Comparable light stimulation of organic nutrient uptake by SAR11 and *Prochlorococcus* in the North Atlantic subtropical gyre

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Abstract

Subtropical oceanic gyres are the most extensive biomes on Earth where SAR11 and *Prochlorococcus* bacterioplankton numerically dominate surface waters depleted in inorganic macronutrients as well as in dissolved organic matter. In such nutrient poor conditions bacterioplankton could become photoheterotrophic, i.e. potentially enhance uptake of scarce organic molecules using the available solar radiation to energise appropriate transport systems. Here, we assessed the photoheterotrophy of the key microbial taxa in the North Atlantic oligotrophic gyre and adjacent regions using ^{33}P-ATP, ^{3}H-ATP and ^{35}S-methionine tracers. Light-stimulated uptake of these substrates was assessed in two dominant bacterioplankton groups discriminated by flow cytometric sorting of tracer-labelled cells and identified using catalysed reporter deposition fluorescence *in situ* hybridization (CARD–FISH). One group of cells, encompassing 48% of all bacterioplankton, were identified as members of the SAR11 clade, whilst the other group (24% of all bacterioplankton) was *Prochlorococcus*. When exposed to light, SAR11 cells took 31% more ATP and 32% more methionine, whereas the *Prochlorococcus* cells took 33% more ATP and 34% more methionine. Other bacterioplankton did not demonstrate light stimulation. Thus, the SAR11 and *Prochlorococcus* groups, with distinctly different light harvesting mechanisms, used light equally to enhance, by approximately one-third, the uptake of different types of organic molecules. Our findings indicate the significance of light-driven uptake of essential organic nutrients by the dominant bacterioplankton groups in the surface waters of one of the less productive, vast regions of the world's oceans - the oligotrophic North Atlantic subtropical gyre.

Introduction

Over half of the bacterioplankton inhabiting the oceans occupy the thin surface layer that is regularly exposed to solar rays. However, beyond CO_2 fixation rates by phototrophs the effect of light on the metabolism of the dominant microorganisms remains uncertain. Photoheterotrophic microorganisms have been defined as microorganisms that use light as their energy source and organic compounds as their carbon source with anoxygenic phototrophs as a typical example (Madigan 2012). However, in the last two decades the use of the term was broadened to incorporate other microorganisms that could harvest light energy as well as utilise organic molecules (reviewed in Beja and Suzuki 2008, Zubkov 2009).

Three main groups of marine photoheterotrophic bacteria were identified: i) chlorophyll-containing cyanobacteria, like *Prochlorococcus* (Chisholm *et al* 1988) and *Synechococcus* (Waterbury *et al* 1979), ii) aerobic anoxygenic bacteriochlorophyll-containing bacteria, such as members of the *Roseobacter* clade (Beja *et al* 2002), and iii) proteorhodopsin-containing bacteria that use a rhodopsin-based system (Beja *et al* 2000) like the SAR11 alphaproteobacterial clade (Giovannoni *et al* 2005a). The ability of those phylogenetic and metabolically diverse bacteria to harness light energy might impose a benefit in a range of physiological functions. However, the effect of light on proteorhodopsin (PR)- containing microorganisms is controversial (Fuhrman *et al* 2008) and its role in SAR11, the most abundant microorganism in the surface ocean (Morris *et al* 2002) remains unresolved (Steindler *et al* 2011). Recently, it was shown that energy starved PR-containing *Candidatus* Pelagibacter ubique HTCC1062, a member of the SAR11 clade, increased the transport of amino acids and cellular ATP content after being exposed to light, but light had no effect during active growth (Steindler *et al* 2011).

The influence of light on the uptake rates of organic molecules by natural photoheterotrophic bacterioplankton populations in the oligotrophic ocean, however, is poorly known (Beja and Suzuki 2008), and has only been evaluated using amino acids as tracers (Church *et al* 2004, Michelou *et al* 2007, Mary *et al* 2008). We previously showed preliminary evidence that light significantly stimulated the transport of amino acids by *Prochlorococcus* and heterotrophic non-pigmented low nucleic acid (LNA)-containing bacterioplankton, as discernible by flow cytometry.

These two groups closed the budget of total bacterioplankton light-enhanced uptake of amino acids (Mary *et al* 2008). On the other hand, Michelou and colleagues (2007) did not evaluate LNA bacteria and could not budget the total bacterioplankton light-enhanced amino acids uptake.

The LNA bacterioplankton population in the surface open ocean includes the SAR11 clade. However, on average 40% of the LNA cells have not yet been phylogenetically assigned further than to Bacteria (Mary *et al* 2008, Hill *et al* 2010, Schattenhofer *et al* 2011). Considering the small size, (0.01-0.05 μm^3, (Rappe *et al* 2002, Malmstrom *et al* 2004) and the streamlined genome (1.3 Mbp, (Giovannoni *et al* 2005b)) of SAR11, they likely have few ribosomes. Therefore, their detection by FISH could be hampered (Amann and Fuchs 2008) even when horseradish peroxidase labelled oligonucleotide probes are used in combination with catalysed reported deposition (CARD) of fluorescently labelled tyramides.

The objective of the present study is to directly compare the light stimulated uptake of two essential biomass building blocks, nucleotides and amino acids, by SAR11 and *Prochlorococcus* in the North Atlantic subtropical gyre. We aim to compare the effect of light on the uptake rates of two simple organic nutrients that have distinctly different transport mechanisms. 5'-nucleotides, such as ATP, are dephosphorylated extracellularly and then the phosphate and adenosine monomers transported (Bengis-Garber and Kushner 1982, Bengis-Garber 1983, Wanner 1996, Sebastian and Ammerman 2011) while amino acids are directly taken up by the cells via high affinity ABC transporters. We hypothesise that light enhances transport of essential nutrients by SAR11 bacteria and *Prochlorococcus,* inhabiting surface waters of the oligotrophic North Atlantic subtropical gyre, in autumn when the water column becomes more stratified and depleted in inorganic macronutrients.

Material and Methods

Sampling and bacterioplankton enumeration

Experimental work was performed on board the Royal Research Ship (R.R.S) *James Cook* (cruise number JC53, October-November 2010) as part of the Atlantic Meridional Transect (AMT) programme, and on board the R.R.S *Discovery* (cruise

number D369, August–September 2011) (Figure 1). At each station, samples were collected from 20 m depth with a sampling rosette of 20 L Niskin bottles mounted on a conductivity-temperature-depth (CTD) profiler. Samples were collected in 1 L thermos flasks (washed with 10% v/v HCl) in the dark and processed immediately. A depth of 20 m was chosen because it represents the mixed layer and it was the shallowest depth unaffected by the ship's movement, including thrusting, that could artificially affect microbial metabolism in nutrient-depleted stratified surface waters. The effect of light on metabolic rates was evaluated using photosynthetically active radiation light spectra (400 – 700 nm), because at 20 m the penetration of UV is low.

Samples (1.6 mL) were fixed with 1% (w/v) paraformaldehyde (PFA) and stained with SYBR Green I DNA dye (Marie *et al* 1997, Zubkov *et al* 2000). Bacterioplankton, the cyanobacteria *Prochlorococcus* and *Synechococcus*, and LNA bacteria (Supplementary Figure 1) were enumerated with a FACSort flow cytometer (Becton Dickinson, Oxford, UK). Yellow-green 0.5 μm and 1.0 μm reference beads (Fluoresbrite Microparticles, Polysciences, Warrington, USA) were used in all analyses as an internal standard for both fluorescence and flow rates. The absolute concentration of beads in the stock solution was determined using syringe pump flow cytometry (Zubkov and Burkill 2006).

