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Light-Stimulated Bacterial Production and Amino Acid Assimilation by Cyanobacteria and Other Microbes in the North Atlantic Ocean[▽]

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We examined the contribution of photoheterotrophic microbes—those capable of light-mediated assimilation of organic compounds—to bacterial production and amino acid assimilation along a transect from Florida to Iceland from 28 May to 9 July 2005. Bacterial production (leucine incorporation at a 20 nM final concentration) was on average 30% higher in light than in dark-incubated samples, but the effect varied greatly (3% to 60%). To further characterize this light effect, we examined the abundance of potential photoheterotrophs and measured their contribution to bacterial production and amino acid assimilation (0.5 nM addition) using flow cytometry. *Prochlorococcus* and *Synechococcus* were abundant in surface waters where light-dependent leucine incorporation was observed, whereas aerobic anoxygenic phototrophic bacteria were abundant but did not correlate with the light effect. The per-cell assimilation rates of *Prochlorococcus* and *Synechococcus* were comparable to or higher than those of other prokaryotes, especially in the light. Picoeukaryotes also took up leucine (20 nM) and other amino acids (0.5 nM), but rates normalized to biovolume were much lower than those of prokaryotes. *Prochlorococcus* was responsible for 80% of light-stimulated bacterial production and amino acid assimilation in surface waters south of the Azores, while *Synechococcus* accounted for on average 12% of total assimilation. However, nearly 40% of the light-stimulated leucine assimilation was not accounted for by these groups, suggesting that assimilation by other microbes is also affected by light. Our results clarify the contribution of cyanobacteria to photoheterotrophy and highlight the potential role of other photoheterotrophs in biomass production and dissolved-organic-matter assimilation.

The discovery of proteorhodopsin-containing bacteria (3), aerobic anoxygenic phototrophic (AAP) bacteria (17), and assimilation of dissolved organic matter (DOM) by cyanobacteria (26) suggests that photoheterotrophy may be common in the oceans. Several studies indicate that *Prochlorococcus* may be able to assimilate components of the DOM pool, in addition to its large contribution to primary production in oligotrophic oceans (10). Church et al. (5, 6) found that leucine incorporation was 48 to 114% higher in light incubations than in the dark in the North Pacific Gyre, where *Prochlorococcus* is well known to be abundant. Other studies had demonstrated that *Prochlorococcus* is responsible for a large fraction of dissolved methionine turnover (~1 nM) and up to 30% of leucine incorporation (20 nM), although the effect of light was not examined (33, 34). This capacity to take up amino acids is consistent with the presence of amino acid transport systems, as revealed by the whole-genome sequence of *Prochlorococcus* (28). However, the role of *Prochlorococcus* in assimilating other organic compounds and the effect of light on this assimilation are still unknown.

Synechococcus may also assimilate components of the DOM pool, as well as being a major contributor to primary production in temperate and tropical waters (20). *Synechococcus* can take up methionine and another important organic sulfur compound, dimethylsulfoniopropionate (DMSP) (21, 31). Although *Synechococcus* cannot incorporate thymidine (11), axenic strains of this cyanobacterium are capable of utilizing urea (7, 24) and amino acids (4, 23, 26), albeit at low rates. In addition, genomic analyses of *Synechococcus* strain WH8102 revealed genes homologous to those for transporting amino acids, oligopeptides, and cyanate (27). There is also evidence for aminopeptidase activity in *Synechococcus* (22). However, Zubkov and colleagues (33) found that *Synechococcus* was responsible for only 3% of methionine turnover in a mesotrophic region of the Arabian Sea. More work is needed to determine the extent of photoheterotrophy by these cyanobacteria.

The goals of this study were to identify the microbial groups responsible for light-dependent leucine incorporation and to examine the effect of light on the uptake of amino acids added at tracer levels. We hypothesize that stimulation of bacterial production by light is due to photoheterotrophy by *Prochlorococcus*. Our results suggest this was in fact the case, but other groups of photoheterotrophic bacteria contributed to the light effect as well.

MATERIALS AND METHODS

Abundance of AAP bacteria, cyanobacteria, and total prokaryotes. The experimental work was done during the North Atlantic Spring Bloom (NASB) project onboard the R/V Seward Johnson from 28 May to 2 July 2005. The cruise track included a transit leg across the Sargasso Sea, from Fort Pierce, FL, to Ponta Delgada, Azores, followed by a 5-week transect survey beginning at 45°N and 20°W. Surface seawater for sampling was collected daily from the ship's underway system from about a 5-m depth during the first transect. Samples during the second leg were collected at various depths with a rosette of Niskin bottles mounted on a conductivity-temperature-depth profiler.

