

Epilithic nitrogen fixation in the rocky littoral zones of Lake Malawi, Africa

Abstract—Rates of epilithic N_2 fixation in the rocky littoral zones of Lake Malawi, determined by in situ incubations and the acetylene reduction method, declined with depth, were highly correlated to light intensity and heterocyst biovolume, and are among the highest observed values for freshwater or marine systems. Daytime N_2 fixation rates were similar between sites at similar depths, except at sediment and nutrient impacted sites, where rates were lower and more variable. Daytime N_2 fixation rates were measurable in all transparent chambers and were negligible in opaque chambers, which indicates that phototrophic diazotrophs (organisms capable of utilizing atmospheric nitrogen) were responsible for all daytime N_2 fixation. Nocturnal N_2 fixation was ~60% of daytime fixation. A model that integrated N_2 fixation over depth and time predicted that epilithic N_2 fixation may contribute up to 35% of N inputs to the epilimnion of Lake Malawi.

Lake Malawi is a large (28,000 km²), deep (>700 m) oligotrophic African rift valley lake located between latitudes 9°5'S and 14°5'S. Although only 30% of the shoreline is composed of rocky substrata, approximately one half of Lake Malawi's 500–1,000+ species of fish reside in the rocky littoral areas (Reinthal 1990; Ribbink et al. 1983), making it a critical habitat for fish biodiversity. The vast majority of these fish are herbivorous, and both ecological studies (Reinthal 1990) and stable isotope studies (Bootsma et al. 1996) indicate that epilithic periphyton is the most important component of their diets. Photosynthetic rates by the epilithic periphyton of Lake Malawi reach maximum values near 1g C m⁻² d⁻¹, which are similar to those that occur on coral reefs (Bootsma 1993). However, rates of nutrient uptake for these algal communities are unknown. The dominance of heterocystous cyanoprokaryotes within the epilithic periphyton community, and recent evidence that ambient total nitrogen concentrations are among the lowest for freshwater or marine systems (~7 μM; Guildford & Hecky 2000), suggest that the process of nitrogen fixation could play an important role in supplying "new" nitrogen to the epilithic periphyton community and epilimnion of Lake Malawi.

The purpose of this study was, first, to determine the spatial dynamics of epilithic N_2 fixation in the rocky littoral zones of Lake Malawi and to estimate its contribution to the N requirements of the epilithic periphyton community. Such information is necessary for a more complete understanding of the factors that control periphyton productivity, which in turn maintains the dense and diverse littoral fish community of Lake Malawi. Second, this study examines the relationships between epilithic N_2 fixation, heterocyst biovolume, light intensity, and chlorophyll concentration, in an attempt to identify the controlling factors and variability in epilithic N_2 fixation rates. Third, this study gives preliminary estimates for the importance of epilithic N_2 fixation in the whole lake nitrogen budget of Lake Malawi and compares rates in

Lake Malawi with freshwater and marine habitats over a wide range of latitudes.

Sites—To accomplish the study objectives, five rocky littoral zone sites were selected in the central and southern portions of Lake Malawi. All experiments were conducted during the dry windy seasons (May–October) of 1997 and 1998. During 1997, two sites were used for study (Maleri South and Maleri North). Because of the presence of crocodiles at these sites during 1998, these sites were abandoned, and experiments were conducted at three new sites (Likoma Island [LI]-Makalawe, LI-Membe, and Thumbi West) during 1998. A more detailed description of the physical habitat at these sites was presented in Higgins (1999) and Ribbink et al. (1983). On average, each site/depth was sampled 2–3 times monthly, with the exception of LI-Makalawe and LI-Membe, which were opportunistically sampled over a 2-d period during May 1998.

Acetylene reduction assays—Epilithic nitrogen fixation was estimated by modification of the acetylene reduction technique (Stewart et al. 1967) to allow for sampling epilithic periphyton communities, in situ, over a depth gradient (2–16 m) by use of SCUBA diving. The modifications are described in detail in Higgins (1999) and include the use of 800-ml, opaque, and ultraviolet-transparent plexiglass chambers fitted with a neoprene seal and skirt and weighted with a lead sock to isolate chamber water from surrounding ambient water. Chambers were placed on rocky surfaces, injected with 100 ml (at surface temperature and pressure) of welder's-grade acetylene, stirred vigorously by use of a built-in stirring device, and incubated for 0.5–2.5 h. After the incubation time, the chamber was stirred vigorously, and a 40-ml aqueous sample was taken by use of a glass 50-ml syringe fitted with a three-way valve. After 10 ml of surface air was drawn in, the syringe was shaken vigorously for 2 min and placed in a water bath (at lake temperature) for ~1 min before the headspace was drawn into a 7-ml vial, which was later analyzed on a Shimadzu GC-8A chromatograph fitted with a 3.05 m × 6.35 mm × 5.34 mm Altech porasil C/phenyl isocyanate mesh 80/100 column. Calibration with ethylene standards was conducted both prior to and after sample analysis each day, and an interlab calibration with known ethylene concentrations and 25 random samples (Freshwater Institute, Winnipeg, Canada). A time-series experiment was conducted, and rates of ethylene production were found to be linear between 0.5 and 2.5 h. Rates of N_2 fixation were calculated by use of a theoretical 4:1 (C_2H_2 : N_2) conversion ratio, which is of similar magnitude to experimentally determined conversion ratios for epilithic algal communities (Reuter et al. 1986; Larkum et al. 1988).

