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„Abundance and diversity of nitrate assimilating bacteria in the deep waters of the Atlantic Ocean“

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Einleitung

Das marine Ökosystem ist ein heterogener Lebensraum, der von verschiedensten physikalischen, chemischen und biologischen Faktoren gekennzeichnet ist und eine dementsprechend vielfältige Organismenwelt aufweist. Prokaryoten machen durch ihre Allgegenwärtigkeit und Häufigkeit den Großteil der marinen Biomasse aus. Abundanzen von 10^5 bis 10^6 Zellen/mL in der Wassersäule des Kontinentalschelfs und den oberen 200 m des offenen Ozeans und 5 x 10^4 Zellen/mL im tiefen Ozean (<200 m) wurden berichtet (Whitman et al., 1998). Prokaryoten spielen daher auch eine wichtige Rolle in den biogeochemischen Zyklen von Kohlenstoff, Sauerstoff, Stickstoff, Phosphor und Kalzium etc. (Lavelle *et al*., 2005). Der Stickstoffkreislauf ist besonders interessant, da die Verfügbarkeit von Sickstoff (N) Ökosystemfunktionen wie Primärproduktion und Dekomposition beeinflussen kann (Zehr und Kudela, 2011). Wegen seiner Vielfalt an chemischen Formen und den dazugehörigen Umwandlungen, die durch mikrobielle metabolische Prozesse durchgeführt werden, ist der N-Kreislauf so komplex wie kein anderer.

Stickstoff kommt in Oxidationsstufen von -3 bis +5 vor und kann im Energiehaushalt als Elektronen-Akzeptor und -Donor verwendet werden (Zehr und Kudela, 2011). In Oberflächengewässern sind gasförmiger Stickstoff und gelöster organischer Stickstoff (dissolved organic nitrogen, DON) neben Nitrat und Ammonium die häufigsten N Formen (Zehr und Kudela, 2011). Diese werden durch biologische Aktivitäten verfügbar gemacht. Durch N_2 Fixierung wird inertes atmosphärisches N-Gas in biologisch verfügbare Verbindungen, wie Ammonium, umgeformt (Zehr und Paerl, 2008). Nur wenige Mikroorganismengruppen können atmosphärisches N_2 fixieren, während die meisten Mikroorganismen N in anderen Formen, wie Nitrat, Nitrit, Harnstoff, Ammonium oder organischen N, aufnehmen (Zehr und Paerl, 2008). In gelöstem organischem Material kommt N meistens als Amin- oder Amid-Gruppen in Aminosäuren und Harnstoff vor. Diese sind labile Stoffe, die von Mikroorganismen leicht aufgenommen und schnell regeneriert werden

(Baker *et al*., 2009), wobei das erste für Organismen verfügbare Aufschlussprodukt Ammonium ist. Die meisten Mikroorganismen verwenden Ammonium als primäre N Quelle, weil es energetisch günstiger ist als Nitrat und daher auch oft nur in geringen oder kaum messbaren Konzentrationen vorhanden ist (Zehr und Kudela, 2011).

Ammonium wird von „Ammonia Oxidizing Bacteria“ (AOB) und „Ammonia Oxidizing Archaea“ (AOA) bei der Nitrifikation als Energiequelle verwendet, indem Ammonium zu Nitrit oxidiert wird (Wuchter *et al*., 2006; Mincer *et al*., 2007). Ammonium-Monooxygenase (*amo*) Gene von Crenarchaeota sind die dominanten Stickstoff-oxidierenden Gene im Ozean (Venter *et al*. 2004). *Amo* Gene sind daher in vielen marinen Lebensräumen sehr verbreitet (Francis *et al*. 2005), einschließlich der photischen Zone (Church *et al*. 2010). Im Gegensatz zu AOA können AOB nur in der aphotischen Zone nitrifizieren, da nitrifizierende Bakterien von Licht inhibiert werden (Zehr und Kudela, 2011). Wegen der biologischen Oxidation von Ammonium zu Nitrit und nachfolgend zu Nitrat nimmt die Nitrat-Konzentrationen mit der Tiefe zu (Sarmiento und Gruber, 2006).

Denitrifikation, der Prozess bei dem Nitrat zu molekularem N umgewandelt wird, kann nur in sauerstoffarmen Regionen, den „oxygen minimum zones“ (OMZ) oder Sedimenten passieren. Nitrat kann wegen seiner hohen Oxidationszahl als Ersatz für Sauerstoff verwendet werden, um organisches Material zu oxidieren (Zehr und Kudela, 2011). Dabei wird es zu molekularem N reduziert und verlässt letztendlich das marine System. Ein weiterer biologischer Prozess bei dem N vom marinen System verloren geht ist die „anaerobic ammonia oxidation“ (Anammox), die ebenfalls in sauerstoffarmen Regionen passiert. Im Anammox-Prozess wird Ammonium zusammen mit Nitrit, direkt zu Stickstoff Gas umgeformt (Zehr und Kudela, 2011). Denitrifikation und Anammox sind biologische Prozesse die dem Ozean N entziehen. Eine weiter Form der Nitrat-Reduktion ist die direkte Umwandlung von Nitrat zu Ammonium, ein Prozess der dissimilatorisch oder assimilatorisch

sein kann (Gardner *et al*., 2006; Allen *et al*., 2001). In beiden Fällen wird Nitrat zu Ammonium reduziert und bleibt dem marinen System erhalten (An und Gardner, 2002). Viele Enzyme des N Kreislaufes wurden bereits identifiziert und die Gene, die diese kodieren werden verwendet, um die mikrobiellen Lebensgemeinschaften die diese Prozesse ausführen zu quantifizieren und phylogenetisch zu charakterisieren.

Diese Studie fokussiert auf Nitrat als anorganischen Nährstoff und dessen Assimilation durch heterotrophe Bakterien in der Tiefsee. Dass Nitrat für marines Phytoplankton ein wichtiger anorganischer Nährstoff ist, ist schon lange bekannt (Zehr und Kudela, 2011), doch erst kürzlich wurde gezeigt, dass auch heterotrophe marine Bakterien Nitrat zum Wachstum und der Synthese von Biomasse verwenden können (Kirchman, 2000; Allen *et al*., 2001). Um einen besseren Einblick in den N-Kreislauf und das N-Budget des Atlantiks zu bekommen, analysierten wir Häufigkeit und Diversität des *nas*A Genes in der Wassersäule des westlichen Atlantik von 64°N bis 50°S von der Oberfläche bis zum Bathypelagial. Das *nas*A Gen kodiert das Enzym Nitrat-Reduktase, verantwortlich für die assimilatorische Nitratreduktion.

Introduction

The marine ecosystem is a heterogeneous environment characterized by contrasting physical, chemical and biological factors, and is inhabited by a diverse set of organisms highly adapted to certain conditions. Prokaryotes account for the largest part of marine biomass due to their ubiquitous occurrence and average cellular abundances of 10^5 to 10^6 cells/mL in the continental shelf and the upper 200 m of the open ocean and 5 x 10^4 cells/mL in the deep ocean (<200 m) (Whitman *et al*., 1998). Due to the high abundance and metabolic diversity, prokaryotes facilitate the biogeochemical cycling of carbon, oxygen, nitrogen,

phosphorus and calcium etc. (Lavelle *et al*., 2005). Nitrogen (N) is of particular importance as its availability can affect the rate of key ecosystem processes such as primary production and decomposition (Zehr and Kudela, 2011). The multitude of chemical forms of N and the corresponding transformation pathways and microbial metabolisms that drive this biochemical cycle makes it more complex than other element cycles. Nitrogen exists in redox states ranging from -3 to +5 and can serve as electron acceptor and donor in energy metabolism (Zehr and Kudela, 2011). The most abundant forms of N in surface oceans are dissolved dinitrogen gas and dissolved organic nitrogen (DON) (Zehr and Kudela, 2011) which are both made available through biological activities. The process of N_2 fixation converts inert atmospheric gas into biologically available ammonium (Zehr and Paerl, 2008). However, only a few groups of microorganisms can perform N_2 fixation while most microorganisms obtain the N from other forms such as nitrate, nitrite, urea, ammonium, or organic N (Zehr and Paerl, 2008). In dissolved organic matter, N usually is present as amine or amide groups in amino acids and urea. These are labile compounds that are easily assimilated by microorganisms and rapidly recycled (Baker *et al*., 2009), whereby ammonium is the first breakdown product that becomes available to other organisms. Most microorganisms use ammonium as primary N source as it is energetically favourable over nitrate and thus, often only present at low or undetectable concentrations (Zehr and Kudela, 2011).

Ammonia is used by ammonia oxidizing Bacteria (AOB) and ammonia oxidizing Archaea (AOA) to harvest energy by oxidizing ammonia to nitrite in the first nitrification pathway (Wuchter *et al*., 2006; Mincer *et al*., 2007). Crenarchaeal ammonia monooxygenase (*amo*) genes are the dominant nitrogen oxidation genes in oceanic waters (Venter *et al*. 2004). It has been reported that *amo* genes are very common in a number of marine environments (Francis *et al*. 2005) including the euphotic zone (Church *et al*. 2010). Contrary to AOA,

nitrification by AOB is only possible in the dysphotic realm because nitrifying Bacteria are inhibited by light (Zehr and Kudela, 2011). Due to these biological processes, nitrate abundance increases with depth in the oceanic water column (Sarmiento and Gruber, 2006).

Denitrification, the process of transforming nitrate to molecular nitrogen occurs only in oxygen-depleted regions of the water column such as the oxygen minimum zones (OMZ) or suboxic to anaerobic sediments. Due to its high oxidation state, nitrate can be used as substitute of oxygen to oxidize organic material (Zehr and Kudela, 2011). It is thereby reduced to molecular nitrogen and eventually leaves the marine system. Another biological pathway of N loss from the system is anaerobic ammonia oxidation (Anammox), which also occurs in oxygen-depleted environments. Anammox is mediated by *Planctomycetes* Bacteria that directly convert ammonium and nitrite to dinitrogen gas (Zehr and Kudela, 2011). Anammox, together with heterotrophic denitrification are sinks of nitrogen from the ocean. Another form of nitrate reduction is the direct transformation of nitrate to ammonium, a process that can be dissimilatory or assimilatory (Gardner *et al*., 2006; Allen *et al*., 2001). In both cases the nitrate is reduced to ammonium and therefore not lost from the system, but rather conserved and made available to organisms at higher trophic levels (An and Gardner, 2002). Many enzymes involved in the nitrogen cycle have been identified and the genes encoding them have been used to quantify and phylogenetically characterize the microbial communities carrying out these processes.

This study focuses on nitrate assimilation by heterotrophic Bacteria in the deep sea. The importance of NO_3^- as an inorganic nutrient source for marine phytoplankton has long been recognized (Zehr and Kudela, 2011). Only fairly recently, however, marine heterotrophic Bacteria have been shown to utilize NO_3^- for growth and biomass synthesis (Kirchman, 2000; Allen *et al*., 2001). To gain better insights into the distribution and abundance of nitrate assimilating Bacteria in the water column of the Atlantic Ocean, we

analyzed samples collected along a transect in the Western Atlantic basin from 64°N to 50°S from the surface to bathypelagic waters. We determined the abundance and diversity of the *nas*A gene, which encodes the enzyme nitrate reductase responsible for assimilatory nitrate reduction in heterotrophic Bacteria.

