nature geoscience

ARTICLES

PUBLISHED ONLINE: 18 SEPTEMBER 2011 | DOI: 10.1038/NGEO1265

Assimilation of upwelled nitrate by small eukaryotes in the Sargasso Sea

Sarah E. Fawcett[1]*, Michael W. Lomas[2], John R. Casey[3], Bess B. Ward[1] and Daniel M. Sigman[1]

Phytoplankton growth is potentially limited by the scarcity of biologically available forms of nitrogen such as nitrate and ammonium. In the subtropical ocean gyres, water column stratification impedes the upward flux of nitrate to surface waters. Phytoplankton in these waters are assumed to rely largely on ammonium and other forms of nitrogen recycled during the breakdown of organic matter. Here, we use flow cytometry to separate prokaryotic and eukaryotic phytoplankton collected from Sargasso Sea surface waters in the summers of 2008 and 2009, and to analyse their respective nitrogen isotope ratios. We show that prokaryotes have a uniformly low ratio of ^{15}N to ^{14}N, $\delta^{15}N$, consistent with their reliance on recycled nitrogen. In contrast, small eukaryotic phytoplankton, less than 30 μm in size, have a higher and more variable $\delta^{15}N$, with a mean value similar to that of nitrate in underlying Subtropical Mode Water. For the summertime Sargasso Sea, we estimate that small eukaryotes obtain more than half of their nitrogen from upwelled nitrate. In addition, our data support the view that sinking material derives largely from eukaryotic, not prokaryotic, phytoplankton biomass.

As an essential nutrient, biologically available nitrogen ('fixed N') has the potential to limit marine productivity and determine phytoplankton community composition. 'New production' is phytoplankton growth in the sunlit upper ocean fuelled by 'new' N: nitrate (NO_3^-) mixed up from the ocean interior, augmented by *in situ* N_2 fixation. Annually, new production balances the export of sinking organic matter from shallow waters, maintaining the sequestration of carbon in the ocean interior[1,2]. In the subtropical ocean, the supply of nitrate from below seems to be slow, such that most phytoplankton growth is thought to be supported by 'recycled' N (refs 3,4) (predominantly ammonium (NH_4^+)).

The Bermuda Atlantic Time-series Study (BATS) site is located at the northern margin of North Atlantic subtropical gyre (the 'Sargasso Sea'), and its summertime conditions are representative of the open subtropical ocean[5]. After higher mixing and productivity in the winter and spring, strong summertime stratification develops at the base of and within the euphotic zone (upper ~100 m; ref. 4), and the wind-mixed surface layer shoals to ≤20 m (ref. 5), resulting in trace nitrate throughout the euphotic zone (Fig. 1a; as low as 0.001–0.01 μM, with sporadic observations of ~0.1 μM; ref. 4). Under these conditions, the recycling of N by euphotic zone biota is thought to be the major N source for phytoplankton growth[1–3].

The photoautotrophic biomass at BATS is comprised of the prokaryotic cyanobacterial genera *Prochlorococcus* and *Synechococcus* (hereafter 'prokaryotes')[6,7] and a number of eukaryotic species (hereafter 'eukaryotes'), predominantly prymnesiophytes and pelagophytes[8]. The N uptake capacities of these different taxa are being investigated (Supplementary Information S3.1), but the low concentrations of all fixed N forms in the Sargasso Sea make it difficult to assess *in situ* N assimilation rates or to determine which taxa (prokaryotes versus eukaryotes) use which N sources.

Nitrogen isotopes in the subtropical ocean

At BATS, N isotope studies have supported the expectation that recycled N fuels most phytoplankton growth. The $\delta^{15}N$ of bulk suspended particulate N (PN) ranges from −3 to 1‰ (refs 9,10), lower than that of nitrate in the underlying Subtropical Mode Water (STMW) ($\delta^{15}N$ of 2–3‰ at 250 m (ref. 11); $\delta^{15}N$, in permil versus atmospheric N_2, $= \{[^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{atm}] - 1\} \times 1{,}000$). Similarly low-$\delta^{15}N$ PN in the Pacific was first interpreted as indicating a substantial input from N_2 fixation[12], which produces N with a $\delta^{15}N$ of −2 to 0‰ (refs 13,14). However, the limited data from BATS indicate that the $\delta^{15}N$ of sinking PN is ~3–4‰ (ref. 9; Fig. 1b), similar to underlying STMW nitrate, suggesting that most of the new N supply is as nitrate from below, with N_2 fixation accounting for only a small fraction of new N input on an annual basis[9]. In this view, the low $\delta^{15}N$ of suspended PN was interpreted as the result of upper ocean N recycling: Zooplankton sustained by upper ocean PN metabolize and excrete ^{15}N-depleted ammonium, the assimilation of which renders phytoplankton low in $\delta^{15}N$ (refs 15,16).

Flow cytometry with nitrogen isotope analysis

Most isotope studies have measured surface ocean PN as a homogenous N pool, but PN includes biologically distinct N-containing particles: diverse living autotrophs and heterotrophs as well as detrital organic matter[17]. By combining flow cytometry with new, high-sensitivity methods for N isotope analysis (the 'persulphate/denitrifier' method[11]), we have characterized the N concentration ([N]) and $\delta^{15}N$ of taxonomically distinct components of the PN suspended in Sargasso Sea surface waters. Samples were collected through the summertime euphotic zone near the BATS site in July 2008 and 2009 (Methods and Supplementary Information S1–S2). Averaging over the euphotic zone, photoautotrophic contribution to bulk PN was 47 ± 9%, consistent with previous findings for particulate carbon[8], and the sorted fractions of *Prochlorococcus*, *Synechococcus* and eukaryotic phytoplankton each comprised roughly a third of photoautotrophic biomass N (Fig. 2a,c).

