Letter

Distribution, sources and sinks of cyanate in the coastal North Atlantic Ocean

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Distribution, sources and sinks of cyanate in the coastal North Atlantic Ocean

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ABSTRACT

Based on reverse genomics and growth of cultured populations, it has been hypothesized that cyanate is utilized as a nitrogen source by ubiquitous groups of marine phytoplankton. Recently a nanomolar method was developed to measure cyanate concentrations in marine and estuarine waters. Here we report the first measurements of cyanate distributions, biological utilization, and production from the coastal North Atlantic Ocean. Cyanate concentrations were highest below the chlorophyll maximum at many stations but were high throughout the water column on the shallow Georges Bank where chlorophyll concentrations were especially high down to the bottom, suggesting production by organic matter degradation or release by phytoplankton. Here we demonstrate that cyanate is produced in senescent algal cultures and through photochemical reactions at rates comparable to production of other labile nitrogen compounds. Cyanate uptake accounted for up to 10% of total N uptake at an oligotrophic mid-Atlantic Bight station. Our results suggest that cyanate may be an important but hitherto overlooked component of the marine nitrogen cycle.

Introduction

Nitrogen (N) limits phytoplankton growth in most marine environments. Consequently, identifying sources and sinks of bioavailable N is critical for estimating oceanic primary and secondary productivity. While many dissolved organic nitrogen (DON) compounds are known to be bioavailable, much of that pool is uncharacterized.[1] Recently it was discovered that some microbes have the genetic capacity to take up and metabolize cyanate (OCN^-), perhaps the simplest DON compound. Genes encoding intracellular cyanate decomposition and a cyanate-specific transporter have been identified in marine cyanobacteria,[2-4] and isolates of *Synechococcus* (WH8102), *Prochlorococcus* (MED4, SB), a coastal dinoflagellate, *Prorocentrum donghaiense*, and some heterotrophic bacteria have been cultured using cyanate as the sole N source.[5-8] It has been hypothesized that the evolution of *Prochlorococcus* strains has been driven by the availability of different N sources. Because *Prochlorococcus* have streamlined genomes likely containing only the genes necessary for survival,[9,10] it is possible that *Prochlorococcus* strains living in the modern ocean and containing cyanate-related genes, utilize this compound in the environment. *Prochlorococcus* and *Synechococcus* account for two thirds of present day oceanic primary production,[9] therefore cyanate utilization could be globally significant and its biogeochemistry may affect global primary and secondary production. Cyanate has also been shown to support nitrification as both a reductant and N source in chemoautotrophic prokaryotic cultures,[11] which could have implications for N speciation in marine systems.

Cyanate is produced abiotically as a result of urea and carbamoyl phosphate decomposition.[12,13] As a simple molecule with chemical linkages common in organic matter, cyanate is likely produced by other largely unexplored biotic and abiotic processes in aquatic

systems such as pyrimidine, protein and peptide decomposition. However, the abundance and distribution of cyanate and its reactivity in marine environments is unknown because, until recently, we lacked a sensitive method to quantify it. Cyanate may have formed spontaneously on the prebiotic Earth,[14,15] and it is possible that cyanate played important roles in early Earth biogeochemistry,[16] contributing to the abiotic synthesis of pyrimidines[17] and adenosine diphosphate (ADP).[18] Cyanobacterial cyanate genes also appear to have evolved early[5] suggesting that cyanate could have also served as an N source for cyanobacteria living on the pre-oxygenated Earth. Understanding cyanate cycling in the modern ocean may therefore give important clues to both present day and early Earth N cycling.

We have developed a method to measure cyanate in seawater,[19] and here we provide the first observations of: 1) cyanate distributions in modern coastal waters, 2) cyanate production through biotic and abiotic processes, and 3) cyanate uptake by natural microbial communities.

