Supplementary methods.

Environmental parameters

Inorganic nutrients — The methods for determining inorganic nutrient concentrations followed Joint Global Ocean Fluxes Study recommendations (Gordon *et al.* 1993). The concentrations of dissolved inorganic nutrients (NH_4^+ , NO_3^- , NO_2^- , PO_4^{3-}) were determined in a TRAACS autoanalyzer immediately after collecting the samples and gentle filtration through 0.2 µm filters (Acrodisc, Gelman Science). NH_4^+ was detected with the indophenol blue method (pH 10.5) at 630 nm. NO_2^- was detected after diazotation with sulfanilamide and *N*-(1-naphtyl)-ethylene diammonium dichloride as the reddishpurple dye complex at 540 nm. NO_3^- was reduced in a copper cadmium coil to nitrite (with imidazole as a buffer) and then measured as nitrite. Inorganic PO_4^{3-} was determined via the molybdenum blue complex at 880 nm.

Dissolved organic carbon (DOC) — Samples for DOC were filtered through rinsed 0.2 μ m polycarbonate filters and sealed in precombusted (450°C for 4 h) glass ampoules after adding 50 μ L of 40% phosphoric acid. The samples were stored frozen at -20°C until analysis back in the lab. DOC concentrations of duplicate samples were determined using a Shimadzu TOC-5000 analyzer. Three-point standard curves, prepared with potassium hydrogen phthalate (Nacalai Tesque, Kyoto, Japan), were used to calculate DOC concentrations. The instrument's performance and the validity of the calibration were determined using reference material of the Hansell CRM program (44–46 μ mol L⁻¹ for the reference samples; n = 3 and 1–2 μ mol L⁻¹ for low carbon water; n = 3). The average analytical precision of the instrument was < 3%.

Dissolved organic nitrogen (DON) — Total dissolved nitrogen (TDN) was analyzed with a TRAACS 800 continuous-flow analysis system following the persulfate oxidation method. DON concentrations were calculated by subtracting the sum of the inorganic nitrogen species from

TDN concentrations. The recovery of DON was estimated on a mixture of 10 different organic compounds containing known concentrations of N (Kramer *et al.* 2005). For this reference material, the recovery efficiency of organic nitrogen was 92% of the calculated organic nitrogen concentration.

Bacterial-related parameters

Prokaryotic abundance determined by flow cytometry — Prokaryotic plankton collected from the different depth layers of the water column were enumerated using flow cytometry. Samples (2 ml) were fixed with 1% paraformaldehyde (final concentration), shock-frozen in liquid nitrogen for 5 min and stored at -80°C (Kamiya *et al.* 2007). Picoplankton cells were stained with SYBR-Green I and enumerated with a FACSCalibur flow cytometer (BectonDickinson) within 2 months. Immediately before analysis, the thawed picoplankton samples were stained with SYBRGreen I at room temperature in the dark for 15 min. Fluorescent microspheres (Molecular Probes) with a diameter of 1 mm were added to all samples as an internal standard. Counts were performed with an argon laser at 488 nm wavelength. Prokaryotic cells were enumerated according to their right angle scatter and green fluorescence. The counting window of the flow cytometer was set to exclude eukaryotic picoplankton.

Prokaryotic heterotrophic production by $[{}^{3}H]$ *leucine incorporation* — Bulk PHP was measured by incubating triplicate 10–40 ml samples and formaldehyde-killed blanks (2% final concentration) with 10 nM $[{}^{3}H]$ -leucine (final concentration, specific activity 160 Ci mmol⁻¹; Amersham) in temperature-controlled incubators in the dark at *in situ* temperature for 4–10 h (Kirchman *et al.* 1985). Incubations were terminated by adding formaldehyde (2% final concentration) before filtering the samples and the blanks through 0.2 µm polycarbonate filters (25 mm filter diameter; Millipore). Subsequently, the filters were rinsed three times with 5% icecold trichloroacetic acid, dried and placed in scintillation vials. Scintillation cocktail (8 ml Canberra-Packard Filter Count) was added, and after 18 h, counted in a liquid scintillation counter (LKB Wallac model 1212). The mean disintegrations per minute (DPM) of the formaldehyde-fixed blanks were subtracted from the mean DPM of the respective samples, and the resulting DPM converted into leucine incorporation rates. Prokaryotic carbon biomass production was estimated using a conservative theoretical conversion factor of 1.55 kg C mol⁻¹ Leu assuming no internal isotope dilution (Kirchman & Ducklow 1993).