The $\delta^{15}N$ of bulk PN ranged from −2 to 0‰ (Fig. 2b,d), similar to previous data interpreted as indicating a system supported by recycled N (refs 9,10). The $\delta^{15}N$ of *Prochlorococcus* (−4 to

[1]Princeton University, Department of Geosciences, Guyot Hall, Princeton, New Jersey 08544, USA, [2]Bermuda Institute of Ocean Sciences, St George's GE01, Bermuda, [3]School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, Hawaii 96822, USA. *e-mail: sfawcett@princeton.edu.

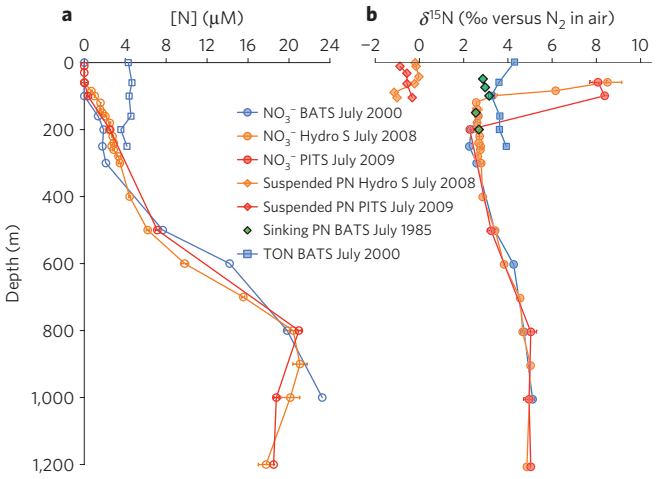

Figure 1 | Important forms of nitrogen in the Sargasso Sea. Depth profiles of summertime N concentrations (**a**) and their corresponding $\delta^{15}N$ values (**b**). **a**, Open circles: [NO_3^-] from BATS July 2000 (blue)[11], Hydrostation S July 2008 (orange, this study), PITS July 2009 (red, this study). Blue squares: [TON] (total organic N, or DON plus the small PN pool) at BATS July 2000 (ref. 11). **b**, Nitrate $\delta^{15}N$ (shaded circles), TON $\delta^{15}N$ (shaded squares), bulk suspended PN $\delta^{15}N$ from Hydrostation S July 2008 (orange diamonds) and PITS July 2009 (red diamonds), $\delta^{15}N$ of sinking PN (green diamonds) from sediment traps at BATS July 1985 (ref. 9). Error bars indicate ±1 standard error of all measurements, including samples from duplicate Niskin bottles, duplicate samples from the same Niskin bottle, and replicate sample analyses.

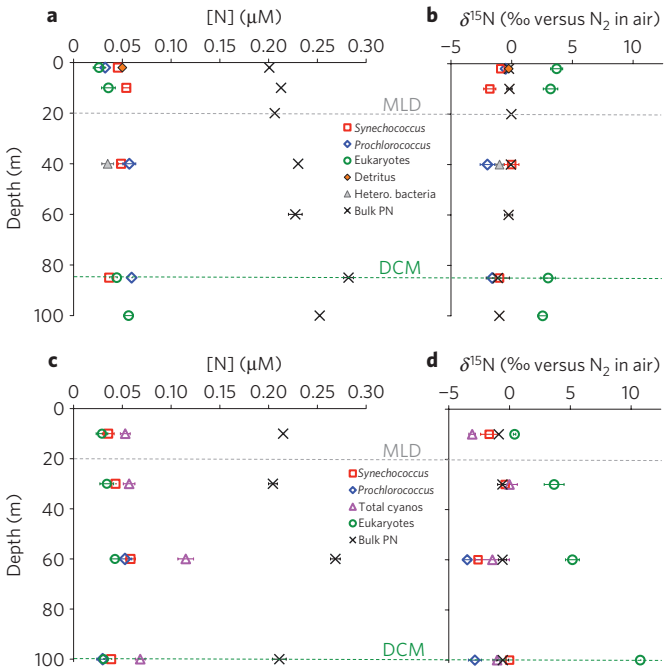

Figure 2 | Abundances and nitrogen isotope ratios of populations sorted by flow cytometry. [N] and $\delta^{15}N$ of flow cytometrically sorted components of the PN from the Sargasso Sea in July 2008 (**a** and **b**) and 2009 (**c** and **d**). The mixed layer depth (MLD; dashed grey line) was ~20 m in both years, and the deep chlorophyll maximum (DCM; dashed green line) was ~85 m in 2008 and ~100 m in 2009. 'Total cyanos' (purple triangles) represents a combined population of *Prochlorococcus* plus *Synechococcus*, sorted and measured independently of the individual genera. 'Detritus' (orange diamonds) denotes dead material (containing no chlorophyll fluorescence or stainable DNA), and 'hetero. bacteria' (grey triangles) refers to heterotrophic bacteria (containing no naturally fluorescing chlorophyll but stainable DNA). Error bars indicate the full range for replicate samples, commonly duplicates, collected, sorted, and analysed independently.

−1‰; Fig. 2b,d) and *Synechoccocus* (−3 to −1‰; Fig. 2b,d) was typically slightly lower than that of bulk PN, consistent with the expectation that prokaryotic phytoplankton use recycled N (NH_4^+ and/or simple organic N forms such as amino acids) as their dominant N source throughout the subtropical euphotic zone. Some amount of the prokaryote N supply may derive from N_2 fixation, although only via recycling, as these taxa apparently cannot fix N_2 (ref. 18). Remarkably, the $\delta^{15}N$ of sorted eukaryotes (Fig. 2b,d) was always higher than bulk $\delta^{15}N$ and prokaryote $\delta^{15}N$, typically by >3‰, pointing to a biogeochemical difference between prokaryotes and eukaryotes.