Samples for AAP bacteria and total prokaryote abundance were preserved and enumerated, following the protocol in Cottrell et al. (8). Each of 30 fields of view was subjected to the following four exposures: 4′,6′diamidino-2-phenylindole (60 ms); bacteriochlorophyll *a* (400 ms); chlorophyll *a* (1,500 ms); and phycoerythrin (50 ms). AAP bacteria were scored as 4′,6′diamidino-2-phenylindole and bacte-

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TABLE 1. Abundances of microbes in surface waters sampled during the NASB expedition[a]

Station	Lat (°N)	Lon (°W)	Abundance (10^4 cells ml^{-1})				
			Prokaryotes	*Prochlorococcus*	*Synechococcus*	AAP	Picoeukaryotes
1-1	29.3	76.1	46.1 (5)	1.3 (2.8)	0.9 (1.9)	5.4 (2.4)	0.1 (0.2)
1-2	29.8	74.9	56.7 (7.6)	0.3 (0.5)	1.4 (2.4)	2.9 (0.1)	0.1 (0.2)
1-3	30.1	71.2	53.1 (3.6)	0.1 (0.1)	1.2 (2.2)	4.5 (0.2)	0.1 (0.2)
1-4	31.7	69.8	58.9 (7.8)	0.2 (0.3)	2.5 (4.3)	4.2 (0.6)	0.1 (0.2)
1-5	32.6	67.0	63.3 (6)	4.3 (6.8)	1.9 (2.9)	8.6 (0.2)	0.1 (0.1)
1-6	33.3	64.8	79.2 (11.6)	2.5 (3.1)	3.6 (4.5)	5.4 (0.2)	0.2 (0.2)
1-7	34.1	62.0	77.8 (1.9)	2.0 (2.6)	1.3 (1.7)	2.7 (0.2)	0.1 (0.2)
1-8	34.8	59.7	56.5 (6.3)	1.9 (3.4)	3.0 (5.2)	3.7 (0.4)	0.3 (0.5)
1-9	35.3	56.8	64.3 (11)	3.1 (4.9)	1.6 (2.4)	8.3 (0.3)	0.1 (0.2)
1-10	35.8	54.3	71.4 (12)	3.0 (4.3)	5.9 (8.3)	4.1 (0.1)	0.2 (0.3)
1-11	36.3	51.3	78.7 (10)	1.5 (1.9)	4.1 (5.2)	3.2 (0.1)	0.3 (0.4)
1-12	36.7	48.7	114.9 (14)	6.8 (5.9)	2.5 (2.2)	7.3 (0.1)	0.3 (0.2)
1-13	37.1	45.6	81.6 (20)	3.8 (4.6)	1.2 (1.4)	4.0 (0.5)	0.1 (0.1)
1-14	37.4	42.9	83.9 (18)	8.4 (10.0)	1.1 (1.3)	3.8 (0.1)	0.1 (0.1)
1-15	37.6	40.1	87.8 (7.3)	3.4 (3.9)	1.0 (1.2)	6.1 (0.0)	0.2 (0.2)
1-16	37.8	37.4	70.6 (13)	0.1 (0.2)	2.3 (3.2)	9.9 (0.0)	0.1 (0.2)
1-17	37.9	34.6	85.3 (12)	6.8 (8.0)	1.2 (1.4)	1.3 (1.3)	0.2 (0.2)
1-18	37.9	32.0	71.8 (23)	6.5 (9.0)	2.8 (3.9)	7.4 (0.4)	0.1 (0.2)
1-19	37.9	29.0	81.1 (13)	0.5 (0.6)	0.3 (0.4)	5.8 (0.7)	0.1 (0.2)
2-3	45.0	20.0	108.0 (12)	1.5 (1.3)	2.9 (2.6)	4.4 (0.4)	1.0 (0.9)
2-5	48.0	20.0	82.3 (20)	1.0 (1.0)	8.4 (8.0)	7.7 (0.2)	2.3 (2.2)
2-7	50.0	20.0	97.8 (11)	0.2 (0.2)	15.2 (15.0)	7.3 (0.2)	1.6 (1.6)
2-13	52.9	20.0	192.0 (36)	0.1 (0.1)	6.3 (4.2)	12.0 (0.1)	1.3 (0.8)
2-14	54.1	17.0	188.9 (24)	0.1 (0.0)	3.5 (2.1)	12.0 (0.0)	0.9 (0.5)
2-23	57.0	16.0	194.0 (49)	0.1 (0.0)	5.4 (3.0)	NA (0.0)	2.9 (1.6)
2-27	58.0	15.5	238.1 (31)	0.1 (0.0)	1.8 (1.0)	NA (0.0)	2.5 (1.4)
2-40	61.1	19.0	131.2 (28)	0.2 (0.1)	4.1 (3.4)	NA (0.1)	0.2 (0.2)
2-48	64.5	25.0	289.0 (77)	0.3 (0.1)	0.9 (0.4)	NA (0.4)	0.9 (0.4)

[a] Values in parentheses indicate standard deviations. Lat, latitude; Lon, longitude.

riochlorophyll *a* positive but chlorophyll *a* and phycoerythrin negative. *Prochlorococcus*, *Synechococcus*, and picoeukaryotes were enumerated using flow cytometry and distinguished by their different size and pigment properties in unstained samples following common procedures (12, 25). For flow-cytometric analyses of total prokaryotes, samples were stained with SYTO 13 (final concentration, 5 μM) for 10 to 15 min at room temperature in the dark and discriminated following procedures described previously (30). Abundances were estimated with a FacsCalibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with an air-cooled argon laser (488 nm, 25 mW).

