrador Seawater (LSW), the Iceland Scotland Overflow Water (ISOW) and the Northeast Atlantic Deep Water (NEADW). The subtropical North Atlantic was sampled during ARCHIMEDES-1 (Nov 2005) and ARCHIMEDES-2 (Nov 2006) and covered a transect from 34°N to 5°S in the eastern North Atlantic basin. The main water masses sampled here were the North Atlantic Central Water (NACW), Central Water (CW), the South Atlantic Central Water (SACW), the Antarctic Intermediate Water (AAIW), the Mediterranean Sea Outflow Water (MSOW), the Subarctic Intermediate Water (SAIW), the Northeast Atlantic Deep Water (NEADW), and the Lower Deep Water (LDW). TRANSAT-2 (May 2003) was conducted in the western North Atlantic basin from 65°N to 37°N. The main water masses on this transect were the Labrador Seawater (LSW), the North Atlantic Deep Water (NADW), and Denmark Strait Overflow Water (DSOW). In total 132 stations were occupied (Fig. 1).

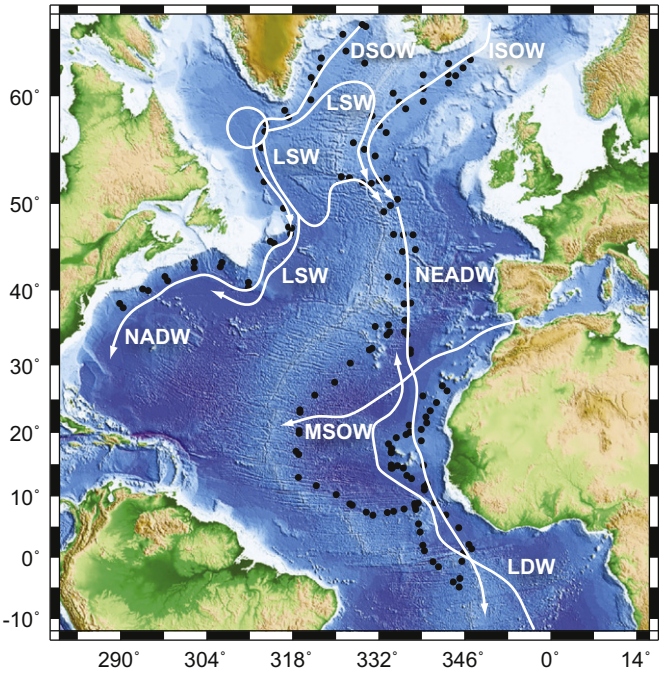

Fig. 1. Map of the occupied stations and flow of the main water masses in the North Atlantic. A total of 662 samples were collected (black dots) during 4 cruises conducted with R/V Pelagia. The flow of the major deep-water masses contributing to the meridional overturning circulation in the North Atlantic are indicated (white lines). Abbreviations of water masses: Iceland Scotland Overflow Water (ISOW), Denmark Strait Overflow Water (DSOW), Labrador Sea Water (LSW), Northeast Atlantic Deep Water (NEADW), North Atlantic Deep Water (NADW), Mediterranean Sea Outflow Water (MSOW), Lower Deep Water (LDW).

2.3. Flow pattern of the main deep-water masses

The flow of the main deep-water masses in the northern North Atlantic is shown in Fig. 1, and the basic physical characteristics of the water masses sampled during this study are summarized in Table 1. The ISOW originates from the Nordic seas, flows across the sill through the Faroe Bank Channel and to the south in the eastern North Atlantic basin. The LSW is formed in the Labrador Sea via deep convection to depths of up to 2000 m and is characterized by a relatively low salinity of ~34.9. A branch of the LSW flows northeast and enters the eastern North Atlantic via the Irminger- and the Iceland basin. A southeast flowing branch of LSW enters the eastern North Atlantic basin via the Charlie Gibbs Fracture Zone at ~52°N carrying with it the typical low-salinity core (Talley and McCartney 1982). The NEADW, characterized by a deep-water salinity maximum (>34.9), is formed mainly by entrainment of ISOW and LSW in the eastern basin south of the Charlie Gibbs Fracture Zone (van Aken, 2000) from where it spreads over the eastern North Atlantic basin.

Table 1
Physical characteristics of the water masses in the eastern, subtropical and western North Atlantic.

