

Stoichiometry of nitrogen and carbon utilization in cultured populations of *Trichodesmium* IMS101: Implications for growth

Abstract—Rates of particulate organic carbon (POC) and particulate organic nitrogen (PON) accumulation, N_2 and CO_2 fixation, and NH_4^+ uptake were measured in cultures of *Trichodesmium* IMS101 growing on an artificial culture medium without added N substrates. Cultures exhibited a doubling time of about 4 d based on the accumulation of PON, POC, chlorophyll *a*, and cell number. Cell-specific rates of N_2 and CO_2 fixation were highest during midday and during the initial stages of the growth cycle.

Up to midlog phase, integrated estimates of CO_2 fixation (based on short-term $^{13}CO_2$ uptake) closely tracked increases in POC. However, after day 8, estimates of C accumulation based on CO_2 fixation exceeded observed POC increases. Within the error of the measurements, N_2 fixation estimates (based on C_2H_2 reduction) also closely tracked, and could largely account for, PON increases over most of the experiment. N demand based on CO_2 fixation also followed PON increases during early to midlog phase but diverged during late exponential phase and thereafter by greater than 100%. Estimates of short-term C fixation rates during and after late exponential phase overestimate net growth of *Trichodesmium* IMS101, whereas estimates of N_2 fixation rates approximated net growth. These observations may partially explain the disparity of estimates of growth in field populations based on $^{14}CO_2$ fixation compared to N_2 fixation.

Ammonium accumulated in the culture medium during the experiment, and rates of NH_4^+ uptake were high throughout the day and over the whole growth cycle. Rates of NH_4^+ uptake generally exceeded N_2 fixation. The turnover of N was therefore much greater than the net accumulation of N in biomass and implies a concurrent high rate of NH_4^+ release.

Species of *Trichodesmium*, a nonheterocystous cyanobacterium, are ubiquitously distributed in tropical and subtropical oligotrophic oceans where they occur as free trichomes or as colonial aggregates (Carpenter 1983). They contribute to new N input in these systems by fixing N_2 (Capone et al. 1997). In contrast to many other nonheterocystous cyanobacterial diazotrophs, nitrogenase synthesis and activity in natural (Capone et al. 1990; Zehr et al. 1993) and cultured (Ohki and Fujita 1988; Chen et al. 1996) populations of *Trichodesmium* exhibit a daily cycle with nitrogenase activity confined to the light portion of the day, paralleling photosynthetic CO_2 fixation.

Several aspects of the biology and ecology of *Trichodesmium* have perplexed researchers over the years. Based on evidence from the natural abundance of stable isotopes and tracer experiments investigating uptake of combined N, it has been assumed that *Trichodesmium* is largely dependent upon N_2 fixation for its N requirements for growth (Carpenter et al. 1997). However, other studies have reported uptake of nitrogenous compounds such as NH_4^+ , NO_3^- , and dissolved organic N (DON) (Mulholland and Capone 1999, and references therein). Some of the discrepancies among studies

assessing combined N use by *Trichodesmium* may be due to the fact that populations in differing physiological states were sampled.

In addition, field studies have often found a large discrepancy in growth rate estimates based on measured rates of CO_2 fixation versus N_2 fixation and relative to the observed C:N ratios (Carpenter 1983; Lipschultz and Owens 1996; Capone et al. 1998). In general, growth rates estimated using the former are often substantially greater than those based on the latter. This has generated concern about the procedures, calculations, and assumptions used to quantify CO_2 and N_2 fixation and consequent growth in natural populations (Lipschultz and Owens 1996).

The main objective of our study was to explore the possible bases for the conflicting evidence of CO_2 versus N_2 -based growth estimates, as well as the capacity for NH_4^+ uptake. In this regard, we examined the relationships among CO_2 , N_2 fixation, and NH_4^+ uptake over the growth cycle of *Trichodesmium* IMS 101 grown under N_2 fixing conditions, and with respect to the net accumulation of POC and PON. Since our cultures were closed systems and combined N sources were not added, C and N use should be balanced over the growth cycle since N_2 fixation ultimately limits net growth rates. We also wanted to determine whether there were changes in the relative uptake of NH_4^+ with respect to N_2 fixation over the course of the diel and growth cycle.

Methods—Culture conditions: Triplicate cultures of *Trichodesmium* IMS101 were grown on an artificial seawater medium depleted in combined N as described by Chen et al. (1996). Cultures were maintained in incubators at 28°C on a 12:12 hour light:dark schedule at the Chesapeake Biological Laboratory (CBL). Light levels in the incubators were about 55 to 65 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) supplied by banks of cool white fluorescent lighting. Cells were transferred using sterile techniques under a laminar flow hood and maintained in exponential growth up until the time of the experiments to prevent excess bacterial accumulation. Bacterial biomass in cultures was estimated as <1% of cell numbers based on microscopic examinations. Accumulation of bacteria was not observed in the medium prior to the addition of *Trichodesmium* IMS101. Cultures were gently swirled, at least 2 times per day, in order to prevent cells and filaments from adhering to the sides of the culture vessels. Prior to each sampling, cultures were swirled to ensure homogenous sampling.