Response of leucine incorporation and amino acid assimilation to irradiance. To examine the effects of light on biomass production, [^{3}H]leucine incorporation was measured in seawater incubated in the light and dark in a deck incubator with running seawater for 6 h. Experiments were conducted in triplicate with [^{3}H]leucine (20 nM; specific activity of 173 Ci/mmol; Amersham) in 5.0 ml of seawater. Samples were placed in clear bags (Whirl Pack, Nasco Fort Atkinson, WI) and placed in the deck incubator covered by a clear acrylic sheet (Plexiglas XT colorless, 3 mm thick; Rohm & Haas), which partially screens out UV irradiance (50% transmission at 375 nm) (data not shown). Killed controls for light and dark treatments consisted of samples to which 5% trichloroacetic acid (TCA) was added before the addition of isotopes. At the end of the incubation, 1.5 ml was transferred from each bag to a 2-ml polypropylene centrifuge tube, terminated by the addition of 5% TCA, and processed using the microcentrifuge method (29). In addition, other 1.5-ml samples were incubated with 20 nM leucine for 1 h at the in situ temperature in the dark. Incubations were terminated and processed by the centrifuge method (29).

Amino acid assimilation by cyanobacteria. We also examined assimilation of leucine (20 nM) and amino acids by cyanobacteria and other microbes separated by flow cytometry. The amino acid mixture consisted of 15 amino acids (TRK 440; specific activity, 40 Ci/mmol; Amersham) commonly found in protein but without asparagine, cysteine, glutamine, tryptophan, and methionine, added to a final concentration of 0.5 nM. Six replicate 4.5-ml samples from depths corresponding to the 30% light level were incubated in the deck incubator for 6 h under 30% light or in the dark. At the end of the incubation, samples were fixed with 2% paraformaldehyde, frozen in liquid nitrogen, and stored at −80°C before analysis by flow cytometry and sorting in the lab. Radiolabeled and stained cells were analyzed and sorted using a FacsCalibur flow cytometer following protocols previously described (33, 34).

RESULTS

Abundance of microbes in surface waters. Total numbers of prokaryotes varied with geographic location (Table 1). Most of these prokaryotes were bacteria, as archaeal abundance was low in surface waters (data not shown). *Prochlorococcus* was present only between 27°N and 47°N, where it constituted on average about 4% of the total prokaryotic community in the surface layer, reaching the highest cell abundance of 8.4×10^4 cells ml^{-1} in the Sargasso Sea (Table 1). Abundance of *Prochlorococcus* declined substantially north of 45°N, to <1% of all cells. In contrast, *Synechococcus* was present in all surface waters, ranging from 0.5% to 15% of total cells. Abundance of AAP bacteria ranged from 1.3×10^4 cells ml^{-1} to 7.36×10^5 cells ml^{-1}, constituting up to 50% of all cells. Picoeukaryote abundances were low at most stations ([0.1 to 2.9] $\times 10^4$ cells ml^{-1}) and reached maximum numbers (1.3×10^4 cells ml^{-1}) at 50°N, where *Synechococcus* was most abundant.

Effects of light on leucine incorporation. Leucine incorporation was greater in the light than in the dark in 19 out of 33 experiments. Rates were up to 6 pmol Leu $liter^{-1}$ h^{-1} higher in the light near the Azores Islands (stations 1-13 to 1-19) (Fig. 1A), but there was no significant effect of light in the Sargasso Sea (30°N, 72°W to 32°N, 66°W). The light effect was also positive from 46°N to 64°N during the second transect, and rates of incorporation were on average 1 to 2 pmol Leu $liter^{-1}$