Activity of the ETS — Electron transport system (ETS) activity was measured following the modifications of the tetrazolium reduction technique as described earlier (Aristegui & Montero 1995). Some minor modifications of the method were made to increase its sensitivity (Baltar *et al.* 2009).

Measurements of prokaryotic extracellular enzymatic activity — The hydrolysis of the fluorogenic substrate analogues 4-methylcoumarinyl-7-amide (MCA)-L-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferyl (MUF)-a-D-glucoside, 4-MUF-b-D-glucoside and MUF-phosphate was measured to estimate potential activity rates of alpha-, beta-glucosidase, aminopeptidase and alkaline phosphatase, respectively (Baltar *et al.* 2009; Hoppe 1983).

The environmental data used in statistical analysis can be found at: <u>http://icomm.mbl.edu/microbis</u>.

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Table S1. Description of the 45 samples from the North Atlantic Ocean selected for bacterial pyrosequencing. LDW: Lower Deep Water, NEADW: North East Atlantic Deep Water, AAIW: Antarctic Intermediate Water, tCW/SACW: transitional/South Atlantic Central Water, NIW: Northern Intermediate Water, LSW: Labrador Sea Water).

Sample ID	Cruise	Station	Longitude °E	Latitude °N	Depth (m)	Water mass
53R	Transat-1	15	-29.13	58.3	1400	LSW
55R	Transat-1	15	-29.13	58.3	500	NIW
112R	Transat-1	27	-25	50.4	4000	LDW
115R	Transat-1	27	-25	50.4	500	NIW
137	Transat-2	27	-38.52	60.9	1700	LSW
138	Transat-2	27	-38.52	60.9	700	LSW
S1	Archimedes-2	11	-20.21	11.98	4000	LDW
S2	Archimedes-2	11	-20.21	11.98	2750	NEADW
S3	Archimedes-2	11	-20.21	11.98	900	AAIW
S4	Archimedes-2	11	-20.21	11.98	500	tCW
S5	Archimedes-2	11	-20.21	11.98	250	tCW
S6	Archimedes-2	11	-20.21	11.98	100	Subsurface
S 7	Archimedes-2	19	-15.19	4.9	4500	LDW
S8	Archimedes-2	19	-15.19	4.9	4000	LDW
S9	Archimedes-2	19	-15.19	4.9	2750	NEADW
S10	Archimedes-2	19	-15.19	4.9	900	AAIW
S11	Archimedes-2	19	-15.19	4.9	500	SACW
S12	Archimedes-2	19	-15.19	4.9	250	SACW
S13	Archimedes-2	19	-15.19	4.9	100	Subsurface
S14	Archimedes-2	25	-13.24	0.81	4500	LDW
S15	Archimedes-2	25	-13.24	0.81	3500	NEADW
S16	Archimedes-2	25	-13.24	0.81	3000	NEADW
S17	Archimedes-2	25	-13.24	0.81	2000	NEADW
S18	Archimedes-2	25	-13.24	0.81	1800	NEADW
S19	Archimedes-2	25	-13.24	0.81	900	AAIW
S20	Archimedes-2	25	-13.24	0.81	750	AAIW
S21	Archimedes-2	25	-13.24	0.81	500	SACW
S23	Archimedes-2	25	-13.24	0.81	100	Subsurface
S24	Archimedes-2	30	-13.24	0.81	2500	NEADW
S26	Archimedes-2	30	-15.91	-4.09	750	AAIW
S27	Archimedes-2	30	-15.91	-4.09	500	tCW
S29	Archimedes-2	30	-15.91	-4.09	100	Subsurface
S30	Archimedes-2	37	-22.05	7.78	2500	NEADW
S31	Archimedes-2	37	-22.05	7.78	1800	NEADW
S32	Archimedes-2	37	-22.05	7.78	750	AAIW
S33	Archimedes-2	37	-22.05	7.78	500	tCW
S36	Archimedes-2	45	-26.0	16.83	2500	NEADW
S38	Archimedes-2	45	-26.0	16.83	900	AAIW
S39	Archimedes-2	45	-26.0	16.83	500	tCW
S40	Archimedes-2	45	-26.0	16.83	250	tCW
S41	Archimedes-2	45	-26.0	16.83	100	Subsurface
S42	Archimedes-2	50	-23.11	21.09	2500	NEADW
S43	Archimedes-2	50	-23.11	21.09	1800	NEADW
S44	Archimedes-2	50	-23.11	21.09	900	AAIW
S47	Archimedes-2	50	-23.11	21.09	100	Subsurface