References

- Allen AE, Booth MG, Frischer ME, Verity PG, Zehr JP, Zani S. (2001). Diversity and detection of nitrate assimilation genes in marine Bacteria. Appl. Environ. Microbiol. 67: 5343-5348

- An S, Gardner WS. (2002). Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). Mar. Ecol. Prog. Ser. 237: 41-50

- Baker KM, Gobler CJ, Collier JL. (2009). Urease gene sequences from algae and heterotrophic Bacteria in axenic and nonaxenic phytoplankton cultures. J. Phycol. 45: 625–34

- Church MJ, Wai B, Karl DM, DeLong EF. (2010). Abundances of crenarchaeal *amo*A genes and transcripts in the Pacific Ocean. Environ. Microbiol. 12: 679–88

- Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. (2005). Ubiquity and diversity of ammonia oxidizing archaea in water columns and sediments of the ocean. Proc. Natl. Acad. Sci. USA 102: 14683-14688

- GardnerWS, McCarthy MJ, An SM, Sobolev D, Sell KS, Brock D. (2006). Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. Limnol. Oceanogr. 51: 558–68

- Kirchman DL. (2000). Uptake and regeneration of inorganic nutrients by marine heterotrophic Bacteria. In: Kirchman DL (ed) Microbial ecology of the oceans. John Wiley & Sons, New York, p. 261-288

- Lavelle P, Dugdale R, Scholes R, Berhe AA, Carpenter E, Codispoti L, et al. (2005). "12. Nutrient cycling". Millennium Ecosystem Assessment: Objectives, Focus, and Approach. Island Press. ISBN 1-55963-228-3. pp 333-347

- Mincer TJ, Church MJ, Taylor LT, Preston C, Kar DM, DeLong EF. (2007). Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environ. Microbiol. 9: 1162-75

- Sarmiento JL and Gruber N. (2006). In: Sarmiento JL and Gruber N. (ed) Ocean Biogeochemical Dynamics. Princeton, New Jersey, pp.189-196

- Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, et al. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66-74

- Whitman WB, Coleman DC, Wiebe WJ. (1998). Prokaryotes: The unseen majority. Proc. Natl. Acad. Sci. USA 95: 6578-6583

- Wuchter C, Abbas B, Coolen MJL, Herfort L, van Bleijswijk J, et al. (2006). Proc. Natl. Acad. Sci. USA 103: 12317-12322

- Zehr JP and Kudela RM. (2011). Nitrogen Cycle of the Open Ocean: From Genes to Ecosystems. Annu. Rev. Mar. Sci. 3: 197-225

- Zehr JP and Paerl HW. (2008). In: Microbial ecology of the oceans Chapter 13: Molecular ecological aspects of nitrogen fixation in the marine environment (Kirchman DL). John Wiley & Sons. DOI: 10.1002/9780470281840.ch13

Zusammenfassung

Es wird angenommen dass heterotrophe Bakterien der Tiefsee durch die Verfügbarkeit von organischem Material limitiert sind. Das Vorkommen von Abbau-resistentem organischem Stickstoff und Phosphor der Tiefsee steht im Gegensatz zu potentiell verfügbarem Nitrat und Phosphat, die in großen Mengen vorhanden sind. Um die potentielle Aufnahme von Nitrat durch Bakterien in der Tiefsee zu bestimmen, wurden Wasserproben von Oberflächengewässern bis zum Bathy- und Abyssopelagial an 51 Stationen eines latitudinalen Transekts von der arktischen bis zur subantarktischen ozeanographischen Provinz des Atlantik genommen. Die Abundanz von Nitrat-assimilierenden Bakterien wurde über das *nas*A Gen (welches die assimilatorische Nitrat-Reduktase kodiert) und quantitativer PCR gemessen. Eine größere Fraktion mutmaßlich Nitrat-assimilierender Bakterien wurde in tieferen Regionen des Bathypelagial der Subantarktis (51%) und der nordatlantischen Drift Provinz (20%) und im tiefen Mesopelagial der südatlantischen Provinz (20%) als im Epipelagial und der arktischen Region (0 – 3%) gefunden. Um die Nitrat-assimilierende Bakteriengemeinschaft phylogenetisch zu charakterisieren, wurde eine „nested“ PCR verwendet, um das *nas*A Gen zu amplifizieren, klonieren und zu sequenzieren. Die Bakteriengemeinschaft, die das *nas*A Gen besitzt, bestand hauptsächlich aus *Gammaproteobakterien* und *Bacteroidetes*, obwohl Unterschiede in der Zusammensetzung der *nas*A-beinhaltenden Gemeinschaft zwischen einzelnen ozeanischen Provinzen und Tiefenschichten festgestellt wurden. Die *nas*A-beinhaltenden Gemeinschaften der arktischen Provinz waren in allen Tiefenschichten ähnlich und signifikant unterschiedlich von den *nas*A-

beinhaltenden Gemeinschaften anderer ozeanographischen Provinzen. Die *nas*A-beinhaltenden Gemeinschaften der Tiefengewässer der nordatlantischen Drift, der nordatlantischen Gyre, der westlichen-tropischen atlantischen und der südlichen atlantischen Provinz waren alle ähnlich zueinander. Allgemein wurden größere Fraktionen mutmaßlich Nitrat-assimilierender Bakterien in Regionen mit organischer Stickstofflimitierung, wie den Tiefengewässern der subtropischen Regionen und der mesopelagischen (sub)tropischen Gewässer, gefunden. Demzufolge hat zumindest ein Teil der heterotrophen Bakterien der Tiefsee das Potential, Limitierung durch organischen Stickstoff durch Aufnahme von frei verfügbarem Nitrat zu kompensieren.

Abstract

Heterotrophic Bacteria in the deep ocean are generally limited by the availability of organic matter. The recalcitrant nature of the organic nitrogen and phosphorus pool in the deep ocean contrasts the high concentrations of potentially available nitrate and phosphate. To determine the potential of deep-water Bacteria to take up nitrate, water samples were taken from the epipelagic to the lower bathypelagic and abyssopelagic waters of the Atlantic at 51 stations along a latitudinal transect from the Arctic to the Subantarctic oceanographic province. The abundance of nitrate assimilating Bacteria was quantified by q-PCR of the *nas*A gene (encoding the assimilatory nitrate reductase). A higher fraction of putatively nitrate assimilating Bacteria was found in the lower bathypelagic waters of the Subantarctic (51%) and the North Atlantic Drift (20%) province and in lower mesopelagic waters of the South Atlantic province (20%) than in the epipelagic waters and the Arctic region (0 – 3%). To phylogenetically characterize the nitrate assimilating bacterial community, nested PCR was used to amplify, clone and sequence the *nas*A gene. The *nas*A gene harboring bacterial community consisted mainly of *Gammaproteobacteria* and *Bacteroidetes*, although differences in the composition of the *nas*A-containing community were observed among oceanic provinces and depth layers. The composition of the *nas*A-containing communities of the Arctic province was similar throughout the water column and significantly different from the *nas*A-containing communities of the other oceanic provinces. The deep-water *nas*A-containing communities of the North Atlantic Drift, the North Atlantic Gyre, the Western Tropical Atlantic and the South Atlantic province were all similar to each other. Overall, higher fractions of putatively nitrate assimilating Bacteria were found in regions where the bioavailability of organic nitrogen is presumably limited such as in the deep waters of the subtropical regions and the mesopelagic (sub)tropical waters. Hence, at least a certain fraction of the heterotrophic Bacteria in the deep ocean has the potential to compensate organic nitrogen limitation by utilizing the readily available nitrate.

Introduction

Heterotrophic Bacteria are utilizing a diverse array of organic compounds consisting of carbon, nitrogen and phosphorus which are usually available in surface waters either via phytoplankton extracellular release or via grazing mediating release of dissolved organic matter (DOM) (Azam *et al*. 1983). In mesopelagic waters, organic P and N are preferentially utilized over C (Benner, 2002). Consequently, organic matter in the deep sea is typically depleted in metabolizable organic N and P (reviewed in Arístegui *et al*. 2009). However, accompanied with a decline in organic N availability with depth in the oceanic water column, the concentrations of inorganic N, essentially NO_3^- is increasing (Sarmiento and Gruber, 2006). Deep ocean NO_3^- concentrations range from 20-45 μM with phosphate concentrations corresponding to the Redfield ratio of inorganic N:P of about 16 (Sarmiento and Gruber, 2006).

Nitrate is used as nitrogen source not only by marine phytoplankton (Zehr and Kudela, 2011) but can also be utilized by marine heterotrophic Bacteria (Kirchman, 2000). The structural genes for nitrate reductase have been characterized in the Gammaproteobacterium *Klebsiella pneumoniae* (Lin *et al*., 1993). The alpha subunit of the nitrate reductase (*nas*A) protein, which contains the active site for nitrate reduction has been characterized in the phototrophic Alphaproteobacterium *Rhodobacter capsulatus* (Moreno-Vivan *et al*., 1992; Blasco *et al*., 1997). Analysis of currently available prokaryotic genome sequences suggests that the *nas*A gene is present in a wide range of Bacteria (Richardson *et al*., 2001). Nitrate assimilation genes of heterotrophic prokaryotes are distinct from those of autotrophs (Allen *et al*., 2001). Heterotrophic NO_3^- assimilation has been confirmed to occur in several distinct clades of Bacteria and in a variety of marine environments such as the South Atlantic Bight, the Barents Sea and the North Pacific Gyre, where heterotrophic Bacteria can account for up

to 40% of the total NO_3^- uptake in surface waters (Kirchman, 2000; Middelburg and Nieuwenhuize, 2000; Allen *et al*., 2001; Allen *et al*., 2002; Joint *et al*., 2002; Ovreas *et al*., 2003).

From the surface to the deep euphotic zone, nitrate becomes an increasingly important N source for heterotrophic Bacteria (Allen *et al*., 2005). Moreover, the distribution and diversity of the *nas*A harboring bacterial community vary among epipelagic oceanic regions with contrasting nutrient regimes and, in general, are strongly related to NO_3^- availability (Allen *et al*., 2005). Polar regions and upwelling areas of the oceans are highly productive due to high concentrations of labile organic and inorganic nutrients, the latter fueling primary production (Sarmiento and Gruber, 2006). Labile organic material supplied by the primary producers provides heterotrophic microorganisms with substrate within the epipelagic layers of the ocean (Jiao *et al*., 2010). In these highly productive areas, particulate organic carbon (POC) export rates can be up to 10 mol C m^{-2} yr^{-2} (Sarmiento and Gruber, 2006), while in the deep waters of gyral and tropical regions refractory forms of organic material low in nitrogen content dominate (Jiao *et al*., 2010) leading to a limitation of deep-water microbial communities in bioavailable organic nitrogen. However, NO_3^- is available at copious concentrations in the deep ocean (Sarmiento and Gruber, 2006) providing potentially an alternative source of N for deep-water heterotrophic microbial communities.

The extent to which deep-water heterotrophic microbial communities are capable of utilizing nitrate, however, has not been investigated yet. We hypothesized that the potential for nitrate utilization increases in heterotrophic Bacteria with depth as the readily available organic nitrogen becomes increasingly depleted. To test this hypothesis, we analyzed the bacterial *nas*A gene abundance and phylogenetically characterized the *nas*A harboring bacterial community along a transect in the Western Atlantic Ocean from 64°N to 50°S from the surface to bathypelagic waters.

Materials and Methods

Sampling site

During the GEOTRACES research cruises-1 to -3, 51 stations were sampled along a latitudinal transect from 64°N to 50°S in the Atlantic Ocean between April and July 2010 on board the R/V *Pelagia* (GEOTRACES-1 and -2) and in February-March 2011 on board the R/V *James Cook* (GEOTRACES-3) (Fig. 1). Sampling was performed with a CTD (conductivity-temperature-depth; Seabird, Bellevue, WA, USA) rosette sampler equipped with 24 25-L Niskin bottles and sensors for chlorophyll fluorescence, turbidity, photosynthetic active radiation and oxygen.

Samples for DNA (2-10 L depending on the depth) were collected at 6 to 8 depths per station, filtered onto 0.2µm pore-size polycarbonate filters, flash-frozen in liquid N_2 and kept at -80°C until DNA extraction. Samples for abiotic parameters and for prokaryotic abundance were taken at 24 depths per station. Five pelagic zones were sampled at different depths: the epipelagic (0–200 m), the upper (200–500 m) and lower (500-1000 m) mesopelagic, the upper (1000–2000 m) and lower (2000-6000 m) bathypelagic layer including abyssopelagic waters, subsequently referred to as lower bathypelagic zone. Water samples were taken in six different oceanographic provinces based on the classification scheme of ecological provinces in the ocean (Longhurst, 1998): the North Atlantic Arctic province (ARCT; 70°N-55°N), the North Atlantic Drift province (NADR; 55°N-40°N), the North Atlantic Gyral province (NAG) comprising the North Atlantic Tropical and Subtropical Gyre province (40°N-12°N), the Western Tropical Atlantic province (WTRA; 12°N-10°S), the South Atlantic Gyre Province (SATL; 10°S-40°S) and the Subantarctic (SANT; 40°S-55°S) province (Fig. 1).

Inorganic nutrient concentration

The concentrations of NO_3^-, NO_2^- and PO_4^{3-} were determined on a TRAACS800 autoanalyzer system immediately after filtering the samples through 0.2 µm filters (Acrodisc, Gelman Science). NO_2^- was measured after diazotation with sulfanilamide and *N*-(1-naphtyl)-ethylene diammonium dichloride as the reddish-purple dye complex at 540 nm (Parsons *et al*., 1984). NO_3^- was reduced in a copper cadmium coil to nitrite with imidazole as buffer and subsequently measured as nitrite. Inorganic PO_4^{3-} was measured via the molybdenum blue complex at 880 nm (Murphy and Riley, 1962).