Methods

Sample Collection and Analysis of N compounds

Samples were collected in the coastal and oligotrophic North Atlantic Ocean aboard the *R/V Henry B. Bigelow* and *R/V Hugh Sharp*, respectively, using a CTD-rosette equipped with twelve Niskin bottles. Water samples for determination of urea, nitrate, and nitrite, ammonium, and cyanate concentrations were collected from Niskin bottles (0.2 μm filtered) and analyzed using an Astoria-Pacific nutrient autoanalyzer during the *Bigelow* cruise.[20] The autoanalyzer was equipped with a waveguide during the *Sharp* cruise to achieve lower detection limits for nitrate and nitrite.[21] Ammoniuim concentrations were analyzed using the manual indophenol method (*Bigelow* cruise)[22] or the manual orthophthaldialdehyde method (*Sharp* cruise)[23].

Photochemical Experiments

Filtered (0.2 μm) water samples were collected from the Dismal Swamp (freshwater site), Elizabeth River (estuarine site), and Virginia Beach oceanfront (coastal oceanic site, Fig. S3 and Table S2). Samples were irradiated in a UV solar simulator for 2, 4, and 8 hours; 8 hours in the solar simulator equates to approximately 10.2 hours of midday winter sunlight[24,25]. Each sample was irradiated in triplicate quartz tubes, one of which was wrapped in aluminum foil as a dark control. Photoproduction rates were calculated as the difference between the mean of the irradiated quartz tubes and the dark control. To account for differences in source material (DOM composition) in the different water samples, we normalized photoproduction rates to the initial absorptivity at 300 nm, which is thought to represent humic substance absorbance,[26] and we report both absolute and normalized photoproduction rates (Table S2).

Culture Experiments

Cultures of two diatoms (*Thalassiosira pseudonana* and *Thalassiosira oceanica*) and one cyanobacterium (*Synechococcus* FWRI isolate CCFCW 502) were grown in batch on f/2 media[27] under fluorescent lighting supplied on a 12 h light/ 12 h dark cycle. The *Thalassiosira* cultures were axenic prior to the experiment, but we microscopically confirmed the presence of bacteria after the cultures had incubated for one week. Non-autofluorescent bacteria were present in the *Synechococcus* cultures both before and during the experiment.

Nitrogen Uptake

Uptake of N from NH_4^+, NO_3^-, NO_2^-, urea, and cyanate was measured at 3 depths at a station (72.2 °W, 31.5 °N) in the oligotrophic North Atlantic during the cruise aboard the *R/V Hugh Sharp* using stable isotopes as tracers.[28,29] Incubations were initiated with the addition of 40 nmol N l^{-1} ^{15}N-labeled substrate, and uptake rates were calculated using a mixing model.[28]

Expanded methods, results, and discussion describing control experiments for cyanate uptake are in the Supporting Information.

Results and Discussion

Vertical profiles of cyanate were measured in the North Atlantic Ocean on the continental slope near the Mid-Atlantic Bight (MAB, Figure S1). Cyanate, urea, nitrite, and ammonium concentrations all exhibited surface minima and subsurface maxima (Fig. 1A). Profiles of this shape typify biological N cycle intermediates and are generally thought to reflect the balance of biological consumption in surface waters, production in subsurface waters as a result of remineralization, and oxidation of organic matter.[30] Therefore, we infer that cyanate is biologically labile. Because cyanate exhibited vertical distributions similar to those of urea, ammonium, and nitrite, it is likely that cyanate production and consumption processes are similar to or linked with those N compounds.

Profiles of ammonium and nitrite generally reflect rates of removal through phytoplankton uptake in surface waters, ammonification and ammonium and nitrite oxidation (nitrification) in subsurface waters, and rates of production through excretion and organic matter degradation.[30,31] Recent evidence suggests that some cultured nitrifying bacteria can also oxidize cyanate when ammonium is unavailable.[11] If this process happens in the environment, it may partially explain the subsurface cyanate maximum as well as the depletion of cyanate below approximately 200 m. The gradual depletion of cyanate in deep waters is likely due to abiotic and/or biotic degradation of cyanate to ammonium and nitrification, but rates of cyanate depletion in the mesopelagic have not yet been measured. Although maximum cyanate concentrations were lower than those of urea, ammonium, and nitrite, cyanate utilization and remineralization may still be quantitatively important if its production and consumption are

tightly coupled, as has been shown for ammonium[30] and labile DON compounds such as dissolved free amino acids, both of which are generally present at submicromolar concentrations in most marine systems.[1]