In several acetylene reduction assays, screens of different mesh sizes were placed over the chambers, to reduce the amount of solar radiation received by the periphyton com-

Table 1. Maximum, minimum, and mean rates of daytime epilithic N₂ fixation at five rocky littoral zone sites in Lake Malawi, Africa.

Site	Nitrogen fixation rate ($\mu\text{g m}^{-2} \text{h}^{-1}$)						Coefficient of variation (%)
	z	n	Max	Min	Mean	SD	
LI-Makalawe (May 1998)	2	3	1,283	1,003	1,105	93	8.4
	5	3	742	557	642	93	14.5
	10	3	862	682	743	102	13.7
LI-Membe (May 1998)	2	3	1,423	1,125	1,276	149	11.7
	5	3	670	479	590	99	16.8
	10	3	560	362	445	103	0.23
Maleri N (May–Oct 1997)	2	28	1,847	843	1,190	291	24.5
	5	6	1,552	782	1,068	371	34.7
	10	10	1,090	146	443	342	77.2
Maleri S (May–Oct 1997)	2	41	2,203	613	1,310	402	30.7
	5	45	1,732	437	782	276	35.3
	10	18	249	1	108	79	73.1
Thumbi West (May–Oct 1998)	2	22	2,918	2,165	2,585	197	7.6
	5	10	1,431	719	1,029	260	25.3
	10	6	530	283	387	93	24.0
	16	12	506	213	327	89	27.2

munities under study. The chambers were allowed to adjust to their new light regime for 15–20 min prior to the initiation of the acetylene reduction assays. The amount of photosynthetically active radiation (PAR) received by the algal communities within each chamber was calculated from in situ light readings, combined with the percentage of transmission through each screen, calculated by use of a LICOR LI-1800 Spectroradiometer within a tub of lake water under low wave conditions. The in situ light readings were taken concurrent to, and directly beside, the acetylene reduction assays by use of a LICOR LI-1000 Data Logger and Quantum Sensor with a flat plate collector.

Algal collection, heterocyst enumeration, and chlorophyll—After most experiments, duplicate or triplicate algal samples were taken from the rock surface within each chamber by use of a periphyton-scraping device, as described by Loeb (1981). The periphyton samples were stored in the dark and returned to the laboratory, where subsamples were taken for chlorophyll *a* analysis and heterocyst enumeration. Chl *a* was extracted from filtered subsamples at -5°C for ~ 14 h by use of an alkaline acetone solution, according to the methods of Stainton et al. (1977), and analyzed fluorometrically. For each site and depth combination, five algal samples were randomly chosen for heterocyst enumeration. A 2-ml subsample was placed in a Utermöhl chamber, and at least 50 fields (or 100 heterocysts) were counted by use of a Zeiss Axioinvert 35 inverted phase microscope with $400\times$ magnification. Each sample to be counted was selected randomly, such that the person counting did not know the site or depth from which it originated.

Daytime N₂ fixation—During daylight hours, rates of nitrogen fixation were easily detectable in all transparent chambers (Table 1), whereas rates in opaque chambers were typically 4%–7% of rates in transparent chambers, which indicates that photosynthetic cyanoprokaryotes were responsible for the majority N₂ fixation rates noted. The dominance of heterocystous forms of *Calothrix* at virtually all sites and

depths studied (Higgins 1999) make it highly probable that they were responsible for the majority of daytime N₂ fixation by the periphyton community. Cyanoprokaryotic endosymbionts capable of N₂ fixation were found in several species of the diatom *Rhopalodia*; however, their total biovolume was generally an order of magnitude less than *Calothrix* heterocyst biovolume (Higgins 1999); therefore, they likely do not contribute largely to the overall fixation rates noted. The range of daytime N₂ fixation rates was $1\text{--}2,920 \mu\text{g N m}^{-2} \text{h}^{-1}$, with variability generally $<30\%$ of the mean value for each site/depth (Table 1). The Maleri Island sites, which are known to be impacted annually by a plume of sediment- and nutrient-rich water extending from the nearby Linthipe River (Bootsma and Hecky 1999), are an exception and have much higher variability, especially at 10 m depth. At the Maleri South site, a 1–2 cm layer of silt covering rocky surfaces was noted below 7 m depth, and rocky surfaces had a relatively low total standing crop of periphyton and biovolume of heterocysts (Higgins 1999).

