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Amino Acid Cycling in Colonies of the Planktonic Marine Cyanobacterium *Trichodesmium thiebautii*

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We examined diel trends in internal pools and net efflux of free amino acids in colonies of the nonheterocystous, diazotrophic cyanobacterium *Trichodesmium thiebautii*, freshly collected from waters of the Caribbean and the Bahamas. The kinetics of glutamate uptake by whole colonies were also examined. While intracolonial pools of most free amino acids were relatively constant through the day, pools of glutamate and glutamine varied over the diel cycle, with maxima during the early afternoon. This paralleled the daily cycle of nitrogenase activity. We also observed a large net release of these two amino acids from intact colonies. Glutamate release was typically 100 pmol of N colony^{-1} h^{-1}. This is about one-fourth the concurrent rate of N_2 fixation during the day. However, while nitrogenase activity only occurs during the day, net release of glutamate and glutamine persisted into the night and may therefore account for a greater loss of recently fixed N on a daily basis. This release may be an important route of new N input into tropical, oligotrophic waters. Whole colonies also displayed saturation kinetics with respect to glutamate uptake. The K_s for whole colonies varied from 1.6 to 3.2 μM, or about 100-fold greater than typical ambient concentrations. Thus, uptake systems appear to be adapted to the higher concentrations of glutamate found within the intracellular spaces of the colonies. This suggests that glutamate may be a vehicle for N exchange among trichomes in the colony.

Pelagic nonheterocystous cyanobacteria of the genus *Trichodesmium* are widely distributed in tropical and subtropical oceans (8, 11), where they can account for a major fraction of planktonic biomass and productivity (12) and fixed N input (4, 10). While rates of N_2 fixation by *Trichodesmium* spp. have been well characterized, little is known of the fate of recently fixed nitrogen. In contrast to several other well-studied nonheterocystous cyanobacteria (17), *Trichodesmium thiebautii* fixes N_2 only during the day, concurrent with oxygenic photosynthesis (6).

In a previous study examining pathways of NH_4^+ incorporation in this organism, we obtained preliminary evidence that diel variability in glutamate and glutamine pools occurred in the colonies (10). We therefore examined intracolonial pools of amino acids to determine whether there were discernible patterns relative to the diel pattern of N_2 fixation. We also determined rates of net amino acid release from intact colonies. We related the observed release to simultaneous estimates of N_2 fixation and the turnover of ambient amino acid pools and considered this release with reference to its potential biogeochemical importance as a source of recently fixed ("new") nitrogen in the upper water column. Finally, the kinetics of glutamate uptake by whole colonies was examined.

MATERIALS AND METHODS

During research cruises aboard the R/V *Columbus Iselin* (University of Miami, Miami, Fla.), primarily in the southeast Bahamas in September 1991 and 1992 and on a longer transect through the northeast Caribbean in January and February 1992 (Fig. 1), colonies of *T. thiebautii* were collected by plankton tows, using a 1-m-diameter, 202-μm-mesh net towed at 1 knot at 1- to 15-m depth. The cod-end filtrates were diluted in an acid-rinsed bucket with filtered (GF/F) seawater. Colonies of the fusiform morphology, typically composed of several hundred parallel trichomes and about 2 to 5 mm in length by 0.5 mm wide, were individually sorted out with disposable plastic inoculating loops and placed in filtered seawater. Disposable plastic gloves were worn during all procedures to minimize amino acid contamination.

Amino acid pools. For analysis of total amino acid pools (inter- and intracellular), samples were collected at various times of the day. At each sampling, 10 colonies were placed in 1 ml of MilliQ water contained in a small plastic centrifuge tube in order to lyse cells and release all free amino acids (FAAs). The tube was then briefly immersed in liquid N_2 and stored frozen until analysis for total FAAs. Upon thawing, colonies (in their original tubes) were sonicated for 2 min to disrupt the cells and heated to 70°C for 1 h to further extract FAAs. The extract was gently filtered (0.2-μm Acrodisc) by syringe prior to analysis.