To determine whether the relationship between cyanate distributions and those of other simple N compounds is consistent across a highly productive coastal environment, cyanate, ammonium, nitrite, and nitrate distributions were examined with respect to salinity, temperature and chlorophyll *a* concentrations in a physically, biologically, and chemically heterogeneous shallow coastal region in the Gulf of Maine (GOM). Vertical profiles were measured at nine stations along a south to north transect from the continental shelf slope, across Georges Bank (GB) and the GOM to the coast of Nova Scotia (Fig. 1B, Fig. S1, Table S1). Cyanate was generally more abundant on GB and in the GOM than in the more oligotrophic Gulf Stream-influenced slope waters. At stations on the continental slope and interior GOM basin, there were cyanate peaks below the chlorophyll maximum, similar to what was observed in the MAB (Fig. 1). However, on GB and at the nearshore station, elevated surface cyanate concentrations were coincident with weak stratification and high surface chlorophyll *a* concentrations. On GB, cyanate and chlorophyll *a* concentrations were also high throughout the water column all the way to the bottom suggesting a possible sedimentary source of cyanate (Fig. 1B). At these stations ammonium, nitrite, and nitrate were depleted in surface waters (Fig. S2).

We evaluated two potential *in situ* sources of cyanate that may in part explain the observed cyanate distributions: organic matter degradation and photoproduction. To determine whether cyanate could be produced by organic matter degradation, cyanate concentrations were measured in cultures of a coastal marine cyanobacterium, *Synechococcus* (strain CCFWC 502), and a coastal and an oceanic strain of a ubiquitous diatom genus, *Thalassiosira,* which is

136 commonly found in the study region,[32] *T. pseudonana* and *T. oceanica*, during exponential and
137 stationary growth phases. While cyanate concentrations always remained near the limit of
138 detection (0.4 nM) in *Synechococcus* cultures, cyanate concentrations in the *Thalassiosira*
139 cultures increased linearly as biomass decreased during late stationary phase (Fig. 2A)
140 suggesting that cyanate was produced during the decay of these organisms, potentially by
141 contaminating bacteria, or that it was released by senescent cells. The lack of cyanate
142 accumulation in *Synechococcus* cultures could have been because it wasn't produced or because
143 its production and consumption were tightly coupled. To our knowledge, the genome of
144 *Synechococcus* CCFWC 502 has not been sequenced, but another *Synechococcus* isolate
145 (WH8102) has been cultured on cyanate as the sole source of N.[33]
146 The vertical zonation of microbial communities with respect to light, physical gradients,
147 and availability of nitrogenous substrates results in similar segregation of nutrient regeneration
148 processes and accumulation of N cycle intermediates by depth within and below the euphotic
149 zone.[34] In vertical profiles collected from the MAB (Fig. 1A), the cyanate maximum was below
150 that of urea indicating that cyanate might be produced from biotic urea decomposition, analogous
151 to the observation that nitrite accumulates below the ammonium maximum as a result of
152 nitrification.[34] There is currently no known mechanism for biotic conversion of urea to cyanate,
153 but abiotic decomposition of biologically produced urea and carbamoyl phosphate have been
154 proposed as mechanisms of cyanate production in marine systems.[33] Because C-N linkages are
155 so common in organic matter it is also likely that there are other pathways of cyanate production
156 and decomposition, both biotic and abiotic, that have yet to be discovered. Many ubiquitous
157 phytoplankton are known to release labile metabolic intermediates during stationary and late
158 exponential phase[1] or when stressed[35] and so it is possible that *T. pseudonana* and *T. oceanica*