Ambient concentrations and turnover rates of methionine and ATP

Ambient concentrations as well as microbial uptake rates of the amino acid methionine and of ATP were measured using isotopic dilution time-series incubations (Zubkov *et al* 2004, Zubkov *et al* 2007), referred to below as bioassays. L-[^{35}S] methionine (specific activity >1000 Ci mmol^{-1}, Hartmann Analytic GmbH, Braunschweig, Germany) was added at a concentration of 0.05 nM and diluted with unlabelled L-methionine (Sigma Aldrich, Dorset, UK) using a dilution series spanning the range 0.05-1.0 nM.

We compared the uptake rate of the phosphate and of the adenosine monomers of ATP by using two radiotracers: α-^{33}P-adenosine 5'-triphosphate (^{33}P-ATP) where the phosphorus in the α position is labelled, and [2,5',8-^{3}H]-ATP (^{3}H-ATP) where the adenosine is labelled. [α ^{33}P]- ATP (specific activity >3000 Ci mmol^{-1}, Hartmann Analytic GmbH, Braunschweig, Germany) was added at a concentration of 0.05 nM or 0.1 nM and diluted with non-labelled ATP-disodium salt hydrate (Sigma Aldrich,

143 Dorset, UK) using a dilution series in the range 0.1-2.0 nM. Incubated samples were
144 fixed after 10, 20, 30 and 40 minutes with 1% (w/v) paraformaldehyde final
145 concentration. [2,5',8-^{3}H]–ATP (specific activity 51.5 Ci mmol^{-1}, PerkinElmer Inc,
146 MA, USA) was added at a concentration of 0.1-1.0 nM. Samples were fixed after 15,
147 30, 45 and 60 minutes. Fixed samples were filtered onto 0.2 µm pore size
148 polycarbonate filters and washed twice with 4 mL of deionised water. Radioactivity
149 retained on the filters was measured as counts per minute using a liquid scintillation
150 counter (Tri-Carb 3100TR, Perkin-Elmer, Beaconsfield, UK). Calculations of
151 substrate concentration, uptake rate and turnover time were performed as described
152 previously (Zubkov *et al* 2007).

153 *Light and dark incubations*

154 The experimental set up for the light and dark uptake measurements was a
155 dark room illuminated only by a very dim light of <1 µmol photons m^{-2} s^{-1}. Light
156 incubation experiments were placed in a 6 L water tank illuminated by a warm white
157 light emitting diode (LED) array (Photon Systems Instruments, Drasov, Czech
158 Republic). Dark uptake experiments were placed in a water tank covered with two
159 layers of black bags that kept the experiment in fully dark conditions. Undetectable
160 CO_2 fixation by eukaryotic cells confirmed the absence of light in the dark experiment
161 (Hartmann, Zubkov pers. com). All experiments were placed in the light and in the
162 dark incubators simultaneously. Temperature in both tanks was maintained within 1^{o}C
163 of sea surface water temperature by circulating water through the tanks using a
164 refrigerated bath (Grant Instruments, Cambridge, UK).

165 In autumn 2010, samples were incubated in polypropylene crystal clear
166 microcentrifuge tubes (Starlab, Milton Keynes, UK) according to Mary et al (2008).
167 The tubes transmitted 72% of the light at 400 nm, increasing approximately linearly to
168 82% at 700 nm (Mary *et al* 2008) . The LED light array was adjusted to an intensity
169 of 500 µmol photons m^{-2} s^{-1}. Therefore, the intensity at which samples were incubated
170 was between 350 and 410 µmol photons m^{-2} s^{-1}. In summer 2011, samples were
171 incubated in borosilicate glass bottles (Pyrex, SciLabware, Staffordshire, UK) at 300
172 µmol photons m^{-2} s^{-1}. Borosilicate glass bottles were soaked overnight with 10% (v/v)
173 HCl, rinsed three times with deionised water, and three times with the seawater
174 sample prior to incubations. There was no difference in the bacterioplankton groups

observed by flow cytometry between samples incubated (10 h) in microcentrifuge tubes and those in bottles (data not shown). For high throughput experiments, microcentrifuge tubes presented the advantage that they are sterile and disposable despite their modest light absorbance. The light intensity of the LED array was measured using a PAR quantum sensor (Skye, Powys, UK), and intensity was chosen within the range of ambient light at 20 m at noon, measured with the PAR sensor mounted on the CTD profiler.

Microbial light and dark uptake

During autumn 2010, light and dark uptake of ATP and methionine were evaluated at 12 and 22 stations, respectively. In each case 3 to 5 independent time points were measured. 0.05 nM L-[^{35}S] methionine and 0.1-0.2 nM of unlabelled methionine, or 0.05-0.1 nM of [α ^{33}P]- ATP and 0.45 - 0.8 nM of unlabelled ATP was placed into tubes and 1.6 mL of seawater was added to the experimental vials. Samples were fixed with 1% (w/v) PFA after 30, 60, 90, 120 and occasionally 150 minutes and processed as described in the previous section. We calculated the difference between the uptake in the light and in the dark in all paired experiments, and applied a one sample t-test, to test if the difference between light and dark uptake was significantly higher than zero. All time points measured were tested independently. If data were not normally distributed (Shapiro-Wilk test), it was natural Log transformed before computing light and dark differences.

In autumn 2010, four 1.6 mL samples were inoculated with 0.5 nM L-[^{35}S] methionine or 0.1-0.4 nM [α^{33}P]-ATP, and incubated for two hours in the light or dark before being fixed with 1% (w/v) PFA as described above. In summer 2011, parallel measurements of [2,5',8-^{3}H]–ATP and [α ^{33}P]- ATP uptake were performed. The drawback of the latter is that the specific activity of the tritium label is low (51.5 Ci mmol^{-1}) and therefore long (10 hours) incubations were required to achieve cell labelling sufficient for detection in sorted cells. As the turnover of the internal ATP is very fast (Chapman and Atkinson 1977, Winn and Karl 1984) long incubations might lead to recycling of ATP. Therefore we compared the uptake rates of both substrates, but chose the ^{33}P-ATP for extensive testing of light-enhanced uptake because of its high specific activity (3000 Ci mmol^{-1}). Between 4.8 and 8 mL of seawater sample was incubated with 0.8 nM [2,5',8-^{3}H]–ATP or 0.3 nM [α ^{33}P]- ATP in borosilicate

glass bottles. To monitor that microbial uptake was linear, subsamples were fixed every one or two hours and filtered as described above. In all experiments performed, bacterioplankton uptake in the light and dark was linear ($r^2 > 0.98$ in eight independent experiments) (see supplementary Figure 2).

Flow cytometric cell sorting of radioactively labelled bacterioplankton cells

Flow cytometric cell sorting of radioactively labelled bacterioplankton cells was performed on board the ship within 12 h of fixation. Cells were flow-sorted from SYBR Green I DNA stained samples (Supplementary Figure 1) by a FACSort flow cytometer (Becton Dickinson, Oxford, UK) using single-cell sort mode at a rate of 10-250 particles s^{-1}. For each experiment, four proportional numbers of cells of total bacterioplankton, LNA bacteria, and *Prochlorococcus* cells were sorted. At three stations, where their abundance was sufficiently high to warrant radiotracer flow sorting (Fig. 1), *Synechococcus* were sorted from stained samples by discriminating them from other cells using their characteristic orange phycoerythrin autofluorescence. Sorted cells were directly collected onto 0.2 µm pore size polycarbonate filters, washed twice with 4 mL deionised water, and the radioactivity retained on the filters radioassayed as described above. The ^{3}H-ATP experiments were counted using an ultra- low-level liquid scintillation counter (1220 Quantulus, Wallac, Finland) to improve the sensitivity of tritium detection. The mean cellular tracer uptake of each group was determined as the slope of the linear regression of radioactivity against the number of sorted cells, resulting in the uptake of an average cell (CPM $cell^{-1}$).