Nitrogen fixation, Chl a, and heterocyst biovolume—Of the parameters known to influence N₂ fixation, the strongest correlation was found with heterocyst biovolume ($R = 0.71$, $P < 0.005$; Fig. 1) and solar insolation (discussed below). Both N₂ fixation rates and heterocyst biovolume showed generally decreasing values with increasing depth at all sites sampled (Fig. 2a–e, Table 1). In contrast to studies on the epilithic periphyton of five northern temperate lakes by Loeb and Reuter (1981), chlorophyll and N₂ fixation were not highly correlated in the epilithic periphyton of Lake Malawi ($R = 0.04$, $P = 0.70$). Studies on coral reefs have shown an inverse relationship between N₂ fixation and chlorophyll, which was attributed to the grazing activity of fish keeping the periphyton community in an early successional stage, decreasing chlorophyll concentrations while increasing rates of primary production and nitrogen fixation (Wilkinson and Sammarco 1983; Larkum 1988). This may also be the case in Lake Malawi, where densities of grazing fish can exceed 10 individuals m^{-2} area (Ribbink et al. 1983). However, be-

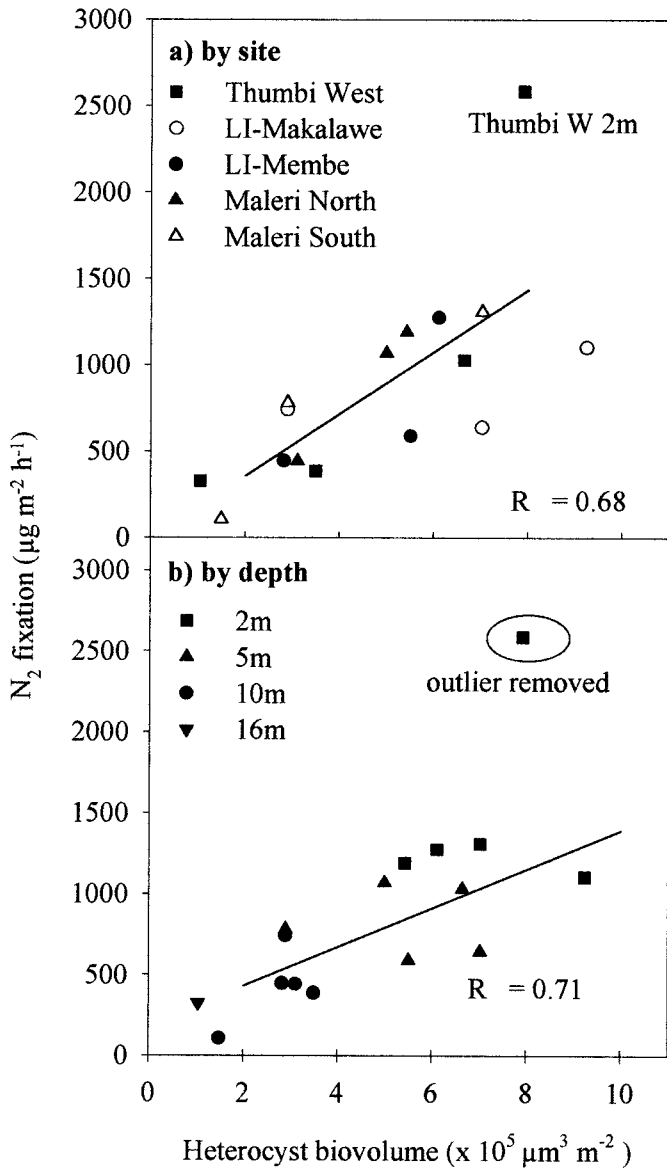


Fig. 1. Epilithic N₂ fixation vs. heterocyst biovolume by (a) site and (b) depth in the rocky littoral zone of Lake Malawi, Africa.

cause cellular chlorophyll concentrations may increase under decreasing light intensities, the use of chlorophyll concentration to estimate algal biomass and, in turn, N₂ fixation, can be complicated in studies that use a depth/light gradient, which was the case in this study.