Location	Wmass[a]	Depth (m)	Range (m)	Temp (°C)	Range (°C)	Salinity	Range	Oxygen (μmol kg^{-1})	Range (μmol kg^{-1})
Eastern	SSL	136	90–150	10.80	6.2–15.7	35.44	34.8–36.2	250.02	213.7–279.8
	O_2-min	722	350–1030	6.91	4.3–10.5	35.13	34.9–35.5	219.71	181.4–266.1
	LSW	1828	1160–2130	3.42	3.1–4.0	34.91	34.9–35.0	283.49	259.6–291.6
	ISOW	2329	810–3100	2.77	1.9–3.5	34.98	35.0–35.0	286.90	276.4–295.7
	NEADW	2839	2540–3300	2.98	2.8–3.1	34.95	34.9–35.0	268.59	249.1–281.8
	LDW	3783	3240–4620	2.66	2.5–2.8	34.93	34.9–34.9	257.56	243.8–281.0
Subtropical	NACW	271	100–510	16.64	11.3–24.1	36.28	35.5–37.4	168.85	92.4–221.5
	CW	174	100–250	14.37	11.8–16.9	35.45	35.2–35.7	112.84	104.4–121.3
	SACW	263	100–500	12.35	6.9–19.7	35.25	34.6–36.4	111.50	92.3–171.2
	O_2-min	344	100–750	11.43	6.6–17.2	35.25	34.8–36.0	70.75	42.5–90.8
	AAIW	861	750–900	5.32	4.4–6.5	34.64	34.5–34.9	128.26	102.4–155.3
	SAIW	871	750–900	6.96	6.5–8.3	34.97	34.9–35.2	111.87	96.2–127.9
	MSOW	938	900–1100	8.28	7.6–9.0	35.35	35.1–35.6	164.02	121.2–197.7
	LSW	2401	2400–2400	3.18	3.2–3.2	34.95	35.0–35.0	256.48	256.5–256.5
	NEADW	2696	1750–5000	3.07	1.9–4.1	34.94	34.8–35.0	236.14	212.2–250.8
	LDW	4039	3400–4990	2.41	2.2–2.6	34.90	34.9–34.9	236.35	223.7–245.1
Western	SSL	100	90–110	8.62	3.5–21.3	35.17	34.6–36.7	260.27	161.1–307.0
	O_2-min	402	180–740	7.75	4.0–10.3	35.06	34.8–35.3	189.20	141.9–274.1
	LSW	1352	710–2130	3.44	3.1–4.1	34.89	34.9–35.0	279.47	257.7–290.6
	NADW	2537	1980–3260	2.97	2.5–3.2	34.92	34.9–34.9	272.04	262.3–282.3
	DSOW	3011	1220–3870	1.90	0.8–2.4	34.89	34.8–34.9	288.02	267.3–309.2

[a] Only samples of clearly identified water masses are included. Numbers represent averages and ranges based on bottle samples. Abbreviations of water masses: Subsurface layer (SSL), O_2-min (Oxygen minimum zone), Labrador Sea Water (LSW), Iceland Scotland Overflow Water (ISOW), North East Atlantic Deep Water (NEADW), Lower Deep Water (LDW), North Atlantic Central Water (NACW), Central Water (CW), South Atlantic Central Water (SACW), Antarctic Intermediate Water (AAIW), Subarctic Intermediate Water (SAIW), Mediterranean Sea Outflow Water (MSOW), Denmark Strait Overflow Water (DSOW).

The NEADW was identified over the entire eastern transect at 2800 m depth with an average salinity of ~34.9 and a temperature of ~3 °C. However, south of the Azores (<36.5°N) the eastern North Atlantic basin is also increasingly influenced by other intermediate and deep water masses originating in the northern subtropical Atlantic or coming from the southern hemisphere. The LDW, characterized by low salinity (34.9) and temperature (2.2–2.8 °C) was sampled at 3900 m depth between 6°N and 32°N but actually may be found up to the Charlie Gibbs Fracture Zone. Two intermediate water masses were found in the subtropical North Atlantic. In the gyre region, the MSOW at 1000 m depth is recognizable by its high salinity (~35.35) and relatively high oxygen concentration, whereas in the region of the North Equatorial Counter Current below 20°N AAIW was present, characterized by lower salinity and temperature than the MSOW.

The DSOW in the western North Atlantic basin is the freshest and densest component of the NADW (Smethie et al., 2000) with a typical temperature of ~2 °C and a salinity >34.8. DSOW originates from north of the sill of the Greenland-Iceland ridge and was found in the entire western transect. The NADW in the western basin, typically found between 2000–3300 m depth, is a result of the entrainment of southward flowing LSW and DSOW and was again identifiable by its salinity maximum (34.90–34.95) and temperature (~3 °C) from ~60°N southwards.

2.4. Microbial heterotrophic production and DIC fixation measurements

Immediately after the recovery of the CTD rosette, samples for microbial heterotrophic production and DIC fixation measurements were collected from the Niskin bottles. Processing of the samples, from collecting water from the Niskin bottles to incubating the samples with the radiolabeled tracers in temperature-controlled incubators, took less than 15 min.

Microbial heterotrophic production was measured by incubating 50 cm^3 of seawater in triplicate with 10 nM [^{3}H]-leucine (final concentration, specific activity 5.809×10^6 MBq mmol^{-1}, Amersham) in the dark at in situ temperature (±1 °C) for 4–7 h. Triplicate formaldehyde-killed blanks were treated in the same way as the samples. Incubations were terminated by adding formaldehyde (2% final concentration) to the samples. Samples and blanks were filtered through 0.2-μm polycarbonate filters (Millipore, 25 mm filter diameter) supported by cellulose acetate filters (Millipore, HAWP, 0.45-μm pore size). Subsequently, the filters were rinsed with 5% ice-cold trichloroacetic acid, dried, 8 cm^3 of scintillation cocktail (FilterCount, Canberra-Packard) added, and after about 18 h counted on board in a liquid scintillation counter (LKB Wallac). The instrument was calibrated with internal and external standards. The blank-corrected leucine incorporation rates were converted into microbial carbon production using the theoretical conversion of 1.55 kg mol^{-1} leucine incorporated (Kirchman, 1993; Simon and Azam, 1989).