Nitrogen fixation: Rates of N_2 fixation were measured by the C_2H_2 reduction method (Capone 1993). Ten milliliters were removed from a well-mixed parent culture and placed into a serum vial. Vials were sealed and 1 ml of C_2H_2 (>99.9%) was added to the headspace. Control assays were

conducted on 10 ml of the culture medium. A time-zero gas sample was taken and the vials were placed in the incubators. Measurements of C_2H_4 production were made by removing 100 μ l of headspace gas from each vial immediately after and at intervals of about 30 min following the C_2H_2 addition. The duration of incubations for N_2 fixation was 2 h. Ethylene concentration in the sample vials was quantified relative to a C_2H_4 standard using a Shimadzu mini-II flame ionization gas chromatograph having a 2 m Hayesep A column. Estimates of N_2 fixation rates were calculated using the equations of Capone (1993) and a conversion factor of 3. Based on several intensive diel studies, short-term assays were extrapolated to daily rates by assuming that cells maintained the hourly rates measured at 0800, 1200, and 1600 for 2, 8, and 2 h, respectively (this study; Mulholland et al. 1999; Mulholland and Capone 1999).

$^{15}NH_4^+$ uptake: Rates of NH_4^+ uptake in the cultures were measured using ^{15}N tracer techniques as outlined in Glibert and Capone (1993) using highly (>98%) enriched ^{15}N substrates. Twenty milliliters of well-mixed parent culture were transferred into acid-cleaned 24 ml scintillation vials and inoculated with 0.03 μ M of $^{15}NH_4^+$. Nutrient samples were collected at the same time for analysis of dissolved NH_4^+ as described below. Vials were returned to the incubator for a 1 h incubation period. ^{15}N incubations were terminated by gently filtering (<125 mm Hg) the contents of the incubation bottles onto precombusted (450°C for 2 to 4 h) GF/F filters and filters were rinsed three times with N-free artificial seawater medium. Time-zero controls were filtered immediately after the ^{15}N addition. Samples were frozen until analysis.

Sample ^{15}N enrichment and total particulate nitrogen mass was measured by mass spectrometry on a Europa Scientific ANCA-SL 20-20 IRMS (isotope ratio mass spectrometer) against a peptone standard. The instrument was calibrated and tuned before each sample run. A full reference sample set (six samples) was analyzed at the beginning and end of each set of 25 samples. Two reference samples were inserted after every fifth sample throughout the sample run to verify instrument performance over the course of sample runs. Reference samples were reproducible to within 0.0001 atom%.

^{13}C uptake: Incubations were set up by placing 25 ml of culture into 25 ml serum vials, sealing these vials, and removing gas bubbles. A 10% enrichment of the bicarbonate pool was made, based on an estimate of 2 mM dissolved HCO_3^- , using a highly enriched ^{13}C substrate (99%). Incubations were 1 h in duration, after which samples were collected onto precombusted GF/F filters, rinsed, and frozen as described above. Extrapolation to daily rates used the same assumption as for N_2 fixation. Measurements were not corrected for respiration.

Inorganic nutrient analysis: Samples were collected and filtered through precombusted GF/F filters daily to measure dissolved NH_4^+ . Filtrate was frozen immediately until analysis. Dissolved NH_4^+ was measured colorimetrically by the phenol hypochlorite method (Solarzano 1969) using a Technicon autoanalyzer II equipped with an Alpkem auto-sampler and software. Concentrations were determined by