Prokaryotic abundance

For the enumeration of prokaryotic cells the standard procedure for flow cytometric enumeration (Del Giorgio *et al*., 1996) was used with modification as described previously (De Corte *et al*., 2012). Briefly, 2 mL samples were fixed with glutaraldehyde (0.5% final concentration), flash-frozen in liquid N_2 and kept at -80°C until analysis. Samples were thawed to room temperature and 0.5 mL subsamples were stained with SYBR Green I (Molecular Probes, Invitrogen, Carlsbad, CA, USA) in the dark for 10 min. The prokaryotes were enumerated on a FACSAria II flow cytometer (Becton Dickinson, Fraclin Lakes, NJ, USA) by their signature in a plot of green fluorescence versus side scatter.

DNA extraction

Filters for DNA were cut in half in the home lab. Half of the filter was again stored at -80°C while the DNA of the other half was extracted with the Mobio UltraClean Soil DNA isolation kit, following the manufacturer's protocol.

*PCR amplification for cloning and sequencing of nas*A *gene*

The *nas*A gene was amplified via nested PCR following the protocol described by Allen *et al*. (2001). The forward primer nas22 and the reverse primer nas1933 were used for

the outer PCR (Table 1). PCR cycles were carried out according to the following protocol: a hot start at 94°C for 5 min was followed by 35 cycles consisting of a DNA denaturation step at 94°C for 10 s, then by the annealing step at 55°C for 15 s and the extension step at 72°C for 1 min. Subsequently, a final extension step was conducted at 72°C for 7 min and a final hold at 4°C. The reaction mixture for each well contained 1x Taq buffer, 0.2 mM dNTP's, 3.5 mM $MgCl_2$, 1 mM of each primer, 1 U Taq Polymerase, 0.2 µM BSA, 2 µL of sample, made up to 25 µL with ultrapure sterile water (Roche). The forward primer nasA964 and the reverse primer nasA1735 were used for the inner PCR amplification (Table 1). PCR cycles were performed as follows: a hot start at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 10 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, and by a final extension at 72°C for 7 min and hold at 4°C. The PCR reaction mixture was the same as for the outer PCR amplification except the $MgCl_2$ concentration was decreased to 2.5 mM and the amount of sample was increased to 3 µL.

After the nested PCR, the amplicons of 18 samples from meso- (250 m), upper bathypelagic (1250 m) and lower bathypelagic (>2000m) waters covering all oceanographic provinces were cloned using the TOPO-TA cloning kit (Invitrogen) following the manufacturer's instructions. Four µL of fresh PCR product were gently mixed with 1 µL salt solution and 1 µL of TOPO Vector and incubated on ice for 35 min. Thereafter, 2 µL of the cold TOPO ligation reaction were added to a vial of One Shot Chemically Competent *E. coli*, gently mixed and incubated on ice for 20 min. After transfer to a round floating rack, the vials were heat shocked by placing the rack in a 42°C water bath for 30 s. Then, 250 µL of SOC (Super Optimal broth with Catabolite repression) medium (~20°C) was added to the cells and the tubes were shaken (200 rpm) at 37°C for 1 h. Three pre-warmed LB plates (containing ampicillin sodium salt, 100 mg L^{-1}) for each sample were treated with 40 µL X-gal. Different amounts (75 µL, 100 µL and 125 µL) of SOC medium with *E. coli* cells were spread out in

each plate to ensure that at least one plate had well spaced colonies. Thereafter, the plates were incubated at 37°C for 20 h. White colonies were picked with sterile toothpicks, gridded on new LB plates and stored at 4°C. A PCR was performed using the M13 forward and reverse primer (Table 1). The PCR amplification was performed as follows: an initial activation step at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, amplification at 60°C for 45 s and extension at 72°C for 1 min. The final extension was at 72°C for 7 min and hold at 4°C. Successful cloning was verified by the presence of a band of the expected size on a 2% agarose gel electrophoresis. The gels were stained with SYBR Gold for 20 min and the band size was determined by comparison with the molecular weight marker Smart Ladder (Eurogentec). Clones containing the *nas*A gene were sent to GATC Biotech for forward and reverse sequencing using the M13 primers. Sequences were analyzed and trimmed with MEGA5 (Tamura *et al*., 2007) and forward and reverse sequences were merged with the program CodonCode Aligner. The sequence data were aligned together with full-length sequences of *nas*A genes from *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes* and *Cyanobacteria* obtained from NCBI. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987) and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic trees were drawn using iTOL (Letunic and Bork, 2007). One representative of a sequence group ≥97% identical is shown in the tree.

Quantitative-PCR (q-PCR)

All q-PCR analyses were performed on a Light Cycler 480 SW 1.5. The amplification products from different clones containing the amplified fragment were run on an agarose gel (1%) and stained with SYBR Gold (Invitrogen) for 20 min. The bands were isolated from the gel and purified using a PCR Gel Extraction MiniKit (5-PRIME). Thereafter, the DNA concentration of the PCR product was quantified on a Nanodrop 2000 spectrophotometer and the amount of PCR product needed for a specific *nas*A gene abundance per µL was estimated according to fragment length and concentration of the PCR product. A standard dilution series was made by diluting the PCR product with Tris buffer (10mM, pH=8.0) and subsequently used as internal standard for quantification. The standard series from 10^7 to 10^2, blanks and samples were added in triplicates to each 96-well qPCR plate (Bio-Rad) and closed with optical tape (Bio-Rad). The reaction mixture for each sample contained 1x Mastermix (LightCycler 480 SYBR Green I Master, Roche), 0.5 mM nasA964 and 0.5 mM nasA1735 primers (Table 1) and filled up to 10 µL with ultrapure sterile water (Roche). Denaturation was conducted in the first step at 95°C for 10 min, followed by a touchdown PCR over 50 amplification cycles each consisting of denaturation at 95°C for 5 s, annealing for 30 s, extension at 72°C for 45 s and measurement at 80°C for 3 s. During the first 5 cycles, the annealing temperature was decreased by 1°C each cycle starting at 65ºC until it reached 60°C. The specificity of the q-PCR reaction was tested on a 2% agarose gel and with the melting curve analysis (65°C - 95°C). PCR efficiency was on average 93±14% and the mean correlation coefficient 0.967±0.017 (n=5).

The *rec*A gene abundance was also measured by q-PCR (Light Cycler 480 SW 1.5) to relate bacterial *nas*A to *rec*A gene abundance. The abundance of *rec*A genes served as a proxy for bacterial abundance as commonly, Bacteria harbor one *rec*A gene per genome (Miller and Kokjohn, 1990). Q-PCR was performed on all samples in triplicate using the primers recAF and recAR (Table 1). The initial denaturation step at 95ºC for 10 min was

followed by 50 cycles consisting on an initial denaturation at 95ºC for 5 s, followed by an annealing step at 53ºC for 5 s, extension at 72ºC for 15 s and a plate read at 74ºC for 3 s. An internal standard for quantification was generated from a PCR amplified *rec*A from an environmental sample. The concentration of DNA in the purified PCR product was measured with a Nanodrop 2000, and a dilution series was prepared as described above for the *nas*A gene. All wells contained 1x Mastermix (LightCycler 480 SYBRGreen I Master, Roche), 0.5 mM of each primer, 1µL of sample and filled up to 10µL with ultrapure sterile water (Roche). The specificity of the PCR amplification was checked on a gel after a melting curve analysis (65ºC-95ºC). PCR efficiencies and correlation coefficients were on average 83±9% and 0.963±0.029, respectively (n=15).

Statistical analysis

Statistical analysis on the variations of abiotic and biotic factors between depth layers and oceanographic provinces was done by two-way ANOVA and the Holm-Sidak method and ANOVA on Ranks following Dunn's test using SigmaPlot 12. The results were considered statistically significant if $P<0.05$. A principal component analysis (PCA) was conducted to test for differences in the phylogenetic composition of nitrate assimilating microbial communities between different depth layers and oceanographic provinces using UniFrac software and the Unifrac significance level (Lozupone *et al*., 2006). The distance-based multivariate analysis for a linear model using forward selection (DISTML forward) was applied to test the relationship between *nas*A gene abundance and biotic and abiotic environmental parameters (Anderson *et al.*, 2004). Single correlation analysis was conducted to test for the relationship between bacterial abundance assessed by flow cytometry and the *rec*A gene abundance.

Results

Physical and chemical variables of the water column

In the epipelagic layer (0-200 m depth), mean water temperature in the different oceanographic provinces increased from the northernmost province (ARCT) (mean ± SD: 5.47 ± 0.81°C) to the WTRA province (24.14 ± 5.68°C) and decreased to 11.77 ± 4.83°C in the southernmost province (SANT) (Suppl. Table 1, Fig. 2A). In the upper and lower mesopelagic waters, mean water temperature increased from the ARCT province (4.97 ± 0.90°C and 4.01 ± 0.30°C, respectively) to the NAG province (17.30 ± 1.88°C and 10.65 ± 3.50°C, respectively). The WTRA province showed lower temperature in the upper mesopelagic waters (10.11 ± 2.07°C) than the SATL province (14.10 ± 2.12°C) (t-test, $P < 0.001$). The lower mesopelagic waters of the SANT province were colder (3.25 ± 0.63°C) than the lower mesopelagic waters of the ARCT province (Mann-Whitney Rank Sum Test $P < 0.05$). In the upper bathypelagic waters, temperature increased from the ARCT province (3.53 ± 0.21°C) to the NAG province (4.77 ± 0.92°C) (Mann-Whitney Rank Sum Test, $P < 0.001$) and then decreased to 2.61 ± 0.37°C in the SANT province (Mann-Whitney Rank Sum Test, $P < 0.001$).

For the lower bathypelagic waters, no differences in the water temperature were detectable (ANOVA on Ranks, $P > 0.05$) among the different oceanic provinces except the lower bathypelagic waters of the SANT province were significantly colder than in the other provinces (ANOVA on Ranks, $P < 0.05$). All oceanographic provinces showed a significant temperature stratification between depth layers (two way ANOVA, $P < 0.05$) except the water column of the ARCT and the SANT province.

Salinity was not significantly different between the depth layers of the ARCT province (ANOVA on Ranks, $P > 0.05$). However, the upper mesopelagic layer exhibited a higher salinity than the lower bathypelagic layer (ANOVA on Ranks, $P < 0.05$) (Suppl. Table 1, Fig.

2B). In the NADR province, the salinity was significantly lower in the lower bathypelagic zone than in the epipelagic and upper mesopelagic waters (ANOVA on Ranks, $P < 0.05$). In the NADR, salinity ranged from 34.91 ± 0.06 to 34.97 ± 0.12 at depth from 1000 m to 4727 m representing North Atlantic Deep Water (NADW). In the WTRA province, salinity of the lower and upper bathypelagic water masses decreased to 34.88 ± 0.14 and 34.88 ± 0.08, respectively, indicating mixing of Antarctic Bottom Water (AABW) with NADW. Generally, the SANT province was characterized by low salinity values (34.29 ± 0.12 and 34.27 ± 0.02 in the lower and upper mesopelagic waters, respectively), indicating the presence of Antarctic Intermediate Water (AAIW) (Fig. 2B).

Nitrate concentrations were generally lower in the epipelagic zone of the oligotrophic subtropical and tropical provinces (0.76 ± 1.22 μmol kg^{-1}, 1.9 ± 1.78 μmol kg^{-1} and 6.50 ± 8.19 μmol kg^{-1} in the NAG, SATL and WTRA, respectively) than in the higher latitude provinces (Suppl Table 1, Fig. 2C). While nitrate concentrations in the ARCT increased from the epipelagic layer from 13.74 ± 1.11 μmol kg^{-1} to 16.61 ± 0.19 μmol kg^{-1} in the upper bathypelagic zone, nitrate increased over the same depth range in the NAG and WTRA from 0.76 ± 1.22 μmol kg^{-1} to 19.88 ± 2.90 μmol kg^{-1} and 6.50 ± 8.19 μmol kg^{-1} to 24.64 ± 5.01 μmol kg^{-1}, respectively, with a maximum of 33.68 ± 1.35 μmol kg^{-1} in the lower mesopelagic zone of the WTRA (Suppl. Table 1, Fig. 2C). Similar to the ARCT, the SANT province exhibited a weak stratification in nitrate concentrations ranging from 11.93 ± 7.51 μmol kg^{-1} in the epipelagic to 32.86 ± 1.15 μmol kg^{-1} in the upper bathypelagic layer (Suppl. Table 1, Fig. 2C).

Prokaryotic abundance as determined by flow cytometry

In the epipelagic layer, prokaryotic abundance was higher in the SANT (4.94 ± 2.86 x 10^5 cells mL^{-1}) and ARCT province (3.18 ± 1.17 x10^5 cells mL^{-1}) than in the oligotrophic NAG province (1.77 ± 0.93 x10^5 cells mL^{-1}) (ANOVA on Ranks, $P < 0.05$) (Suppl. Table 1,

Fig. 3A). The SANT and ARCT province exhibited a higher prokaryotic abundance than the NAG province (ANOVA on Ranks, $P < 0.05$) in all depth layers. Prokaryotic abundance decreased with depth by two orders of magnitude from highest mean abundances in the epipelagic zone to lowest prokaryotic abundance in the lower bathypelagic zone of the NAG province ($8.37 \pm 1.97 \times 10^3$ cells mL^{-1}) (Fig. 3A).