In order to differentiate between intercellular (interfilamental) and intracellular (intrafilamental) FAA pools, 30 to 50 colonies were placed in 125-ml polycarbonate bottles containing 100 to 125 ml of filtered seawater. After completion of the addition of colonies, duplicate 1-ml samples of the aqueous phase were taken, being careful to avoid any colonies or free filaments, filtered through a 0.2-μm Acrodisc, and rapidly frozen and stored until analysis. Immediately after this sample was taken, the polycarbonate bottle was vigorously shaken by hand for about 5 to 10 s, or until no intact colonies remained. Free filaments were generally obvious through the bottle. A second set of 1-ml samples was taken, again gently filtering the samples through a 0.2-μm Acrodisc to minimize cell lysis, and frozen until analysis. The amino acids released to the medium upon disaggregation represent our estimate of intercellular pools and were subtracted from estimates of total FAAs to

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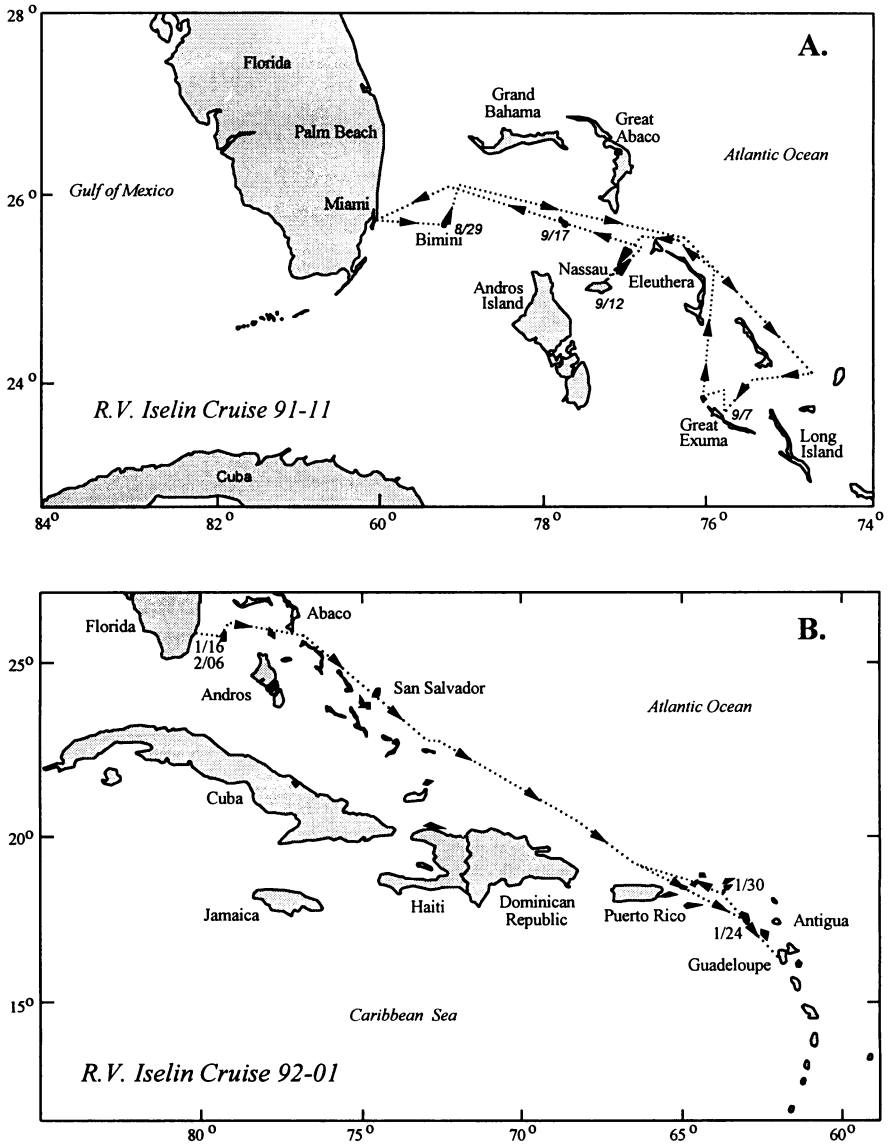

FIG. 1. General area of sampling, with way points (A) and cruise track (B) for September 1991 and January to February 1992 cruises, respectively.

estimate intracellular pools. Disaggregated filaments maintain both cellular integrity (7) and nitrogenase activity if disaggregated under N_2 (5, 37).