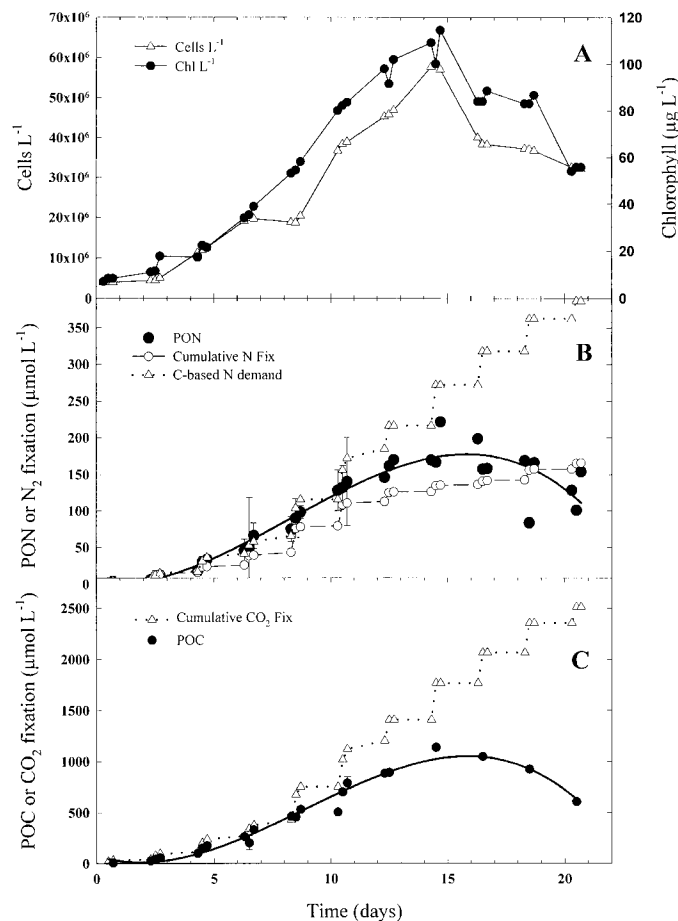


Fig. 1. Growth curves for *Trichodesmium* IMS101 growing on medium without added N in terms of (A) cell numbers and chlorophyll; (B) PON accumulation, integrated N_2 fixation, and cumulative N demand from C fixation using a 6.5:1 C:N ratio; and (C) POC accumulation and integrated $^{13}CO_2$ uptake. Replicate samples were not analyzed for $^{13}CO_2$ uptake after day 10 because of high biomass accumulation. In (A) we simply connected the points to represent trends. Third-order polynomial regressions were used to represent trends in panels B and C.

comparison with standard curves ($R^2 > 0.99$). In addition to replicates, repeat analyses were made on several samples to verify reproducibility of measurements to within 2%.

Results—Growth rate: The growth curve of *Trichodesmium* IMS 101 showed the typical batch culture pattern of lag and exponential growth phases (Fig. 1). Cultures reached maximal density and then biomass began to decrease in all cultures. This phenomenon has been commonly observed in these cultures. Both natural and cultured populations of *Trichodesmium* exhibit bloom and crash cycles. There is some evidence that the rapid decrease in cell number may be due to a lysogenic phase that enters lytic phase when cell densities become high (Ohki 1999). During exponential phase, cell doubling times for POC, PON, chlorophyll *a* (Chl *a*), and cell number were about 3 to 5 d (Fig. 1, Table 1). Ammonium accumulated in the culture medium over the course of the growth cycle to about 2.5 μ M (Fig. 2). The C:N ratio

Table 1. Summary of C and N accumulation and turnover times, growth parameters, and ratios of cellular pools for *Trichodesmium* IMS101 growing on medium without added N sources. (N = 14 for all sample parameters.)

Rates	Parameter used for calculation	Avg.	SE	Units	
Max. POC or PON accumulation	POC	599	73	$\mu\text{mol C L}^{-1}$	
	PON	105	13	$\mu\text{mol N L}^{-1}$	
Avg. daily C accumulation	POC rate	96		$\mu\text{mol C L}^{-1} \text{d}^{-1}$	
	CO ₂ fixation	156*		$\mu\text{mol C L}^{-1} \text{d}^{-1}$	
	(cumulative)	180†		$\mu\text{mol C L}^{-1} \text{d}^{-1}$	
Avg. daily N accumulation	PON rate	18		$\mu\text{mol N L}^{-1} \text{d}^{-1}$	
	N ₂ fixation	13*		$\mu\text{mol N L}^{-1} \text{d}^{-1}$	
	(cumulative)				
Avg. NH ₄ ⁺ uptake	NH ₄ ⁺ uptake	18‡	1	$\mu\text{mol N L}^{-1} \text{d}^{-1}$	
Volume specific rates	CO ₂ fixation	12,003	1,872	$\text{nmol C L}^{-1} \text{h}^{-1}$	
	N ₂ fixation	711	141	$\text{nmol N L}^{-1} \text{h}^{-1}$	
	NH ₄ ⁺ uptake	743	51	$\text{nmol N L}^{-1} \text{h}^{-1}$	
	CO ₂ fixation	515	6.5	$\text{fmol C cell}^{-1} \text{h}^{-1}$	
Cell specific rates	N ₂ fixation	32	7.1	$\text{fmol N cell}^{-1} \text{h}^{-1}$	
	NH ₄ ⁺ uptake	33	3.3	$\text{fmol N cell}^{-1} \text{h}^{-1}$	
	POC	6.2		days	
	CO ₂ fixation	1.3		days	
Pool turnover time	Chlorophyll	5.7		days	
	PON	5.9		days	
	N ₂ fixation	6.1		days	
	NH ₄ ⁺ uptake	3.2		days	
	Cells	0.12		d^{-1}	
	Intrinsic growth rate (μ)	Cells	4.1		days
Chlorophyll		5.4		days	
POC		3.9		days	
CO ₂ fixation		2.2		days	
PON		3.3		days	
N ₂ fixation		2.8		days	
Doubling times		N:Chl	1,841	38	$\text{nmol N } \mu\text{g chl}^{-1}$
		Chl:Cell	2.1	0.1	$\mu\text{g chl cell}^{-1}$
	CO ₂ :N ₂ fixation	28§	10		
		9.6	0.3		
	PC:PN	6.2	0.2		
	N ₂ fixation/(N ₂ fixation + NH ₄ ⁺ uptake)	45¶		%	
		30#		%	

* Based on 12 h of fixation distributed 2:8:2 h around mid-day.

† Based on 12 h of fixation distributed 4:4:4 h around mid-day.

‡ Based on 24 h of uptake.

§ Mid-day estimate during exponential growth.

|| Cumulative estimate over a growth or diel cycle.

¶ Instantaneous.