DIC fixation was measured via the incorporation of ^{14}C-bicarbonate (3.7×10^6 Bq, Amersham) in 50 cm^3 seawater samples. Triplicate samples and formaldehyde-fixed blanks were incubated in the dark at in situ temperature for 60–72 h. Incubations were terminated by adding formaldehyde (2% final concentration) to the samples, filtered onto 0.2-μm polycarbonate filters and rinsed with 10 cm^3 of ultra-filtered seawater (<30 kDa). Subsequently, the filters were fumed with concentrated HCl for 12 h. The filters were then processed as described above and counted in the scintillation counter for 10 min. The resulting mean disintegrations per minute (DPM) of the samples were corrected for the mean DPM of the blanks and converted into organic carbon fixed over time and corrected for the natural DIC concentration as measured by continuous-flow analysis (Stoll et al., 2001).

2.5. Quality control of DIC fixation measurements

We checked the potential contamination of the ^{14}C-bicarbonate with organic ^{14}C in lab experiments. 22.2×10^6 Bq of ^{14}C bicarbonate

were acidified and bubbled with clean air for 2 h to remove the inorganic bicarbonate. Subsequently, the pH was raised again to a pH ~8.2. Seawater was pre-filtered through a 0.8-μm polycarbonate filter and 20 cm^3 were transferred to sterile conical tubes. The sparged radiolabeled tracer was added to triplicate samples and blanks and mixed. The blanks were fixed with formaldehyde (2% final concentration) prior to adding the tracer. Live samples and blanks were incubated at room temperature in the dark for 3 h. Thereafter, the samples were filtered and fumed over concentrated HCl for 12 h. The filters were dried, placed in scintillation vials, and 8 cm^3 of scintillation cocktail (FilterCount, Canberra-Packard) was added. After 18 h the samples were counted in a liquid scintillation counter (LKB Wallac).

3. Results and discussion

3.1. Methodological considerations

We tested a batch of the ^{14}C-bicarbonate for potential contamination with organic ^{14}C. The live incubations (31 ± 8 DPM, n=3) and the formaldehyde-killed controls (23 ± 3 DPM, n=3) in these lab experiments were not significantly different (Mann-Whitney-U test, $P<0.2$) and were in the same range as the average blank measurements during the cruises (36 ± 22 DPM, n=343). In contrast to an earlier report on the impurity of commercially available ^{14}C-bicarbonate solutions (Williams et al., 1972), this suggests that our DIC fixation rates are not biased due to organic ^{14}C-labeled impurities of the added ^{14}C-bicarbonate. Due to the variability in the DPM of live incubations and blanks of ship-board measurements, the analytical error of the method is ± 5%.

Different incubation times had to be used for microbial heterotrophic activity and dark DIC fixation to obtain reliable results. While tritiated leucine with its high specific activity required only about 4–7 h of incubation time, ^{14}C-bicarbonate amended samples required 60–72 h of incubation due to the high isotope dilution with the natural DIC. However, these different incubation times most likely do not pose a problem as it has been shown that the leucine incorporation rates as well as the oxygen consumption in these deep waters is linear over a period of at least 90 h (Reinthaler et al., 2006b).

There are uncertainties in the conversion factors used to convert the microbial leucine uptake into carbon biomass. Empirical conversions factors depend on the method used to calculate the incorporated carbon from cell growth and leucine uptake (Ducklow et al., 1992; Kirchman, 1992). However, averaged conversion factors from different oceanic regions are generally in the range of 0.1–3 kg C mol^{-1} leucine (Alonso-Saez et al., 2007; Ducklow et al., 2002; Bjørnsen and Kuparinen, 1991). Conversion factors for dark-ocean heterotrophic microbes are not available (Nagata et al., 2010), but assuming that conversion factors are generally low in oligotrophic and cold subantarctic regions, we used a conservative theoretical conversion factor of 1.55 kg C mol^{-1} leucine. Moreover, the theoretical conversion factor assumes a fixed ratio of leucine to protein, which has been shown to be rather stable over a wide range of environments (Simon and Azam, 1989). Regardless of the conversion factor applied, the pattern of leucine incorporation rate with depth remains unaffected, indicating a decrease by 2–3 orders of magnitude from the subsurface layer to the bathypelagic North Atlantic (Fig. 2 and Table 3).