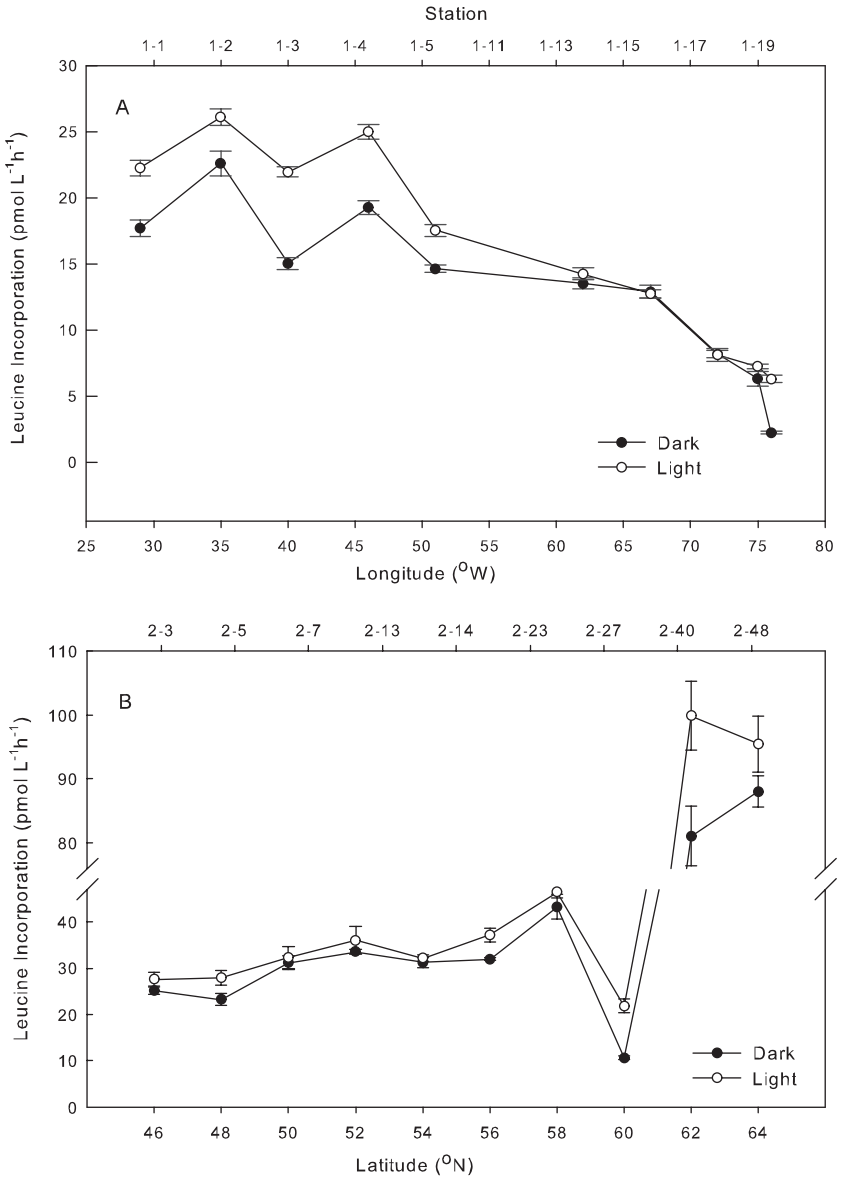

FIG. 1. Bacterial production (leucine incorporation) along a transect from Fort Pierce, FL (76°W), to the Azores Islands (29°W) (A) or from the Azores (46°N) to Iceland (66°N) (B). Light incubations were done at 30% of surface irradiance. Error bars represent standard deviations.

h^{-1} higher with light from 46°N to 58°N (Fig. 1B). Further north, where bacterial production was high (>20 pmol Leu $liter^{-1}$ h^{-1}), light stimulated leucine incorporation the most. Rates were up to 100 pmol Leu $liter^{-1}$ h^{-1} in 30%-light incubations of waters from the coast of Iceland (station 2-40) (Fig. 1B).

Light-stimulated leucine incorporation between Fort Pierce and the Azores was compared with the abundance of *Prochlorococcus* and *Synechococcus* (Fig. 2). Rates of light-induced incorporation were highest at the stations where *Prochlorococcus* was most abundant, accounting for up to 8% of the prokaryotic community, for example, at station 1-13. There was no significant light-stimulated production off the coast of Bermuda (32°N, 62°W), where the abundance of *Synechococcus* and *Prochlorococcus* was $<2 \times 10^4$ cells ml^{-1}. However, at 29°N, 76°W, light-stimulated leucine incorporation was high even though the abundance of *Synechococcus* and *Prochlorococcus* was low ($<2 \times 10^4$ cells ml^{-1}) (Fig. 2). The correlation between *Prochlorococcus* and light-stimulated production was significant ($r = 0.70$; $P < 0.05$; $n = 10$), while it was not for *Synechococcus* ($r = 0.24$; $P > 0.05$; $n = 10$). The correlation between abundance of AAP bacteria and light-stimulated production was also not significant ($r = 0.15$; $P > 0.05$; $n = 10$).

Assimilation of leucine by cyanobacteria. Cyanobacteria and other cells were separated by flow cytometry to determine leucine assimilation per cell (20 nM addition) (Fig. 3). *Prochlorococcus* had greater assimilation per cell than other prokaryotes at the stations where they were abundant (Fig. 3A and B). Assimilation per cell was up to threefold higher for *Prochlorococcus* than assimilation by the other groups we exam-