directly released cyanate or that they released urea, carbamoyl phosphate, or other labile DON compounds that then degraded to cyanate, possibly by prokaryotic heterotrophs. Phytoplankton release could explain the elevated cyanate concentrations correlated with high chlorophyll fluorescence on GB and at the nearshore end of the GOM transect. Prokaryotic organic matter degradation could also explain the observed cyanate accumulation below the subsurface chlorophyll maxima, as well as production of cyanate in the diatom cultures. GOM coastal waters experience dense algal blooms[36] which produce large amounts of labile dissolved organic matter including cyanate and/or cyanate precursors. Cyanate can then accumulate in place or nearby, depending on the rate of its production and circulation patterns.

Cyanate photoproduction was observed in all samples, and rates ranged from 0.4 to 14 nM h^{-1} (Fig. 2B, Table S2), which are similar in magnitude to ammonium and amino acid photoproduction rates.[37] Photoproduction of cyanate could have contributed to the elevated surface cyanate concentrations on GB and at the nearshore end of the GOM transect, particularly if biotic uptake was lower than photoproduction as has been observed for other simple organic compounds.[38] High cyanate concentrations near the coast relative to continental slope waters (Fig. 1B) could also indicate terrestrial cyanate sources, such as urban, industrial, and agricultural runoff and/or decomposition of N compounds therein (such as urea and organic matter) to cyanate.[12,39] Cyanate is not monitored in industrial or municipal wastewater discharges[40] so it is not known whether they are significant sources of cyanate to receiving waters. However, urea discharged from agricultural, urban, and wastewater sources[39] could contribute cyanate to estuarine and coastal systems.

To evaluate whether microbial assemblages can utilize cyanate, and whether cyanate N contributes substantially to microbial N uptake, community cyanate uptake rates were compared

182 with those of nitrate, nitrite, ammonium, and urea, at three depths at an oligotrophic station in the
183 North Atlantic (Fig S1). Cyanate and total N uptake were higher near the surface (cyanate
184 concentrations less than 1 nM) than at the chlorophyll fluorescence maximum where cyanate
185 concentrations were highest (Fig. 3A). Cyanate contributed up to 10% of total measured
186 community N uptake, and cyanate uptake rates were comparable to those of nitrate and nitrite but
187 lower than those of ammonium and urea (Fig. 3B, Table S3). Cyanate turnover times were 1.6
188 and 76 hours in surface waters and at the chlorophyll maximum (103 m), respectively, and were
189 shorter than turnover times calculated for nitrate and nitrite (Table S3).