In order to compare light-enhanced uptake between stations the absolute uptake of an average LNA bacteria and *Prochlorococcus* cell (nmol $cell^{-1}$ h^{-1}) was computed using the uptake of an average sorted cell, the total uptake in a given light or dark experiment and the microbial uptake rates at ambient concentrations as follows:

(uptake of sorted cells, CPM $cell^{-1}$ / total uptake, CPM L^{-1}) * microbial uptake rate, nmol L^{-1} h^{-1}

The total amount of substrate incorporated by microorganisms (total uptake, CPM L^{-1}) was measured by filtering three subsamples from the sorting experiment onto 0.2 µm pore size polycarbonate filters and measuring the radioactivity retained

on the filters. To compute the absolute uptake in the light the microbial uptake rate at ambient concentration, which was measured in the dark, was multiplied by a light/dark factor determined in parallel for each experiment (Figure 2). The uptake rate of methionine and ATP per *Prochlorococcus* and LNA cell was multiplied by the number of molecules in 1 mol (Avogadro constant).

Flow cytometric cell sorting for catalysed reporter deposition fluorescence in situ *hybridization* (CARD-FISH)

CARD-FISH was performed on sorted cells to identify the bacterioplankton groups in which uptake rates were measured. Triplicate 1.6 mL samples were fixed with PFA (1% w/v final concentration), for 1 h at room temperature, subsequently flash frozen in liquid nitrogen, and stored at -80°C. Aliquots were thawed on ice and stained with SYBR Green as described above. LNA bacteria and *Prochloroccocus* cells were sorted on a sterilized FACScalibur flow cytometer (BD, Oxford, UK) with sheath fluid filtered through a 0.1 µm cartridge filter (Pall corporation, NY, USA). Approximately $1x10^5$ cells were sorted and directly filtered onto polycarbonate filters (type GTTP, 13 mm diameter, 0.2 µm pore size, Millipore, Eschborn, Germany) placed on top of a cellulose acetate support filter (0.45 µm pore size, Sartorius, Goettingen, Germany). CARD-FISH was performed as previously described (Pernthaler *et al* 2004) with the following modifications: cells were permeabilised with lysozyme (10 mg mL^{-1}) in 0.05 M EDTA, pH 8.0; 0.1 M Tris-HCl, pH 8.0 buffer for 1 h and subsequently for 30 min with 60 U achromopeptidase (Sigma Aldrich, Dorset, UK) per mL of buffer (10 mM NaCl, 10 mM Tris-HCl, pH 8.0) at 37°C. Filters were hybridized overnight at 46°C with horseradish peroxidase-labelled oligonucleotide probes (Biomers, Ulm, Germany) at varying formamide concentrations depending on the probe used (Supplementary Table 1). Probes (50 ng μL^{-1}) and buffer were mixed in a 1:300 ratio. The probe-delivered horseradish peroxidase was detected with fluorescently labelled tyramide Alexa488 at a ratio of 1:1000 in amplification buffer. All cells were stained with 4,6-diamidino-2-phenylindole (DAPI) (1 mg mL^{-1}). Hybridized and DAPI-stained cells were enumerated using an Axioskope II epifluorescence microscope (Zeiss, Jena, Germany).

Results

Microbial uptake of the organic molecules ATP and methionine, in surface waters of the North Atlantic Ocean, was significantly higher in light incubations compared to dark incubations (Figure 2a and b). Total microbial ^{33}P-ATP uptake in the light was 27 ± 2% higher than in the dark (n=12, all experiments) (Figure 2a). For methionine it was 19 ± 2% higher in the light than in the dark (in 20 out of 22 experiments). For both substrates, a significant increase in uptake was detected within 30 min of light exposure (t-test, $p<0.001$). All consecutive time points (up to 150 min) were also significantly higher in the light than in the dark (t-test, $p<0.001$). Flow sorting of labelled cells showed that bacterioplankton were primarily responsible for the light-enhanced uptake. There was also a good agreement in the proportion that light increased uptake of an average bacterioplankton cell (27 ± 4 %, ATP and 21 ± 3%, methionine), and of the total microorganisms retained on filters (values shown above). No statistical difference was detected between measurements (ATP: t-test $p=0.55$, methionine t-test $p<0.17$, f-statistic higher than f-critical for both substrates) (Supplementary Figure 3 a, b).

Uptake rates of *adenosine and phosphorus moieties*

In order to investigate if microorganisms consume ATP as an organic phosphorus source or as a nucleoside precursor (adenosine) we compared the uptake rates of ATP using ^{33}P-ATP and ^{3}H-ATP tracers. Parallel measurements with both tracers, showed that for the same added concentration of tracer the turnover time of the pool of ^{3}H-ATP was significantly faster than for ^{33}P-ATP (Supplementary Figure 4). The total microbial uptake rate was significantly higher for ^{3}H-ATP (0.243 ± 0.04 nM ATP day^{-1}) than for ^{33}P-ATP (0.098 ± 0.019 nM ATP day^{-1}, p-value <0.05). However, the concentration of nucleotides in seawater measured with both substrates was statistically similar (0.262 ± 0.06 nM for ^{33}P-ATP and 0.259 ± 0.05 nM for ^{3}H-ATP respectively, p-value > 0.05) showing applicability of both tracers for bioassay of ambient ATP concentration. ATP concentration and uptake rates measured with ^{33}P-ATP in the same region in 2010 but later in the autumn were higher (0.69 ± 0.29 nM and 0.18 ± 0.10 nM day^{-1} respectively, Figure 3a, b). The methionine concentrations and uptake rates were in the same range as ATP, 0.24 ± 0.13 nM and 0.27 ± 0.19 nM day^{-1} respectively (Figure 3a,b).

Molecular identification of flow sorted cells

CARD-FISH was performed on flow-sorted cells to identify the groups for which uptake rates were measured. High nucleic acid (HNA) containing bacteria, based on SYBR Green DNA staining, that had virtually undetectable chlorophyll autofluorescence, were phylogenetically affiliated with *Prochlorococcus,* in agreement with our previously reported results (Zubkov *et al* 2007). An average of 88 ± 4% (n=10) of total DAPI-stained cells conferred signals with the *Prochlorococcus*-specific probe PRO405 (West *et al* 2001). Interestingly, almost all the LNA bacteria comprised cells affiliated to the SAR11 clade. Thus, 93 ± 6% (n=9) of total DAPI stained cells (Table 1) were positively identified using a set of probes that target different regions of the SAR11 rRNA ((Morris *et al* 2002) and this study, Supplementary Table 1). Consequently, from this point onwards we refer to LNA bacteria as SAR11. *Prochlorococcus* and SAR11 numerically dominated bacterioplankton in surface waters of the North Atlantic subtropical gyre: the abundance of the former was, on average, 2.2 ± 0.9 x 10^5 cells mL^{-1} while the abundance of the latter was 4.2 ± 1.1 x 10^5 cells mL^{-1} (Figure 1b), representing 24 ± 5% and 48 ± 6% of total bacterioplankton, respectively.