The concentration of individual amino acids was determined by high-performance liquid chromatography (HPLC), using precolumn derivitization with *o*-phthalaldehyde (24). The following 20 amino acids were separated and quantified in each sample: Asp, Glu, Asn, Ser, Gln, His, Gly, Thr, Arg, Tau, Ala, Tyr, Trp, Met, Val, Phe, Ile, Leu, Orn, and Lys. Samples were derivatized by mixing 10 μl of *o*-phthalaldehyde reagent with 500 μl of sample, and this mixture was injected after 2 min. FAAs were separated on a reverse-phase, C_{18}, 5-μm column and quantified fluorometrically (excitation at 340 to 380 nm; emission, >418 nm). This analysis employed a binary gradient composed of 0.025 N Na acetate (pH 7.3) with 1% tetrahydrafuran and 0.05% Brij (eluent A) and 100% HPLC-grade methanol (eluent B). The gradient was adjusted to separate amino acids of interest but was essentially linear from 20 to 70% (eluent B) over 30 min. Individual amino acid peaks were identified by comparison with retention times from a mixture of pure amino acid standards. Integrated peak areas of samples were quantified by comparison with external standards. For the samples collected in September 1991, analyses were performed on board. For January and September 1992 cruises, samples were returned frozen to the Chesapeake Biological Laboratory for analysis. Subsamples of the cells were also analyzed for total proteins.

Amino acid release. Thirty to 50 colonies were placed in several 125-ml light or darkened polycarbonate bottles containing 100 to 125 ml of filtered seawater. At zero time, 1 ml was carefully removed by autopipette (avoiding intact colonies), placed in a microcentrifuge tube, quickly frozen in liquid N_2, and stored frozen in the tube until analysis for amino acids as described above. Bottles were incubated under saturating

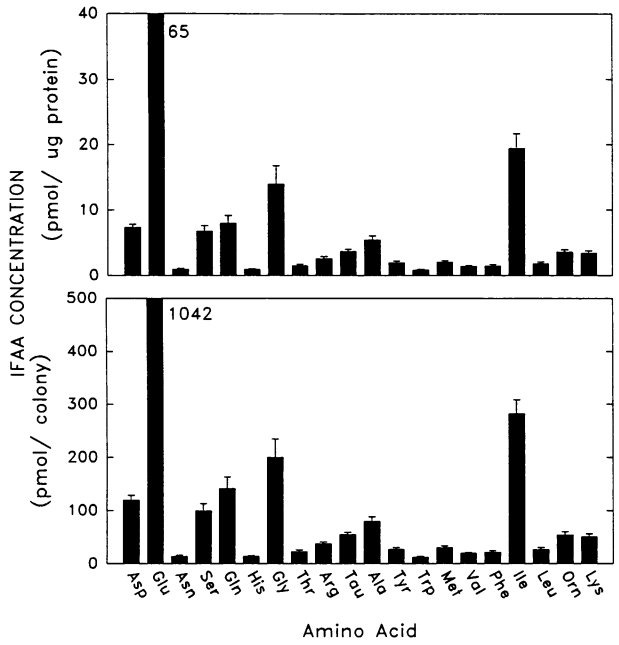

FIG. 2. Mean internal FAA (IFAA) pool (± standard error) of burst whole colonies of *T. thiebautii*. Samples taken at various times of day during the September 1991 cruise were averaged and expressed on a protein (top) or whole colony (bottom) basis.