Daily integrated.

of particulate material ranged between about 5 and 10 (average \pm SE: 7.2 ± 0.32 ; $n = 24$) (Fig. 2). Higher PC:PN ratios were observed during lag and stationary phase growth, whereas PC:PN ratios of about six were characteristic of exponential growth (Fig. 2).

C and N utilization over a growth cycle: Cell-specific rates of CO₂ fixation were high through the initial and exponential growth phase and decreased thereafter (Fig. 3). In general, rates were highest in the afternoon, relative to morning or midday. Cell-specific rates of N₂ fixation were also higher at the beginning of the experiment and decreased after cultures reached their maximum cell density (Fig. 3). Midday N₂ fixation exceeded morning or afternoon rates. NH₄⁺ uptake rates, on a cell basis, were also greater at the outset of

the experiment, but the reduction in uptake was less dramatic than for CO₂ or N₂ fixation (Fig. 3).

On a volumetric basis, CO₂ and N₂ fixation showed the same diel pattern and peaked between days 8 and 10 (Fig 4). NH₄⁺ uptake continued to increase up to day 16 (Fig. 4). N₂ fixation as a percent of total N use decreased from more than 30% of the sum of N uptake (N₂ fixation + NH₄⁺ uptake) in exponential phase to <14% of the daily N uptake after exponential phase.

The accumulation of POC and PON were plotted with estimates of integrated C and N₂ fixation (Fig. 1). Integrated daily CO₂ fixation (not corrected for dark respiration) followed POC accumulation during early log phase but exceeded POC accumulation by more than 50% during late log and stationary phase. Averaged over the exponential phase,

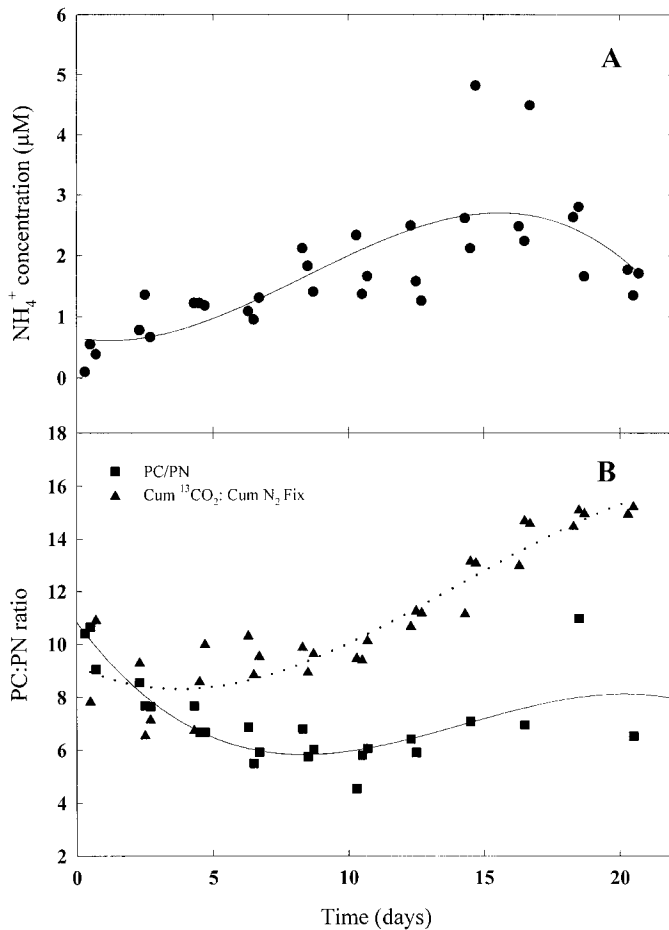


Fig. 2. Concentration of (A) NH_4^+ in the growth medium and (B) PC:PN ratio of cell biomass and the cumulative $^{13}\text{CO}_2$ uptake: N_2 fixation ratio of *Trichodesmium* IMS101 growing in medium without added N sources. Measurements were generally made at 0800, 1200, and 1600 h every 2 d over the course of a growth cycle. Third-order polynomial regressions were used to represent trends.

POC accumulation equaled about $96 \mu\text{mol C L}^{-1} \text{d}^{-1}$, whereas the estimate based on integrated $^{13}\text{CO}_2$ uptake was about $156 \mu\text{mol C L}^{-1} \text{h}^{-1}$. Estimates of N_2 fixation based on C_2H_2 reduction tracked PON accumulation well, although through the exponential phase it underestimated PON accumulation. However, after exponential phase, it was roughly equivalent. As for C fixation, integrated N demand based on CO_2 fixation followed PON accumulation closely during early log phase but greatly exceeded PON accumulation during late log and thereafter. The rate of N accumulation during log phase based on PON accumulation was about $18 \mu\text{mol N L}^{-1} \text{h}^{-1}$, whereas C_2H_2 reduction based PON accumulation amounted to about $13 \mu\text{mol N L}^{-1} \text{h}^{-1}$, or about 73% of the observed rate.