*rec*A *and nas*A *gene abundance determined by qPCR*

Prokaryotic abundance determined by flow cytometry was tightly related to *rec*A gene abundance determined by q-PCR ($y = 1592.2x^{0.52}$; $R^2 = 0.68$, Fig. 4). Within the individual depth layers, however, and in contrast to prokaryotic abundance, *rec*A gene abundance was not significantly different among the oceanographic provinces (two way ANOVA, $P > 0.05$) except in the epipelagic layer (Fig. 3B). In the epipelagic layer of the NAG province, the *rec*A gene abundance was significantly higher than in the NADR and the ARCT province (two way ANOVA, $P < 0.05$) and the *rec*A gene abundance in the SATL was significantly higher than the NADR province (two way ANOVA, $P < 0.05$). Overall, the mean *rec*A gene abundance was highest in the epipelagic layer of the SATL province ($2.17 \times 10^4 \pm 0.00$ *rec*A mL^{-1}).(Suppl. Table 1, Fig. 3B). Similar to prokaryotic abundance determined by flow cytometry, *rec*A gene abundance decreased by two orders of magnitude from the epipelagic to the bathypelagic zone throughout the Atlantic (Fig. 3B).

The *nas*A gene in the epipelagic layer was more abundant in the SANT ($3.32 \pm 1.93 \times 10^2$ *nas*A mL^{-1}) than in the SATL province ($1.21 \times 10^2 \pm 0.00$ *nas*A mL^{-1}) and the NADR province ($0.46 \pm 0.09 \times 10^2$ *nas*A mL^{-1}) (two way ANOVA, $P < 0.05$) (Suppl. Table 1, Fig. 3C). In the upper mesopelagic layer, the *nas*A gene abundance in the SATL province was significantly higher than in the NAG, the NADR, the ARCT and the WTRA province (two way ANOVA, $P < 0.05$). Within the lower bathypelagic layer, *nas*A gene abundance of the

ARCT province was significantly higher than in the other oceanic provinces (two way ANOVA, $P < 0.05$). In the ARCT province, *nas*A gene abundance was highest in the epipelagic zone ($1.11 \pm 0.71 \times 10^2$ *nas*A mL^{-1}), decreasing towards the upper bathypelagic zone ($0.19 \pm 0.15 \times 10^2$ *nas*A mL^{-1}) and increasing again in the lower bathypelagic zone ($0.42 \pm 0.45 \times 10^2$ *nas*A mL^{-1}) (Fig. 3C).

The relative contribution of putatively nitrate assimilating Bacteria to the total Bacteria, expressed as the ratio of *nas*A to *rec*A gene abundance, was low in the epipelagic zone (0.013 ± 0.004) and increased with depth to a maximum of 0.178 ± 0.230 in lower bathypelagic waters (Fig. 3D). The lowest *nas*A:*rec*A ratios (0.005 ± 0.007) were found in the epipelagic waters of the NAG province (Fig. 5), due to the high abundance of *rec*A genes there ($1.81 \pm 1.36 \times 10^4$ *rec*A mL^{-1}). In epipelagic waters, the highest *nas*A:*rec*A ratio was found in the SANT province (0.033 ± 0.010, Fig. 5) associated with high *nas*A gene abundance ($3.32 \pm 1.93 \times 10^2$ *nas*A mL^{-1}) and also high *rec*A gene abundance ($1.27 \pm 0.28 \times 10^4$ *rec*A mL^{-1}). The highest *nas*A:*rec*A gene abundance ratio obtained in the Atlantic ocean (0.513 ± 0.636) was found in the lower bathypelagic waters of the SANT province (Fig 5). In the ARCT province, the *nas*A:*rec*A gene abundance ratio ranged from 0.013 ± 0.006 in the epipelagic to 0.032 ± 0.024 in the upper bathypelagic (Fig. 3D, Fig. 5). The NADR province also exhibited low *nas*A:*rec*A gene abundance ratios throughout the water column, except in the lower bathypelagic waters where this ratio increased to 0.200 ± 0.246 (Fig. 3D, Fig. 5). The *nas*A:*rec*A gene abundance ratio in the meso- and bathypelagic waters of the NAG, WTRA and SATL province ranged from 0.066 ± 0.033 to 0.202 ± 0.140, with highest ratios in the lower mesopelagic waters of the SATL province (Fig. 3D).

*Phylogenetic community composition of nas*A *harboring Bacteria*

The phylogeny of the *nas*A gene containing Bacteria was assessed from three depth layers (mesopelagic: 250 m, upper bathypelagic: 1250 – 1750 m and lower bathypelagic:

2500 – 4500 m) at 6 stations (see Fig. 1) covering all oceanographic provinces. In total, 413 clones were sequenced resulting in 81 operational taxonomic units (OTUs). Most of the *nas*A harboring OTUs (54 OTUs) were affiliated to the *Gammaproteobacteria*, 16 OTUs to *Bacteroidetes*, one OTU to *Aphaproteobacteria* and 10 remained unknown (Fig. 6). The most abundant OTU (76 clones) was a bathypelagic (1250 – 4500 m) *Gammaproteobacterium* which occurred in all provinces except the ARCT. The second most abundant OTU (52 clones) was affiliated to *Bacteroidetes* and was present in all depth layers of the SANT province. Most *nas*A harboring OTUs (86.3%) were confined to specific oceanographic provinces, while the remaining 13.7% (all from the class *Gammaproteobacteria*) were distributed over more than one oceanographic province. Also, most OTUs (64) occurred in one specific depth layer, among them 22 OTUs were confined to the mesopelagic layer, 21 OTUs to the upper bathypelagic and 21 OTUs to the lower bathypelagic (Fig. 7). Only 17 OTUs (or 13.77%) occurred in more than one depth layer (Fig. 7). The *nas*A harboring bacterial community composition of the ARCT province was not stratified and differed from that of the other provinces ($p < 0.001$, Unifrac level of significance) (Fig. 8). Also, the nasA containing bacterial community of the SANT province showed only a weak stratification with depth ($p < 0.1$ between meso- and upper-bathypelagic, Unifrac level of significance). In contrast, the NADR, the NAG, the WTRA and the SATL province exhibited a strong stratification of the *nas*A harboring Bacteria with depth ($p < 0.001$, Unifrac level of significance). The *nas*A harboring bacterial community present in the deep waters of the gyral and tropical provinces were compositionally similar ($p > 0.05$, Unifrac level of significance) (Fig. 8).

Discussion

*Prokaryotic abundance determined by flow cytometry versus rec*A *gene abundance*

Generally, the prokaryotic abundance decreased from epipelagic to lower bathypelagic waters in all oceanographic provinces (Fig. 3A), in accordance with previous studies from the North and South Atlantic (Reinthaler *et al*., 2006; Schattenhofer *et al*., 2009; De Corte *et al*., 2012). The *rec*A gene encodes the recombinase A protein and has a unique role in DNA damage repair as synaptase in homologous recombination and operating abilities. It is maintained across species and genus lines and is universally present in Bacteria (Miller and Kokjohn, 1990). The fact that the *rec*A gene, in contrast to the 16SrRNA gene, has remained a single-copy gene in Bacteria makes it a suitable proxy of bacterial abundance (Lin *et al*., 2006). The *rec*A gene also occurs in Archaea but was not targeted with the primers used in this study (Table 1), as determined by cloning and sequencing of the *rec*A gene (data not shown).

Bacterial abundance determined by q-PCR quantification of the *rec*A gene showed similar patterns as prokaryotic abundance determined with flow cytometry (compare Fig. 3A and 3B), but was one order of magnitude lower (Fig. 4). This discrepancy in the absolute abundance obtained with the two methods is probably mainly associated to the efficiency of the DNA extraction, which has been reported to be as low as 14.9 ± 16.0% (Mean ± S.D.) for the commercial kit used in this study (Mumy and Findlay, 2004), close to our one order of magnitude difference between flow cytometric cell counts and the *rec*A q-PCR approach. Additionally, the presence of a certain, albeit variable proportion of archaeal cells not targeted with the *rec*A primers, might explain some of the differences between the two methods. Determining the abundance of *Euryarchaeota* and *Crenarchaeota* with catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) in the North Western Atlantic revealed that *Crenarchaeota* and *Euryarchaeota* together can contribute more than 50% to the total picoplankton abundance in deep waters (Herndl *et al*., 2005; Teira *et al*., 2006). Regardless of the differences in absolute numbers, the correlation between prokaryotic abundance determined via flow cytometry and the abundance of *rec*A genes determined via

qPCR ($y = 1592.2\ x^{0.52}$; $R^2 = 0.6754$) indicates that the use of *rec*A gene abundance is a suitable proxy for total bacterial abundance in the water column (Fig. 4).

Distribution of nitrate assimilating Bacteria and nitrate concentrations in the Atlantic Ocean

The *nas*A to *rec*A ratio increased with depth (Fig. 3D). Allen *et al*. (2005) reported an 8-fold increase in *nas*A-containing Bacteria in the epipelagic Norwegian coastal waters and the Barents Sea (5 m compared to 80 m depth). In the present study, the lowest percentage of nitrate assimilating Bacteria was found in epipelagic waters (<200 m depth) (0 – 2%) as compared to meso- and bathypelagic waters (Fig. 3C, Fig. 5). Also low contributions of *nas*A gene containing Bacteria to bacterial abundance (estimated by *rec*A gene abundance) were found throughout the water column of the ARCT province (1 – 3%) (Fig. 5). In the epipelagic waters, low *nas*A:*rec*A gene ratios correspond to low NO_3^- concentrations (6.815 ± 0.316 µmol kg^{-1}) (DISTML test, P = 0.044, cumulative $r^2 = 0.5807$, Table 2, Fig. 2C), while high *nas*A:*rec*A ratios were measured in deep waters where NO_3^- concentrations were high (> 9 µmol kg^{-1} below 200 m depth). Despite the high NO_3^- concentrations in the deep waters throughout the Atlantic, the contribution of the *nas*A containing bacterial community to the total bacterial community varies across oceanographic provinces and depth layers. In the lower bathypelagic waters of the SANT province, 51% of the total bacterial community contain the *nas*A gene and therefore, could be assigned to putatively nitrate assimilating Bacteria. Further 'hotspots' of nitrate assimilating Bacteria are found in the lower bathypelagic waters of the NADR province and the lower mesopelagic waters of the SATL province (Fig. 3C), where nitrate assimilating Bacteria contribute on average 20% to the total bacterial community (Fig. 5). A surprisingly low percentage of *nas*A containing Bacteria was found in the lower bathypelagic waters of the NAG and the WTRA provinces (0.1% and 0.09%, respectively, Fig. 3C), although nitrate concentrations are high (19.6 ± 1.64 µmol kg^{-1}

and 21.9 ± 4.02 μmol kg^{-1}, respectively). However, in contrast to epipelagic waters, the abundance of deep-water nitrate assimilating Bacteria does not correlate to NO_3^- concentrations, but relates to leucine incorporation in the upper bathypelagic waters, bacterial abundance and latitude (Fig. 3A, B) in the lower bathypelagic waters (DISTML test, P=0.002, 0.001, 0.01, and cumulative r^2=0.2346,0.419,0.468, respectively) (Table 3). This suggests that the heterotrophic bacterial community in the deep waters of the Atlantic might fuel its biomass production with organic carbon-rich material and its N-requirements at least partly by nitrate.

Nitrate assimilating bacterial community linked to primary production and organic matter export

The cruise track followed the water masses forming the Deep Western Boundary Current (DWBC) and the major water masses of the thermohaline circulation system. The temperature and salinity distribution across the western Atlantic Ocean indicated deep-water formation in the northern and the southernmost latitudes (Fig. 2 A, B). The first four stations in the ARCT province were characterized by the presence of Denmark Strait Overflow Water (DSOW), which further south mixed with Labrador Sea Water (LSW) and Iceland Scotland Overflow Water (ISOW) to form the DWBC at the Charlie Gibbs Fracture Zone. The DSOW, the LSW and the ISOW together form the North Atlantic Deep Water (NADW) in the NADR province (Smethie *et al*., 2000). Deep water formation has also been determined in the SANT province as Antarctic Bottom Water (AABW) and Antarctic Intermediate Water (AAIW). The North Atlantic Ocean, the Antarctic Convergence and equatorial upwelling regions are regions of high annual net primary production (NPP), particulate (POM) and dissolved organic matter (DOM) export (Longhurst *et al*., 1995; Antoine *et al*., 1996; Palmer and Totterdell, 2001). The produced organic matter is categorized in three forms: labile, semi-labile and refractory organic matter (Jiao *et al*. 2010). Labile DOM is taken up by

heterotrophic microorganisms within hours or days, while semi-labile DOM persists for months to years (Bauer *et al*., 1992; Kirchman *et al*., 2001) and accounts for the largest part of the DOM pool that is exported, while refractory DOM is the most persistent form of organic matter and can be stored for millennia in the ocean's interior (Hopkinson *et al*., 2005). In contrast to labile and semi-labile DOM that can provide nitrogen for heterotrophic Bacteria, refractory DOM is low in N content, as nitrogen and phosphorus are preferentially remineralized relative to carbon (Hopkinson *et al*., 1996). Remineralization of organic nitrogen leads to high concentrations of nitrate potentially representing a readily available N source in the deep ocean.