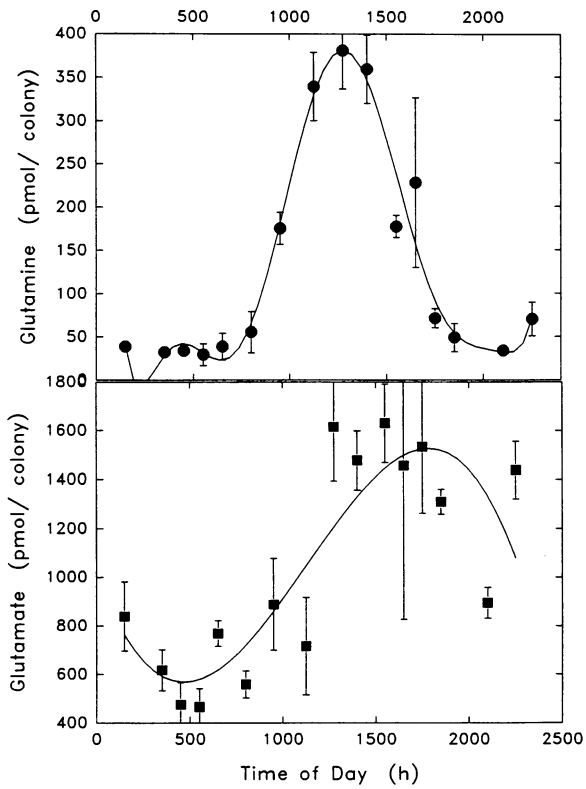

FIG. 3. Time-dependent variability in FAA pools in *T. thiebautii* colonies. Samples were from the September 1991 cruise. Curves were fitted by using higher-order polynomials, ninth for the upper graph and third for the lower graph. Values are means ± standard errors.

light (fluorescent lights, 325 microeinsteins m^{-2} s^{-1}) in a flowing-seawater incubator at ambient surface water temperatures (about 26°C). Samples were also removed at 0.5, 1, and 2 h and similarly preserved. Samples removed at 2 h were filtered through a 0.2-μm Acrodisc filter to remove loose filaments which were visible at that point. At the initiation of the time course experiments, a second set of *Trichodesmium* colonies in polycarbonate bottles was vigorously shaken to disaggregate the colonies. A 1-ml sample of the solution was immediately removed, filtered through an Acrodisc, and frozen, while another unfiltered sample was also taken (cells) and frozen. All procedures were performed with acid-washed glassware and while wearing plastic gloves.

Uptake kinetic studies. Uptake kinetics were estimated by incubation of 20 colonies with 0.2 μCi of ^{14}C-glutamate (specific activity, 293 mCi $mmol^{-1}$; New England Nuclear) for 2 h. Incubations were conducted with concentrations of added glutamate ranging from 0.07 to 12.5 μM, subsequently corrected for the ambient concentration (usually about 20 nM). Incubations were run under saturating fluorescent light at surface seawater temperatures (see above). Data were plotted by using a Lineweaver-Burke transformation (S/v versus S, where S is the substrate concentration and v is velocity) (19), and the K_s was derived from the x intercept.

Nitrogenase activity assays. Nitrogenase activity was estimated by the acetylene reduction assay as previously described (3, 6).

Protein assays. Protein content of colonies was assayed by the Pierce bicinchoninic acid protein assay.

RESULTS

During the September 1991 cruise, sampling was primarily restricted to waters within an area north and west of San Salvador Island, The Bahamas (Fig. 1). Wind stress was minimal and there were dense populations of *Trichodesmium* spp. in the upper water column. For samples of whole colonies collected at various times of the day, the FAA pool (intra- plus intercellular) averaged 152 ± 11 pmol μg of $protein^{-1}$ or 2.4 ± 0.15 nmol $colony^{-1}$ (± standard error; n = 38). Glutamate was the largest FAA pool overall, accounting for 43% of the total FAAs (Fig. 2). Isoleucine, glycine, glutamine, aspartate, and serine followed in importance, accounting for 12.8, 9.2, 5.2, 4.8, and 4.5%, respectively, of the FAA pool.

In examining the FAA pools at various times of the day, while no variation was noted in most of the pool constituents (data not shown), obvious trends were noted in both glutamate and glutamine pools (Fig. 3). During most of the night, glutamine remained below 50 pmol $colony^{-1}$ (<4 pmol μg of $protein^{-1}$), whereas much higher levels were found during daylight hours (Fig. 3). Glutamine levels were highest near midday; the glutamine pattern was similar to that for rates of nitrogenase activity (Table 2; see also reference 6). Glutamate also showed trends over the diel cycle but with much greater variability (Fig. 3). Maximal values occurred somewhat later in the afternoon compared with those for glutamine. The lowest glutamate concentrations (<0.5 nmol $colony^{-1}$) occurred in the very early morning. The ratio of glutamine to glutamate, previously used as a measure of N sufficiency (14), also showed a distinct diel pattern (Fig. 4). Whereas values remained below 0.1 through most of the evening and early morning, values above 0.3 were noted through midday.