Possible explanations for high eukaryote $^{15}N/^{14}N$

One set of hypotheses for the high eukaryote $\delta^{15}N$ involves the loss of low-$\delta^{15}N$ N from these phytoplankton, such as via the excretion of NO_2^- or NH_4^+. In this N-depleted euphotic zone, autotrophic N excretion seems unlikely (Supplementary Information S3.2.1–S2). Regardless, NO_2^- efflux does not have the required isotopic effect (Supplementary Information S3.2.1). With respect to NH_4^+ release (for instance, in the case of mixotrophy by eukaryotes), heterotrophic bacteria are far more likely to excrete NH_4^+ than are autotrophic phytoplankton, as they do not photosynthesize. However, we measure a $\delta^{15}N$ of −1.0‰ for sorted heterotrophic bacteria (see below; Fig. 2b), which is very similar to the $\delta^{15}N$ of cyanobacteria, implying that some small amount of NH_4^+ excretion by the eukaryotic phytoplankton would not significantly increase their biomass $\delta^{15}N$. As yet, no compelling loss-based explanation has been recognized for the high eukaryote $\delta^{15}N$.

We conclude that the eukaryotes are using a high-$\delta^{15}N$ N source, distinct from that supporting prokaryote production. Possible high-$\delta^{15}N$ N sources include dissolved organic nitrogen (DON) and nitrate. DON has a concentration of 4–5 μM (Fig. 1a; ref. 19) and an annual average $\delta^{15}N$ of 3.9‰ (Fig. 1b), similar to mean eukaryote $\delta^{15}N$[11]. However, the high $\delta^{15}N$ of DON itself is best explained by breakdown to simpler N forms (for example, amino acids and NH_4^+) by N isotope discriminating reactions such as peptide hydrolysis and deamination[20–22]. We know of no reason why eukaryotes would be able to consume bulk DON by a non-fractionating mechanism that is unavailable to prokaryotes[18,23,24]. Finally, bulk DON rarely reaches the $\delta^{15}N$ of 5.2‰ observed for eukaryotes at 60 m in 2009, and never approaches 12.7‰, as observed at 100 m (Fig. 2d; ref. 11).

Nitrate could be supplied by *in situ* nitrification (oxidation of recycled NH_4^+ to nitrite and then nitrate) or by upward mixing of STMW nitrate. Nitrification seems to be minor in the euphotic zone near BATS (ref. 25), although some fraction of the very low-level summertime ambient nitrate may derive from it[4]. Any euphotic zone nitrification that does occur is unlikely to produce nitrite or nitrate with a $\delta^{15}N$ consistently higher than that its NH_4^+ substrate, largely because the isotope effect of ammonium assimilation is expected to be lower than that of ammonium oxidation at very low ambient [NH_4^+] (refs 26,27; Supplementary Information S3.2.3). Thus, even if euphotic zone nitrification were significant, the assimilation of its products by eukaryotes does not explain their clear $\delta^{15}N$ elevation relative to prokaryotes.

Assimilation of new nitrate by eukaryotic phytoplankton

The high eukaryote $\delta^{15}N$ most probably derives from 'new' nitrate mixed into the euphotic zone from below. At the typical depth of winter mixing (150–300 m; ref. 5), nitrate $\delta^{15}N$ is 2–3‰ (Fig. 1b). Owing to isotope discrimination during nitrate assimilation, the $\delta^{15}N$ of biomass produced at any given time should be lower than

that of ambient nitrate. This preferential ^{14}N-nitrate assimilation is evident in the elevated nitrate δ^{15}N at the base of the euphotic zone (Fig. 1b; ref. 11). However, the nitrate concentration of $<0.02\,\mu M$ typical of most of the summertime euphotic zone[4] is below the half-saturation constant for nitrate assimilation ($\sim$0.02–0.03 μM; ref. 28), a condition that probably minimizes isotope discrimination during consumption[29]. Moreover, regardless of any variation in this isotope discrimination, as a given increment of nitrate is assimilated, the δ^{15}N of integrated biomass produced from it will converge on the source nitrate δ^{15}N (Supplementary Information S3.3). Thus, assimilation of new nitrate by eukaryotes would imprint them with a higher mean δ^{15}N than if they assimilated only recycled N. Although diatoms have previously been recognized as important nitrate assimilators[30], they are not a significant fraction of the biomass in our collected $<30\,\mu m$ eukaryote pool[5,8,31]; our results indicate a broad tendency among small eukaryotes, which dominate the Sargasso Sea eukaryote population, to assimilate nitrate. The mean cellular sizes of our sorted prokaryotes (particularly *Synechococcus*) and eukaryotes are not highly distinct[8], raising the possibility of a deeper biochemical origin for their N source difference.

Although we do not understand the mechanism controlling this N source difference, there are potential explanations. Given that it is less energetically expensive to assimilate reduced N forms[32], prokaryotic phytoplankton may outcompete eukaryotes for recycled N. Alternatively, eukaryotes may indeed compete successfully for low-level nitrate, perhaps because of their uncoupling of nitrate transport from nitrate reduction (Supplementary Information S3.4). Mechanistic arguments aside, our observation of eukaryotic dominance of nitrate assimilation is consistent with the seasonal pattern of phytoplankton succession in the Sargasso Sea, from eukaryotes after deep winter mixing to *Synechococcus* and then *Prochlorococcus* with intensifying stratification and N depletion into the summer[5,8].