It has been shown that microorganisms from the bathypelagic realm have evolved metabolic strategies as an adaptation to the low reactivity of deep sea DOM (Eichinger *et al*., 2006). In this context, utilization of inorganic nitrogen in the oligotrophic deep sea may be an adaptation to the low amount of organic N in the deep-sea.

The ARCT province exhibits low *nas*A:*rec*A ratios even in deep waters probably due to its high labile and semi-labile organic matter content compared to the deep water regions towards the south (Palmer and Totterdell, 2001). By the time organic material is transported to the NADR province in the NADW, it is degraded to a more refractory form by microbial activity (Jiao *et al*., 2010) accompanied with increasing concentrations of nitrate (Fig. 2C) leading to a higher potential of heterotrophic Bacteria to assimilate nitrate from 40°N southwards as indicated by higher *nas*A gene abundance and a higher *nas*A:*rec*A gene ratio in the NADR than in the ARCT province (Fig. 3C, D). The Southern Ocean also is an area of high primary production with seasonal phytoplankton blooms and high organic matter export rates (Palmer and Totterdell, 2001). The *nas*A-containing community of the SANT province was distinctly different from that of the other oceanic provinces and weaker stratified. In contrast to the ARCT province, the SANT province exhibited a high relative abundance of putatively nitrate assimilating Bacteria (Fig. 5). This might be because deep-water formation

in the Southern Ocean occurs mainly in the Weddell and Ross Seas (Talley, 1999), which is further south than the SANT province. Water mass and organic matter transport from the Southern Ocean to the northern SANT province where samples were taken (≈40°S), is estimated to occur within 50 years (England, 1995). Organic matter arriving from the Southern Ocean is refractory and the nitrate assimilating bacterial community present there might be adapted to use NO_3^- as nitrogen source, in a similar way as the community of the deep waters of the NADR province (Fig. 5).

*Composition of the nas*A *harboring bacterial community*

*Nas*A genes are widespread among different phylogenetic groups in diverse environments (Allen *et al*., 2001). The majority of nitrate assimilating Bacteria found in the South Atlantic Bight, the Barents Sea and the North Pacific Gyre were affiliated to *Gammaproteobacteria* (Allen *et al*., 2001), similar this study. Most OTUs were restricted in their distribution to specific oceanic provinces and/or specific depth layers suggesting a highly adapted *nas*A harboring bacterial community (Figs. 6, 7). The most abundant *nas*A harboring OTU (a *Gammaproteobacteria*) however, was found in all oceanic provinces and depth layers, except the ARCT and the upper 250 m layer. Another highly abundant *nas*A harboring OTU from the *Bacteroidetes* clade was confined to the SANT province but was present in all depth layers.

Regionally distinct *nas*A containing communities have been reported previously using a fingerprinting technique, although differences with depth (5 m *versus* 80 m) could not be deciphered (Allen *et al*., 2005). In contrast to the former study, the PCA analysis of the clone libraries indicates a *nas*A harboring community in the ARCT province distinctly different from all the other provinces but lacking depth stratification (Fig. 8). The lack of stratification of putatively nitrate assimilating communities in the ARCT province might be related to the

deep-water formation in this province and the corresponding weak stratification of the water column (Fig. 2A,B). The *nas*A containing bacterial community of the SANT province also displayed weak stratification supporting the notion that the extent of stratification of the bacterial community is linked to the physical structure of the water column. In contrast, the strong stratification of the water column of the NADR, NAG, WTRA and the SATL provinces was reflected in the stratification of the *nas*A harboring bacterial communities (Fig. 8). The deep-water *nas*A harboring bacterial communities of these provinces from 40°N to 40°S were similar to each other (Fig. 8).

Conclusion

Taken together, the composition and abundance of the putatively nitrate assimilating heterotrophic bacterial community vary among different oceanic provinces and depth layers in the Atlantic Ocean, linked to water mass transport, epipelagic primary production and, as a consequence of that, organic matter export. The nitrate assimilating community composition from the Arctic province differs significantly from other oceanographic provinces as it is not stratified with depth. The communities of the other oceanographic provinces exhibit a depth-related distribution. Collectively, our results point to the utilization of nitrate as a nitrogen source in deep-water heterotrophic Bacteria throughout the Atlantic Ocean, where up to 51% of the bacterial community can potentially assimilate nitrate and thereby, circumvent the limitation in organic nitrogen sources in the deep ocean.

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References

- Allen AE, Booth MG, Frischer ME, Verity PG, Zehr JP, Zani S. (2001). Diversity and detection of nitrate assimilation genes in marine Bacteria. Appl Environ Microbiol 67: 5343-5348

- Allen AE, Booth MG, Verity PG, Frischer ME. (2005). Influence of nitrate availability on the distribution and abundance of heterotrophic Bacterial nitrate assimilation genes in the Barents Sea during summer. Aquat Microb Ecol 39: 247-255

- Allen AE, Howard-Jones MH, Booth MG, Frischer ME, Verity PG, Bronk DA, Sanderson MP. (2002). Importance of heterotrophic Bacterial assimilation of ammonium and nitrate in the Barents Sea during summer. J Mar Syst 38: 93-108.

- Anderson MJ, Ford RB, Feary DA, Honeywill C. (2004). Quantitative measures of sedimentation in an estuarine system and its relationship with intertidal soft-sediment infauna. Mar Ecol Prog Ser 272:33-48

- Antoine D, André JM, Morel A. (1996). Oceanic primary production: 2. Estimation at global scale from satellite (Coastal Zone Color Scanner) chlorophyll. Global Biogeochem Cycles 10: 57-69

- Aristegui J, Gasol JM, Duarte CM, Herndl GJ. (2009). Microbial oceanography of the dark ocean's pelagic realm. Limnol Oceanogr 54: 1501-1529

- Azam F, Fenchel T, Field JG, Gray JS, Meyerreil LA, Thingstad F. (1983). The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10: 257-263

- Bauer JE, Williams PM, Druffel ERM. (1992). 14C activity of dissolved organic carbon fractions in the north-central Pacific and Sargasso Sea. Nature 357: 667-670

- Benner R. (2002). Chemical composition and reactivity, p. 59-90. In D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Elsevier Science.

- Blasco R, Castillo F, Matinez-Luque M. (1997) The assimilatory nitrate reductase from the phototrophic bacterium, *Rhodobacter capsulatus* E1F1, is a flavoprotein. FEBS Lett 414: 45 49

- Casey JR, Lomas MW, Mandecki J, Walker DE. (2007). *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. Geophys Res Lett 34: L10604

- De Corte D, Sintes E, Yokokawa T, Reinthaler T, Herndl GJ. (2012). Links between viruses and prokaryotes throughout the water column along a North Atlantik latitudinal transect. ISME J 12: 1751-7362

- Del Giorgio P, Bird DF, Prairie YT, Planas D. (1996). Flow cytometric determination of Bacterial abundance in lake plankton with the green nucleic acid stain SYTO 13. Limnol Oceanogr 41: 783-789.

- Eichinger M, Poggiale JC, Van Wambeke F, Lefévre D, Sempéré R. (2006). Modelling DOC assimilation and Bacterial growth efficiency in biodegradation

experiments: a case study in the Northeast Atlantic Ocean. Aquat Microb Ecol 43: 139-151

- England MH. (1995). The Age of Water and Ventialation Timescales in a Global Ocean Model. J Phys Oceanogr 25: 2756-2777

- Felsenstein J. (1985). Confidence-limits on phylogenies – an approach using the bootstrap. Evolution 39: 783-791

- Francis CA, Beman JM, Kuypers MMM. (2007). New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J 1: 19-27

- Herndl GJ, Reinthaler T, Teira E, van Aken H, Veth C, Pernthaler A, Pernthaler J. (2005). Contribution of Archaea to Total Prokaryotic Production in the Deep Atlantic Ocean. Appl Environ Microbiol 71: 2303-2309

- Hopkinson CS, Fry B, Nolin AL. (1996). Stoichiometry of dissolved organic matter dynamics on the continental shelf of the northeastern U.S.A. Cont Shelf Res 17: 473-489

- Hopkinson CS, Vallino JJ. (2005). Efficient export of carbon to the deep ocean through dissolved organic matter. Nature 433: 142-145

- Howarth RW. (1988). Nutrient limitation of net primary production in marine ecosystems. Annu Rev Ecol Syst 19: 89-110

- Jiao N, Herndl GJ, Hansell DA, Benner R, Kattner G, Wilhelm SW, Kirchman DL, Weinbauer MG, Luo T, Chen F, Azam F. (2010). Microbial production of

recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nature Rev Microbiol 8: 593-599

- Joint I, Henriksen P, Fonnes GA, Bourne D, Thingstad TF, Riemann B. (2002). Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquat Microb Ecol 29:145-159

- Kirchman DL, Meon B, Ducklow HW, Carlson CA, Hansell DA, Steward GF. (2001). Glucose fluxes and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. Deep-Sea Res. II 48: 4179-4197

- Kirchman DL. (2000). Uptake and regeneration of inorganic nutrients by marine heterotrophic Bacteria. In: Kirchman DL (ed) Microbial ecology of the oceans. John Wiley & Sons, New York, p 261-288

- Könneke M, Bernherd A, De la Torre J, Walker C. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543-546

- Letunic I, and Bork P. (2007). Interactive tTree Of Live (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23: 127-128

- Lin Z, Kong H, Nei M, Ma H. (2006). Origins and evolution of the recA/RAD51 gene family: Evidence for ancient gene duplication and endosymbiotic gene transfer. Proc Natl Acad Sci USA 103: 10328-10333

- Longhurst A, Sathyendranath S, Platt T, Caverhill C. (1995). An estimate of global primary production in the ocean from satellite radiometer data. J Plankton Res 17: 1245-1271

- Longhurst AR. (1998). Ecological Geography of the Sea. Academic Press: San Diego, CA, USA

- Lozupone C, Hamady M, and Knight R. (2006). UniFrac – an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics 7: 371. doi:10.1186/1471-2105-7-371

- Middelburg JJ, and Nieuwenhuize J. (2000). Nitrogen uptake by heterotrophic Bacteria and phytoplankton in the nitrate-rich Thames estuary. Mar Ecol Prog Ser 203: 13-21

- Miller RV, and Kokjohn TA. (1990). General Microbiology of recA: Environmental and Evolutionary Significance. Annu Rev Microbiol 44: 365-94

- Moore LR, Rocap G, Chisholm SW. (1998). Physiology and molecular phylogeny of coexisting Prochlorococcus ecotypes. Nature 67: 393-464

- Moreno-Vivan C, Soler G, Castillo F. (1992). Arginine catabolism in the phototrophic bacterium Rhodobacter capsulatus E1F1. Eur J Biochem 204: 531-537

- Murphy J, Riley JP. (1962). A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta, 27: 31-36

- Mumy KL, and Findlay RH. (2004). Convenient determination of DNA extraction efficiency using an external DNA recovery standard and quantitative-competitive PCR. Journal Microbiol Meth 57: 259-268

- Ovreas L, Bourne D, Sandaa RA, Casamayor EO, Benlloch S, Goddard V, Smerdon G, Heldal M, Thingstad TF. (2003). Response of Bacterial and viral communities to nutrient manipulations in seawater mesocosms. Aquat Microb Ecol 31: 109-121

- Parsons T, Maita Y, Lalli C. (1984). A manual of chemical biological methods for seawater analysis. Pergamon Press, Oxford, UK 173pp

- Palmer JR and Totterdell IJ. (2001). Production and export in a global ocean ecosystem model. Deep-Sea Res 48: 1169- 1198

- Reinthaler T, van Aken H, Veth C, Arístegui J, Robinson C, Williams PjleB, Lebaron P, Herndl GJ. (2006). Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnol Oceanogr 51: 1262-1273

- Richardson DJ, Berks BC, Russell DA, Spiro S, Taylor CJ. (2001). Functional, biochemical and genetic diversity of prokaryotic nitrate reductases. Cell Mol Life Sci 58: 165-178