Whole-colony FAA pools were also determined during the January 1992 transect. While glutamate and glutamine concen-

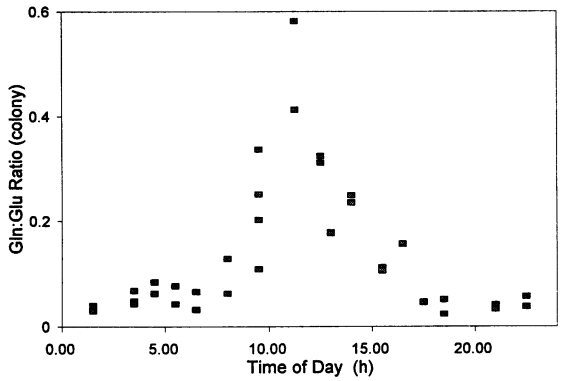

FIG. 4. Diel variation in glutamine-to-glutamate ratio in colonies of *T. thiebautii*, September 1991.

trations were comparable (1.3 and 0.24 nmol colony^{-1}, respectively; $n = 24$), there was no clear indication of a diel trend in either pool (data not shown). However, the *Trichodesmium* populations sampled on this transect, which spanned a much greater geographical range (Fig. 1B), were much sparser and dispersed at most stations.

On the September 1991 and January 1992 cruises, we also partitioned pools of glutamate and glutamine between intra- and interfilamental components (Table 1). For glutamate, 82 to 100% (average, 92%) was associated with the filaments, rather than with interfilamental spaces (on the basis of release upon disaggregation). During the September 1991 cruise, the trend in intracellular glutamate paralleled that noted in total pools. The bulk of glutamine (82 to 100%; average, 96%) was also found within, rather than among, trichomes. Assuming a cylindrical colony size of 5 by 0.5 mm and 10% interfilamental spaces (7), intracellular concentrations of glutamate and glutamine were calculated to be about 350 and 50 μM, respectively, while concentrations in the interfilamental voids were estimated to be about 230 and 33 μM, respectively.

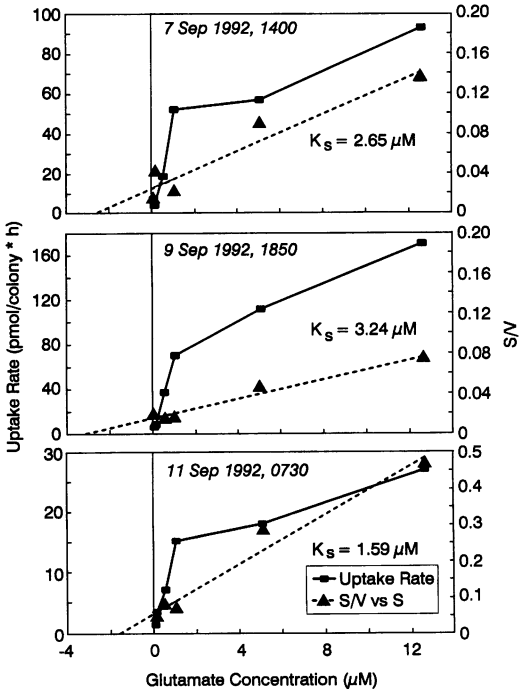

FIG. 5. Uptake kinetics of glutamate for whole colonies of *T. thiebautii*, September 1992.

On the September 1992 cruise, the concentration dependence of glutamate uptake was examined in several experiments. Over the concentration range tested (up to 12 μM), while uptake rates fell off with increasing glutamate concen-

TABLE 1. Pools of glutamate and glutamine in colonies of *T. thiebautii*[a]