190 The distribution of cyanate and the similarity in magnitude of production and community
191 uptake rates relative to those of other dissolved N compounds suggests that cyanate may be an
192 important component of the marine nitrogen cycle and that its production and consumption are
193 tightly coupled. Here we provide the first comprehensive set of measurements comparing the
194 distributions of cyanate to those of other biogeochemically important N compounds in the ocean.
195 We also demonstrate for the first time that cyanate can be produced via a biological source and
196 photoproduction, and that cyanate uptake may be quantitatively important in the environment.
197 However, many questions remain regarding the biotic and abiotic sources and sinks of cyanate in
198 disparate marine environments, the organisms and biochemical pathways that produce and
199 consume cyanate in the present day ocean, regional and seasonal trends in cyanate
200 biogeochemistry, and its possible role in the evolution of life.
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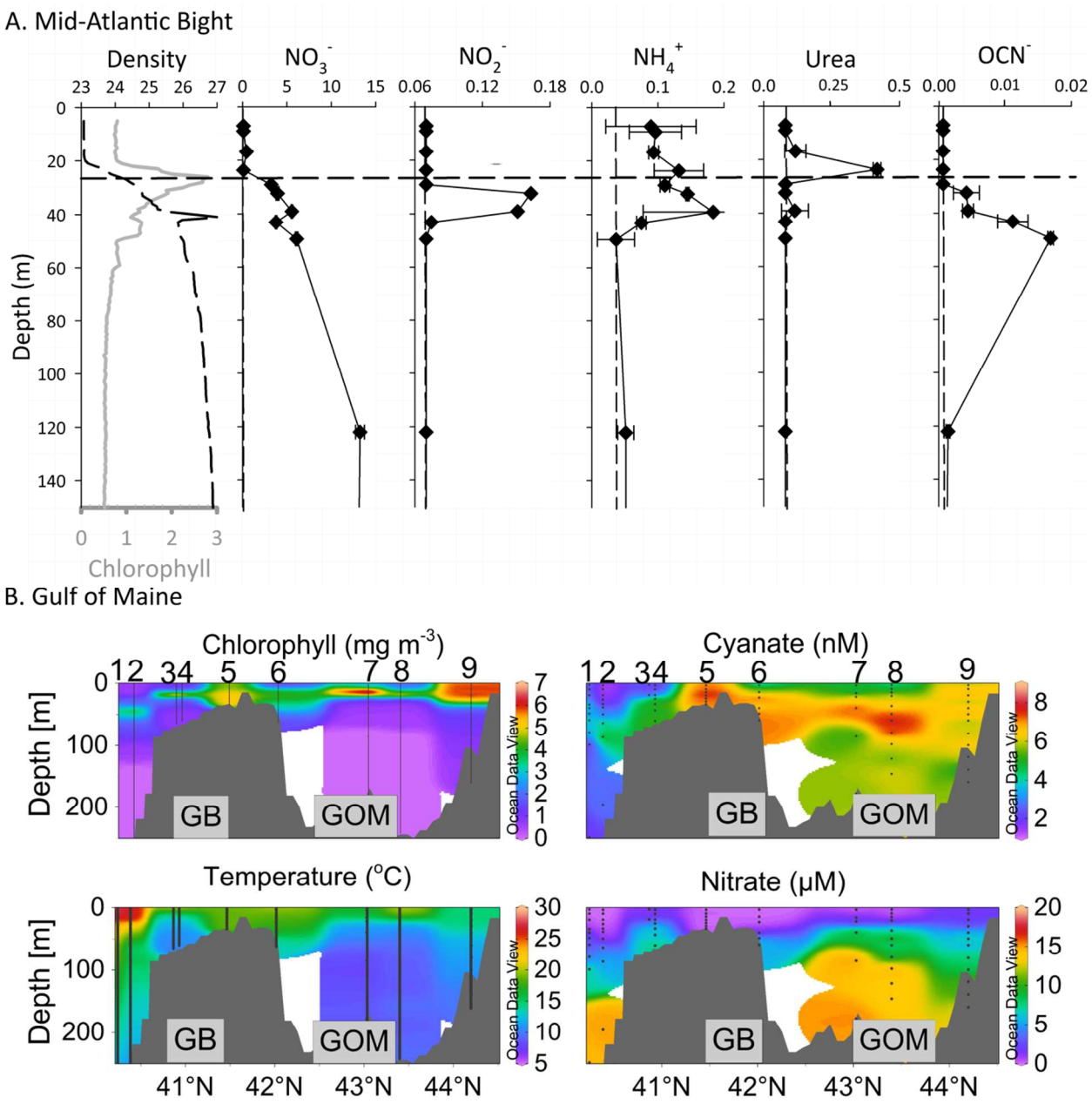

Figure 1. Cyanate Distribution in the Coastal North Atlantic. A) Vertical profiles of density (black dashed line, sigma theta, kg m^{-3}), chlorophyll *a* (grey solid line, mg m^{-3}), nitrate (NO_3^-) (µM), nitrite (NO_2^-) (µM), ammonium (NH_4^+) (µM), urea (µM), and cyanate (OCN^-) (µM) from a Mid-Atlantic Bight station. The dashed vertical lines are the method detection limits (S/N=3), and the dashed horizontal line indicates the depth of the chlorophyll maxima. Concentrations

209 below the detection limit were plotted as equal to the detection limit. Error bars are ± 1 standard
210 deviation. B) Chlorophyll *a* concentrations (mg m^{-3}), temperature (^{o}C), cyanate concentrations
211 (nM) and NO_3^- concentrations (μM) along a transect across the Gulf of Maine from a nearshore
212 station, across the Gulf of Maine (GOM) and over Georges Bank (GB). Grey lines (temperature
213 and chlorophyll) and dots (NO_3^- and OCN^-) represent sampling locations, and the colored
214 contours represent interpolations of the given parameters between those data points. See Fig. S1
215 for station map.