SAR11 and Prochlorococcus uptake rates

To assess whether light increased the transport of simple organic molecules into SAR11 cells, tracer labelled cells were flow-sorted and their cellular tracer uptake was compared to tracer uptake by flow-sorted *Prochlorococcus* cells. On average, SAR11 cells had lower ATP uptake rates than *Prochlorococcus* cells, while methionine uptake rates for the two cell types were comparable (Table 2). SAR11 ^{33}P-ATP uptake was, on average, significantly lower than *Prochlorococcus* both in the light and the dark. Similarly, ^{3}H-ATP uptake by SAR11 cells was significantly lower than ^{3}H-ATP uptake by *Prochlorococcus* cells in both the light and dark. For both SAR11 and *Prochlorococcus* the uptake rate of the adenosine moiety was two to three times faster than the uptake rate of the phosphorus groups of ATP (Figure 4). However, the SAR11 methionine uptake rates were, on average, comparable to those of *Prochlorococcus*, both in the light and dark (Figure 5). On average, SAR11 and *Prochlorococcus* accounted for 43% and 68% of total bacterioplankton ATP and methionine uptake, respectively (Supplementary Figure 3c and d).

The cyanobacteria *Synechococcus* depicted a significantly higher uptake of ATP than SAR11 and *Prochlorococcus* (Figure 6, Table 2), contributing to up to 35% of the bacterioplankton ATP uptake. However, the *Synechococcus* uptake rate was not enhanced by light (Figure 6). The uptake of other non-sorted groups, mainly HNA containing bacteria with low scatter (HNA-ls, Supplementary Figure 1), was not significantly different in the light or dark (paired t-test $p > 0.05$, Supplementary Figure 5). The uptake of the HNA-ls group was calculated by subtracting the sum of *Prochlorococcus* and SAR11 groups from the total bacterioplankton uptake.

Effect of light on SAR11 and Prochlorococcus bacterioplankton

SAR11, as well as *Prochlorococcus,* showed significant light-enhanced ^{3}H-ATP, ^{33}P-ATP (Figure 4) and methionine uptake (Figure 5) (paired t-test, p-value<0.001 for both populations and substrates). On average, SAR11 increased uptake of ATP in the light by 1.4 ± 0.3 and 3.4 ± 1.7 x 10^3 molecules cell^{-1} hour^{-1} for ^{33}P-ATP and ^{3}H-ATP respectively, and by 2.9 ± 0.4 x 10^3 molecules cell^{-1} hour^{-1} for methionine. *Prochlorococcus* showed a similar increase in ATP (2.8 ± 0.5 and 6.0 ± 1.9 x 10^3 molecules cell^{-1} hour^{-1} for ^{33}P-ATP and ^{3}H-ATP respectively), and methionine (3.1 ± 0.7 x 10^3 molecules cell^{-1} hour^{-1}) uptake in the light. The lower absolute values of light-stimulated substrate uptake in SAR11 compared to *Prochlorococcus* reflect the lower absolute uptake rates of the former. However, light stimulated uptake of both substrates amounted to a similar proportion in both SAR11 and *Prochlorococcus*. Thus, exposure to light stimulated SAR11 uptake of ^{3}H-ATP, ^{33}P-ATP and methionine by 29 ± 20 %, 35 ± 6%, and 32 ± 5%, respectively (Figure 7a, b). For *Prochlorococcus* the corresponding values were 34 ± 10%, 33 ± 5%, and 34 ± 4%, respectively (Figure 7c, d).

The total light-enhanced ATP and methionine uptake was budgeted to assess if the groups that were not sorted contributed to the light-enhanced uptake. The average light-enhanced uptake of a cell was multiplied by its abundance relative to total bacterioplankton. The contribution of the SAR11 population to the light- enhanced ATP uptake was 17% while for *Prochlorococcus* it was 9%, which equals the bacterioplankton light-enhanced uptake. Similarly, for methionine the contribution of the SAR11 population to the light-enhanced uptake was 16% and 9% for

365 *Prochlorococcus,* which also accounts for the total bacterioplankton light- enhanced
366 uptake. Therefore, SAR11 and *Prochlorococcus* were the two bacterioplankton
367 groups for which uptake was enhanced when exposed to light in the oligotrophic
368 North Atlantic gyre.

369

370 **Discussion**

371 Here we present experimental evidence, collected in two consecutive years
372 that in the oligotrophic North Atlantic Ocean subtropical gyre *Prochlorococcus*
373 cyanobacteria and LNA bacterioplankton, solely comprising SAR11
374 alphaproteobacteria cells, use light to enhance their uptake of simple organic
375 substrates, and do so in equal measure.

376 Taxonomic identification of LNA bacteria, as discriminated by flow
377 cytometry, has so far remained incomplete (Mary *et al* 2006, Hill *et al* 2010,
378 Schattenhofer *et al* 2011). We show here that virtually all cells in the LNA
379 bacterioplankton are phylogenetically affiliated with the SAR11 clade (Table 1). We
380 improved the detection of SAR11 in the LNA fraction by the application of a
381 combination of six different HRP-labelled probes and a helper oligonucleotide probe
382 (Fuchs *et al* 2000) specifically targeting different positions in the SAR11 rRNA
383 (Supplementary Table 1). This approach significantly increased the number and
384 intensity of the probe-conferred signals, which allowed better quantification of the
385 clade. The simultaneous comparison of the *in situ* light-driven uptake of two organic
386 molecules, and the combination with their phylogenetic identification revealed that,
387 despite significant differences in absolute uptake rates (Figure 4 and 5), the relative
388 response to light by natural populations of SAR11 and *Prochlorococcus* was
389 remarkably similar (Figure 7).

390 *Light-enhanced uptake of nucleotides and amino acids by* Prochlorococcus *and*
391 *SAR11*

392 The aerobic oxygenic cyanobacterium *Prochlorococcus* has a chlorophyll
393 based light harvesting complex and fixes CO_2 (Chisholm *et al* 1988, Chisholm *et al*
394 1992). However, in the oligotrophic surface ocean, *Prochlorococcus* might invest in
395 cyclic photophosphorylation and channel the energy generated to heterotrophically

import essential building blocks such as nucleotides (Figure 4b; Michelou *et al* 2011) and different essential amino acids (Figure 5b; (Zubkov *et al* 2003, Church *et al* 2004, Michelou *et al* 2007, Mary *et al* 2008). The other common marine cyanobacterium *Synechococcus* is also able to take up organic molecules but its photoheterotrophy is less consistent. For example, a higher proportion of *Synechococcus* cells than of SAR11 cells took up ATP in the Eastern Mediterranean Sea, depleted in phosphate (Sebastian *et al* 2012). In the present study it was shown that *Synechococcus* cells took up significantly more ATP than either SAR11 and *Prochlorococcus* cells (Table 2). However, the *Synechococcus* ATP uptake rate was light insensitive (Figure 6) while the uptake of amino acids by *Synechoccocus* cells did increase when exposed to light (Mary *et al* 2008). These differences in light stimulation are probably a result of differential uptake of organic molecules by *Synechococcus* (Cuhel and Waterbury 1984, Willey and Waterbury 1989, Beja and Suzuki 2008) that warrant further field investigation.