Nitrogen fixation versus light model—The results of the screen experiments indicated that rates of nitrogen fixation per heterocyst were strongly dependent on the amount of PAR reaching the periphyton community ($R^2 = 0.75$, $P < 0.001$; Fig. 3). Nonlinear regression was used to fit these data to the relationship modified from Platt et al. (1980) by Lewis and Levine (1984),

$$N = N_s(1 - e^{-(\alpha I/N_s)})e^{-(\beta I/N_s)} + D,$$

where N is the amount of nitrogen fixed per heterocyst bio-

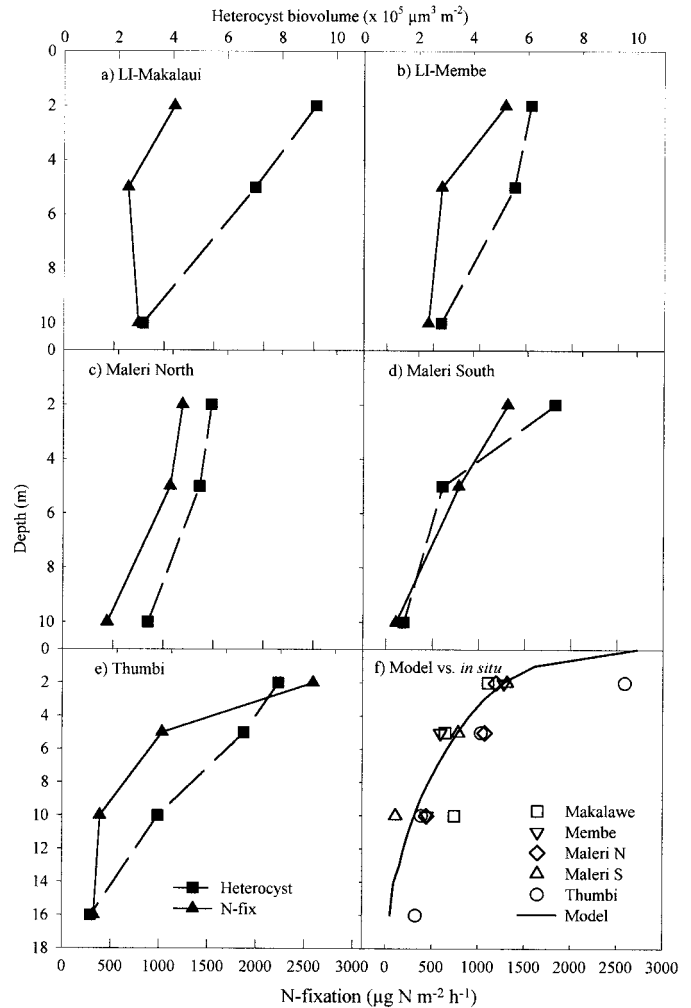


Fig. 2. (a–e) Epilithic N₂ fixation and heterocyst biovolume vs. depth curves for five rocky littoral zone sites in Lake Malawi, Africa. All sites were sampled during the windy season (May–October). Maleri North and Maleri South were sampled during 1997, whereas the remaining sites were sampled during 1998. (f) In situ nitrogen fixation rates for all sites, compared with model estimates (see text for description).

volume per unit time, N_s is the parameter representing maximum nitrogen fixation per heterocyst biovolume in the absence of light inhibition, I is the light intensity (PAR), α is the slope of the light-limited and linear part of the N-fixation versus PAR curve, β is the parameter for light inhibition, and D is the fixation rate for heterocysts in the dark (Levine and Lewis 1987). Because light inhibition of nitrogen fixation under high light intensities was not evident during any of the experiments and fixation rates in the dark (during daylight hours) were negligible, the parameters β and D were set to zero.

The N₂ fixation model, which integrates the relationships between N₂ fixation per heterocyst versus light, solar irradiance, light extinction, and heterocyst biovolume can be used in simulations to calculate N₂ fixation over a wide variety of spatial and temporal ranges. Simulated depth profiles of daily N₂ fixation for 1 June 1997 (windy season, lowest