It is not known whether microbes taking up DIC are exclusively chemoautotrophs, thus we assumed that simultaneous uptake of leucine is low and would not affect our conclusions. All our microbial production measurements were done under atmospheric pressure conditions and hence might be biased. At the moment, it is unclear whether decompression of deep-sea microbes leads to an over- or underestimation of the *in situ* activity, as contradictory results have been reported (Jannasch and Wirsen, 1982; Tamburini et al., 2003).

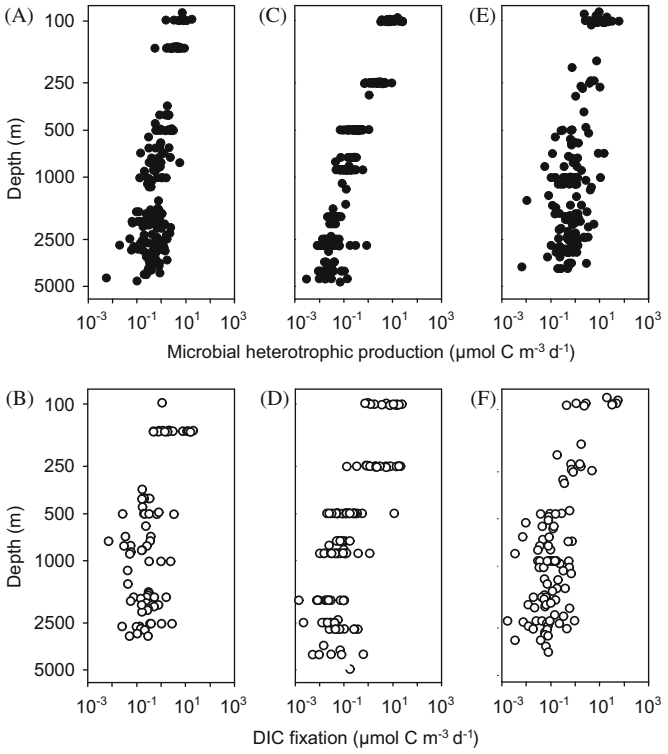

Fig. 2. Microbial heterotrophic production (filled circles) and dark DIC fixation (open circles) in (A, B) the eastern North Atlantic, (C, D) the subtropical North Atlantic and (E, F) the western North Atlantic basin.

3.2. Spatial patterns of DIC fixation and heterotrophic production

On average, dark DIC fixation rates decreased from 3.33 μmol C m^{-3} d^{-1} at 100 m depth to 0.08 μmol C m^{-3} d^{-1} at 3000 m depth. In comparison, microbial heterotrophic production decreased exponentially with depth from 7.45 μmol C m^{-3} d^{-1} at 100 m depth to 0.07 μmol C m^{-3} d^{-1} at 3000 m depth (Table 2). The variability of DIC fixation and microbial heterotrophic production at the different depths was higher in the north than in the subtropical North Atlantic (Fig. 2 and Table 3) and much of the variability in DIC fixation was found in the upper mesopelagic waters, particularly in the oxygen minimum zone. While relationships with environmental parameters (salinity, temperature, oxygen, inorganic nutrients and TOC) were weak (data not shown), the generally large variability in the dataset suggests that the dark ocean is not a homogeneous environment and 'hotspots' of microbial activity occur throughout the water column. Over the 8000-km-long eastern transect, microbial heterotrophic production decreased in the bathypelagic realm by 85% (from ~0.7 to 0.1 μmol C m^{-3} d^{-1}) from the northern stations (~60°N to 40°N) to the subtropical region (~20°N to 5°S) (Fig. 3A). The overall north–south pattern for DIC fixation was less pronounced, although a core of very low DIC fixation in the bathypelagic of the subtropical Atlantic was apparent (Fig. 3A). Along the western North Atlantic transect, relatively high rates of DIC fixation and microbial heterotrophic production were measured in the bathypelagic realm associated with LSW representing a young water mass (Reinthaler et al., 2006b) (Fig. 3B).

3.3. Relationship of dark ocean DIC fixation and microbial heterotrophic production

The mean ratios of DIC fixation : microbial heterotrophic production ranged from 0.2 to 4.4 in the defined water masses of the eastern basin of the North Atlantic, and most of the water masses exhibited a higher DIC fixation than microbial heterotrophic production (Fig. 4A, B). In contrast, in the western basin of the North Atlantic, DIC fixation was higher than microbial heterotrophic production only in the subsurface waters as indicated by the DIC fixation : heterotrophic production ratio >1 (Fig. 4C). In the deep water masses of the western North Atlantic basin the ratio ranged from 0.2 to 0.4 (Fig. 4C).