- Saitou N, and Nei M. (1987). The neighbour-joining method – a new method for reconstructiong phylogenetic trees. Mol Biol Evol 4: 406-425

- Sarmiento JL and Gruber N. (2006). In: Sarmiento JL and Gruber N. (ed) Ocean Biogeochemical Dynamics. Princeton, New Jersey

- Schattenhofer M, Fuchs BM, Amann R, Zubkov MV, Tarran GA, Pernthaler J. (2009). Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. Environ Microbiol 11: 2078-2093

- Schlesinger WH. (1991). Biogeochemistry: An Analysis of Global Change. Academic Press

- Smethie WM, Fine RA, Putzka A, Jones EP. (2000). Tracing the flow of North Atlantic Deep Water using chlorofluorocarbons. Journal of Geophysical Research 105C6: 14,297-14,323

- Talley LD. (1999). Some aspects of ocean heat transport by the shallow, intermediate and deep overturning circulations. AGU Geophysical Monograph 112

- Tamura K, Nei M, Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbour-joining method. Proc Natl Acad Sci USA 101: 11030-11035

- Tamura K, Dudley J, Nei M, Kumar S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596-1599

- Teira E, Lebaron P, van Aken H, Herndl GJ. (2006). Distribution and Activity of Bacteria and Archaea in the Deep Water Masses of the North Atlantic. Limnol Oceanogr 51: 2131-2144

- Van Aken HM. GEOTRACES, the hydrography of the Western Atlantic Ocean, cruises 64PE319, 64PE321, and 74JC057

- Vitousek PM and Howarth RW. (1991). Nitrogen limitation on land and in sea: How can it occure? Biogeochem 13: 87-115

- Zehr JP and Kudela RM. (2011). Nitrogen Cycle of the Open Ocean: From Genes to Ecosystems. Annu Rev Mar Sci 3: 197-225

- Zehr JP and Paerl HW. (2008). In: Microbial ecology of the oceans Chapter 13: Molecular ecological aspects of nitrogen fixation in the marine environment (Kirchman DL). John Wiley & Sons. DOI: 10.1002/9780470281840.ch13

- Zehr JP, Mellon MT, Zani S. (1998). New nitrogen fixing microorganisms detected in oligotrophic oceans by the amplification of nitrogenase (nifH) genes. Appl Environ Microbiol 64: 3444-3450

Tables and Figures

Table 1. Primers used to amplify the *nas*A gene and the *rec*A gene fragments for q-PCR and cloning and sequencing.

Method	Primer	Sequence (5' to 3')	Annealing temp.	Reference
PCR	nas22	TGYCCNTAYTGYGGNGT	55°C	Allen *et al*. 2001
	nas1933	CARTGCATNGGNAYRAA	55°C	Allen *et al*. 2001
	nasA964	CARCCNAAYGCNATGGG	55°C	Allen *et al*. 2001
	nasA1735	ATNGTRTGCCAYTGRTC	55°C	Allen *et al*. 2001
Cloning	M13 forward	GTAAAACGACGGCCAG	60°C	Invitrogen
	M13 reverse	CAGGAAACAGCTATGAC	60°C	Invitrogen
qPCR	nasA964	CARCCNAAYGCNATGGG	65°C	Allen *et al*. 2001
	nasA1735	ATNGTRTGCCAYTGRTC	65°C	Allen *et al*. 2001
	recAF	TGTGCNTTTATWGATGCNGAGCATGC	53°C	Holmes *et al*. 2004
	recAR	CCCATGTCNCCTTCKATTTCNGCTTT	53°C	Holmes *et al*. 2004

Table 2. Multivariate multiple regression analysis with forward selection (DISTML forward) performed on biotic and abiotic parameters to explain the *nas*A gene abundance in depth layers through the water column.

Depth layers	Variable	pseudo-F	P	r^2	cumulative
Epipelagic (n=26)	Latitude	24.3839	0.001	0.504	0.504
	NO3	4.2091	0.044	0.0767	0.5807
	RecA	7.4068	0.015	0.1056	0.6863
Upper Meso (n=46)	RecA	19.5865	0.001	0.308	0.308
	Latitude	14.6352	0.001	0.1757	0.4837
	Temp	8.8675	0.002	0.09	0.5737
Lower Meso (n=27)	NO3	12.3157	0.003	0.33	0.33
	RecA	5.6681	0.03	0.128	0.458
	Latitude	5.8169	0.029	0.1094	0.5674
	bac	8.5997	0.01	0.1216	0.689
	PP	5.1394	0.025	0.0611	0.7502
	Temp	4.9633	0.043	0.0501	0.8081
Upper Bathy (n=50)	PP	14.7098	0.002	0.2346	0.2346
Lower Bathy (n=76)	bac	53.4415	0.001	0.4193	0.4193
	Latitude	6.7751	0.01	0.0493	0.4687

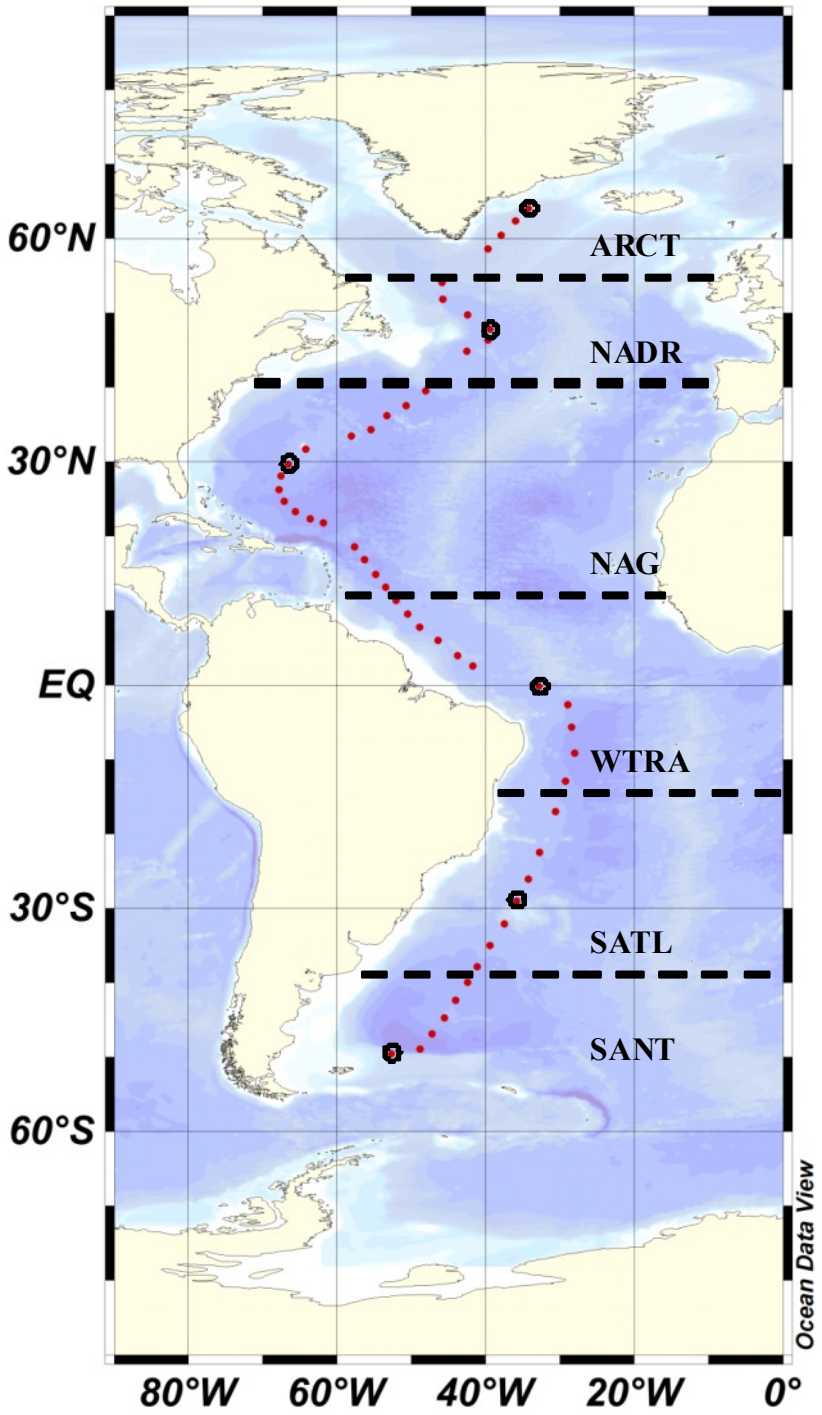

Figure 1. Cruise track and sampling stations (indicated by dots) occupied during the GEOTRACES cruises 1, 2 and 3 in April/July 2010 and February/March 2011. Dashed horizontal lines denote borders between oceanic provinces as defined by Longhurst (1998). Encircled dots indicate stations where samples were taken for establishing clone libraries.

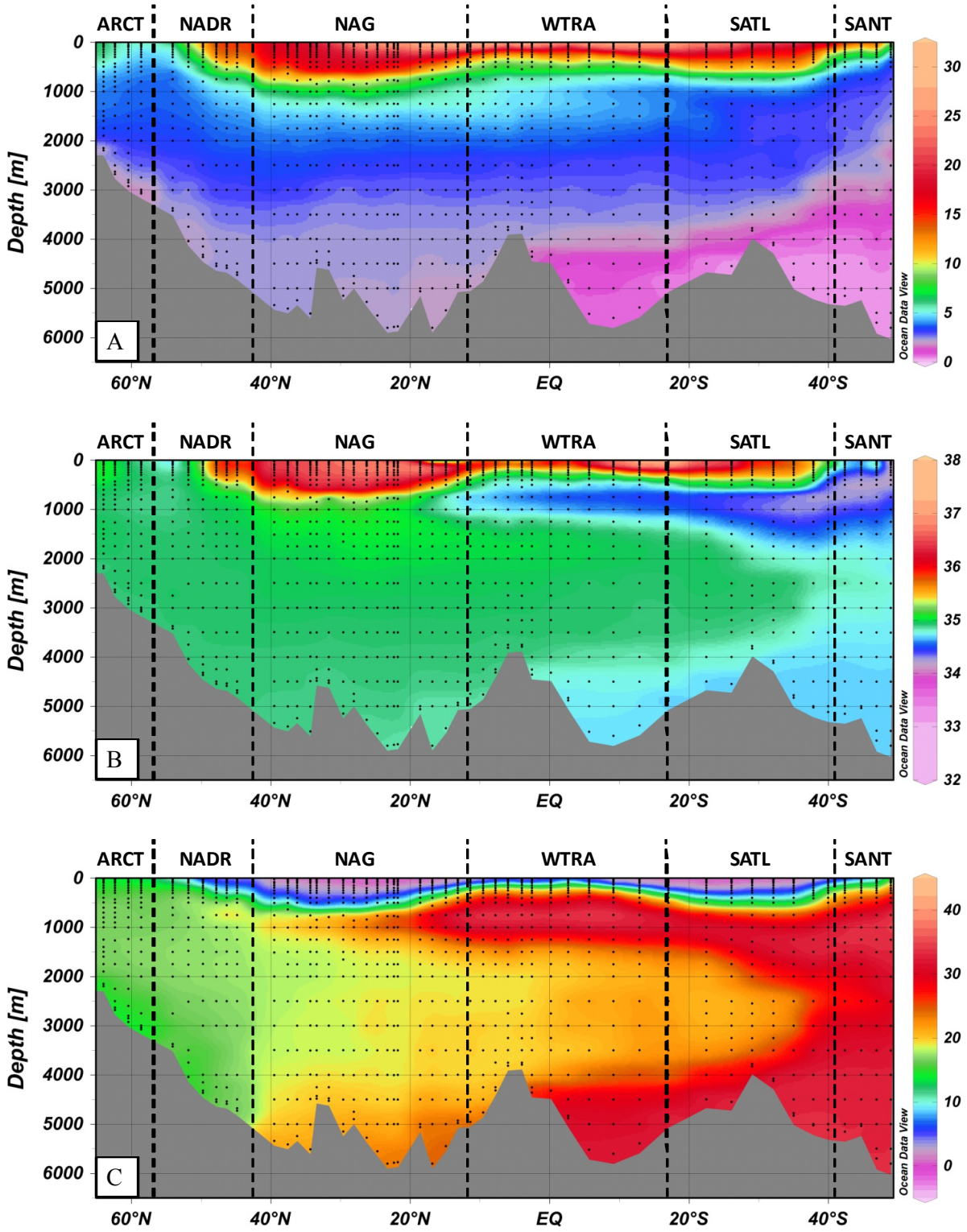

Figure 2. (A) Temperature (in °C), (B) salinity and (C) nitrate concentrations (µmol kg^{-1}) throughout the western Atlantic Ocean from 64°N to 45°S from the epi- to the lower bathypelagic waters. Dashed lines indicate boundaries between oceanographic provinces: North Atlantic Arctic province (ARCT), North Atlantic Drift province (NADR), North Atlantic Gyral province (NAG), Western Tropical Atlantic province (WTRA), South Atlantic Gyral Province (SATL) and the Subantarctic province (SANT) (Longhurst, 1998).