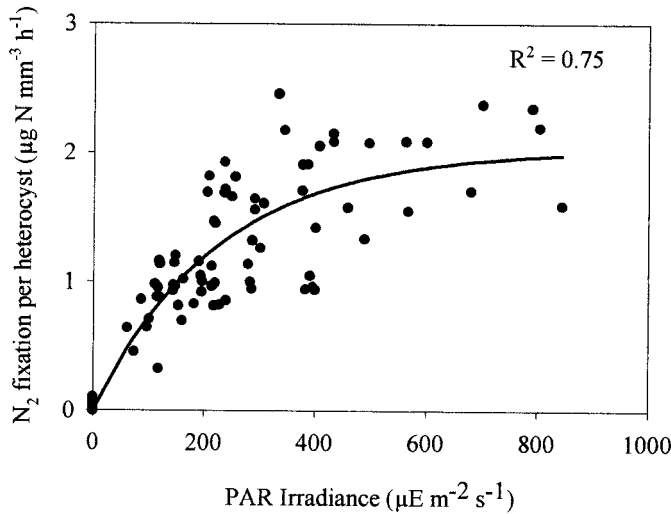


Fig. 3. Epilithic N_2 fixation per heterocyst biovolume vs. PAR, as determined by in situ screen experiments (see text) at 2 and 5 m depths at Maleri South during June 1997. The R^2 value refers to the relationship between in situ screen measurements (dots) and the N-fixation equation (line) modified from Platt et al. (1980).

solar insolation), and 1 January 1997 (rainy season, highest solar insolation), were calculated by use of a mean light extinction coefficient of 0.2 ($n = 18$, range 0.16–0.25), cloudless solar data generated by Fee's (1990) model (14°S latitude), and the relationship between heterocyst biovolume and depth (Higgins 1999). This simulation predicts that the maximum difference in nitrogen fixation rates due to seasonal differences in solar radiation is ~15% of the annual mean value. The 1 June 1997 simulation was also used to compare model estimates with in situ estimates of hourly N_2 fixation at midday for 2, 5, and 10 m depths (Table 1, Fig. 2f). The 1 June 1997 simulation for 2 m depth predicted a N_2 fixation rate of 1,490 $\mu\text{g N m}^{-2} \text{h}^{-1}$, which fell within the range of N_2 fixation rates for each site, with the exception of LI-Makalawe and Thumbi West (Table 1, Fig. 2f) and agreed with the site-averaged in situ N_2 fixation rate of 1,500 $\mu\text{g N m}^{-2} \text{h}^{-1}$. The model predicted values of 910 and 370 $\mu\text{g N m}^{-2} \text{h}^{-1}$ for 5 and 10 m depths, which agreed closely with the site-averaged in situ estimates of 950 and 410 $\mu\text{g N m}^{-2} \text{h}^{-1}$.

Patch-specific and nocturnal rates of nitrogen fixation—Above 1 m depth at all sites, and from 0–5 m depth at Maleri South, visually distinct patches of epilithic periphyton dominated by *Calothrix* or *Cladophora* were observed throughout the sampling season (May–October). At other sites and depths, the epilithic periphyton community was more homogenous and was dominated by heterocystous *Calothrix* spp (Higgins 1999; Higgins unpubl. data). The biomass of *Calothrix* patches were dominated by heterocystous *Calothrix* species, whereas the biomass of *Cladophora* patches were codominated by *Cladophora*, *Calothrix*, and a variety of epiphytic diatoms (Higgins 1999). *Calothrix* patches at 2 m depth had significantly higher rates of nitrogen fixation ($1,450 \pm 540 \mu\text{g N m}^{-2} \text{h}^{-1}$; $X \pm \text{SD}$; $n = 6$) than *Cladophora* patches ($720 \pm 90 \mu\text{g N m}^{-2} \text{h}^{-1}$, $X \pm \text{SD}$; $n = 6$)

Table 2. Nocturnal rates of epilithic N_2 fixation at Maleri South 2 m depth (24 Jun 97; 1-h incubation at midnight) and Thumbi West 1 m depth (13 Sep 98; 12-h incubation sundown to sunrise). See text for description.

Site	Depth (m)	N_2 fixation ($\mu\text{g m}^{-2} \text{h}^{-1}$)	Patch type
Maleri I South	2	718	<i>Cladophora/Calothrix</i>
	2	899	<i>Calothrix</i>
	2	948	<i>Calothrix</i>
Thumbi West I	1	383	<i>Cladophora</i>
	1	492	<i>Cladophora</i>
	1	349	<i>Cladophora</i>
	1	982	<i>Calothrix</i>
	1	721	<i>Calothrix</i>

during daylight hours (t -test, $P = 0.03$). The relative patchiness in the epilithic periphyton community structure at shallow depths of the Maleri Island sites is likely responsible for the higher variability in N_2 fixation rates noted (Table 1), whereas the impacts of sediment accumulation account for the high variability at deeper depths.