If the collected phytoplankton recorded the entire annual cycle, the δ^{15}N of their nitrate supply would be the concentration-weighted mean δ^{15}N of STMW nitrate down to the depth of winter mixing (2–3‰). However, individual eukaryote cells should integrate over only a period of days, so their nitrate supply has a higher δ^{15}N, best approximated by nitrate at the base of the summertime euphotic zone. At the deep chlorophyll maximum (DCM), nitrate δ^{15}N was 6.2‰ in 2008 and 8.4‰ in 2009 (Fig. 1b; at 85 m and 100 m, and $[NO_3^-]$ of 0.49 μM and 0.36 μM, respectively). The δ^{15}N of a given sample of eukaryotes can be higher than this because the collected cells may have acquired nitrate that has already undergone further δ^{15}N elevation by assimilation in the euphotic zone; this can explain the extraordinarily high δ^{15}N (12.7‰) of the eukaryote sample from 100 m in 2009.

On average, the consumption of the subsurface nitrate supply is likely to progress upward, such that isotope discrimination during nitrate assimilation might be expected to cause higher eukaryote δ^{15}N in shallower samples. Depth variations in the relative importance of nitrate uptake may explain the lack of such an upward δ^{15}N increase in our data, and indeed the opposite trend in 2009: eukaryotes in the surface mixed layer may rely more on recycled N than those below the mixed layer. Alternatively, upper ocean circulation is chaotic, such that a given sampling may capture a time when the mixed layer has a more recently delivered parcel of nitrate-bearing water than the underlying euphotic zone. For instance, the passage of Hurricane Bertha over the BATS site may have affected nutrient supply two weeks before our sampling in July 2008, although the hydrographic, nutrient, and cell abundance data are typical of July climatology. Finally, as noted above, the isotope effect of nitrate assimilation is likely to decrease under very low nitrate concentrations, minimizing the isotopic signal of progressive nitrate assimilation above the DCM. Although continued sampling is required to characterize and understand the variation, the high mean δ^{15}N of our eukaryotes requires nitrate assimilation, regardless of isotope effect (Supplementary Information S3.3).

Our interpretation requires that subsurface nitrate is transported upward into the euphotic zone and even the surface mixed layer, across their underlying summertime thermoclines. This transport may be driven by purely physical mechanisms: hydrographic studies have documented summertime transport of nitrate into the subtropical euphotic zone associated with mesoscale eddies[33] and related phenomena[34]. Biologically facilitated physical transport has also been suggested[35]. Finally, direct biological transport has been demonstrated in some systems[30], and recent data from near Hawaii suggest that this mechanism transports nitrate into the mixed layer[34]. Future work coupling isotope measurements with intensive hydrographic data may distinguish among these processes.

Summertime eukaryote and community *f*-ratios

At an ecosystem level, the relative importance of new versus recycled N for phytoplankton production is traditionally quantified as the '*f*-ratio', the ratio of nitrate assimilation (that is, new production) to total N assimilation[1,2]. Assuming parallel cycling of carbon and N, the *f*-ratio is also the fraction of net primary production resulting in carbon export from the euphotic zone. Given the nutrient-poor conditions at BATS during the summer, a low *f*-ratio is expected[2].

Our δ^{15}N measurements of sorted components of the suspended PN allow a non-incubation approach for estimating the *f*-ratio. An *f*-ratio for each phytoplankton group is estimated from its δ^{15}N, and these are then assembled into a community *f*-ratio (Supplementary Information S3.5). This exercise is highly dependent on the δ^{15}N assigned to new and recycled N. We assume that *Prochlorococcus* and *Synechococcus* together assimilate only recycled N, and we use the δ^{15}N of nitrate at the DCM as a measure of the July nitrate supply to the euphotic zone. The assumptions for these end-members and our exclusion of N_2 fixation as a source of low-δ^{15}N N are chosen to err on the side of underestimating the *f*-ratio (Supplementary Information S3.5).

We calculate eukaryote-specific *f*-ratios of 0.60 and 0.67 for July 2008 and 2009, respectively, and total community *f*-ratios of 0.15 and 0.23. Despite the biases in our calculations to underestimate the *f*-ratio, our community estimates are much higher than those based on comparison of annually integrated primary production and sediment trap-derived organic carbon export at BATS, which imply an annual *f*-ratio of 0.06 and a still lower summertime value[5]. Our estimates are lower than the highest annual estimates derived from geochemical tracers (0.36 ± 0.12; ref. 36; Supplementary Information S3.5), but July is the most nutrient-poor period at BATS, when the lowest *f*-ratio is expected[1,2,4], and thus our summer estimates support the geochemically derived annual *f*-ratio estimates.

Nitrogen isotopes of other sorted samples

In July 2008, a heterotrophic bacterial population sorted from 40 m contributed 0.035 μM N ($\sim$15% of bulk PN) with a δ^{15}N of -1.0‰, whereas detrital material from the surface comprised 0.05 μM N ($\sim$25% of bulk PN), with a δ^{15}N of -0.3‰ (Fig. 2a,b). The similarity of heterotrophic bacteria to cyanobacterial δ^{15}N suggests that bacteria also assimilate predominantly recycled N. The recycled N consumed by the bacteria is probably in the form of simple organic compounds liberated during their extracellular degradation of organic matter[37]. The δ^{15}N of detritus suggests that it derives largely from prokaryotic phytoplankton and/or heterotrophic bacteria, which is consistent with the dominance of these combined N pools relative to eukaryotes and with the expectation that prokaryotic phytoplankton preferentially enter the microbial loop[38]. The minimal δ^{15}N elevation in the detrital pool implies that isotope fractionation during degradation typically