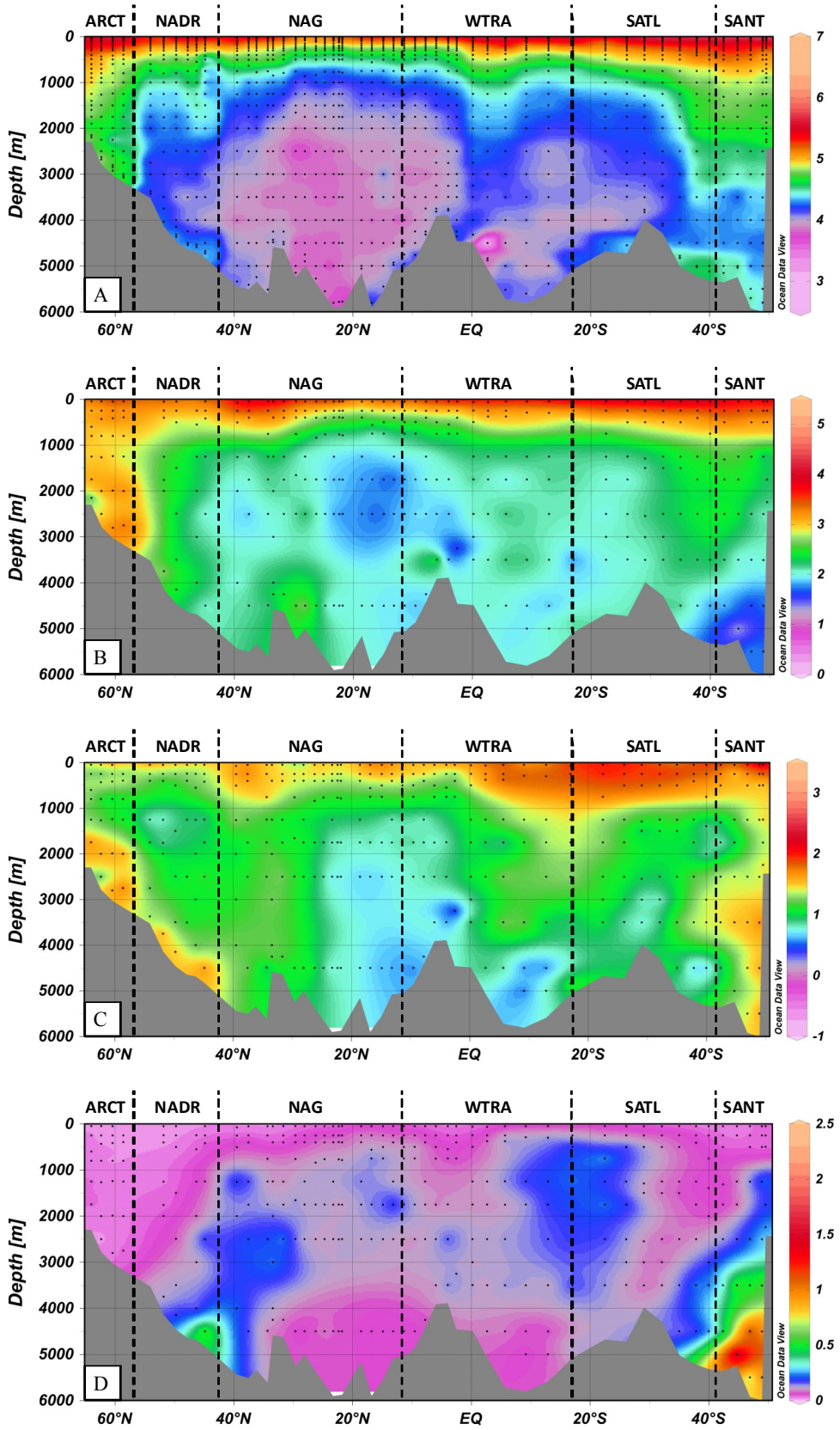

Figure 3. Latitudinal and depth distribution of (A) prokaryotic abundance (log cells mL^{-1}), (B) *rec*A gene abundance (log gene abundance mL^{-1}), (C) *nas*A gene abundance (log gene abundance mL^{-1}), and (D) *nas*A:*rec*A gene ratio along the transect in the western Atlantic Ocean.

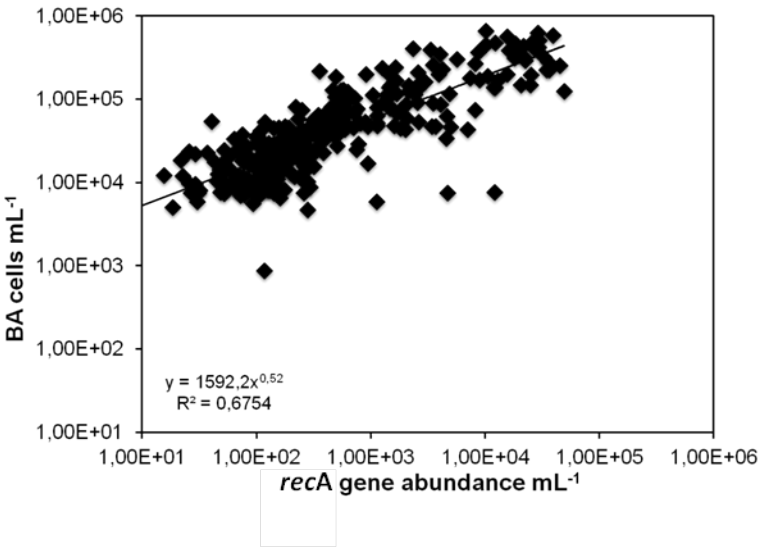

Figure 4. Correlation between *rec*A gene abundance determined by q-PCR and prokaryotic abundance determined by flow cytometry throughout the Atlantic.

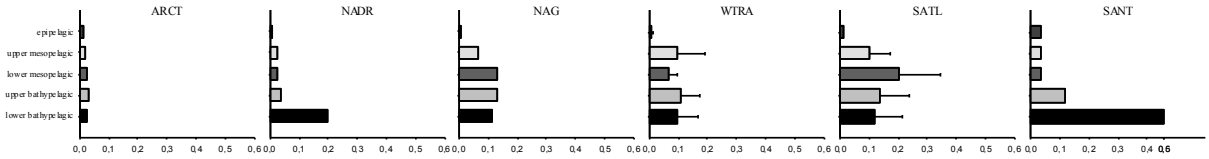

Figure 5. Average ± standard deviation of *nas*A:*rec*A gene ratio at specific depth layers (epipelagic, upper mesopelagic, lower mesopelagic, upper bathypelagic and lower bathypelagic) in the different oceanic provinces (ARCT, NADR, NAG, WTRA, SATL and SANT) of the western Atlantic .

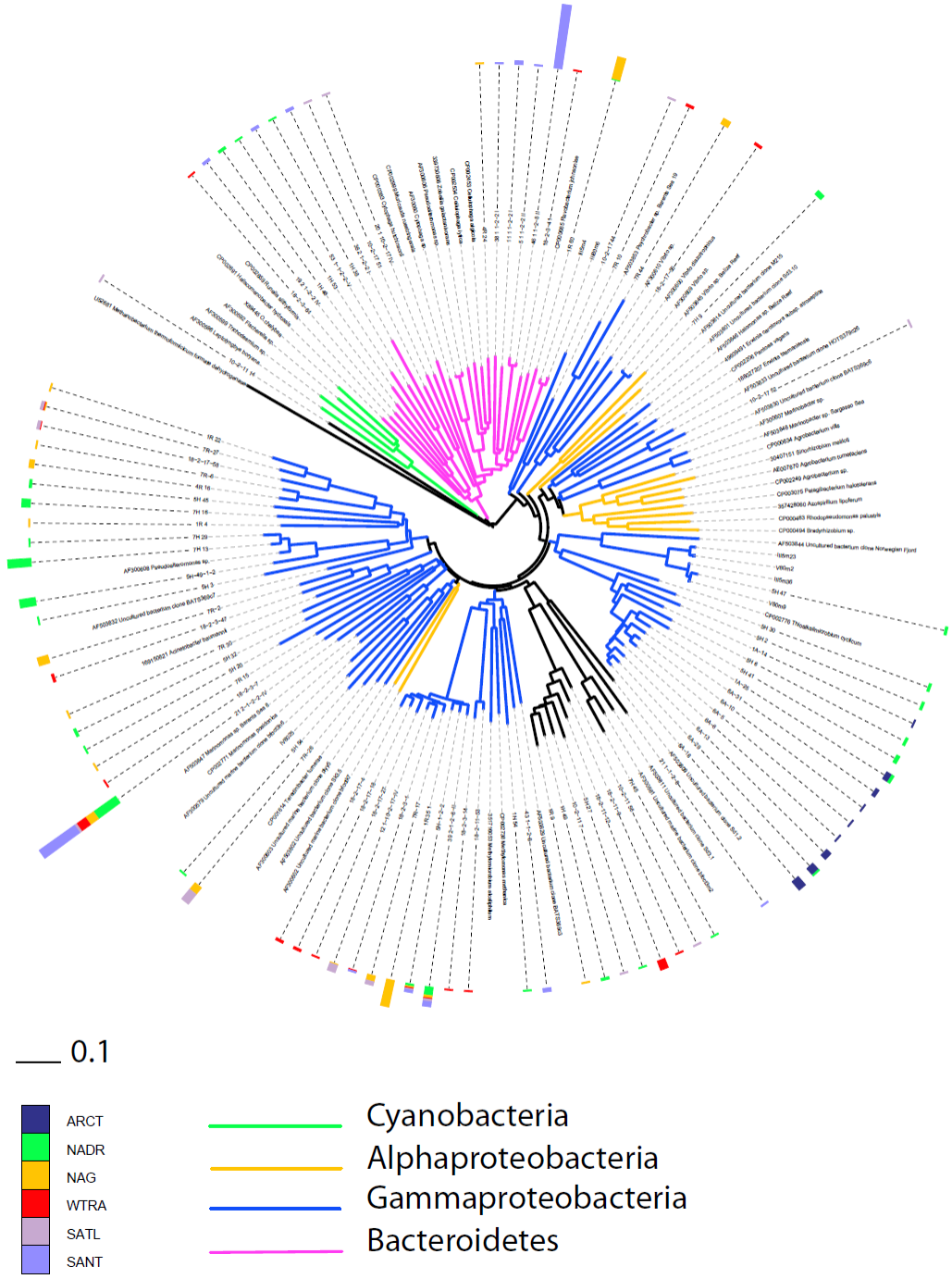

Figure 6. Neighbor-Joining phylogenetic tree from the *nas*A gene sequences. One representative of sequence group ≥ 97% identical is shown.