The mean rate of nocturnal nitrogen fixation in *Calothrix* patches, from 1–2 m depth, was double that for *Cladophora* patches, was ~60% of the daytime averages for nitrogen fixation (Tables 1, 2), and was an order of magnitude larger than rates measured in opaque chambers during the day. Although the difference in N_2 fixation rates between daytime dark box and nocturnal dark box experiments was unexpected, there are a number of plausible explanations. First, nocturnal N_2 fixation by nonheterocystous diazotrophs might occur primarily at night, when O_2 inhibition is reduced (i.e., the temporal separation of N_2 fixation and O_2 evolution processes). Second, the length of incubation during daytime dark box experiments may have been insufficient to allow a shift in adenosine triphosphate (ATP) and reductant pathways, which differ between day and night within heterocystous diazotrophs (Stewart 1973).

Importance of epilithic N_2 fixation to littoral and pelagic N cycles—The strong relationship between N_2 fixation nor-

Table 3. Estimated annual inputs of N to the epilimnion of Lake Malawi. All rates, except N_2 fixation, are from Bootsma and Hecky (1999). Pelagic N_2 fixation calculated from Hendzel's (1999) estimates.*

Source	($\text{mmol m}^{-2} \text{yr}^{-1}$)	N input (% of total)
Atmosphere	150	29–45
Riverine	90–200	17–61
Epilithic N_2 fixation	40–120	8–36
Pelagic N_2 fixation	50	10–15
Other	?	—
Total	330–520	—

* Annual pelagic N-fixation rate estimates calculated from rates for Nov–Dec on the basis of Hendzel (1999). Rates for Jan–Oct estimated from Nov–Dec rates (Hendzel 1999) under the assumption of a proportional relationship between N-fixation and heterocyst biovolume and a 10-fold decrease in heterocyst biovolume during Jan–Oct relative to Nov–Dec (Patterson and Kachinjika 1995).

Table 4. Benthic N₂ fixation rates from freshwater and marine habitats over a latitudinal gradient. For comparative purposes, all rates assume a 3:1 theoretical (C₂H₂:N₂) conversion ratio.

Location	Latitude	$\mu\text{g m}^{-2} \text{h}^{-1}$	$\text{mg m}^{-2} \text{d}^{-1}$	$\text{g m}^{-2} \text{yr}^{-1}$	Notes*	Reference
Freshwater Lakes						
Spring Lake (NWT CAN)	63 N	560	11	0.6	a	Bergmann and Welch (1990)
Crater Lake	42 N	1,265	15	3	b	Loeb and Reuter (1981)
Castle Lake	41 N	107	1	0.3	b	Loeb and Reuter (1981)
Donner Lake	39 N	120	1	0.3	b	Loeb and Reuter (1981)
Lake Tahoe	39 N	140	2	0.3	b	Loeb and Reuter (1981)
Fallen Leaf Lake	38 N	41	<1	<0.1	b	Loeb and Reuter (1981)
Lake Malawi	14 S	1,990	31	13	c	This study (mean rate)
Lake Malawi	14 S	3,890	61	25	d	This study (max rate)
Lake Malawi	14 S	11,300	155	75	e	This study (including substrata tortuosity)
Freshwater Streams						
Desert Stream (AZ-USA)	33 N	5,820	145	16	f	Grimm and Petrone (1997)
Coastal Marine						
Arctic (NWT CAN)	65 N	1,050	21	3	g	Chapin et al. (1991)
Great Britain	55 N	600	8	1	h	Jones (1992)
California	40 N	675	16	3	i	Bebout et al. (1987)
North Carolina	35 N	1,550	35	6	j	Paerl et al. (1996)
Coral reef (Marshall Is.)	10 N	8,120	190	70	k	Wiebe et al. (1975)
Great Barrier Reef	20 S	3,920	47	17	l	Larkum et al. (1988) (<i>Calothrix</i> patch)
Great Barrier Reef	20 S	440	5	2	m	Larkum et al. (1988) (average over reef)
Great Barrier Reef	20 S	1,872	23	8	n	Larkum (1988) (average over reef, grazed)
Average of Coral Reefs	—	—	68	25		Capone (1983)