Table S2. Water mass properties (depth, mean and range of potential temperature and salinity) of the different water type of the North Atlantic (tCW/SACW: transitional/South Atlantic Central Water, AAIW: Antarctic Intermediate Water, NIW: Northern Intermediate Water, LSW: Labrador Sea Water, NEADW: North East Atlantic Deep water, LDW: Lower Deep Water) and number of samples (n).

Water mass	Depth (m)	Pot .Temp.* (°C)	Range (°C)	Salinity	Range	n
Subsurface	100 - 150	16.7	14.2 - 20.6	35.97	35.45 - 37.06	6
tCW/SACW	250 - 500	9.9	7.4 – 13.2	35.05	34.66 - 35.62	9
NIW	500 - 750	7.1	7.0 - 7.1	35.09	35.05 - 35.12	2
AAIW	750 - 900	5.6	4.6 - 6.7	34.70	34.50 - 34.99	8
LSW	1200 - 2100	3.3	3.0 - 3.5	34.89	34.87 - 34.92	3
NEADW	1750 - 4000	3.0	2.2 - 4.0	34.95	34.90 - 35.03	12
LDW	4000 - 5000	1.9	1.5 – 2.3	34.88	34.84 - 34.92	5

* Pot. Temp.: potential temperature

Table S3. ANOSIM test between water masses (tCW/SACW: transitional/South Atlantic Central Water, AAIW: Antarctic Intermediate Water, NIW: Northern Intermediate Water, LSW: Labrador Sea Water, NEADW: North East Atlantic Deep water, LDW: Lower Deep Water) based on the relative abundance of all OTUs (97%) except the singletons.

Pairwise tests between water masses	R-value	Significance level	Actual permutations
Subsurface, AAIW	0.444	0.007	999
Subsurface, tCW/SACW	0.228	0.034	999
Subsurface, NEADW	0.722	0.002	999
Subsurface, LDW	0.744	0.006	462
Subsurface, LSW	0.772	0.012	84
Subsurface, NIW	0.51	0.036	56
AAIW, tCW/SACW	0.115	0.062 (n.s.*)	999
AAIW, NEADW	0.428	0.001	999
AAIW, LDW	0.796	0.002	999
AAIW, LSW	0.874	0.006	165
AAIW, NIW	0.716	0.022	45
tCW/SACW, NEADW	0.759	0.001	999
tCW/SACW, LDW	0.878	0.001	999
tCW/SACW, LSW	0.937	0.005	220
tCW/SACW, NIW	0.709	0.018	55
NEADW, LDW	0.744	0.006	462
NEADW, LSW	0.89	0.002	455
NEADW, NIW	0.937	0.011	91
LDW, LSW	0.508	0.036	56
LDW, NIW	0.545	0.048	21
LSW, NIW	1	0.1 (n.s.*)	10