Date	Time (h)	Glutamate (nmol colony^{-1})		Cells/total (%)	Glutamine (nmol colony^{-1})		Cells/total (%)
		Intercellular	Intracellular		Intercellular	Intracellular	
1991							
9 Sept.	0483	0.050 (2)	0.375 (2)	88	0.018 (2)	0.228 (2)	93
9 Sept.	0800	0 (2)	0.666 (2)	100	0 (2)	0.453 (2)	100
5, 6 Sept.	1100	0.069 (2)			0.07 (2)		
6 Sept.	1250	0.012 ± 0.006 (4)			0.016 ± 0.002 (4)		
9 Sept.	1450	0.071 (2)	1.843 (2)	96	0.033 (2)	0.153 (2)	82
3 Sept.	1625	0.076 (2)	1.126 (2)	94	0.011 (2)	0.399 (2)	97
9 Sept.	2150	0.124 (2)	0.933 (2)	88	0.023 (2)	0.117 (2)	84
Avg		0.057 ± 0.016 (7)	0.988 ± 0.248 (5)	93	0.025 ± 0.009 (7)	0.270 ± 0.067 (5)	91
1992							
27 Jan.	0550	0.057 (2)	0.489 (2)	90	0.010 (2)	0.115 (2)	92
22 Sept.	0650	0.091 ± 0.009 (4)	1.117 ± 0.013 (4)	92	0.006 ± 0.006 (4)	0.155 ± 0.068 (4)	98
19 Sept.	1025	0.026 ± 0.017 (4)	0.561 ± 0.145 (4)	97	0 (4)	0.206 ± 0.045 (3)	100
27 Sept.	1150	0.113 (2)	0.504 (2)	82	0 (2)	0.122 (2)	100
20 Sept.	1320	0.084 ± 0.015 (4)	1.230 ± 0.240 (3)	93	0 (2)	0.336 ± 0.045 (3)	100
19 Sept.	1615	0.069 ± 0.010 (4)	0.729 (2)	92	0.015 ± 0.014 (4)	0.742 (2)	98
21 Sept.	2000	0.146 (2)	1.424 (2)	91	0.001 (2)	0.054 (2)	99
21 Sept.	2200	0.113 (2)	1.075 (2)	91	0 (2)	0.094 (2)	100
Avg		0.087 ± 0.013 (8)	0.891 ± 0.129 (8)	91	0.004 ± 0.002 (8)	0.228 ± 0.079 (8)	98

[a] Where indicated, values are ± standard error. Number in parentheses is number of samples or, for averages, number of experiments.

TABLE 2. Rates of N_2 fixation and glutamate and glutamine release from colonies of *T. thiebautii*[a]

Date	Time (h)	N_2 fixation (pmol of N colony^{-1} h^{-1}, ± SE)	pmol of N colony^{-1} h^{-1} (± SE)			Glu + Gln release (% of N_2/fixation)
			Glutamate release		Glutamine release (light)	
			Light	Dark		
1991						
9 Sept.	0445	0 (3)	44 ± 23 (2)	13.8 ± 4.6 (2)	5.7 ± 9.4 (2)	
4 Sept.	0810	454 ± 108 (3)	−0.4 (1)	−0.4 (1)	9.0 (1)	2
31 Aug.	0945	108 ± 30 (3)	108 ± 7 (2)		49.0 ± 14.5 (2)	145
6 Sept.	1030	752 ± 86 (3)	37 ± 0 (2)	21.8 ± 3.8 (2)	−7.0 ± 8.3 (2)	4
2, 8 Sept.	1200	621 ± 60 (7)	118 ± 28 (4)	17.6 ± 15.4 (2)	34.9 ± 8.0 (4)	25
30 Aug., 2 Sept.	1300	479 ± 74 (4)	121 ± 9 (4)		26.4 ± 10.2 (4)	31
3 Sept.	1415	330 ± 20 (3)	60 ± 11 (2)		9.0 ± 9.0 (2)	21
3 Sept.	1935	0 (3)	102 (1)		9.0 (1)	
4 Sept.	2100	0 (3)	198 ± 105 (2)	98.8 ± 12.8 (2)	21.7 ± 14.7 (2)	
Avg		305 ± 97 (9) 457 ± 92 (6) (light only)	88 ± 20 (9)	30 ± 18 (5)	18 ± 6 (9)	34
1992						
27 Jan.	0530	0 (3)	45 ± 8 (2)		19.5 ± 19.5 (2)	
22 Jan.	0630	0 (3)	73 ± 17 (2)		69.1 ± 14.1 (2)	
19 Jan.	1000	266 ± 48 (3)	155 ± 28 (2)		59.0 ± 59.0 (2)	80
27 Jan.	1130	340 ± 70 (3)	59 ± 26 (2)		17.5 ± 17.5 (2)	22
20 Jan.	1300	326 ± 136 (3)	25 ± 25 (2)		9.0 ± 2.0 (2)	10
19 Jan.	1600	159 ± 25 (3)	150 ± 14 (2)		28.5 ± 8.5 (2)	112
21 Jan.	2200	0 (3)	6 ± 6 (2)		19.3 ± 8.7 (2)	
Avg		156 ± 41 (7) 273 ± 24 (4) (light only)	73 ± 13 (7)		32 ± 5 (7)	67

[a] Number in parentheses in number of samples or, for averages, number of experiments.

tration, uptake did not appear to be fully saturated. Half-saturation constants, which are likely to be minimal estimates, were about 2 to 3 μM (Fig. 5).