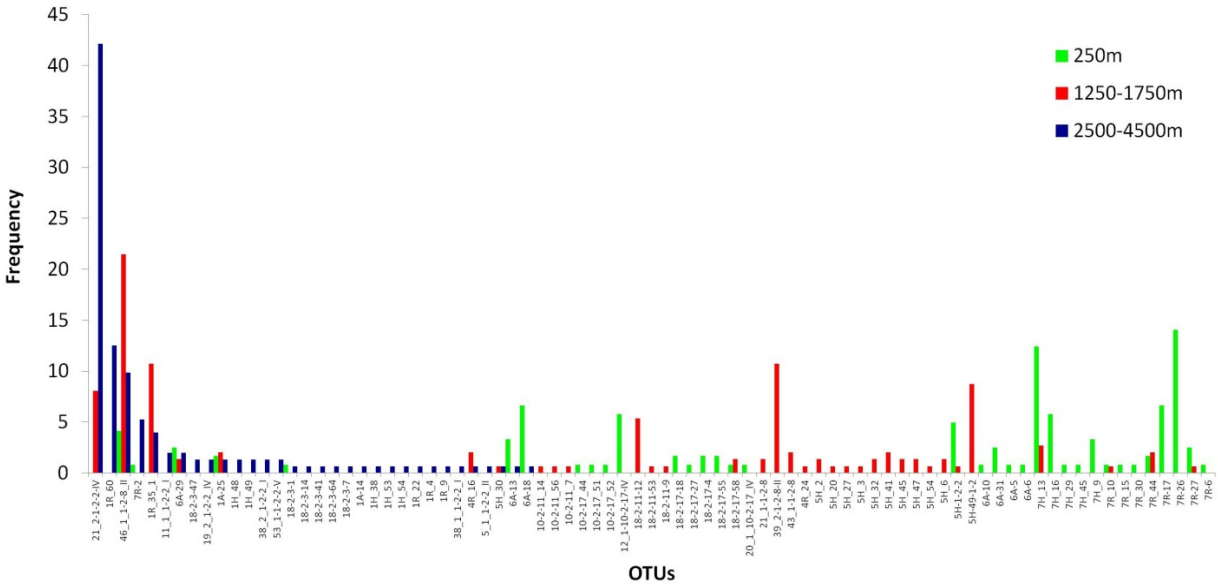

Figure 7. Frequency of different OTUs at specific depths (250m, 1250 – 1750m, 2500 – 4500m)

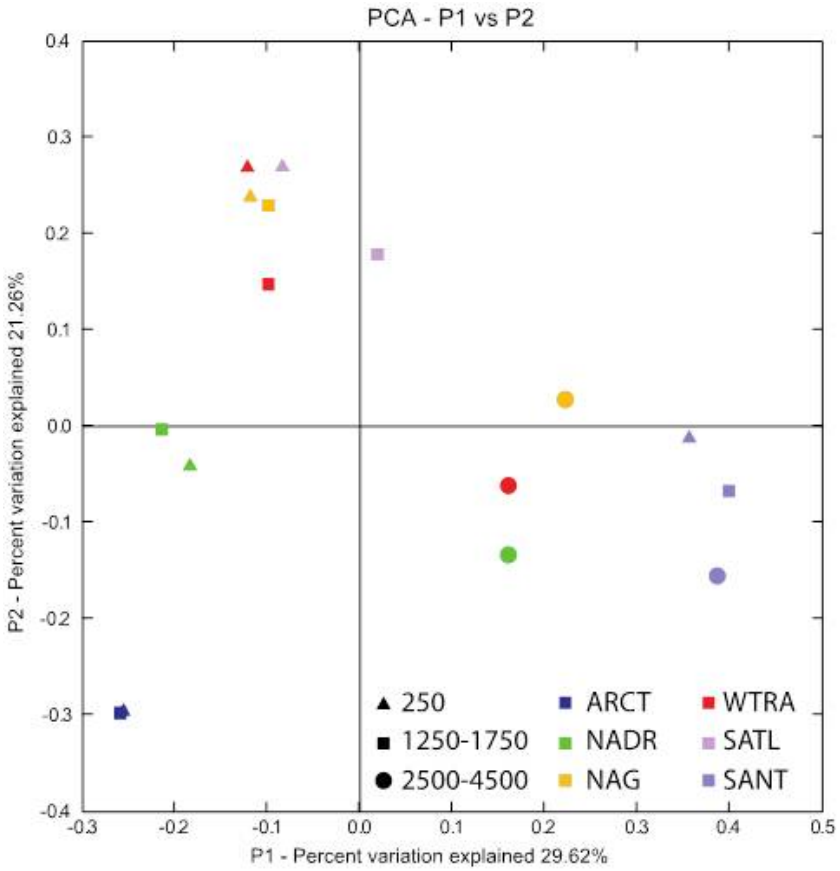

Figure 8. PCA analysis of the *nas*A gene clone libraries obtained at different provinces and depth layers in the Atlantic Ocean.

Supplementary Table 1. Average (av) and standard deviation (sd) of abiotic and biotic parameters of the different oceanic provinces and depth layers. Abbreviations: PA, prokaryotic abundance

EPI		Temp [°C]	Salinity	PA [N x 10^4mL^{-1}]	NO_3 [µmol kg^{-1}]	*nas*A [gene mL^{-1}]	*rec*A [gene mL^{-1}]	*nas*A/*rec*A
ARCT	av	5.47	34.96	31.8	13.74	66.1	10219.23	0.013
	sd	0.81	0.11	11.7	1.11	66.05	14598.31	0.006
NADR	av	11.39	35.4	24.2	7.11	45.53	4562.22	0.01
	sd	3.73	0.55	13.4	4.66	8.7	458.83	0
NAG	av	22.58	36.42	17.7	0.76	53.6	18055.32	0.005
	sd	4.05	0.9	9.26	1.22	62.04	13586.31	0.007
WTRA	av	24.14	36.01	25.6	6.5	50.34	10246.71	0.007
	sd	5.68	0.68	13.7	8.19	21.91	6620.88	0.004
SATL	av	21.12	36.17	34.6	1.9	120.53	21688.89	0.01
	sd	3.77	0.57	13.1	1.78	0	0	0
SANT	av	11.77	34.56	49.4E	11.93	331.76	12673.33	0.033
	sd	4.83	0.49	28.6E	7.51	192.95	2798.91	0.01
average		16.08	35.59	30.6	6.99	111.31	12907.62	0.013
sd		1.65	0.26	6.8	3.2	71.26	6451.52	0.004
Upper Meso		**Temp [°C]**	**Salinity**	**PA [N x 10^4mL^{-1}]**	**NO_3 [µmol kg^{-1}]**	***nas*A [gene mL^{-1}]**	***rec*A [gene mL^{-1}]**	***nas*A/*rec*A**
ARCT	av	4.97	34.98	14.4	15.95	33.11	2223.02	0.022
	sd	0.9	0.08	6.94	0.79	17.76	1754.76	0.016
NADR	av	8.91	35.26	8.23	14.75	16.16	1036.01	0.025
	sd	4.02	0.43	2.8	4.56	9.46	550.55	0.026
NAG	av	17.3	36.41	5.19	6.9	39.7	1169.51	0.067
	sd	1.88	0.32	1.51	5.42	33.73	1714.14	0.047
WTRA	av	10.11	34.97	7.82	27.2	54.7	793.08	0.098
	sd	2.07	0.26	9.4	4.96	48.39	701.89	0.095
SATL	av	14.1	35.41	11.9	8.57	105.19	1306.24	0.102
	sd	2.12	0.3	3.42	5.61	58.85	813.9	0.072
SANT	av	5.28	34.27	16.8	24.74	68.48	2266.67	0.033
	sd	2.24	0.2	4.23	4.51	26.44	1031.47	0.01
average		10.11	35.22	10.7	16.35	52.89	1465.75	0.058
sd		1.01	0.12	2.9	1.78	18.62	520.12	0.033

Continuation of Supplementary Table 1.

Lower Meso		Temp [°C]	Salinity	PA [N x 10^4mL^{-1}]	NO_3 [µmol kg^{-1}]	*nas*A [gene mL^{-1}]	*rec*A [gene mL^{-1}]	*nas*A/*rec*A
ARCT	av	4.01	34.91	8.68	16.51	50.63	1378.23	0.028
	sd	0.30	0.03	2.66	0.18	69.95	1481.92	0.015
NADR	av	5.64	34.97	4.00	18.36	9.28	389.65	0.025
	sd	2.04	0.10	1.77	1.82	7.10	216.10	0.007
NAG	av	10.65	35.41	2.77	21.07	19.10	169.21	0.133
	sd	3.50	0.50	1.21	8.10	22.11	218.49	0.041
WTRA	av	5.90	34.59	3.50	33.68	11.61	196.49	0.066
	sd	1.22	0.10	1.24	1.35	7.44	148.23	0.033
SATL	av	6.90	34.52	5.98	24.33	62.33	411.02	0.202
	sd	2.89	0.29	2.00	6.44	13.21	189.08	0.140
SANT	av	3.25	34.29	9.87	31.27	27.92	1038.80	0.035
	sd	0.63	0.12	2.49	3.06	11.13	476.82	0.024
average		**6.06**	**34.78**	**5.8**	**24.20**	**30.14**	**597.23**	**0.081**
sd		**1.27**	**0.17**	**0.6**	**3.12**	**24.20**	**516.31**	**0.049**
Upper Bathy		**Temp [°C]**	**Salinity**	**PA [N x 10^4mL^{-1}]**	**NO_3 [µmol kg^{-1}]**	***nas*A [gene mL^{-1}]**	***rec*A [gene mL^{-1}]**	***nas*A/*rec*A**
ARCT	av	3.53	34.92	5.67	16.61	39.23	1691.20	0.032
	sd	0.21	0.02	2.17	0.19	43.71	1333.95	0.024
NADR	av	3.86	34.93	2.55	17.11	7.39	215.27	0.035
	sd	0.44	0.03	1.08	0.31	4.52	63.69	0.019
NAG	av	4.77	35.01	1.35	19.88	10.86	113.74	0.134
	sd	0.92	0.06	0.440	2.90	8.17	142.57	0.103
WTRA	av	4.35	34.88	1.86E	24.64	14.84	134.55	0.108
	sd	0.41	0.14	0.723E	5.01	12.98	82.91	0.063
SATL	av	3.33	34.63	2.83	29.16	18.57	173.31	0.136
	sd	0.38	0.21	1.41	4.10	9.74	94.14	0.104
SANT	av	2.61	34.58	6.03	32.86	20.51	293.45	0.119
	sd	0.37	0.13	1.23	1.15	25.38	173.32	0.144
average		**3.74**	**34.82**	**3.4**	**23.38**	**18.57**	**436.92**	**0.094**
sd		**0.24**	**0.07**	**0.6**	**2.03**	**14.73**	**500.77**	**0.049**

Continuation of Supplementary Table 1.

Lower Bathy		Temp [°C]	Salinity	PA [N x 10^4mL^{-1}]	NO_3 [µmol kg^{-1}]	*nas*A [gene mL^{-1}]	*rec*A [gene mL^{-1}]	*nas*A/*rec*A
ARCT	av	2.27	34.91	5.07	14.63	146.75	4606.41	0.028
	sd	0.66	0.02	1.86	1.19	126.25	2148.21	0.015
NADR	av	2.55	34.91	1.57	16.23	32.15	248.11	0.200
	sd	0.46	0.01	0.43	0.85	31.24	287.38	0.246
NAG	av	2.55	34.91	0.84	19.64	12.29	284.43	0.113
	sd	0.48	0.04	0.2	1.64	9.58	819.24	0.097
WTRA	av	2.35	34.88	1.14	21.91	11.39	558.76	0.096
	sd	0.79	0.08	0.4	4.02	11.45	2301.84	0.071
SATL	av	1.93	34.82	1.76	25.76	15.60	152.23	0.118
	sd	1.05	0.10	0.90	4.85	12.17	87.08	0.096
SANT	av	1.14	34.72	2.96	31.36	33.56	150.64	0.513
	sd	0.85	0.05	0.98	1.94	21.74	144.98	0.636
average		**2.13**	**34.86**	**2.22**	**21.59**	**41.96**	**1000.10**	**0.178**
sd		**0.23**	**0.03**	**0.6**	**1.63**	**45.25**	**1011.01**	**0.230**

Curriculum Vitae

Paul Alan Steiner

Education

2013	Diploma thesis at the Department of Marine Biology, University of Vienna; Diploma title: Abundance and diversity of nitrate assimilating bacteria in the deep waters of the Atlantic Ocean
2006-2013	Diploma study of Biology/Ecology with specification in marine biology at the University of Vienna
2004-2005	Civil Service at the AKH Linz (Hospital pharmacy)
2000-2004	Bundes Oberstufen Realgymnasium (BORG) Linz Honauerstraße 24
1996-2000	Akademisches Gymnasium, Linz
1992-1996	Primary School, VS9, Linz

Publications

‘Temporal dynamics in the free-living bacterial community composition in the coastal North Sea’
Eva Sintes, Harry Witte, Karen Stodderegger, Paul Steiner, Gerhard J. Herndl (2012). FEMS Microbiology Ecology. DOI: 10.1111/1574-6941.12003

Marine and biological field courses

2012	MEDEA II cruise, on board of the RV *Pelagia* (Texel/The Netherlands – Iceland)
2011	Practical course on marine biology, Texel/The Netherlands
2011	Practical course for protection of sea turtles (*Caretta caretta*) in the Mediterranean Sea (Fethiye, Turkey)
2011	Marine biological field course on the mediterranean fauna and flora; Centre for Marine Research (Rovinj, Croatia)
2011	Tropical excursion at the field station La Gamba (Costa Rica)
2010	Marine biological field course (Piran, Slovenia)
2010	Biology and systematics of neotropical amphibians (French Guyana)
2009	Practical course on terrestrial ecology of Siberia (from the steppe to the tundra)

Pesonal skills

Languages	German (first language), English (fluent)
Computer skills	MS Office, Sigma Plot, Ocean Data View (ODV)

Additional work experience

Rock/Funk/HipHop band – Drums

Background music group - Percussion

Mango farm (Australia) – Worker

Apple farm (New Zealand) – Worker

Office ‘Ziviltechnik Geologie’ (Linz, Austria) – Trainee

Steel industry VOEST (Linz, Austria) – Trainee

Interests

Music, art