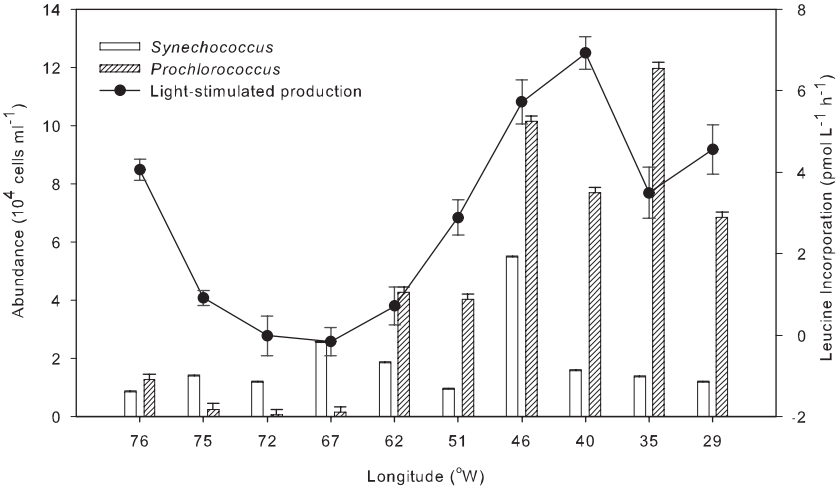

FIG. 2. Abundance of cyanobacterial groups and light-stimulated leucine incorporation rate along the Fort Pierce-to-Azores transect from 76°W to 29°W. Light-stimulated leucine incorporation was calculated by subtracting the dark leucine incorporation from leucine incorporation at 30% of surface light. Error bars represent standard deviations.

ined. Leucine assimilation by *Prochlorococcus* was on average 160% higher in the light than in the dark. Assimilation by prokaryotes other than cyanobacteria was also higher in the light than in the dark but only by 22% (Fig. 3A). Leucine assimilation by *Synechococcus* was not as high, but uptake per cell was comparable to that of other prokaryotes (Fig. 3C and D). Leucine assimilation by this cyanobacterial group was 44% higher in the light than in the dark.

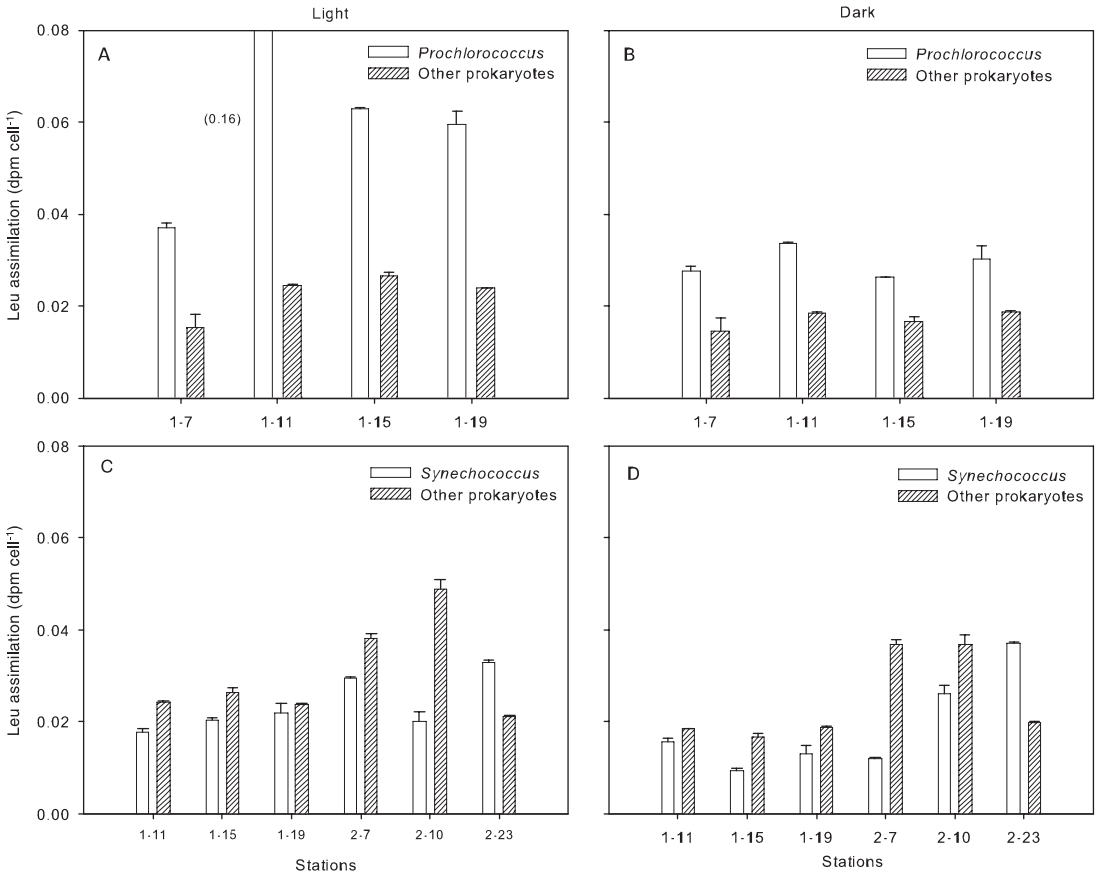

FIG. 3. Leucine assimilation rates for *Prochlorococcus* (A and B) or *Synechococcus* (C and D) and prokaryotes other than cyanobacteria (Other prokaryotes). Cell samples were taken from depths corresponding to the 30% light level and incubated in the dark (B and D) or at 30% of surface light irradiance (A and C) with 20 nM [^{3}H]leucine. Error bars indicate standard errors of three measurements.