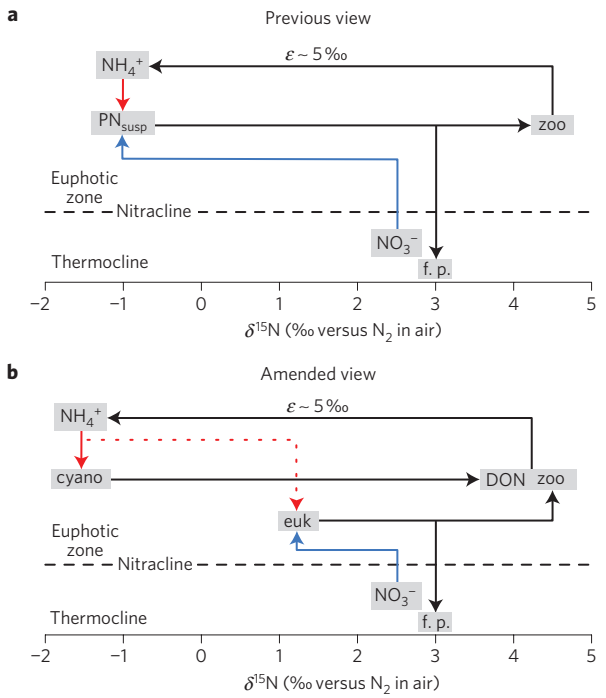

Figure 3 | Changes to our view of Sargasso Sea nitrogen cycling. Previously understood mean annual view of N fluxes and their δ^{15}N in the surface of the Sargasso Sea (**a**) and our amended view (**b**). Blue arrows represent the new N source (subsurface nitrate, δ^{15}N of 2–3‰); red arrows show recycled N (mostly ammonium); red dashed arrow indicates that eukaryotes get less of their N from recycled sources than from new nitrate. 'PN_{susp}' refers to bulk suspended PN, 'cyano' to prokaryotic phytoplankton, 'euk' to eukaryotic phytoplankton, 'DON' to dissolved organic nitrogen, 'zoo' to herbivorous copepod zooplankton, and 'f.p.' to faecal pellets produced by them, approximating export. For comparison with **a**, we extrapolate our July findings to an annual average, so as to use the same nitrate supply δ^{15}N. Thus, eukaryote δ^{15}N in **b** reflects its observed position relative to new nitrate and recycled N sources, rather than the measured July eukaryote δ^{15}N. This schematic neglects N_2 fixation, which has been estimated to represent a small fraction of the annual N supply to the BATS euphotic zone[9,11].

occurs after organic matter has been solubilized to DON, such that isotopic elevation occurs in DON and not PN (refs 11,22).

The ^{15}N/^{14}N of sinking and suspended particles

The traditional view of N fluxes in the Sargasso Sea (Fig. 3a) has been that the export of high-δ^{15}N zooplankton faecal material from the euphotic zone balances the δ^{15}N of the STMW nitrate supply. In this view, the preferential excretion of ^{14}N-rich recycled N during heterotrophic metabolism lowers the δ^{15}N of bulk PN relative to the subsurface nitrate supply. However, work to date indicates that the δ^{15}N of faecal pellets is not as elevated as the zooplankton producing them[39] and may instead resemble the δ^{15}N of the food source[40,41]. Thus, it is unclear how the consumption by herbivorous zooplankton of bulk suspended PN with a δ^{15}N of ~ −2–0‰ could yield the export flux δ^{15}N of 3–4‰ measured near BATS (ref. 9).

The δ^{15}N of sorted eukaryotes averaged over the euphotic zone was 3.0‰ in 2008 and 5.0‰ in 2009, with a mean δ^{15}N similar to the previously measured sinking flux. Sinking flux δ^{15}N is therefore consistent with faecal pellets produced by zooplankton that have preferentially consumed eukaryotic phytoplankton, without the need for an unrealistically large δ^{15}N increase (~5‰) during the passage of phytoplankton biomass N through the zooplankton gut (Fig. 3b). Indeed, studies indicate selective feeding by herbivorous zooplankton on phytoplankton size classes dominated by eukaryotes[42,43]. A simple isotope-driven end-member mixing calculation indicates that *Prochlorococcus* and *Synechococcus* contribute less than half (roughly a third) of sinking PN or carbon[44] (Supplementary Information S3.6), despite the importance of prokaryotes at BATS (>65% of photoautotrophic biomass N in this study). Thus, our data suggest that the eukaryotic phytoplankton contribution to export is disproportionately larger than their contribution to total net primary production, a long-held view[45] that has recently been challenged[46]. Given that export production drives carbon sequestration in the ocean interior, and that this study was conducted in a region where prokaryotic phytoplankton are as dominant as anywhere in the ocean, our results suggest that the biological pump in nutrient-poor subtropical gyres (~60% of oceanic surface area) is driven mostly by eukaryotic phytoplankton. With regard to paleoceanographic studies, our data indicate a disconnect between prokaryote-dominated biomass in the low-latitude surface ocean and the organic matter sinking to the seabed, which we find to be predominantly of eukaryotic origin. Although small eukaryotes dominate the Sargasso Sea eukaryote pool[5,8,31], previous work suggests that the large eukaryotes also assimilate nitrate and are preferentially incorporated into sinking particles[30,45], indicating the same eukaryotic dominance of the sinking flux in more productive ocean regions as well.