In the non-pigmented heterotrophic bacterium SAR11 it is likely that light stimulation of methionine and nucleotides uptake rates is linked to the functioning of a proteorhodopsin (PR) proton pump. The PR gene is broadly distributed within the SAR11 clade (Giovannoni *et al* 2005a, Campbell *et al* 2008). However, expression levels are not consistently linked to dark or light conditions (Giovannoni *et al* 2005a, Cottrell and Kirchman 2009, Steindler *et al* 2011). The expression of PR even after long periods in the dark might enable cells to respond quickly when light becomes available. SAR11, together with *Prochlorococcus,* significantly increased their uptake of ATP and methionine within the first 30 minutes of incubation, both in samples taken at dawn after long periods in the dark and at midday under bright sunlight (Figure 2, 4 and 5). The short time response suggests that light has a direct photophysiological effect on these bacterial cells rather than an indirect one through uptake of organic molecules exuded by photo-stimulated phytoplankton (Karl *et al* 1998, Church *et al* 2004).

The relative influence of light on the transport of methionine, and adenosine and phosphorus monomers from nucleotides was also remarkably similar (Figure 7), despite significantly different uptake rates between molecules and bacterial groups (Figure 4 and 5), and different uptake mechanisms.

The uptake of ^{33}P-ATP involves the extracellular dephosphorylation by alkaline phosphatase (E.C. 3.1.3.1) (Wanner 1996), or 5'-nucleotidase (E.C. 3.1.3.5) (Bengis-Garber 1985) and the uptake of the phosphate groups. The uptake of the ^{3}H-ATP could either reflect the transport of the intact molecule or of the ribose backbone. Likely, ^{3}H-ATP is taken up by the cells after the phosphate groups have been cleaved from the ribose (Bengis-Garber 1983, Bengis-Garber 1985). Bacterial membranes, with the exception of the ones from obligate intracellular bacteria, seem not to be permeable to the intact ATP molecule (Daugherty *et al* 2004, Schmitz-Esser *et al* 2004). Moreover, if ^{3}H-ATP was taken up with the phosphate groups, as AMP or ADP, then the uptake rates would be comparable between ^{33}P-ATP and ^{3}H-ATP, as we used α-^{33}P-ATP. However, our results show that *Prochlorococcus* and SAR11 inhabiting the oligotrophic North Atlantic gyre take up the adenosine monomers two to three times faster than the phosphate groups (Figure 4, Supplementary Figure 4). This indicates that they might be scavenging nucleotides as a nucleoside source rather than for phosphorus (Casey *et al* 2009, Michelou *et al* 2011), which is rather taken from the inorganic phosphate pool (Zubkov *et al* 2007). The coupling between the hydrolysis of nucleotides and uptake could be loose, and not all hydrolysed phosphate might be taken up by the cells (Ammerman and Azam 1985). The energy harnessed from light may be directed to enhance the enzymatic activity of the 5'-nucleotidase or alkaline phosphatases that results in a higher uptake of both phosphate and adenosine by *Prochlorococcus* and SAR11. Genes encoding phosphatases with potential 5′-nucleotidase activity are known from *Prochlorococcus* and *Synechococcus* (Moore *et al* 2005, Scanlan *et al* 2009, Kathuria and Martiny 2011), and in SAR11 (Gilbert *et al* 2008, Kathuria and Martiny 2011).

Clearly, *Prochlorococcus* and SAR11 have a different evolutionary history and lifestyle. However, in surface waters of the oligotrophic ocean they have converged to benefit from abundant light to import sparse organic molecules at a comparable magnitude. Under such nutrient-depleted conditions both autotrophic or heterotrophic metabolism converge on photoheterotrophy, allowing microorganisms to use a resource as abundant as light and direct it to the necessary energy-dependent functions like nutrient acquisition. The energy harnessed from light, by either PR or chlorophyll, can be directed to several cellular processes to promote an increase in enzymatic activity for dephosphorylating nucleotides (Figure 4 and 6) and a general

uptake of organic matter (Figure 5 and 6; (Michelou *et al* 2007, Mary *et al* 2008, Steindler *et al* 2011))

It seems plausible that photoheterotrophy benefits microorganisms inhabiting the least productive ecosystems, such as the North (Figure 1) or South Atlantic subtropical gyres (Michelou *et al* 2007, Mary *et al* 2008) or the North Pacific Ocean (Church *et al* 2004), but might not be significant in more productive seas (Alonso-Saez *et al* 2006, Ruiz-Gonzalez *et al* 2012). When nutrient availability is high, as in coastal waters, SAR11 uptake of amino acids could even be inhibited by light (Alonso-Saez *et al* 2006, Ruiz-Gonzalez *et al* 2012), or have no effect, as in *Candidatus* Pelagibacter ubique cells during active growth (Steindler *et al* 2011). A PR-based light–harvesting could provide SAR11 cells with extra energy to import organic compounds in oceanic areas where dissolved organic matter as well as essential inorganic macronutrients, like nitrogen and phosphorus, are present at exceedingly low concentrations.

Measuring energy harvesting and storage in bacterioplankton cells is challenging (Zubkov 2009). Therefore, the costs and benefits of utilising light energy by aerobic anoxygenic bacteria and PR-containing bacteria were estimated using bioenergetic models (Kirchman and Hanson 2012). The net energy gain seems to be sufficient to meet maintenance costs by the former and insufficient to meet maintenance costs of the latter except under situations when high light intensities are combined with a large number of PR molecules imbedded in the cell membrane. Such a situation is plausible in the subtropical North Atlantic gyre, where light intensities are regularly high (up to 800 μmol photons m^{-2} s^{-1} measured when samples for this study were taken) and the extra energy harvested from light could advantage PR-containing microorganisms like SAR11.

It is likely then, that PR- or Chl a- based photoheterotrophy provides a large fraction of cellular energy requirements for the SAR11 clade and *Prochlorococcus* to outperform other bacterioplankton groups in essential nutrient acquisition in the least productive and most extensive ecosystems on Earth like the North Atlantic subtropical gyre.

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717

718 **Table 1:** Composition of bacterioplankton in flow cytometrically sorted groups as
719 relative abundance (% DAPI stained cells) with oligonucleotide probes detailed in
720 Supplementary Table 1.
721

Sample		LNA		*Prochlorococcus*	
Lat (°N)	**Long (°W)**	**EUBI-III**	**SAR11-mix**	**EUB I-III**	**PRO405**
34.21	29.72	n.d	n.d	93	88
32.43	31.8	n.d	84	94	83
31.73	32.56	92	92	n.d.	82
28.11	36.51	93	98	95	90
23.77	41.11	88	97	93	86
21.21	39.29	90	96	94	86
16.19	35.8	88	98	94	85
13.47	33.95	90	96	97	95
7.82	30.16	80	89	94	93
4.8	28.16	84	84	93	93
	mean ± SD	88 ± 4	93 ± 6	94 ± 1	88 ± 4

722 n.d not determined
723

724 **Table 2:** Average ATP and methionine uptake rate of Bacterioplankton (Bpl),
725 SAR11, *Prochlorococcus* (Pro) and *Synechococcus* (Syn) in the light, dark and light-
726 enhanced uptake.