TABLE 2. Leucine assimilation per cell and per biovolume for picoplankton groups in surface waters from Fort Pierce, FL, to the Azores islands

Group	Leu assimilation per cell, 10^{-17} mol cell^{-1} h^{-1} (SE)	Biovolume (μm^{-3})[a]	Leu assimilation per biovolume, 10^{-17} mol μm^{-3} h^{-1} (SE)	No. of samples
Other prokaryotes[b]	2.65 (0.54)	0.037	71.73 (14.5)	7
Prochlorococcus	7.06 (3.83)	0.131	53.93 (29.3)	4
Synechococcus	2.50 (0.72)	0.449	5.57 (1.6)	5
Picoeukaryotes	6.92 (1.08)	2.000	3.46 (0.5)	2

[a] Cell size for prokaryotes other than cyanobacteria was estimated by epifluorescence microscopy. Sizes for *Prochlorococcus, Synechococcus* and picoeukaryotes were taken from the work of Zubkov et al. (36).
[b] Prokaryotes other than *Prochlorococcus* and *Synechococcus*.

TABLE 3. Contributions to leucine assimilation (20 nM addition) by cyanobacteria and other microbial groups[a]

Group	Leucine assimilation, pmol liter^{-1} h^{-1} (SE)			% by group[c] (SE)	No. of samples
	Light	Dark	Light − dark[b] (SE)		
Total community	25.92 (3.67)	22.50 (3.76)	3.42 (0.85)	100 (25)	7
Prochlorococcus	4.63 (1.11)	1.88 (0.27)	2.75 (1.08)	80 (30)	4
Synechococcus	1.46 (0.96)	1.05 (0.52)	0.41 (0.62)	12 (7)	7
Other[d]	21.82 (0.89)	20.52 (0.48)	1.30 (0.75)	38 (8)	7

[a] Values are averages for all stations.
[b] Difference in leucine incorporation between light and dark incubations.
[c] Percentage of the difference between light and dark incubations accounted for by the indicated group.
[d] Difference between total community and cyanobacteria (*Prochlorococcus* and *Synechococcus*). Values were calculated per sample, and then percentages were averaged for all stations.

Prochlorococcus had the highest per-cell assimilation rates, 2.6-fold higher than those of other prokaryotes (Table 2). Picoeukaryotes had the second highest per-cell rates (6.92×10^{-17} mol Leu cell^{-1} h^{-1}), whereas *Synechococcus* had the lowest (2.50×10^{-17} mol Leu cell^{-1} h^{-1}). In contrast, rates of leucine assimilation per cell volume for *Prochlorococcus* and other prokaryotes were not significantly different. *Synechococcus* and picoeukaryotes had much lower assimilation rates per cell volume than other prokaryotes and *Prochlorococcus* (Table 2).

Prochlorococcus accounted for on average 18% of total leucine assimilation in the light, ranging from 13% to 24% (Fig. 4A). In the dark, leucine assimilation by *Prochlorococcus* accounted for only 5% to 14% of total assimilation. The percentage of total leucine assimilation by *Synechococcus* was much lower in both the light and dark, ranging from <1% to 10% of the total. *Prochlorococcus* had on average higher rates of assimilation than expected based on its abundance when incubated both at 30% light and in the dark (Fig. 4A). The contribution of *Synechococcus* to <10% of the total leucine assimilation was close to that expected based on its abundance, which was also on average <10% of the prokaryotic community (Fig. 4A).

We examined the contributions of *Prochlorococcus*, *Synechococcus*, and other potential photoheterotrophs to the light effect by combining data from the light-dark incubations and flow cytometry (Table 3). There was high variability among stations in rates of leucine incorporation and in cyanobacterial abundance. The average light-stimulated incorporation by the total community was 3.57 pmol Leu liter^{-1} h^{-1}, while that for *Prochlorococcus* was on average 2.76 pmol Leu liter^{-1} h^{-1} and for *Synechococcus* 0.7 pmol Leu liter^{-1} h^{-1}. About 80% of the light-stimulated incorporation can be accounted for by *Prochlorococcus* and 12% by *Synechococcus* (Table 3). The difference between the total leucine assimilation and assimilation by the cyanobacteria suggests that 38% of the light-stimulated rate was the result of microbes other than cyanobacteria.

Amino acid assimilation by cyanobacteria. Uptake of a 0.5 nM mixture of 15 amino acids by cyanobacteria was compared with uptake by other prokaryotes (Fig. 5). Assimilation per cell for all groups was on average higher in the light than in the dark. *Prochlorococcus* and *Synechococcus* were able to assimilate low concentrations of the amino acids at per-cell rates comparable to those of other prokaryotes. The average assimilation per cell in the light for both cyanobacterial groups was 0.04 ± 0.01 dpm cell^{-1}, while for other prokaryotes it was 0.06 ± 0.02 dpm cell^{-1}. *Synechococcus* assimilation rates were twofold higher in the light (Fig. 5C) than in the dark (Fig. 5D).