As previously reported (6), rates of N_2 fixation showed a distinct diel pattern (Table 2). No activity could be detected before dawn or after sunset. High rates of glutamate release were detected, with glutamine release typically less than one-third the rate of glutamate release. Low rates were noted in the early morning, before the onset of nitrogenase activity. Release rates remained high even after cessation of nitrogenase activity. During periods of active nitrogenase activity, glutamate and glutamine release accounted for about 30% of the concurrent rate of N_2 fixation in 1991 (Table 2). Integrated over the day, more than one-half of the recently fixed N may have been released as glutamate or glutamine.

DISCUSSION

Internal pools of glutamate and glutamine in *T. thiebautii* appear to be highly dynamic over the diel cycle, and they parallel the daily pattern of photosynthesis, nitrogenase synthesis (6, 35), and activity (6, 37). Maxima in internal glutamine pools preceded the peak in glutamate pools, consistent with a glutamine synthetase-glutamate synthase pathway of primary nitrogen incorporation into *Trichodesmium* spp. This reinforces our earlier conclusion based on relative levels of glutamine synthase and glutamate dehydrogenase activities, glutamate and glutamine uptake, and preliminary analysis of glutamate and glutamine pools (10).

Induction of *nif* expression and synthesis of nitrogenase appear to occur during predawn hours (6, 36), apparently before photosynthesis is initiated. This is in contrast to studies with a culture of *Trichodesmium* sp. in which light appeared to be a prerequisite for nitrogenase synthesis and activity (26). The external and/or internal factors contributing to this switch-on of N_2 fixation in natural populations of *Trichodesmium* spp. are as yet unknown. However, because glutamate and glutamine are downstream products of N_2 fixation, shown to be implicated in the control of N metabolism through the *ntr* gene system in many bacteria (16), their absolute or relative internal concentrations may play a role in the regulation of nitrogenase synthesis in *Trichodesmium* spp.

In contrast to *T. thiebautii*, amino acids in *Gloeothece* sp. varied little between periods of N_2 fixation compared with nonfixing periods (15). However, *Gloeothece* sp. released Gly, Ser, and Ala during periods of active fixation (dark periods), reassimilating these compounds during light periods (15).

The ratio of Gln/Glu has been used to assess the N status of algae (14), including cyanobacteria (15). Ratios at or below 0.05 were found in *Gloeothece* sp. even during periods of active N_2 fixation (15), indicative of severe N limitation. In contrast, ratios in N_2-fixing cultures of *Anabaena variabilis* are high (25). In *Trichodesmium* spp., ratios were greater than 0.2 through much of the day, suggestive of N sufficiency. Ultrastructural studies have shown that granules of the N storage product, cyanophycin, are generally observed, providing further evidence of N sufficiency (34).

While a net efflux of dissolved organic nitrogen (DON), and certain amino acids, occurs from *T. thiebautii* colonies, our evidence also demonstrates a capacity for uptake of glutamate and glutamine (Fig. 5; also, reference 10). The ability of some cyanobacteria, including diazotrophs, to assimilate exogenous amino acids has been recognized for some time (1). In addition, microheterotrophs (bacteria, protozoans, etc.) commonly associated with *Trichodesmium* spp. may be responsible for amino acid assimilation (29). Indeed, amino acid leakage may promote such associations.

Bergman and Carpenter (2) reported that not all filaments within a *Trichodesmium* colony have nitrogenase present (cf. reference 31). Individual trichomes within a colony may operate independently with respect to N nutrition by turning nitrogenase activity on and off as required by N demand. Alternatively, actively fixing filaments may provide a source of combined N to adjacent, nonfixing filaments such as occurs between heterocysts and vegetative cells of heterocystous cyanobacteria. As early products of N_2 fixation, both glutamine and glutamate would be appropriate vehicles for such exchange. Release of these amino acids may be a consequence of intracolonial exchange. That estimated K_s values for glutamate uptake (2 to 3 μM) were about 100-fold greater than ambient levels of glutamate (20 nM) indicates a low-affinity system which may be useful at the elevated concentrations of glutamate estimated for the interfilamental spaces (230 μM).