Our data suggest that small eukaryotic phytoplankton are more readily removed to the deep ocean than are cyanobacteria. The PN that sinks into the deep ocean is remineralized to nitrate, which is eventually resupplied to surface waters. We have also found that the eukaryotes are more reliant on the subsurface nitrate supply, whereas the prokaryotes are more completely recycled within the surface ocean and, in turn, rely mostly on the N forms recycled there (for example, NH_4^+). The internal consistency between uptake and remineralization in each group raises the possibility that the N uptake strategy of a given phytoplankton group is partially guided by the fate of its biomass.

Methods

Field collections. Samples were collected aboard the R/V *Atlantic Explorer* at Hydrostation S (32° 10′ N, 64° 34′ W) on cruise HS1113 in July 2008 and at PITS station (31° 35′ N, 64° 10′ W) on BATS cruise B248 in July 2009. Seawater was collected for PN and nitrate (NO_3^-) at the surface, 10 m, 40 m, 85 m and 100 m in 2008; and 10 m, 30 m, 60 m, and 100 m in 2009. Five 12 l Niskin bottles were tripped at each depth to collect sufficient PN for later isotope analysis. Additional Niskin bottles were tripped at regular intervals from below the euphotic zone down to 1,000 m (see Supplementary Information S1.1) and subsampled for later measurement of [NO_3^-] and NO_3^- δ^{15}N. For PN collections, 8 l of seawater from each of the shallower Niskin bottles was passed through a 47 mm 0.4 µm polycarbonate filter under gentle vacuum filtration. Filters were stored in 5 ml acid-washed cryovials with ~4 ml of 0. 2 µm pre-filtered seawater and 200 µl of 10% formaldehyde solution (see Supplementary Information S1.1 and S1.2), then flash frozen for later flow cytometric sorting. In 2008, bulk PN was collected by filtering 8 l seawater aliquots onto precombusted glass fibre filters; total N content and δ^{15}N was analysed with an elemental analyser (NC2500 Carlo Erba) interfaced through a ConFlo III with a ThermoFischer Scientific DeltaPlusXL mass spectrometer. In 2009, bulk PN was collected on 0.4 µm polycarbonate filters and processed in the same manner as sorted samples.

Flow cytometric sorting. Samples were thawed and agitated to quantitatively remove fixed cells from the filters (recoveries were >95%, Supplementary Information S1.2.1), and the resuspended cells were then filtered through a 30 µm mesh to remove any large particles or chains of cells that could clog the flow cytometer sorting tip. Samples were sorted for *Prochlorococcus*, *Synechococccus*, eukaryotic algae, heterotrophic bacteria, and 'detritus'. Autotrophs were identified and sorted unstained according to ref. 47 (see Supplementary Information S1.2.2). A pool of heterotrophs containing bacteria and archaea (hereafter simply 'bacteria') were identified by nucleic acid staining with the cyanine dye SYBR Green I (Invitrogen, Carlsbad, CA) and gated with side scatter, green fluorescence (relative nucleic acid content; 530/40 nm), and red fluorescence to discriminate low nucleic acid content heterotrophic bacteria from *Prochlorococcus*. 'Detritus' was defined as non-fluorescing small particles in SYBR Green stained samples. All post-acquisition analysis was performed using FCS Express (DeNovo Software, Los Angeles, CA).

A high-speed jet-in-air Influx Cell Sorter (Cytopeia, Seattle, WA) was used for all analysis and sorts. A 100 mW blue (488 nm) laser (Coherent, Santa Clara, CA) run at full power was used for excitation of SYTO-13 stained and autofluorescent cells (see Supplementary Information S1.2.3 for sort conditions). With these sort conditions and event rates kept below 2×10^4 s^{-1}, software abort rates were <1%, sort purity was better than 95%, and mean recovery from secondary sorts was 98.1 ± 1.1%. Sorted cells were deposited gently into 5 ml polystyrene Falcon tubes (BD Biosciences, San Jose, CA).

Oxidation of organic N to NO_3^-. Sorted cell populations were filtered onto 25 mm 0.2 μm polycarbonate filters by gentle vacuum filtration. Both sorted and bulk PN filters were placed in combusted 12 ml Wheaton vials and 1–2 ml of ultra-high purity deionized water was added. Vials were sonicated for 60 min to dislodge cells from filters into the deionized water, after which filters were removed. Cellular N content and δ^{15}N were measured by coupling persulphate oxidation of organic N to NO_3^- with chemiluminescent analysis and the 'denitrifier method'[11] (see Supplementary Information S1.2.4). The final N content and δ^{15}N of these samples was corrected for the N blank associated with the reagents. N content was converted to N concentration ([N]) using the seawater volume filtered for each PN sample, corrected for volume loss during flow cytometry (see Supplementary Information S2 for methods validation).

Water column [NO_3^-] and nitrate δ^{15}N. Water samples were collected for [NO_3^-] from the surface to 1,000 m and for δ^{15}N of NO_3^- from 60 m to 1,000 m at Hydrostation S in 2008 and PITS in 2009. [NO_3^-] was determined by reduction to nitric oxide followed by nitric oxide measurement with a chemiluminescent detector[48] (Teledyne model #200 EU), in a configuration with a detection limit of ~0.025 μM.

The δ^{15}N of NO_3^- was measured using the 'denitrifier method'[49] for quantitative bacterial conversion of nitrate to nitrous oxide followed by the isotopic analysis of the nitrous oxide product. The isotopic composition of the nitrous oxide was measured by gas chromatography-isotope ratio mass spectrometry using a modified ThermoFinnigan GasBench II and DeltaPlus[50] (see Supplementary Information S1.2.5).