		Average uptake rate				
		Light		**Dark**	**Light enhanced**	**Light enhanced**
		x10³ molecules cell⁻¹ hour⁻¹				*Percentage*
	^{33}P-ATP					
Group	Bpl	7.7 ± 0.8	>*	5.6 ± 0.6	2.1 ± 0.6	36 ± 4%
	SAR11	3.5 ± 0.6	>**	2.1 ± 0.3	1.4 ± 0.3	35 ± 6%
	PRO	7.4 ± 1.0	>**	4.6 ± 0.6	2.8 ± 0.5	33 ± 5%
	Syn	96.7 ± 38	<	166 ± 68	No light enhancement	
	^{3}H-ATP					
Group	SAR11	8.2 ± 1.8	>	4.8 ± 0.2	3.4 ± 1.7	29 ± 20%
	PRO	18.0 ± 0.9	>	11.9 ± 2.1	6.0 ± 1.9	34 ± 10%
	Methionine					
Group	Bpl	11.9 ± 1.5	>**	8.7 ± 1.1	3.1 ± 0.5	21 ± 3%
	SAR11	8.8 ± 1.1	>**	5.8 ± 0.8	2.9 ± 0.4	32 ± 5%
	PRO	8.8 ± 1.4	>**	5.7 ± 1.1	3.1 ± 0.7	34 ± 4%

727 * p-value <0.05, ** p-value <0.01

728

729 **Figure legends**

730 **Figure 1: (a)** Map showing the study area in the North Atlantic Ocean during the
731 AMT20 cruise in autumn 2010 and the LINK cruise in summer 2011. Symbols
732 indicate the stations at which light/dark sorting experiments were carried out. The
733 boundaries of the oceanic provinces were identified with the *Synechococcus*
734 distribution (Hartmann *et al* 2012) indicated with short dash lines. **(b)** Latitudinal
735 distribution of the abundance of SAR11 and *Prochlorococcus* during autumn 2010.
736 Error bars show standard errors.

737 **Figure 2:** Scatter plot comparison of the total bacterioplankton uptake of **(a)** ATP and
738 **(b)** methionine in the light and dark. Error bars show standard errors of independent
739 time points measurements. The light grey line indicates the unity line.

740 **Figure 3:** Latitudinal distribution of bacterioplankton uptake rates and bioavailable
741 methionine and ATP along the AMT20 transect during autumn 2010. **(a)** Bioavailable
742 concentration and **(b)** total bacterioplankton uptake rates of methionine and ^{33}P-ATP.
743 Error bars show standard errors.

744 **Figure 4:** Scatter plot comparison of **(a)** SAR11 and **(b)** *Prochlorococcus* ^{3}H-ATP
745 and ^{33}P-ATP uptake rates in the light and dark. Uptake in the light was significantly
746 higher ($p<0.05$) than in the dark in **(a)** 17 of 21 and **(b)** 16 of 20 experiments. The
747 light grey line indicates the unity line.

748 **Figure 5:** Scatter plot comparison of **(a)** SAR11 and **(b)** *Prochlorococcus* methionine
749 uptake rates in the light and dark. Uptake in the light was significantly higher
750 ($p<0.05$) than in the dark in **(a)** 16 of 20 and **(b)** 15 of 19 experiments. The light grey
751 line indicates the unity line.

752 **Figure 6:** ^{33}P-ATP uptake rate of SAR11, *Prochlorococcus* (Pro) and *Synechococcus*
753 (Syn) in three selected stations, showing uptake rate in the light and in the dark.

754 **Figure 7:** Comparison of relative light-enhanced uptake rates of nucleotides and
755 amino acids by **(a, b** – correspondingly**)** SAR11 and **(c, d)** *Prochlorococcus*:
756 latitudinal variability (left column) and mean values (right column). The hatched
757 pattern corresponds to SAR11.

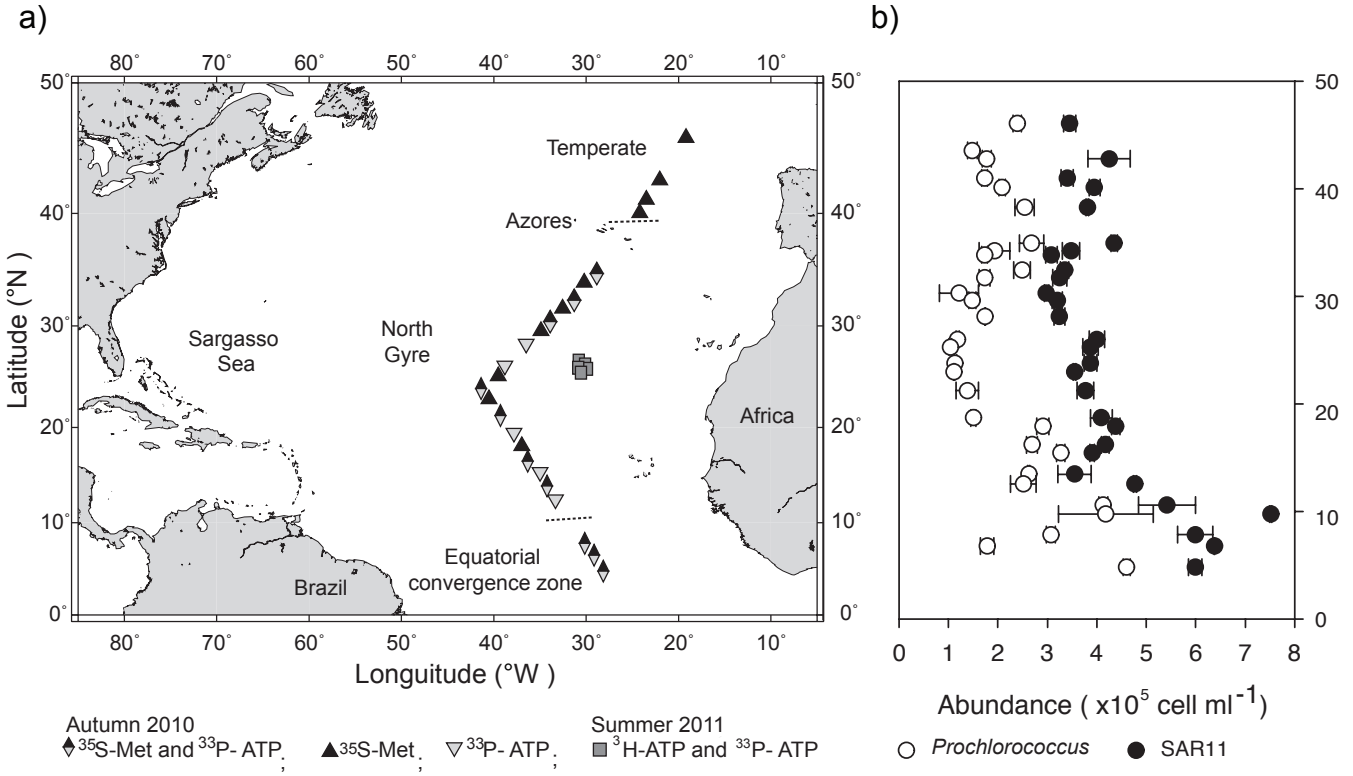
a)
b)
Temperate
Azores
North
Gyre
Sargasso
Sea
Africa
Brazil
Equatorial
convergence zone
Latitude (°N)
Longuitude (°W)
Autumn 2010
35S-Met and 33P- ATP;
35S-Met;
33P- ATP;
Summer 2011
3H-ATP and 33P- ATP
Abundance (x10^5 cell ml^-1)
Prochlorococcus
SAR11