The latitudinal decline in microbial heterotrophic production in the NEADW from 53°N to 5°S might be the combined effect of the decreasing bioavailability of dissolved organic carbon (DOC) as the NEADW ages by about 20 years from its main source of origin in the Greenland-Iceland-Norwegian Sea to the equator as indicated by chlorofluorocarbon inventories (Fine et al., 2002) and a decreasing supply of export primary production (Antia et al., 2001). The concurrent decline in DIC fixation might be related to the decreasing availability of reduced inorganic compounds used as an energy source such as ammonia, recently identified as an energy source for at least some *Crenarchaeota* (Könneke et al., 2005; Wuchter et al., 2006). Microbial heterotrophic production was 2.2 ± 1.0 times higher in the western Atlantic transect than in the northeastern basin, possibly due to the proximity of the shelf in the south and the young Labrador Sea Water in the north. This resulted in an overall DIC fixation : microbial heterotrophic production ratio of <1 (Fig. 4A, C). Calculating the grand average of DIC fixation in the North Atlantic suggests that, overall, DIC fixation in the meso- and bathypelagic realm is about 1.3 ± 0.2 (mean $\pm$ s.e., n=226) times higher than microbial heterotrophic production. Depending on the leucine to carbon conversion factor used to calculate microbial heterotrophic production, the DIC fixation : microbial heterotrophic production ratio may vary between 0.7 and 20. Despite this variation in the ratio, our data suggest regional differences in the balance of chemoautotrophic and heterotrophic processes in the dark open ocean. However, if the assumption of generally low conversion factors in the dark ocean is correct (Nagata et al., 2010) than, DIC fixation should exceed microbial heterotrophic production in most parts of the eastern North Atlantic.

Table 2
Reduced major axis regression models describing depth-dependence of microbial metabolism using $\log_{10}$ transformed microbial heterotrophic production (MHP) and DIC fixation (DIC_f) in the three North Atlantic regions.

Location	Type	Slope	Error[a]	Intercept	Error[a]	R^2	*N*
Eastern	MHP	−1.05	0.05	3.02	0.15	0.56	70
Subtropical	MHP	−1.78	0.03	4.49	0.10	0.90	302
Western	MHP	−1.18	0.07	3.52	0.19	0.52	158
North Atlantic[b]	MHP	−1.56	0.04	4.25	0.12	0.56	530
Eastern	DICf	−1.29	0.13	3.35	0.37	0.33	78
Subtropical	DICf	−1.86	0.14	4.70	0.38	0.51	131
Western	DICf	−1.59	0.12	4.05	0.35	0.57	80
North Atlantic[b]	DICf	−1.60	0.08	4.10	0.22	0.47	289

MHP and DICf in $\mu mol\ C\ m^{-3}\ d^{-1}$. Depth in regression models is in meters. *N* - number of data points used in the regressions.

[a] Error of the slope and the intercept, respectively.
[b] Model based on the combined data from all three regions.

3.4. Importance of DIC fixation in the dark ocean

Current estimates of net phytoplankton production center around 40 and 20 $mmol\ C\ m^{-2}\ d^{-1}$ for the mesotrophic and the oligotrophic open North Atlantic, respectively (Behrenfeld and Falkowski, 1997). Generally however, the phytoplankton production exported into the mesopelagic layer amounts to less than 30% of the net phytoplankton production in the euphotic layer (Antia et al., 2001; Lutz et al., 2002). To compare DIC fixation in the dark ocean to export primary production, we integrated dark DIC

Table 3
Daily rates of microbial heterotrophic production (MHP) and DIC fixation (DIC_f) in the main water masses of the eastern, subtropical and western North Atlantic.

Location	Wmass[a]	Depth (m)	MHP[b] ($\mu mol\ C\ m^{-3}\ d^{-1}$)	s.e.	*N*	DIC_f ($\mu mol\ C\ m^{-3}\ d^{-1}$)	s.e.	*N*
Eastern	SSL	136	4.87	0.48	39	5.28	1.63	16
	O2-min	722	0.97	0.11	37	0.39	0.12	20
	LSW	1828	0.44	0.05	37	0.31	0.05	16
	ISOW	2329	1.12	0.27	21	0.72	0.45	3
	NEADW	2839	0.50	0.08	24	0.54	0.30	9
	LDW	3783	0.51	0.10	17			
Subtropical	NACW	271	5.76	1.21	34	9.57	3.81	12
	CW	174	3.89	2.30	2	0.54	0.20	2
	SACW	263	4.48	0.82	37	5.36	1.41	23
	O2-min	344	2.80	0.49	56	2.71	0.77	26
	AAIW	861	0.18	0.02	27	0.22	0.09	11
	SAIW	871	0.17	0.02	10	0.14	0.05	4
	MSOW	938	0.09	0.02	7	0.12		1
	NEADW	2696	0.04	0.00	82	0.18	0.04	15
	LDW	4039	0.03	0.01	23	0.21	0.14	4
Western	SSL	100	12.26	1.86	33	21.99	7.30	9
	O2-min	402	3.53	1.23	12	0.70	0.18	10
	LSW	1352	1.36	0.32	33	0.19	0.04	19
	NADW	2537	0.88	0.17	23	0.24	0.09	10
	DSOW	3011	0.97	0.24	18	0.21	0.09	7

N – number of measurements.
s.e. – standard error.

[a] For details of water mass (Wmass) see Section 2.3 and Table 1.
[b] MHP was calculated using 1.55 $kg\ C\ mol^{-1}$ leucine and DIC_f represents ^{14}C-bicarbonate uptake corrected for the ambient DIC concentration.