Global R = 0.597; Significance level = 0.001; Number of permutations: 999

*n.s.: non-significant

Table S4. Characteristics of the 100 most abundant OTUs (# of OTUs; ID, identification
number; percent of contribution to the 100 most abundant OTUs; affiliation)

# 0TU	Cluster ID	% to the 100 first	Affiliation
010		OTUss	
1	Alphaproteobacteria_03_1	29,72	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SAR11
2	Alphaproteobacteria_03_2	7,87	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SAR11
3	ProteobacteriaNA_03_1	7,06	Bacteria; Proteobacteria
4	Gammaproteobacteria_03_8	5,85	Bacteria;Proteobacteria;Gammaproteobacteria
5	Alphaproteobacteria_03_21	3,08	Bacteria; Proteobacteria; Alphaproteobacteria
6	Gammaproteobacteria_03_29	2,93	Bacteria;Proteobacteria;Gammaproteobacteria
7	Gammaproteobacteria_03_47	2,88	Bacteria;Proteobacteria;Gammaproteobacteria
8	Gammaproteobacteria_03_44	2,69	Bacteria;Proteobacteria;Gammaproteobacteria
9	ProteobacteriaNA_03_6	1,52	Bacteria;Proteobacteria
10	Actinobacteria_03_4	1,54	Bacteria;Actinobacteria;Actinobacteria
11	ProteobacteriaNA_03_8	1,52	Bacteria;Proteobacteria
12	Alphaproteobacteria_03_53	1,46	Bacteria;Proteobacteria;Alphaproteobacteria
13	Alphaproteobacteria_03_26	1,41	Bacteria;Proteobacteria;Alphaproteobacteria
14	Verrucomicrobia_03_3	1,33	Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales
15	Gammaproteobacteria_03_52	1,11	Bacteria;Proteobacteria;Gammaproteobacteria
16	Alphaproteobacteria_03_258	1,03	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales
17	BacteriaNA_03_2	0,89	Bacteria
18	Alphaproteobacteria_03_20	0,81	Bacteria;Proteobacteria;Alphaproteobacteria
19	Deltaproteobacteria_03_2	0,75	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales; Nitrospinaceae; Nitrospina
20	Verrucomicrobia_03_6	0,73	Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales
21	Betaproteobacteria_03_1	0,87	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Ralstonia
22	Gammaproteobacteria_03_10	6 0,72	Bacteria;Proteobacteria;Gammaproteobacteria
23	Betaproteobacteria_03_11	0,74	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Diaphorobacter
24	ProteobacteriaNA_03_14	0,64	Bacteria;Proteobacteria
25	BacteriaNA_03_15	0,62	Bacteria
26	Alphaproteobacteria_03_17	0,60	Bacteria;Proteobacteria;Alphaproteobacteria
27	Gammaproteobacteria_03_78	0,57	Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales
28	Deferribacteres_03_6	0,61	Bacteria;Deferribacteres
29	ProteobacteriaNA_03_12	0,53	Bacteria;Proteobacteria
30	BacteriaNA_03_6	0,49	Bacteria
31	Alphaproteobacteria_03_94	0,45	Bacteria; Proteobacteria; Alphaproteobacteria
32	Alphaproteobacteria_03_/1	0,44	Bacteria; Proteobacteria; Alphaproteobacteria
33	Alphaproteobacteria_03_261	0,40	Bacteria; Proteobacteria; Alphaproteobacteria; Knizobiales
34	Gammaproteobacteria_03_12	8 0,41	Bacteria; Proteobacteria; Gammaproteobacteria
35	Gammaproteobacteria_03_/	0,41	Bacteria; Proteobacteria; Gammaproteobacteria; Alteromonadales; Pseudoalteromonadaceae; Pseudoalteromona
36	Alphaproteobacteria_03_2/	0,45	Bacteria; Proteobacteria; Alphaproteobacteria; Spningomonadales; Spningomonadaceae
3/	Gammaproteobacteria_03_11	5 0,39 0,20	Bacteria; Proteobacteria; Gammaproteobacteria
38 20	Alphaproteobacteria_05_50	0,39	Bacteria, Proteobacteria, Alphaproteobacteria
39 40	DacteriaNA_05_10	0,39	Bacteria
40	DacterialNA_05_14	0,58	Dacteria Protochastoria: Commence constante
41	Gammaproteobacteria_03_19	0,45	Bacteria, Proteobacteria, Gammaproteobacteria
42	Deformible to a citeria_05_40	0,38	Bacteria, Proteobacteria, Galilliapioteobacteria
43	BacteriaNA 03 40	0,37	Bacteria
44	Alphaproteobacteria 03 116	0,50	Bactaria: Protachactaria: Alphanrotachactaria
40 46	Verrucomicrobia 03 17	0,30	Bacteria: Vertucomicrohia: Vertucomicrohiae: Vertucomicrohiales: Vertucomicrohiaceae
-10 ∆7	Alphaproteobacteria 03 150	0,35	Bacteria, venuconneroba, venuconnerobac, venuconnerobales, venuconnerobaceae
48	Alphaproteobacteria 03 75	0.33	Bacteria: Proteobacteria: Alphaproteobacteria
49	Cyanobacteria 03 1	0.32	Bacteria:Cvanobacteria:True Cvanobacteria:Prochlorales
		0,02	