Figure 1

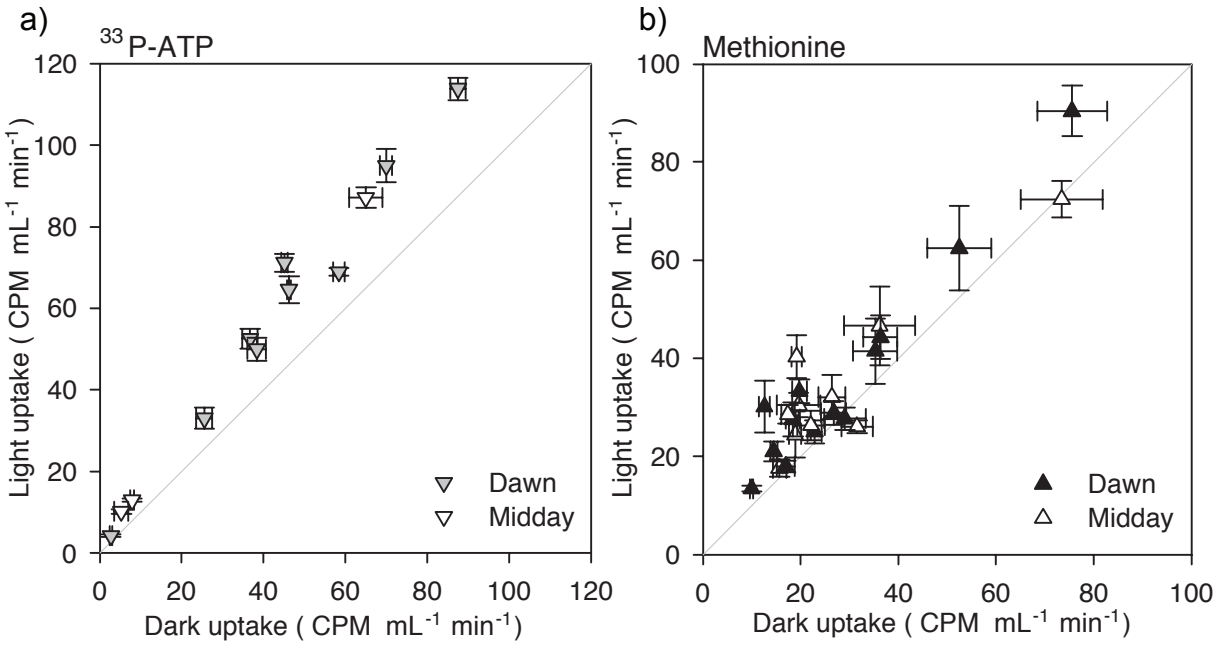
a)
33 P-ATP
Light uptake (CPM mL-1 min-1)
Dark uptake (CPM mL-1 min-1)
Dawn
Midday
b)
Methionine
Light uptake (CPM mL-1 min-1)
Dark uptake (CPM mL-1 min-1)
Dawn
Midday

Figure 2

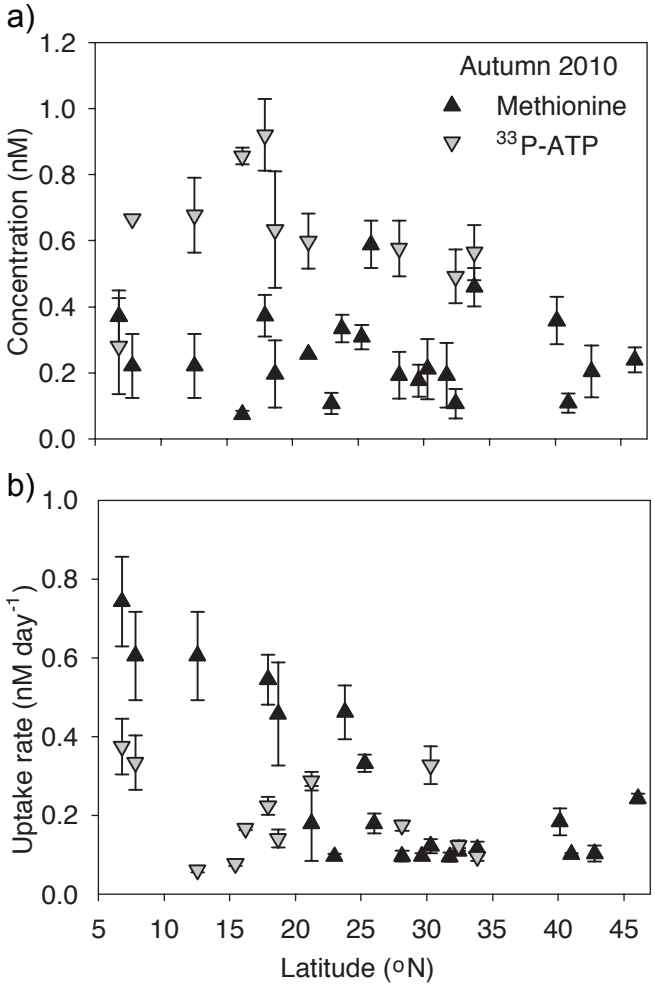
a)
Autumn 2010
Methionine
33P-ATP
Concentration (nM)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
b)
Uptake rate (nM day-1)
1.0
0.8
0.6
0.4
0.2
0.0
5
10
15
20
25
30
35
40
45
Latitude (°N)

Figure 3

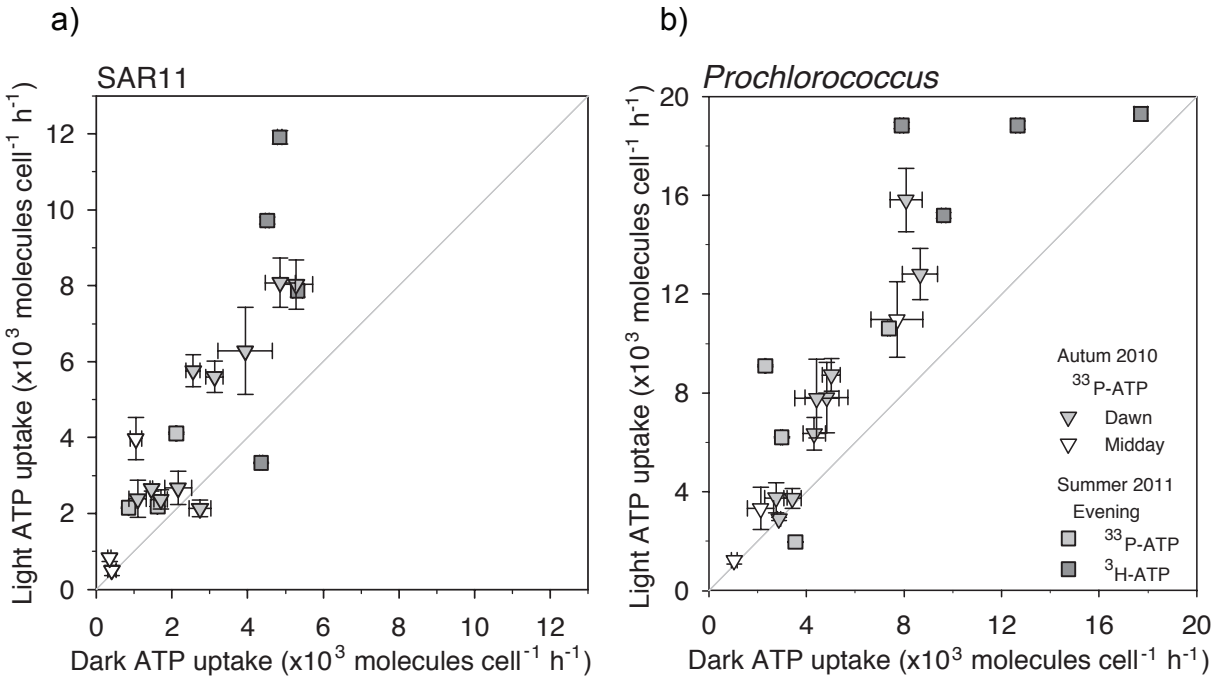
a)
SAR11
Light ATP uptake (x10^3 molecules cell^{-1} h^{-1})
Dark ATP uptake (x10^3 molecules cell^{-1} h^{-1})
0
2
4
6
8
10
12
b)
Prochlorococcus
Light ATP uptake (x10^3 molecules cell^{-1} h^{-1})
Dark ATP uptake (x10^3 molecules cell^{-1} h^{-1})
0
4
8
12
16
20
Autum 2010
^{33}P-ATP
Dawn
Midday
Summer 2011
Evening
^{33}P-ATP
^{3}H-ATP