N_2 fixation could support a turnover time of about 6.1 d and a cellular doubling time of about 4.2 d (Table 1), comparable to the turnover and rates derived from POC, PON, cell, and Chl *a*. Turnover and doubling times based on $^{13}\text{CO}_2$ uptake (extrapolated to 12 h) were considerably shorter (1.3 and 0.9 d; Table 1). Turnover time for NH_4^+ relative to PON was about 3.2 d, with a doubling time of 2.2 d.

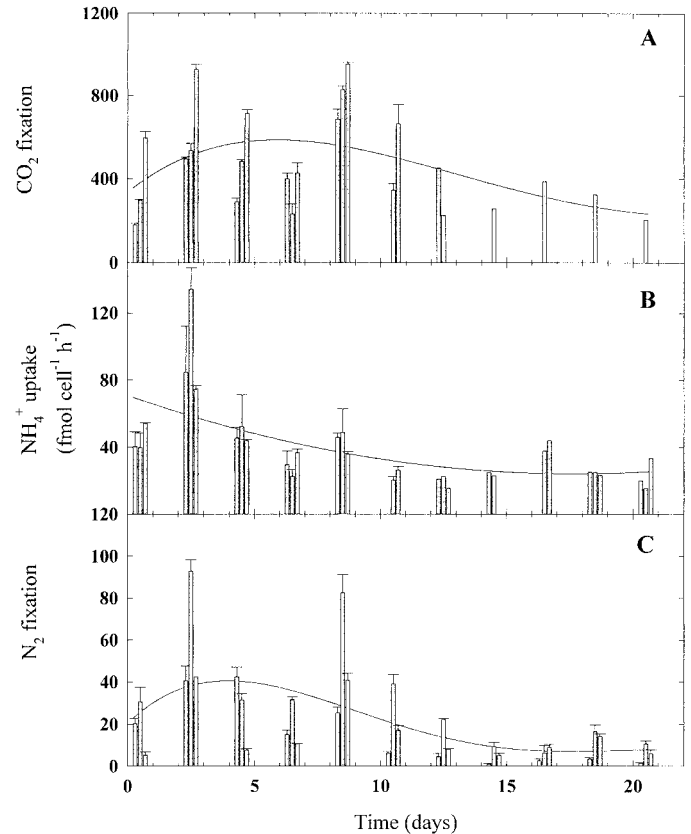


Fig. 3. Cell-specific rates of (A) $^{13}\text{CO}_2$ uptake, (B) NH_4^+ uptake, and (C) N_2 fixation over a growth cycle in *Trichodesmium* IMS101 growing on medium without added N sources. Error bars represent \pm standard error for three replicates. Bars represent measurements made generally at 0800, 1200, and 1600 h on each day as shown. Second- (B) or third- (A), (C) order polynomial regressions were used to represent trends.

Discussion—Our results confirm that *Trichodesmium* IMS101 growing on medium without added N uses CO_2 and N_2 to meet their absolute demand for growth and that during the early stages of population growth, accumulation of C and N biomass could be accounted for by measured rates of N_2 and CO_2 fixation. However, as populations grow, NH_4^+ is regenerated into the culture medium, NH_4^+ uptake increases, and the stoichiometry of CO_2 and N_2 fixation changes relative to the observed C and N biomass. Although cells are able to simultaneously take up NH_4^+ and fix N_2 , causing additional N turnover, this uptake does not support net accumulation of biomass or growth. Rates of CO_2 fixation exceed rates of POC accumulation after log phase growth when there are high rates of N turnover from NH_4^+ uptake relative to N_2 fixation. Culture biomass declined after log phase. This observation is typical of *Trichodesmium* populations and may be the result of a lysogenic phage that enters its lytic cycle when cell biomass is high or when cells experience changes in environmental conditions (Ohki 1999).

Stoichiometry of CO_2 and N_2 fixation—Because rates of CO_2 and N_2 fixation measured in these cultures (Table 1) agreed well with measurements made in natural populations