The percentage of total amino acid assimilation by *Prochlorococcus* was closer to what was expected based on its abundance than was the case for leucine assimilation (Fig. 4B), as indicated by the values being closer to the one-to-one line. *Prochlorococcus* contributed up to 24% of total leucine assim-

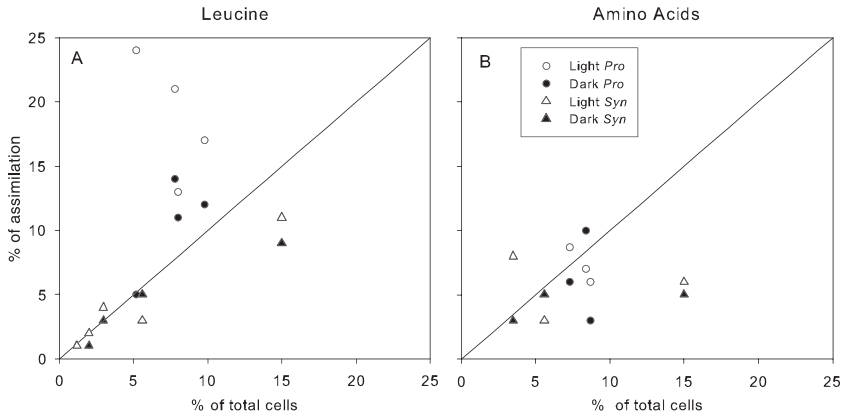

FIG. 4. Percent assimilation of [^{3}H]leucine (A) or ^{3}H-labeled amino acids (B) versus percentage of total prokaryotic abundance for the indicated cyanobacterial group. The diagonal represents the one-to-one line for *Prochlorococcus* (*Pro*) and *Synechococcus* (*Syn*).

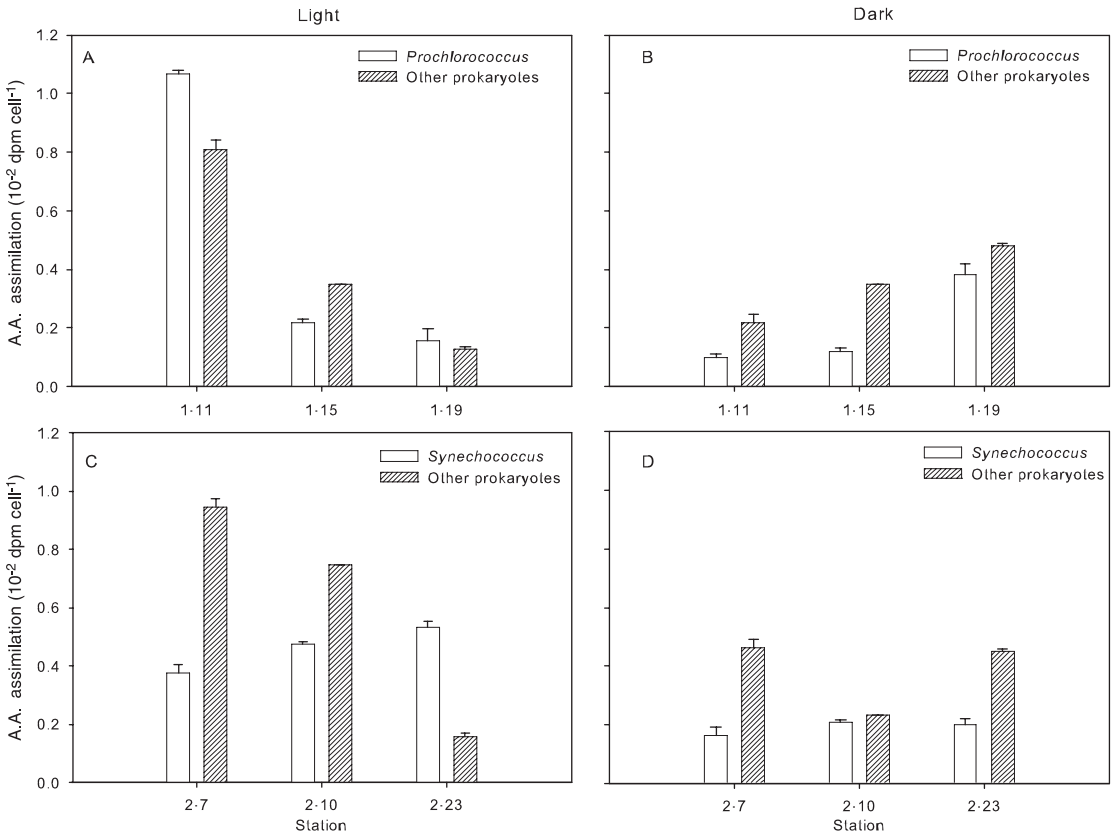

FIG. 5. Rates of amino acid (A.A.) assimilation per cell for *Prochlorococcus*, *Synechococcus*, and other prokaryotes. Samples were incubated in the dark (B and D) or at 30% of surface light irradiance (A and C). Error bars indicate standard errors of three measurements.

ilation but only 10% of total amino acid assimilation. Total amino acid assimilation by *Synechococcus* was similar to that by *Prochlorococcus* (Fig. 4B). However, the contribution to total amino acid assimilation by *Synechococcus* was higher than its average contribution to the total assimilation of leucine (Fig. 4A and B).