Figure 4

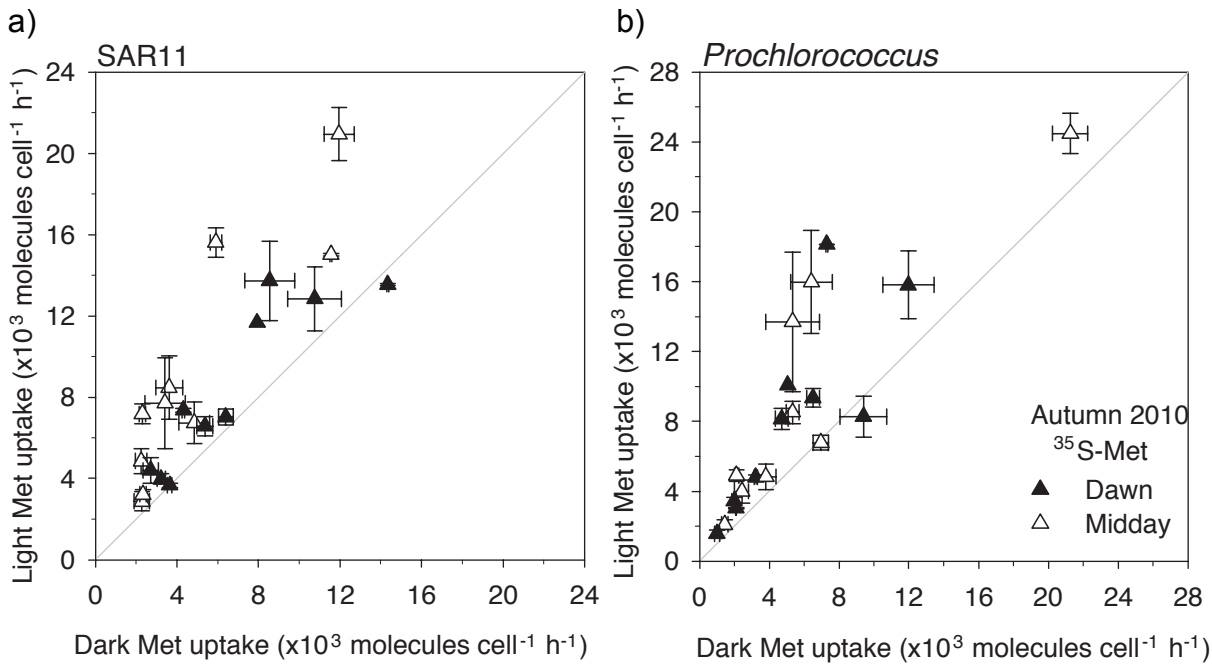
a)
SAR11
Light Met uptake (x10^3 molecules cell^{-1} h^{-1})
Dark Met uptake (x10^3 molecules cell^{-1} h^{-1})
0
4
8
12
16
20
24
b)
Prochlorococcus
Light Met uptake (x10^3 molecules cell^{-1} h^{-1})
Dark Met uptake (x10^3 molecules cell^{-1} h^{-1})
0
4
8
12
16
20
24
28
Autumn 2010
^{35}S-Met
Dawn
Midday

Figure 5

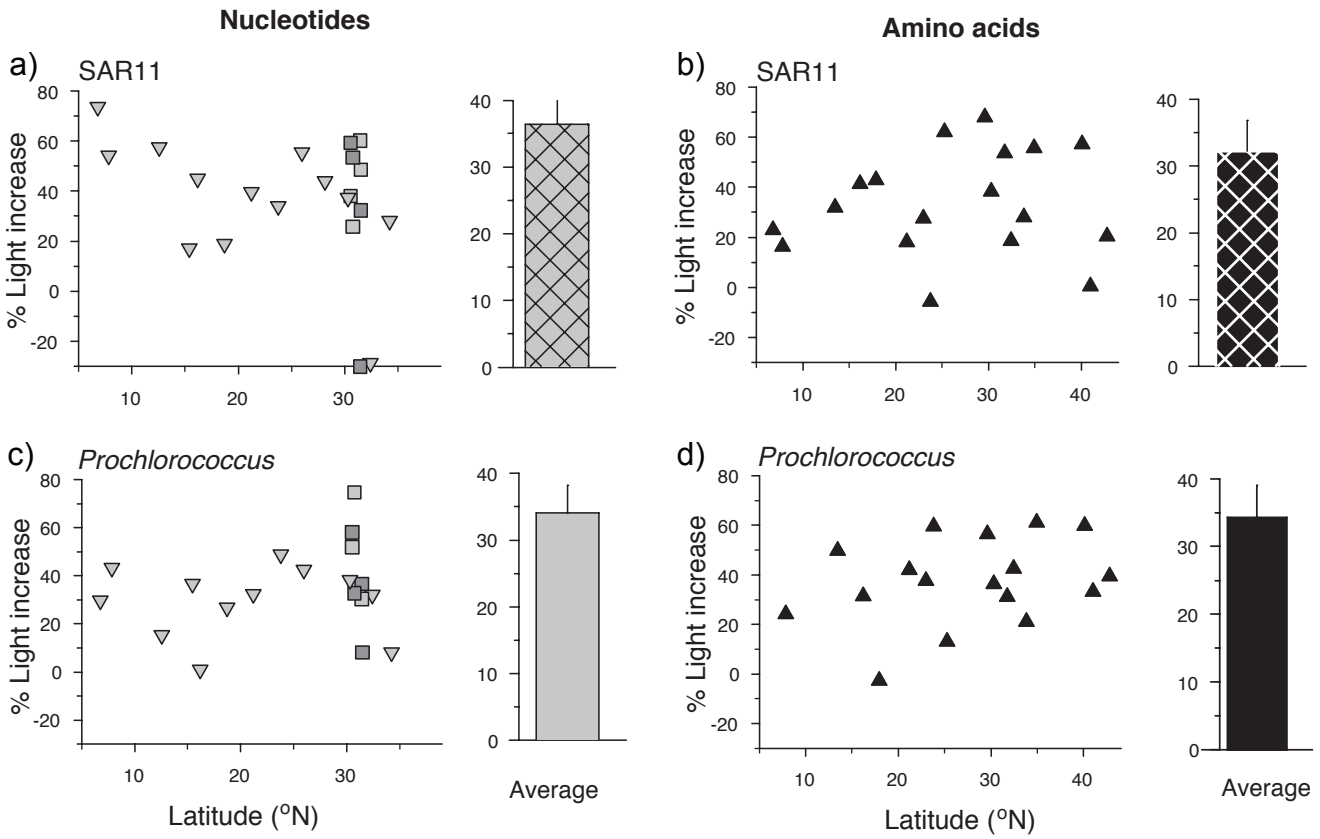
Nucleotides
Amino acids
a)
SAR11
b)
SAR11
c)
Prochlorococcus
d)
Prochlorococcus
% Light increase
Latitude (°N)
Average

Figure 6