50 Gammaproteobacteria_03_130 0,31 Bacteria; Proteobacteria; Gammaproteobacteria 0,30 51 ProteobacteriaNA_03_7 Bacteria; Proteobacteria 52 Gammaproteobacteria_03_270 0,30 Bacteria; Proteobacteria; Gammaproteobacteria 53 Alphaproteobacteria_03_169 0,29 Bacteria; Proteobacteria; Alphaproteobacteria Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales; Ectothiorhodospiraceae 54 Gammaproteobacteria_03_9 0,28 55 0,28 Bacteria:Proteobacteria ProteobacteriaNA_03_22 56 Deferribacteres_03_11 0,27 Bacteria; Deferribacteres 57 0,28 Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae Betaproteobacteria_03_3 58 BacteriaNA_03_18 0,26 Bacteria 59 Verrucomicrobia_03_45 0,25 Bacteria; Verrucomicrobia; Opitutae; Opitutales; Opitutaceae; Opitutus 60 BacteriaNA_03_4 0,24 Bacteria 61 Alphaproteobacteria_03_79 0,24 Bacteria; Proteobacteria; Alphaproteobacteria 0,23 62 Alphaproteobacteria_03_52 Bacteria; Proteobacteria; Alphaproteobacteria 63 Verrucomicrobia_03_29 0,23 Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales 0,23 64 BacteriaNA_03_41 Bacteria 65 0,24 Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Sphingobium Alphaproteobacteria_03_18 0,21 66 Gammaproteobacteria_03_206 Bacteria; Proteobacteria; Gammaproteobacteria 67 Gammaproteobacteria_03_25 0.21 Bacteria; Proteobacteria; Gammaproteobacteria Alphaproteobacteria_03_14 0,21 68 Bacteria; Proteobacteria; Alphaproteobacteria 69 BacteriaNA_03_35 0,21 Bacteria 70 0,20 Alphaproteobacteria_03_242 Bacteria; Proteobacteria; Alphaproteobacteria 71 Alphaproteobacteria_03_155 0,20 Bacteria; Proteobacteria; Alphaproteobacteria 72 ProteobacteriaNA_03_25 0,20 Bacteria; Proteobacteria 73 Gemmatimonadetes 03 3 0.18 Bacteria:Gemmatimonadetes 74 0,19 Bacteria; Proteobacteria; Gammaproteobacteria; Alteromonadales; Alteromonadaceae; Alteromonas Gammaproteobacteria_03_15 75 Deltaproteobacteria_03_106 0,19 Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales; Nitrospinaceae; Nitrospina 0,19 76 Deferribacteres_03_7 Bacteria; Deferribacteres 0,18 Bacteria; Proteobacteria; Gammaproteobacteria 77 Gammaproteobacteria_03_83 78 Deferribacteres_03_4 0,18 Bacteria; Deferribacteres 79 0,17 Verrucomicrobia_03_28 Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales 0,18 80 Betaproteobacteria_03_9 Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae 0,17 81 Deltaproteobacteria 03 122 Bacteria:Proteobacteria:Deltaproteobacteria 0,17 82 Bacteria; Proteobacteria; Alphaproteobacteria Alphaproteobacteria_03_32 83 Alphaproteobacteria_03_329 0,17 Bacteria; Proteobacteria; Alphaproteobacteria 84 Gemmatimonadetes_03_1 0,17 Bacteria;Gemmatimonadetes 85 0,17 Gammaproteobacteria_03_46 Bacteria; Proteobacteria; Gammaproteobacteria 86 Actinobacteria_03_32 0,15 Bacteria; Actinobacteria; Actinobacteria 87 0,16 Alphaproteobacteria 03 264 Bacteria; Proteobacteria: Alphaproteobacteria 88 0,16 Bacteria; Verrucomicrobia; Opitutae; Opitutales; Opitutaceae; Opitutus Verrucomicrobia_03_19 89 0,14 Gammaproteobacteria_03_198 Bacteria; Proteobacteria; Gammaproteobacteria 90 0,15 Alphaproteobacteria_03_195 Bacteria; Proteobacteria; Alphaproteobacteria 91 0,15 Bacteria;Proteobacteria;Deltaproteobacteria Deltaproteobacteria_03_74 92 0,15 Bacteria BacteriaNA_03_38 93 ProteobacteriaNA_03_51 0,14 Bacteria;Proteobacteria 0,15 94 Bacteroidetes_03_18 Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales 95 0,13 Bacteria; Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae Planctomycetes_03_6 Alphaproteobacteria_03_280 0,12 Bacteria; Proteobacteria; Alpha proteobacteria; Rickettsiales; SAR1196 97 BacteriaNA_03_21 0,13 Bacteria 98 BacteriaNA_03_75 0,14 Bacteria 99 Actinobacteria_03_37 0,13 Bacteria; Actinobacteria; Actinobacteria 100 Gammaproteobacteria_03_32 0.12 Bacteria; Proteobacteria; Gammaproteobacteria