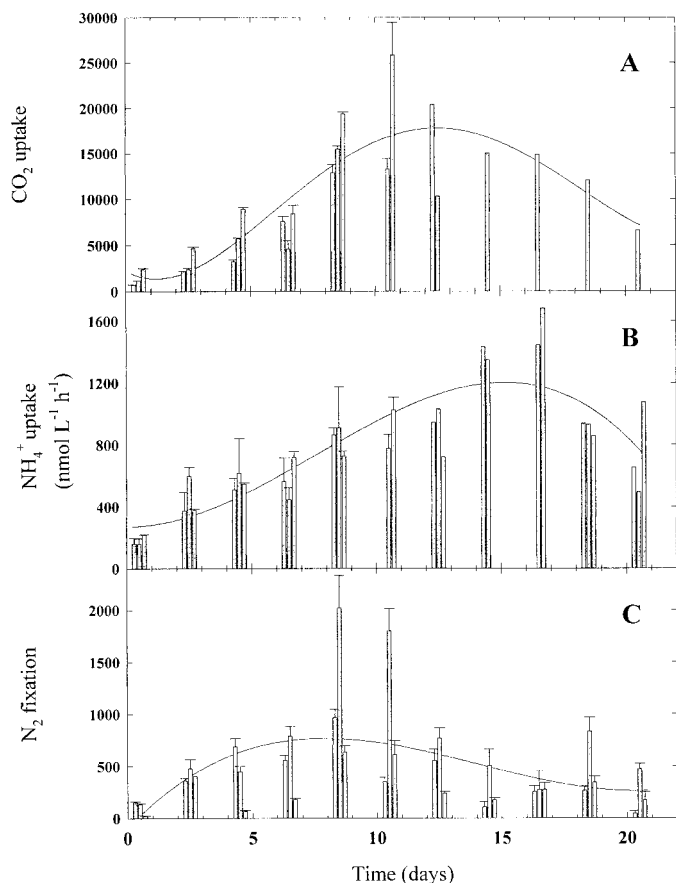


Fig. 4. Volume-specific rates of (A) $^{13}\text{CO}_2$ uptake, (B) NH_4^+ uptake, and (C) N_2 fixation over a growth cycle in *Trichodesmium* IMS101 growing on medium without added N sources. Error bars represent \pm standard error for three replicates. Bars represent measurements made generally at 0800, 1200, and 1600 h on each day as shown. Third-order polynomial regressions were used to represent trends.

of *Trichodesmium* spp., we believe that these culture systems are good model systems for physiological studies. By way of comparison, rates of N_2 fixation observed in natural populations of *Trichodesmium* from the Caribbean Sea range from 10 to 75 $\text{fmol N cell}^{-1} \text{h}^{-1}$ (Capone et al. 1994; Glibert and Bronk 1994), and reported CO_2 fixation rates range from 100 to over 1,000 $\text{fmol C cell}^{-1} \text{h}^{-1}$.

We reasoned that it would be useful to compare the accumulation of biomass C and N with short-term estimates of CO_2 fixation and N_2 fixation in cultures of *Trichodesmium* in order to provide insights into these problems in a more defined system. We also wanted to assess the quantitative validity of the C_2H_2 reduction method against a measure of N accumulation independent of bottle effects.

As a preliminary exercise, we first attempted to compare the biomass accumulation reported in two earlier studies of *Trichodesmium* where determinations of C_2H_2 reduction had been used, namely those of Prufert-Bebout et al. (1993) and Chen et al. (1996) (Table 2). Each used *Trichodesmium* IMS101. Prufert-Bebout et al. (1993) used seawater-based

medium in their culture studies, and Chen et al. (1996) grew the isolate in a defined medium.

We integrated the reported rates of C_2H_2 reduction (fig. 3 in Prufert-Bebout et al. 1993; fig. 2 in Chen et al. 1996) and compared them with the estimate of Chl *a* accumulation over the growth cycle, converting it to N using the value we obtained in our study (about 1.9 $\mu\text{mol N } \mu\text{g chlorophyll}^{-1}$, Table 1). We were unable to closely reconcile C_2H_2 reduction-based estimates of N_2 fixation for the Prufert-Bebout et al. (1993) study (Table 2) with chlorophyll-based N accumulation; N_2 fixation could only account for about 8% of the chlorophyll-based N accumulation. One reason for this discrepancy may have been that the seawater-based medium used in the earlier study contained unquantified N sources, such as DON, that could be used for growth by *Trichodesmium* in these cultures (Capone et al. 1994; Glibert and Bronk 1994; Mulholland and Capone 1999). Estimates of N_2 fixation using the C_2H_2 reduction technique were much closer to the observed chlorophyll-based N accumulation in the Chen et al. (1996) study in which N_2 fixation accounted for about 60% of the chlorophyll-based N accumulation. In our study, the integrated N_2 fixation was slightly less than, but closely parallel to, the increase in PON (Fig. 1). This indicates that the C_2H_2 reduction method, if properly applied, can be an accurate proxy for N_2 fixation.

We did not correct for dark respiration in our culture experiments. Generally, daily rates of CO_2 fixation are extrapolated by simply scaling short-term assays to 10 or 12 h (e.g. Carpenter and McCarthy 1975; Carpenter and Price 1977), without accounting for nighttime respiration. Dark respiration accounted for between 7 and 30% of gross CO_2 fixation (Li et al. 1980; Kana 1992; Carpenter and Roenneberg 1995) in three field studies where it was considered. These respiration rates agree well with our results until late exponential phase growth when the cumulative CO_2 fixation rate exceeded POC accumulation by more than 50%. At the same time, N_2 fixation fell far short of meeting the C-based N demand during this time.

In natural populations, there are other reasons that cells may fix additional CO_2 relative to N_2 . Excess C fixation may support ballasting of cells within the water column (Romans et al. 1994) or support high respiration rates to reduce intracellular O_2 concentrations that might otherwise inhibit nitrogenase activity (Gallon et al. 1996). A second factor possibly contributing to the increasing disparity between CO_2 uptake and POC accumulation could be release of soluble organic C to the medium. We did not monitor DOC production in these experiments.