* (a) Daily rates from max hourly rates under the assumption of a 24-h insolation period. Annual rate assumes 12 h d⁻¹ and a 90-d ice-free season. (b) Daily and annual rates assume 12 h d⁻¹ and 200 d yr⁻¹. (c) Hourly rate from averaged mean rates from 2 m depth at 5 sites (Table 1). Daily and annual rates calculated from a computer model that includes changes in daily and seasonal solar irradiance and nocturnal fixation. (d) Maximal hourly rate from Table 1 (present study). (e) Mean hourly, daily, and annual rates scaled to include lake bottom topography, such that rates reflect actual amount of fixation within orthogonal projection of lake bottom. (f) Hourly rate calculated as average of mean rates (Jul–Dec) from Fig. 3 (Grimm and Petrone 1997). Daily and annual rates from table 6 and Fig. 8 in Grimm and Petrone (1997), which include nocturnal fixation. (g) Annual rate assumes 120-d insolation period. (h) Daily rate represents mean daytime rate × 12 h + 18% of daytime rate × 12 h⁻¹. Annual rate assumes 180 d yr⁻¹. (i) Daily rate from mean diurnal rate × 24 h. Annual rate assumes 180 d yr⁻¹. (j) Daily rates from mean diurnal rate × 24 h from Figs. 6 and 7 (Paerl et al. 1996). Annual rate assumes 180 d yr⁻¹. (k) Hourly rate averaged over reef types under full daylight intensity. Daily rates from mean day and night rates multiplied by 24 h. Annual rates assume 365 d yr⁻¹. (l) Mean hourly rate from *Calothrix* patch in intertidal reef flat. Daily and annual rates assume 12 h d⁻¹ and 365 d yr⁻¹. (m) Rates from median values in table 6 (Larkum et al. 1988) are averaged across reef and include substrata tortuosity. (n) Median rates averaged over highly grazed reef area and include substrata tortuosity.

malized to heterocyst biovolume and PAR, and between heterocyst biovolume and depth, allowed the creation of a model that integrates N₂ fixation over depth and time. The agreement between model estimates and in situ estimates for N₂ fixation allowed us to estimate daytime N₂ fixation throughout the littoral zone. From a maximal carbon fixation rate of 1 g m⁻² d⁻¹ (Bootsma 1993) and a “Redfield” C:N ratio of 5.68 (molar), the calculated 24-h N demand of periphyton would be 170 mg N m⁻² d⁻¹. From model estimates of daily N₂ fixation at shallow depths (1–2 m), N₂ fixation would contribute between 8% and 12% of the total 24-hr N demand of epilithic periphyton and between 13% and 18% if nocturnal N₂ fixation were included.

To estimate the importance of epilithic N₂ fixation to the N budget of Lake Malawi, we employed our model to spatially and temporally integrate N₂ fixation rates over a depth gradient (under the assumption of a 45° slope) for a single year. Within the model, we multiplied area estimates over a range of factors (2–5.7), to adjust for roughness of the substrata (Higgins 1999), then extrapolated the results over the entire rocky littoral zone area in Lake Malawi, as determined from literature sources (Ribbink et al. 1983) and aerial surveys (Cooley unpubl. data). Although our model uses estimated daily PAR under 100% cloudless conditions from

Fee’s (1990) model, we calculated depth-integrated rates under 70% cloudless conditions and found only a 10% difference in model output (due to light saturation at shallow depths). On the basis of these assumptions, the N₂ fixation model estimates that 4–20 × 10⁴ kg N lake⁻¹ yr⁻¹, or 40–120 mmol N m⁻² yr⁻¹, is biologically fixed by epilithic diazotrophs. This estimate for epilithic N₂ fixation is similar in magnitude to those for precipitation and riverine inputs to Lake Malawi and suggests that epilithic N₂ fixation may be a significant source of N to the epilimnion (Table 3). Furthermore, because epilithic N₂ fixation rates do not vary substantially between seasons, the relative importance of epilithic N₂ fixation increases during the dry-windy season, when riverine and atmospheric inputs of N and pelagic N₂ fixation are much reduced.

Interlake comparisons—Because this study represents the first epilithic N₂ fixation estimates from a tropical lake, epilithic N₂ fixation rates from Lake Malawi are presented along with benthic N₂ fixation rates from both marine and freshwater ecosystems that encompass a wide latitudinal range (Table 4). Summertime hourly N₂ fixation rates do not appear to follow a strong latitudinal gradient for freshwater systems. During the summer insolation period, somewhat lower hour-

ly rates in temperate regions are made up for by longer day lengths. As a result, daily rates in arctic regions (during summer months) may be similar to those in more temperate or tropical regions. Because of seasonal differences in solar irradiance patterns and water temperature, however, annual N_2 fixation rates in tropical regions are often an order of magnitude higher than those in temperate regions. Nonetheless, the importance of benthic N_2 fixation to the nitrogen budget in these systems is related to seasonal trends in primary production and N demand and therefore does not necessarily vary over latitudes. For example, Bergmann and Welch (1990) estimated that N_2 fixation accounted for up to 28% of the N budget in some arctic lakes, similar to our estimates for the epilithon of Lake Malawi (Table 3). Epilithic N_2 fixation rates do not show any strong differences between freshwater and marine habitats. Hourly, daily, and annual N_2 fixation rates in the rocky littoral areas of Lake Malawi are similar to those in coral reef habitats. This similarity in N_2 fixation can be at least partially attributed to similarities in primary productivity (Bootsma 1993), grazing rates (Wilkinson and Sammarco 1983; Larkum et al. 1988; Andre 1999), and nutrient availability (Howarth et al. 1988; Bootsma and Hecky 1999).