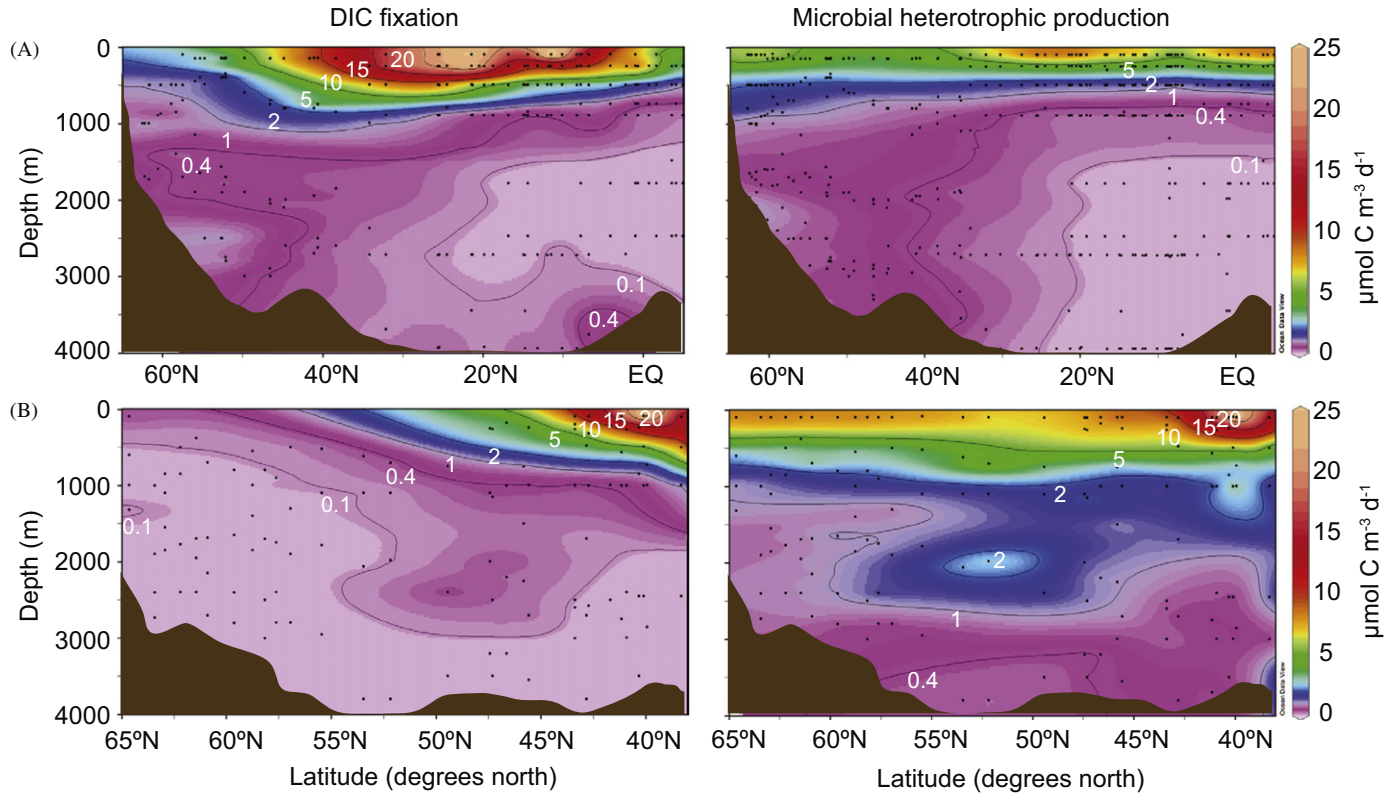

Fig. 3. Microbial DIC fixation (DIC_f) and heterotrophic production (MHP) in the North Atlantic. Contour plots of DIC_f and MHP in the eastern North Atlantic basin from 65°N to 5°S (**A**), and the western North Atlantic basin from 65°N to 38°N (**B**). Dots indicate the latitude and depths where samples were collected. Black contour lines and related numbers indicate rates in µmol C m^{-3} d^{-1}. The data were plotted with the VG gridding algorithm in Ocean Data View (Schlitzer, 2007).