216

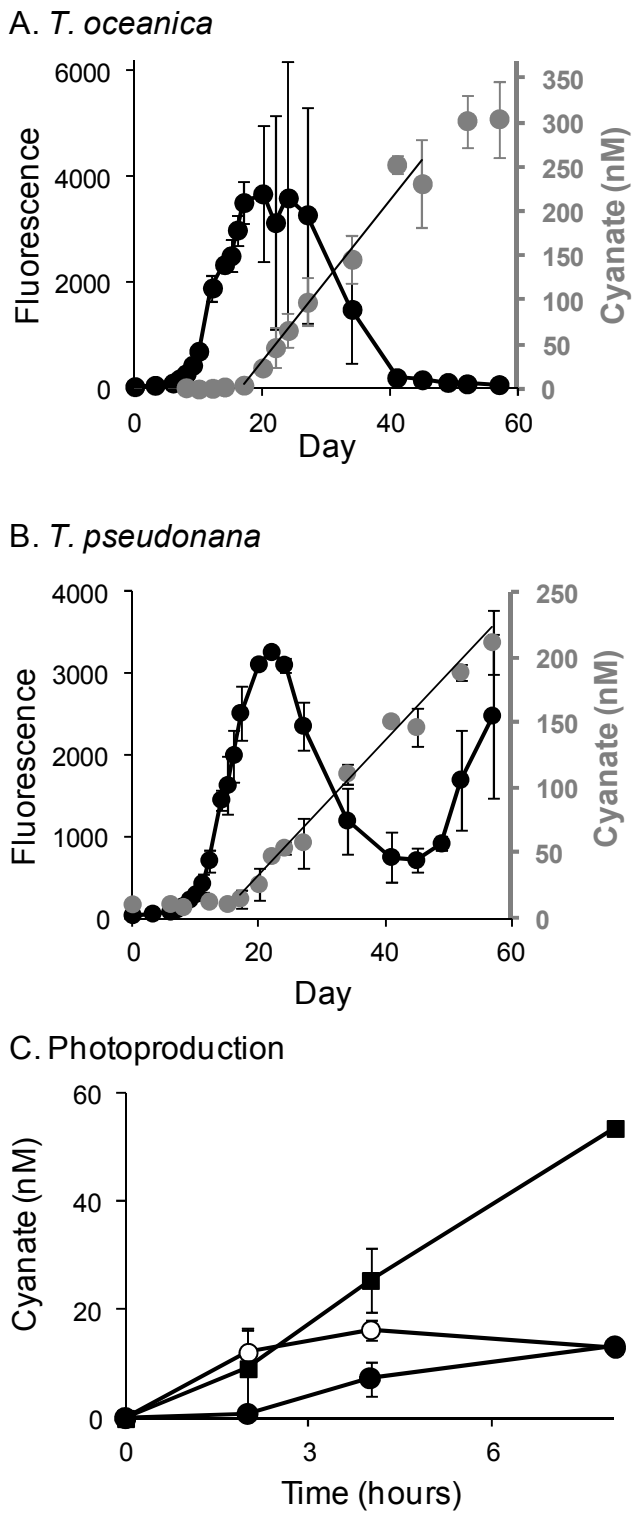

Figure 2. Cyanate Production. Production of cyanate in cultures of A) *Thalassiosira oceanica* and B) *Thalassiosira pseudonana*. *In vivo* fluorescence was used as a proxy for biomass and is

222 shown in black, and cyanate concentrations are shown in grey. Error bars are ± 1 standard
223 deviation (n=2). Cyanate production rates during the linear portions were 5 and 9 nM d^{-1} in *T.*
224 *pseudonana* and *T. oceanica* cultures, respectively (r^2 0.97 and 0.93, respectively; slope p-values
225 <0.0001). C) Photochemical production of cyanate in fresh (open circles), estuarine (squares),
226 and coastal oceanic (closed circles) sterile (0.2 μm filtered) water where cyanate concentrations
227 were calculated as the difference between the mean of the irradiated tubes and the dark controls.
228 Error bars are ± 1 standard deviation (n=2).