DISCUSSION

Our study adds to several lines of evidence that demonstrate the potential for *Prochlorococcus* to utilize amino acids (5, 6, 33, 35). Church et al. (5) observed that leucine incorporation responded to irradiance and attributed the effect to *Prochlorococcus* because of its high abundance in the North Pacific Gyre, although the contribution of *Prochlorococcus* to light-enhanced assimilation was not quantified. Zubkov et al. (33, 35) found that *Prochlorococcus* assemblages were capable of assimilating both methionine and leucine at ~1-nM and 5-nM concentrations, respectively, but the effect of light was not examined. We were able to measure the specific contribution of *Prochlorococcus* to light-stimulated bacterial production and amino acid assimilation in the North Atlantic Ocean and found evidence that other microbes are also involved in the light-stimulated assimilation of these compounds.

Synechococcus, another abundant phototroph in the upper ocean (19), might also be able to assimilate some organic compounds. Zubkov et al. (33) found that the contribution of *Synechococcus* to methionine uptake was <5% in the Arabian Sea, and methionine uptake per *Synechococcus* cell was only 30% of the activity of other bacterial cells. In contrast, our results indicate that *Synechococcus* was able to take up leucine (20 nM) and a mixture of amino acids (0.5 nM) at rates comparable to those for other bacteria, consistent with results in previous studies (26, 32). Malmstrom et al. (21) demonstrated that *Synechococcus* was able to assimilate DMSP at rates higher than those for other bacteria and accounted for about 20% of DMSP assimilation in the northwest Atlantic Ocean and the Gulf of Mexico. Vila-Costa et al. (31) showed that not only *Synechococcus* but also *Prochlorococcus* and many eukaryotic phytoplankton, including diatoms, also take up DMSP.

Uptake of amino acids by *Prochlorococcus* and *Synechococcus* during our study was a relatively small fraction (2% to 10%) of total uptake by the community. However, the abundance of these cyanobacteria was also low, reaching only 10% of prokaryotes in the waters we sampled. In oceanic regimes where the abundance is higher, amino acid uptake by cyanobacteria is potentially large. The assimilated ^{3}H-labeled amino acids in our experiments were probably used for biomass synthesis and incorporated into macromolecules, most likely protein, because the formaldehyde treatment has the same effect on cells as TCA (16). Cyanobacteria may rely on external sources for some amino acids (auxotrophy), but *Prochlorococcus* and *Synechococcus* have all of the genes necessary to synthesize amino acids (27, 28). Regardless, dissolved free

amino acid utilization could account for a high percentage of the bacterial carbon and nitrogen demand (13, 14, 18) and thus could be a large component of DOM fluxes in the oceans (15).

Light stimulated leucine assimilation even where *Prochlorococcus* and *Synechococcus* abundances were low, suggesting that other microbes are involved in the light effect. The unexplained light-stimulated leucine incorporation was probably not due to uptake by picoeukaryotes, which accounted for only about 2.6% of total uptake. More likely, the unexplained light effect was due to AAP bacteria (17) and proteorhodopsin-bearing bacteria (3). During our study, AAP bacterial abundances reached 50% of total prokaryotic abundance in some surface waters, but there was no correlation between AAP bacteria and light-enhanced production. However, Alonzo-Saez et al. (2) found significant light enhancement in leucine incorporation by *Roseobacter* bacteria, some of which are phototrophs (1). Proteorhodopsin has been found in several bacterial groups, including SAR11 and SAR86 (3, 9). During our study, SAR11 bacteria made up more than 40% and SAR86 up to 17% of all prokaryotes (data not shown). Proteorhodopsin-containing bacteria, perhaps AAP bacteria, and other potential photoheterotrophic prokaryotes could explain the large fraction of light-induced assimilation unaccounted for by cyanobacteria.

Evaluating the effect of light on leucine and amino acid incorporation provides insight into light-driven heterotrophic biomass production and DOM assimilation in oceanic ecosystems. Where they are abundant, *Prochlorococcus* and *Synechococcus* might play a more important role in the cycling of DOM than previously thought. However, during our study, DOM uptake by cyanobacteria and picoeukaryotes was not enough to account for the total stimulation by light. These results emphasize the potential role of other photoheterotrophic microbes in light-stimulated uptake of amino acids and possibly other compounds. The results from this study stress the need for further work to identify the different microbial groups responsible for light-affected processes.

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