Growth and C:N mass balance—Generation times in our cultures growing in a defined medium were about 4 d based on several parameters (cell numbers, chlorophyll, POC, and PON). These results are comparable to the earlier results of Chen et al. (1996). Somewhat longer doubling times, about 8 d, and poor agreement between estimates of N_2 fixation and biomass accumulation were reported in culture studies using seawater-based medium (see above, Prufert-Bebout et al. 1993; Mulholland and Capone 1999). The growth rates of these cultures overlapped with the more rapid growth rates reported for field populations (Carpenter 1983; Lip-

Table 2. Estimates of N accumulation in culture studies using *Trichodesmium* IMS101 based on Chl *a*, assuming a conversion factor of 1,941 nmol N $\mu\text{g chl}^{-1}$, or from integrated N_2 fixation estimates. We had to assume a value for the number of days at particular biomass levels and these are reported in column 3. Based on the light period length, we assume 14 h of N_2 fixation per day for Prufert-Bebout et al. (1993) and 12 h of N_2 fixation per day for Chen et al. (1996).

Time from inoculation (days)	Assumed period (days from inoculation)	Total days	Chl <i>a</i> ($\mu\text{g L}^{-1}$)	C_2H_2 reduction (light period)		N_2 fixation (nmol N L^{-1})
				(nmol N $\mu\text{g chl}^{-1} \text{h}^{-1}$)	(nmol N $\text{L}^{-1} \text{h}^{-1}$)	
From Prufert-Bebout et al. (1993): (Fig. 3)						
16	16–18	3	5	4.5	22.5	630
23	19–24	6	25	4.5	112.5	6,300
27	25–28	4	50	8	400	14,933
31	29–31	3	80	1	80	2,240
34	32–33	2	160	0	0	0
Total N_2 fixed						24,103
Total PN accumulation (from chl <i>a</i>)						285,355
From Chen et al. (1996):						
4	4–5	2	5	300	1,500	24,000
6	6–7	2	10	130	1,300	20,800
9	8–10	3	20	70	1,400	33,600
12	11–13	3	30	120	3,600	86,400
15	14–16	3	80	80	6,400	153,600
18	17–19	3	130	40	5,200	124,800
21	20–21	2	170	30	5,100	81,600
Total N_2 fixed						524,800
Total PN accumulation (from Chl <i>a</i>)						303,765

schultz and Owens 1996). Similarly, intrinsic growth rates (μ) were about 0.12 d^{-1} , comparing well with the 0.07 d^{-1} reported by Ohki et al. (1986) for NIBB 1067 grown on an N-free, defined medium. CO_2 fixation, although closely matching POC accumulation for the first 10 d, rapidly diverged from POC accumulation later during exponential growth and thereafter (Fig. 1). This is a very interesting observation that may bear on field observations of a poor stoichiometric balance between C-based compared to N-based estimates of growth. We speculate that respiration increased over the growth cycle and was responsible for the oxidation of the excess (relative to POC accumulation) reduced C generated each day.

NH_4^+ uptake—An alternative explanation for higher C-based growth rates, relative to growth rate estimates from N_2 fixation, in natural populations is the potential to use combined N sources (Lipschultz and Owens 1996). Recent results have shown that the capacity of natural and cultured populations of *Trichodesmium* to take up combined N is greater than previously thought (Mulholland and Capone 1999; Mulholland et al. 1999).

In some earlier studies measuring N uptake by natural populations of *Trichodesmium*, no N uptake was observed, although in others, high rates of N uptake were reported (table 5 in Mulholland and Capone 1999, and references therein). In these previous studies, as well as several more recent efforts using cultured populations of *Trichodesmium*, high rates of NH_4^+ uptake were measured, and the source of the NH_4^+ appears to be direct release from *Trichodesmium* filaments (Mulholland and Capone 1999; Mulholland et al. 1999). There are several possible explanations for the lack of N uptake in earlier field studies. For example, when nat-

ural populations of *Trichodesmium* are encountered in the field, there is no way to ascertain their prior nutritional history or what stage of growth they are in at the time of sampling.

In our system, N_2 fixation accounted for the observed increase in N biomass (or net growth). NH_4^+ pools did accumulate over the growth cycle (Fig. 2), presumably from excess N_2 fixation. Moreover, the capacity to assimilate NH_4^+ was present throughout the growth cycle, and its importance relative to N_2 fixation and total N turnover increased over time. In parallel, gross CO_2 fixation increased markedly over net CO_2 fixation. We speculate that NH_4^+ uptake was supporting much of this excess productivity, but that the respiration that must have accounted for the consumption of this photosynthate occurred with the release of N over each diel period.

In nature, allochthonous NH_4^+ may contribute to net growth. Although surface waters in the tropical oligotrophic ocean gyres are generally very low in combined N, NH_4^+ and dissolved organic N (DON) are rapidly recycled to support much of the apparent N demand of phytoplankton primary production (Eppley and Peterson 1979; Bronk et al. 1994). During so-called blooms of *Trichodesmium*, dissolved N pools can become elevated as a result of recent N input by N_2 fixation (Devassy 1987; Karl et al. 1992).

Implications—Although our results may explain some of the observed discrepancies in C and N budgets for cultured populations growing in closed systems, extrapolations from cultured to natural systems should be made with caution. The culture systems used in these studies were closed, and cells were grown under low light and high or saturating Fe and P levels. These conditions are different from those found

in natural systems, where concentrations of these elements are low and light levels in the upper euphotic zone are high. C and N use under different light levels and Fe and P concentrations should be examined to determine how relationships vary with the nutrient regime.