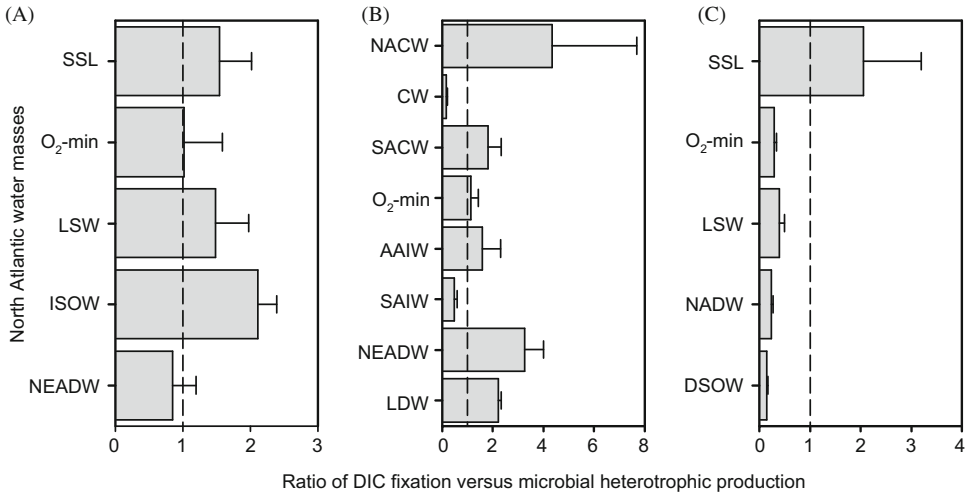

Fig. 4. Ratio of DIC fixation versus microbial heterotrophic production (MHP) in (A) the eastern North Atlantic, (B) the subtropical North Atlantic and (C) the western North Atlantic basin. Broken lines indicate a ratio of DIC_f : MHP of 1. The error bars indicate the standard deviation. See Fig. 1 for water mass abbreviations.

fixation from 125 to 3000 m depth of 80 complete profiles. Depth-integrated DIC fixation rates in the North Atlantic deep waters range from 1.8 to 3.2 mmol C m^{-2} d^{-1} corresponding to 15–53% of export phytoplankton production (Table 4).

We used the regression models from Table 2 to derive depth-integrated DIC fixation rates for the mesopelagic (integration of data between 125–1000 m) and bathypelagic (integration of data between 1000–3000 m) of the different trophic regions. Comparing these data to the depth-integrated particulate organic carbon loss derived from a recent model based on sediment trap data (Antia et al., 2001) suggests that our DIC fixation rates amount to ~7% of POC loss in the mesopelagic layer and between 8–36% of POC loss in the bathypelagic layer of the mesotrophic northern North Atlantic. Due to the lower surface productivity in the oligotrophic subtropical Atlantic, DIC fixation in the meso- and bathypelagic realms each account for ~28% of the loss with depth of phytoplankton-derived POC in this region (Table 5). Other than the allochthonously derived organic carbon undergoing substantial reworking during its descent in the ocean's interior, DIC fixation of chemoautotrophic microbes

represents a newly produced, non-sinking source of organic carbon for the deep ocean.

The energy sources for chemoautotrophic microbes in the oxygenated ocean's interior remain enigmatic. Based on the abundance of the archaeal *amoA* gene, a subunit of one of the key enzymes in ammonia oxidation, recent studies suggest that mesophilic Crenarchaea play a major role in ammonia oxidation in the pelagic ocean (Francis et al., 2005). The distribution of the archaeal *amoA* and the crenarchaeal 16S rRNA gene in the meso- and bathypelagic water masses of the North Atlantic suggests, however, that probably not all Crenarchaeota are chemoautotrophic nitrifiers but some use organic carbon sources as well (Agogué et al., 2008; Varela et al., 2008). Furthermore, single-cell analysis using catalyzed reporter deposition fluorescence in situ hybridization combined with microautoradiography showed that mainly Bacteria assimilate DIC in the bathypelagic realm of the subtropical North Atlantic, while DIC fixation by Crenarchaeota is restricted to the mesopelagic layer (Varela et al., in prep.)

Possible energy sources other than ammonia in the oxygenated dark ocean include molecular hydrogen (H_2) or nitrous oxide (N_2O), potentially abundant gases in the dark ocean albeit with unknown supply rates. The few available measurements of molecular hydrogen in the ocean's interior indicate very low concentrations of H_2 ranging from 0.04–0.2 μmol H_2 m^{-3} (Conrad and Seiler, 1988). Nitrous oxide concentrations in the deep North Atlantic are slightly higher (~10 μmol N_2O m^{-3}) (Walter et al., 2006); however, its energy yield to drive chemoautotrophic processes is low. Regardless the nature of the energy source used by chemoautotrophic microbes in the ocean's interior, our dark DIC fixation rates obtained for the North Atlantic suggest that DIC fixation represents a hitherto unrecognized sink of inorganic carbon and a source of non-sinking autochthonously produced organic matter.

Table 4
Depth integrated dark ocean DIC fixation compared to export primary production in the North Atlantic. DIC fixation (DIC_f; mmol C m^{-2} d^{-1}) was integrated over complete profiles in the range of 125 to 3000 m for the mesotrophic northern and the oligotrophic subtropical North Atlantic.

	Mesotrophic		Oligotrophic	
	DIC_f	%NPP[a]	DIC_f	%NPP[b]
Mean	1.84	15.3	3.20	53.3
s.e.	0.42	3.5	0.81	13.5
N	51		29	

[a] Integrated dark ocean DIC fixation as percentage of export production, assuming 30% export from the euphotic zone (Antia et al., 2001; Lutz et al., 2002) using 40 mmol C m^{-2} d^{-1} surface phytoplankton net primary production (NPP) (Behrenfeld and Falkowski, 1997).