Figure S1. Temperature – salinity diagram based on all the samples. Different symbols indicate the different water masses (tCW/SACW: transitional/South Atlantic Central Water, AAIW: Antarctic Intermediate Water, LSW: Labrador Sea Water, NEADW: North East Atlantic Deep Water, LDW: Lower Deep Water).







Figure S2 B. Rarefaction curves for mesopelagic samples.





Figure S2 C. Rarefaction curves for bathypelagic samples.



Figure S3. Evenness (Gini index) for each bacterial OTUs (97%) except the singletons from (A) subsurface, (B) mesopelagic and (C) bathypelagic samples vs. the rank (based on the relative abundance) of that OTU. A Gini index of 1 would indicate a very uneven OTU whereas a Gini index of 0 would indicate a perfectly even OTU.



Figure S4. Non-metric multidimensional analysis based on relative abundance of (A) all pyrotags except the singletons, (B) abundant pyrotags (frequency > 1% within a sample) and (C) rare pyrotags (frequency < 0.1% within a sample). Individual samples were affiliated to their respective water-mass. Superimposed circles represent clusters of samples at similarity values of (A) 30 and 40%, (B) 60 and 80% and (C) 20 and 30% (Bray-Curtis similarity). The final solution was based on 25 iterations with a final stress of (a) 0.10 and (b) 0.13.



Figure S5. Percent of similarity (calculated through SIMPER analysis) between depth zones.