Additionally, nutrient dynamics in nature are very different from those in culture systems due to the effects of containment. In culture systems, NH_4^+ is retained and can accumulate in the culture medium for use by the cultured organism. By contrast, in natural systems, NH_4^+ and DON released from cells or regenerated within colonial associations may diffuse away from cells and become part of the ambient nutrient pool where it is available for use by other organisms.

Despite the obvious limitations of culture systems, our results provide some useful insights into some of the enigmatic facets of *Trichodesmium* physiology and ecology. More rigorous analyses of net growth rates of natural and cultured populations that include an analysis of respiratory losses are required. We have shown that there are changes in the stoichiometry of C and N use in this culture over the growth cycle. We assert that similar changes may occur in populations as they develop in nature and that because the physiological status of cells collected in nature is uncertain, such changes have direct relevance to the interpretation of field data.

Margaret R. Mulholland¹ and Douglas G. Capone²

Chesapeake Biological Laboratory
University of Maryland Center for Environmental Sciences
Solomons, Maryland 20688

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¹ Present address: Marine Sciences Research Center, State University of New York Stony Brook, Stony Brook, New York 11794-5000 (mmulholland@notes.cc.sunysb.edu).

² Present address: Wrigley Institute for Environmental Studies and Department of Biological Sciences, University of Southern California, Los Angeles, California 90089

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The role of DON and the effect of N:P ratios on occurrence of cyanobacterial blooms: Implications from the outgrowth of *Aphanizomenon* in Lake Kinneret

Abstract—Supply (or loading) ratios of biologically available nitrogen and phosphorus, N:P, have often been suggested as the major determinants for the presence or absence of N_2 fixing cyanobacteria in aquatic environments. Increasing evidence that some components of the dissolved organic nitrogen (DON) pool can play an active role in supplying N nutrition either directly or indirectly to phytoplankton implies that this source of N must be considered in any attempt to apply the N:P resource ratio approach to predict or explain phytoplankton population composition. For example, the unprecedented bloom of *Aphanizomenon ovalisporum* that occurred in Lake Kinneret from mid-September through October 1994 derived most of the N required for growth directly or indirectly from DON rather than from N_2 -fixation. This would suggest that factors other than apparent low N:P ratios were important in causing the outgrowth of the cyanobacteria. The present analysis of the *Aphanizomenon* bloom in Lake Kinneret emphasizes (1) the need to include the DON pool as a potential source of available N for planktonic microbiota; (2) at least for some cyanobacteria, the presence of heterocysts does not necessarily imply active nitrogen fixation; and (3) the development of diazotrophic cyanobacterial blooms in nature is generally due to a multiplicity of environmental factors.

In both marine and freshwaters considerable amounts of nitrogen (N) are associated with the pool of dissolved organic nitrogen (DON), which frequently greatly exceeds the concentrations of total dissolved inorganic nitrogen, DIN (Antia et al. 1991). There is now compelling evidence that some components of the DON pool can play an active role in supplying N nutrition directly or indirectly to phytoplankton and bacteria. The capability of some algae to derive N directly from organic N compounds such as amino acids and purines has long been known (see review by Antia et al. 1991). Probably in most cases, indirect supply of N from

the DON pool is more important for phytoplankton. For example, studies by Palenik and Morel (1990) showed the presence of L-amino acid oxidases on the surface of algal cells that serve to liberate NH_4 , which is subsequently transported to the cell interior. Urea (one of the more frequently measured components in the DON pool) is almost ubiquitously used by phytoplankton as a nitrogen source, often preferentially to NO_3 (McCarthy 1972; McCarthy et al. 1982). It has also been shown that some components of indigenous freshwater and marine DON pools such as purines, amino acids, and amino sugars are susceptible to relatively rapid bacterial degradation, giving rise to NH_4 or urea (Berman et al. 1999). Moreover, the hydrolytic action of UV and visible light irradiation is known to degrade DON, yielding nitrogen-rich compounds (including NH_4) that are biologically available (Bushaw et al. 1996). A further indication of the dynamic nature of N cycling in aquatic environments is the fact that not only are some DON components degraded and/or taken up by phytoplankton and bacteria, but that considerable quantities of DON compounds may be directly excreted by algae (Bronk et al. 1994) or released by bacteriophage, viruses, or grazers of various size, shape, and dietary preference (Antia et al. 1991; Turk et al. 1992).

Supply (or loading) ratios of biologically available nitrogen and phosphorus, N:P (Schindler 1977), or, alternatively, the environmental ratios of total nitrogen:total phosphorus, TN:TP (Smith 1983) have often been proposed as the major determinants for the presence or absence of N_2 -fixing cyanobacteria, mainly for lakes but also elsewhere (see recent reviews by Hyenstrand et al. 1998 and Smith and Bennet 1999). A simpler criterion for defining limiting N levels sufficiently low to stimulate the development of N_2 -fixing cyanobacteria was proposed by Horne and Commins (1987)