We conclude that benthic nitrogen fixation is a quantitatively significant contribution to the nitrogen requirement of the epilithic periphyton and to dependant herbivores, as well as to the whole-lake nitrogen budget. The predictive model of N_2 fixation should be tested through further studies on Lake Malawi and other tropical lakes.

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High-resolution metal gradients measured by in situ DGT/DET deployment in Black Sea sediments using an autonomous benthic lander

Abstract—DET (Diffusive equilibration in thin films) and DGT (diffusive gradients in thin films) have been deployed in situ using an autonomous benthic lander to measure concentrations and induced fluxes of Fe and Mn (DET/DGT) and trace metals (DGT) in pore waters at millimeter spatial resolutions. The newly developed deployment system is described, and based on these first results, its strengths and weaknesses are discussed. Deployments were made in the Western Black Sea in shelf sediments overlain by well-oxygenated water at a water depth of 77 m. Maxima of the redox-sensitive metals at 4 and 8 cm deep within the sediment indicated that two zones of reduction dominated the geochemistry. Sharp, but systematic, features were superimposed on this general picture and were well replicated in the profiles of Mn, Co, and Cd, but the sharp features in the Fe profile were offset from those of the others elements by several millimeters. Detection of this functional discrimination between Fe and Mn as regulators of trace metals would not have been possible using more conventional sampling procedures.

The sediment–water interface is chemically and microbially the most active site in natural waters, with steep gradients in physical, chemical, and biological properties (Santschi et al. 1990). Over the last 20 yr, the spatial resolution of measurements of solute concentrations in pore waters has been improved to a submillimeter scale for a limited range of determinands (e.g. O_2 , NO_3^- , pH, pCO_2) through the development of microelectrodes and optrodes (Larsen et al. 1997; Glud et al. 1999). These techniques have demonstrated the existence of concentration gradients across the sediment–water interface over millimeter and submillimeter depth scales (Cai and Reimers 1993). Although the resolution of conventional (Carrigan et al. 1985) slicing/squeezing and dialysis procedures has been improved (Shaw et al. 1990; Aller et al. 1998) and voltametric electrodes have been used for Mn and Fe and other solutes (e.g., I^- and $S(-II)$) (Bren-

del and Luther 1995; Luther et al. 1998), only the emerging techniques of DET (diffusive equilibration in thin films) and DGT (diffusive gradients in thin films) have provided high-resolution data for a wide range of components that include trace metals other than Fe and Mn (Davison et al. 2000).

DET is directly comparable to more traditional “peeper” systems, except that diffusive equilibrium is attained between solutes in the pore waters and in a thin film of gel. The thinness (<1 mm) of the film results in much faster diffusive equilibration than with traditional peepers. DET has been used to measure Ca^{2+} , Mg^{2+} , Na^+ , K^+ , $Fe^{2/3+}$, Mn^{2+} , Cl^- , SO_4^{2-} , NO_3^- , alkalinity, and ΣCO_2 in pore waters at a resolution of 1–2 mm (Davison et al. 1994; Brunnegard 1997; Mortimer et al. 1998; Zhang et al. 1999) and Fe at a resolution of 400 μm (Fones et al. 1998) and in two dimensions (Shuttleworth et al. 1999).

DGT (Davison et al. 2000) uses an extra backing layer of gel impregnated with chelating resin. Metal continuously diffuses across the outer layer of gel (95% water) and accumulates on the chelating resin. After deployment, the resin gel layer is sliced and eluted with acid, and metals are determined by atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS). Deployment for a given time at a known temperature allows calculation of the mean flux to the resin gel during deployment. If pore waters are effectively buffered by rapid resupply from a local source, such as a desorbing solid phase, the measured flux can be quantitatively interpreted as a concentration (Zhang et al. 1995; Harper et al. 1998).

DET/DGT assemblies were initially deployed in situ in shallow waters by SCUBA divers (Davison et al. 1994; Zhang et al. 1995; Brunnegard 1997) or placed by hand (Davison et al. 1997). For deeper waters, they were usually deployed in retrieved sediment cores. A lander was developed for the deployment of cylindrical DET assemblies in