[b] Integrated DIC fixation as percentage of export production, assuming 30% export from the euphotic zone using 20 mmol C m^{-2} d^{-1} surface NPP. s.e. – standard error of the mean; *N* – number of complete profiles.

4. Summary and conclusions

Dark-ocean DIC fixation amounts to about 15–53% of the phytoplankton-derived POC entering the dark ocean, assuming 30% of export production leaving the euphotic zone. DIC fixation (125–3000 m) ranges from 1 mmol C m^{-2} d^{-1}, using the model based on our data (Table 2), and 2.5 mmol C m^{-2} d^{-1} taking into account the local variability (Table 4). Assuming that this autotrophy is fuelled exclusively by ammonia as an energy source, our measured inorganic carbon fixation of 1–2.5 mmol C m^{-2} d^{-1} would result in the oxidation of 10–25 mmol NH_3 m^{-2} d^{-1}, according to the nitrification stoichiometry where one carbon atom is fixed for ~10 nitrogen molecules oxidized. This ammonia has to be provided by the sinking PON flux, or by excretion by zooplankton. The grand average of export POC flux in the North Atlantic entering the mesopelagic ocean is 5.2 mmol C m^{-2} d^{-1} (Antia et al., 2001) or about 0.5 mmol N m^{-2} d^{-1} assuming a sinking POC/PN ratio of around 10 (Trull et al., 2008). Although this is only a rough estimate on the potential supply of PON and, after ammonification, of ammonia potentially available, it indicates that the supply of ammonia as an energy source, based on sediment trap data, is 20–50 times lower than needed to explain the measured deep-water autotrophy. Other pathways to supply ammonia in the deep ocean might be excretion by diel vertically migrating zooplankton which is equivalent to 8–45% of the PON flux leaving the euphotic zone (mean values for a variety of environments; see Table 4 in Steinberg et al., 2002). There might be also energy sources other than ammonia available to fuel DIC fixation in the dark ocean. The concentrations and formation rates of H_2 and N_2O are most likely not sufficient to provide these additional energy sources to cover our measured DIC fixation rates. Hence, additional energy sources are required that have yet to be identified to meet the energy requirements of the dark ocean's DIC fixation, as Burd et al. (2010) have described for energy sources required to meet the organic carbon demand in the dark ocean.

Table 5
DIC fixation compared to loss of sinking particulate organic carbon (POC) with depth in the dark ocean of the North Atlantic. The depth-integrated loss of POC was derived from the model of Antia et al. (2001), which is based on a regional analysis of sediment trap data from the North Atlantic between 33°N and 54°N. The algorithm derives the particulate organic carbon flux from surface net primary production (NPP, mmol C m^{-2} d^{-1}) and depth (*z*, *m*) and three constants:
POC – flux (mmol C m^{-2} d^{-1}) = 0.3122 $NPP^{1.77}$ $z^{-0.68}$.
As productivity input we assumed an average NPP of 40 mmol C m^{-2} d^{-1} in the mesotrophic region (eastern and western North Atlantic) and 20 mmol C m^{-2} d^{-1} in the oligotrophic region (subtropical North Atlantic) (Behrenfeld and Falkowski, 1997). Depth-integrated DIC fixation (DIC_f) rates for the meso- and bathypelagic of the different trophic regions in the North Atlantic were calculated from the intercepts and slopes given in Table 2. The resulting rates represent regional estimates of DIC fixation in the dark ocean of the North Atlantic because station-to-station variability is smoothed by the fit.

	Trophic Region	Meso[a] (mmol C m^{-2} d^{-1})	Bathy[b] (mmol C m^{-2} d^{-1})	Meso	Bathy
				DIC_f as % of POC flux	
POC flux	Mesotrophic	6.07	1.78		
	Oligotrophic	1.03	0.30		
DIC_f	Mesotrophic (Eastern)	0.39	0.37	6.5	35.8
	Mesotrophic (Western)	0.42	0.13	6.9	8.4
	Oligotrophic (Subtropical)	0.47	0.09	26.5	28.7

[a] Mesopelagic data integrated data from 125–1000 m depth.
[b] Bathypelagic data integrated data from 1000–3000 m depth.

Unlike phytoplankton-derived POC, this freshly produced organic carbon is a non-sinking source of dark-ocean organic carbon production not captured by sediment traps, as free-living microbes are too small to sink (Buesseler et al., 2007). Taken together, our results suggest that DIC fixation is an important microbial process in the dark ocean representing a fresh carbon source potentially fueling the meso- and bathypelagic organic carbon demand of the heterotrophic microbial communities. For accurate rate measurements of dark-ocean microbial activity it will be necessary to firmly establish the conversion factors and consider potential pressure effects; however, the major open question to be resolved now is the nature of the energy source fueling dark-ocean chemoautotrophy. It has been shown in the past that the identification of functional genes in microbes paves the way to assess the metabolic biogeography of microbes in the ocean. In this respect, the analysis of metagenomic datasets from dark-ocean prokaryotic communities seems a good starting point to reveal novel metabolic pathways capable of using hitherto unconsidered energy sources.

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