Received 4 October 2010; accepted 16 August 2011; published online 18 September 2011

References

1. Dugdale, R. C. & Goering, J. J. Uptake of new and regenerated forms of nitrogen in primary production. *Limnol. Oceanogr.* **12,** 196–206 (1967).
2. Eppley, R. W. & Peterson, B. J. Particulate organic matter flux and planktonic new production in the deep ocean. *Nature* **282,** 677–680 (1979).
3. Menzel, D. W. & Ryther, J. H. The annual cycle of primary production in the Sargasso Sea off Bermuda. *Deep-Sea Res.* **6,** 351–367 (1960).
4. Lipschultz, F. A time-series assessment of the nitrogen cycle at BATS. *Deep-Sea Res. II* **48,** 1897–1924 (2001).
5. Steinberg, D. K. *et al.* Overview of the US JGOFS Bermuda Atlantic Time-series Study (BATS): A decade-scale look at ocean biology and biogeochemistry. *Deep-Sea Res. II* **48,** 1405–1447 (2001).
6. Chisholm, S. W. *et al.* novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* **334,** 340–343 (1988).
7. Waterbury, J. B., Watson, S., Guillard, R. R. L. & Brand, L. E. Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. *Nature* **277,** 293–294 (1979).
8. DuRand, M. D., Olson, R. J. & Chisholm, S. W. Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep-Sea Res. II* **48,** 1983–2003 (2001).
9. Altabet, M. A. Variations in nitrogen isotopic composition between sinking and suspended particles: Implications for nitrogen cycling and particle transformation in the open ocean. *Deep-Sea Res.* **35,** 535–554 (1988).
10. Altabet, M. A. A time-series study of the vertical structure of nitrogen and particle dynamics in the Sargasso Sea. *Limnol. Oceanogr.* **34,** 1185–1201 (1989).
11. Knapp, A. N., Sigman, D. M. & Lipschultz, F. N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic Time-series Study site. *Glob. Biogeochem. Cycles* **19,** 1–15 (2005).
12. Saino, T. & Hattori, A. Geographical variation of the water column distribution of suspended particulate organic nitrogen and its ^{15}N natural abundance in the Pacific and its marginal seas. *Deep-Sea Res. I* **34,** 807–827 (1987).
13. Carpenter, E. J., Harvey, H. R., Fry, B. & Capone, D. G. Biogeochemical tracers of the marine cyanobacterium *Trichodesmium*. *Deep-Sea Res. I* **44,** 27–38 (1997).
14. Minagawa, M. & Wada, E. Nitrogen isotope ratios of red tide organisms in the East China Sea—a characterization of biological nitrogen-fixation. *Mar. Chem.* **19,** 245–259 (1986).
15. Checkley, D. M. Jr & Miller, C. A. Nitrogen isotope fractionation by oceanic zooplankton. *Deep-Sea Res.* **36,** 1449–1456 (1989).
16. Montoya, J. P., Carpenter, E. J. & Capone, D. G. Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. *Limnol. Oceanogr.* **47,** 1617–1628 (2002).
17. Rau, G. H., Teyssie, J. L., Rassoulzadegan, F. & Fowler, S. W. ^{13}C/^{12}C and ^{15}N/^{14}N variations among size-fractionated marine particles—implications for their origin and trophic relationships. *Mar. Ecol.-Prog. Ser.* **59,** 33–38 (1990).
18. Moore, L. R., Post, A. F., Rocap, G. & Chisholm, S. W. Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Limnol. Oceanogr.* **47,** 989–996 (2002).
19. Hansell, D. A. & Carlson, C. A. Biogeochemistry of total organic carbon and nitrogen in the Sargasso Sea: Control by convective overturn. *Deep-Sea Res. II* **48,** 1649–1667 (2001).
20. Macko, S. A., Estep, M. L. F., Engel, M. H. & Hare, P. E. Kinetic fractionation of stable nitrogen isotopes during amino-acid transamination. *Geochim. Cosmochim. Acta* **50,** 2143–2146 (1986).
21. Silfer, J. A., Engel, M. H. & Macko, S. A. Kinetic fractionation of stable carbon and nitrogen isotopes during peptide-bond hydrolysis—experimental evidence and geochemical implications. *Chem. Geol.* **101,** 211–221 (1992).
22. Knapp, A. N., Sigman, D. M., Lipschultz, F., Kustka, A. & Capone, D. G. Interbasin isotopic correspondence between upper-ocean bulk DON and subsurface nitrate and its implications for marine nitrogen cycling. *Glob. Biogeochem. Cycles.* (in the press).
23. Zubkov, M. V., Fuchs, B. M., Tarran, G. A., Burkill, P. H. & Amann, R. High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Appl. Environ. Microbiol.* **69,** 1299–1304 (2003).
24. Wawrik, B., Callaghan, A. V. & Bronk, D. A. Use of inorganic and organic nitrogen by *Synechococcus spp.* and diatoms on the West Florida shelf as measured using stable isotope probing. *Appl. Environ. Microbiol.* **75,** 6662–6670 (2009).
25. Lomas, M. W. & Lipschultz, F. Forming the primary nitrite maximum: Nitrifiers or phytoplankton. *Limnol. Oceanogr.* **51,** 2453–2467 (2006).
26. Hoch, M. P., Fogel, M. L. & Kirchman, D. L. Isotope fractionation associated with ammonium uptake by a marine bacterium. *Limnol. Oceanogr.* **37,** 1447–1459 (1992).
27. DiFiore, P. J., Sigman, D. M. & Dunbar, R. B. Upper ocean nitrogen fluxes in the Polar Antarctic Zone: Constraints from the nitrogen and oxygen isotopes of nitrate. *Geochem. Geophys. Geosyst.* **10,** Q11016 (2009).
28. Harrison, W. G., Harris, L. R. & Irwin, B. D. The kinetics of nitrogen utilization in the oceanic mixed layer: Nitrate and ammonium interactions at nanomolar concentrations. *Limnol. Oceanogr.* **41,** 16–32 (1996).
29. Granger, J., Sigman, D. M., Lehmann, M. F. & Tortell, P. D. Nitrogen and oxygen isotope fractionation during dissimilatory nitrate reduction by denitrifying bacteria. *Limnol. Oceanogr.* **53,** 2533–2545 (2008).
30. Villareal, T. A. *et al.* Upward transport of oceanic nitrate by migrating diatom mats. *Nature* **397,** 423–425 (1999).
31. Goericke, R. Response of phytoplankton community structure and taxon-specific growth rates to seasonally varying physical forcing in the Sargasso Sea off Bermuda. *Limnol. Oceanogr.* **43,** 921–935 (1998).
32. Cochlan, W. P. & Harrison, P. J. Inhibition of nitrate uptake by ammonium and urea in the eucaryotic picoflagellate *Micromonas pusilla* (Butcher) Manton et Parke. *J. Exp. Mar. Biol. Ecol.* **153,** 143–152 (1991).
33. McGillicuddy, D. J. Jr *et al.* Influence of mesoscale eddies on new production in the Sargasso Sea. *Nature* **394,** 263–266 (1998).
34. Johnson, K. S., Riser, S. C. & Karl, D. M. Nitrate supply from the deep to near-surface waters of the North Pacific subtropical gyre. *Nature* **465,** 1062–1065 (2010).
35. Katija, K. & Dabiri, J. O. A viscosity-enhanced mechanism for biogenic ocean mixing. *Nature* **460,** 624–626 (2009).
36. Jenkins, W. J. Studying subtropical thermocline ventilation and circulation using tritium and ^{3}He. *J. Geophys. Res.* **103,** 15817–15831 (1998).
37. Fenchel, T., King, G. M. & Blackburn, T. H. *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling* (Elsevier Academic Press, 1998).
38. Ducklow, H. W., Purdie, D. A., Williams, P. J. L. & Davies, J. M. Bacterioplankton—a sink for carbon in a coastal marine plankton community. *Science* **232,** 865–867 (1986).
39. Altabet, M. A. & Small, L. F. Nitrogen isotopic ratios in fecal pellets produced by marine zooplankton. *Geochim. Cosmochim. Acta* **54,** 155–163 (1990).
40. Montoya, J. P., Wiebe, P. H. & McCarthy, J. J. Natural abundance of ^{15}N in particulate nitrogen and zooplankton in the Gulf Stream region and warm-core ring 86a. *Deep-Sea Res. I* **39,** S363–S392 (1992).
41. Tamelander, T., Soreide, J. E., Hop, H. & Carroll, M. L. Fractionation of stable isotopes in the Arctic marine copepod *Calanus glacialis*: Effects on the isotopic composition of marine particulate organic matter. *J. Exp. Mar. Biol. Ecol.* **333,** 231–240 (2006).
42. Harbison, G. R. & McAlister, V. L. Filter-feeding rates and particle retention efficiencies of 3 species of *Cyclosalpa* (Tunicata, Thaliacea). *Limnol. Oceanogr.* **24,** 875–892 (1979).