229

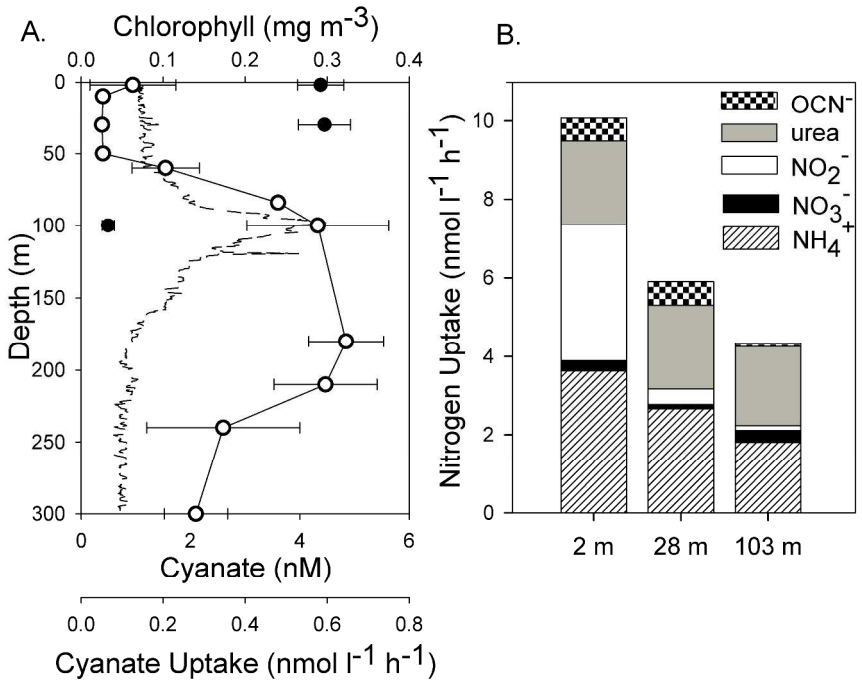

Figure 3. Cyanate and Total Nitrogen Uptake in at the North Atlantic Oligotrophic Station. A) Cyanate uptake (closed circles), cyanate concentration (open circles), and chlorophyll fluorescence (dashed line). Error bars are ± 1 standard deviation (n=3). B) Total N uptake at each depth as the sum of ammonium (diagonal lines), nitrate (solid black), nitrite (solid white), urea (solid grey), and cyanate (black and white checked) uptake.

237 ASSOCIATED CONTENT

238 Expanded Methods, Expanded Results and Discussion, Figures S1-3 and Tables S1-3. This
239 material is available free of charge via the Internet at http://pubs.acs.org.

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244 **Author Contributions**

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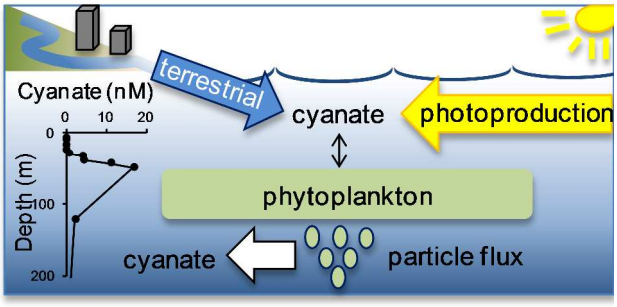

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375 Brittany Widner, Margaret R. Mulholland, and Kenneth Mopper