The ability of *Trichodesmium* spp., as nonheterocystous cyanobacteria, to reconcile O_2-sensitive N_2 fixation with concurrent photosynthesis has been the topic of considerable discussion and research (6, 9, 16, 36). Photorespiration is an established metabolic capability in *Trichodesmium* spp. (21, 23) which may contribute to mitigating O_2 inhibition of nitrogenase (21). As for higher plants, the involvement of the amino acids glycine and serine in the glycolate pathway of photorespiration is known for some cyanobacteria (32). Relatively high levels of these two amino acids in *Trichodesmium* spp. (9 and 4.5% of FAA pool for glycine and serine, respectively) may serve as evidence for the presence of this pathway.

A large positive efflux of glutamate and glutamine from the colony was noted and could account for greater than 50% of the daily integrated rate of N_2 fixation. Glibert and Bronk (18) have also recently assessed the direct release of ^{15}N-DON by *T. thiebautii* after exposure to $^{15}N_2$. Using this independent approach, they, too, could account for about one-half of the recently fixed N as released DON, with greater than half of this (25% of total) in a low-molecular-weight (<10,000) fraction.

Photosynthetic carbon uptake on a per-colony basis based on $^{14}CO_2$ during September 1991 was about 16 nmol of C colony^{-1} h^{-1} (5), a relatively high value compared with those previously reported (9). Average glutamate and glutamine release amounted to about 0.5 nmol of C colony^{-1} h^{-1}, or 3% of photosynthetic production. Shimura et al. (33) similarly reported extracellular release rates of 2 to 8% in light incubations of *T. thiebautii*. The loss of recently fixed N by *Trichodesmium* spp. has direct implications for growth rate calculations. While the C/N ratio of *Trichodesmium* spp. typically ranges from 4 to 5 (9), C fixation/N fixation ratios are generally higher (e.g., 35 with the current data of 16 nmol of C colony^{-1} h^{-1} for C fixation and an N fixation rate of 0.46 nmol colony^{-1} h^{-1}, per Table 2). Similarly, C doubling times based on a C content of 10 μg of C colony^{-1} and the above-mentioned C fixation rate assumed for 10 h per day amount to 5.2 days. This compares with an N doubling time of 37.5 days on the basis of the average daylight N fixation rate in September projected over 10 h and an N content of 2.4 μg colony^{-1}. The loss of glutamate and glutamine, if not accounted for, aggrevates this situation in that N doubling times based on N_2 fixation rates uncorrected for release would be underestimates. Further work is required to account for and reconcile these discrepancies.

Several recent studies indicate that *Trichodesmium* sp. in the world's oceans may be a much more important contributor to overall N inputs (i.e., "new" N) than previously thought (11, 13, 22). Assuming a density of one colony per liter, typical for the upper 1 m (11), one may calculate daily release rates of glutamate and glutamine to be about of 1.6 and 0.3 nmol liter^{-1} day^{-1}, respectively. Ambient seawater concentrations of glutamate and glutamine were about 20 and 1 nM, respectively. Thus, this input may account for pool turnover of 8 and 30% per day. This is relatively small compared with directly determined rates of glutamate and glutamine turnover times, which range from minutes to hours (35). However, the input of amino acids from *Trichodesmium* spp. represents a new N input distinct from the bulk of the water column amino acid flux which is recycled.

The pathway(s) of C and N recently fixed by *Trichodesmium* spp. to higher trophic levels is unresolved. In the open ocean, cyclopoid and calanoid zooplankton do not graze on *Trichodesmium* spp. (28), presumably because of the presence of chemical feeding deterrents (20). Harpactacoid copepods, such as *Macrosetella* spp., found in the open ocean are apparently insensitive to the chemical deterrents, and both feed and live on *Trichodesmium* colonies (20, 27). However, the quantitative significance of this pathway remains to be determined. The substantial effluxes of glutamine and glutamate with subsequent assimilation by heterotrophs and photoheterotrophs (29, 30, 35) may be the primary pathway of transfer of this C and N to higher trophic levels.

High rates of glutamate and glutamine release relative to N_2 fixation rates, along with relatively high Gln:Glu ratios indicative of N sufficiency, suggest an excess capacity for N_2 fixation with respect to cellular demands for N. While N_2 fixation is an energetically costly process, the localization of this phototrophic diazotroph in the upper layers of the tropical seas places it in an environment of very high photon flux. Release of DON may also indicate growth limitation by factors other than light, C, or N.

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