43. Schnetzer, A. & Steinberg, D. K. Natural diets of vertically migrating zooplankton in the Sargasso Sea. *Mar. Biol.* **141,** 403 (2002).
44. Lomas, M. W. & Moran, S. B. Evidence for aggregation and export of cyanobacteria and nano-eukaryotes from the Sargasso Sea euphotic zone. *Biogeosciences* **8,** 203–216 (2011).
45. Michaels, A. F. & Silver, M. W. Primary production, sinking fluxes and the microbial food web. *Deep-Sea Res.* **35,** 473–490 (1988).
46. Richardson, T. L. & Jackson, G. A. Small phytoplankton and carbon export from the surface ocean. *Science* **315,** 838–840 (2007).
47. Casey, J. R. *et al.* Phytoplankton taxon-specific orthophosphate (Pi) and ATP utilization in the western subtropical North Atlantic. *Aquat. Microbial. Ecol.* **58,** 31–44 (2009).
48. Braman, R. S. & Hendrix, S. A. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection. *Anal. Chem.* **61,** 2715–2718 (1989).
49. Sigman, D. M. *et al.* A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.* **73,** 4145–4153 (2001).
50. Casciotti, K. L., Sigman, D. M., Hastings, M. G., Böhlke, J. K. & Hilkert, A. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. *Anal. Chem.* **74,** 4905–4912 (2002).

Acknowledgements

We thank A. Babbin, S. Bell, J. Granger, A. Knapp, H. Ren and L. Treibergs, the staff of the Bermuda Institute of Ocean Sciences and the captain and crew of the R/V *Atlantic Explorer*. We also thank B. Plessen at GeoForschungsZentrum, Potsdam, for analysis of bulk PN samples from July 2008. N. Levine pointed us towards the possible impacts of Hurricane Bertha. This work was supported by the Charrock Foundation, by the Siebel Energy Grand Challenge of Princeton University, and by the US NSF through grants OCE-0752161 (M.W.L.), OCE-0452162 (B.B.W.) and OCE-0447570 (D.M.S.). This is BIOS contribution no. 2028.

Author contributions

M.W.L. and D.M.S. suggested the research area, S.E.F., M.W.L., B.B.W. and D.M.S. planned the project, S.E.F. and J.R.C. performed most of the work, and all authors wrote the paper, led by S.E.F.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/naturegeoscience. Reprints and permissions information is available online at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.E.F.