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Nitrogen fixation by periphyton and plankton on the Amazon floodplain at Lake Calado

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Abstract. Nitrogen fixation by periphyton and plankton was measured on the Amazon floodplain using the acetylene reduction method calibrated with ^{15}N–N_2. The average ratio (± SD) of moles C_2H_4 reduced per mole N_2–N fixed was 3.4 ± 0.7, similar to other studies. Periphyton and plankton had high rates of light-dependent nitrogen fixation, with dark nitrogen fixation averaging 26% of the average rates in the light. The average daily (24 h) rates for periphyton nitrogen fixation in 1989 and 1990 were 1.79 and 0.51 mmol N_2–$N \cdot m^{-2} \cdot d^{-1}$ respectively, which are comparable to summer rates in many temperate cyanobacterial assemblages. Nitrogen fixation was depressed at NO_3^- concentrations as low as 0.5 μM, and was below detection limits at concentrations of 4 μM, which occurred during periods of river flooding. Planktonic nitrogen fixation rates were high (0.5–0.8 mmol N_2–$N \cdot m^{-2} \cdot d^{-1}$) during the high-water and drainage phases of the annual hydrograph when the floodplain waters were draining towards the river (low NO_3^-), but rates were undetectable (< 0.05 mmol N_2–$N \cdot m^{-2} \cdot d^{-1}$) when there was river flooding (high NO_3^-). Nitrogen fixation by periphyton and plankton in 1989–1990 accounted for approximately 8% of previously reported total annual nitrogen inputs to the floodplain at Lake Calado.

Introduction

The Amazon's floodplain is one of the most productive and fertile regions of the Amazon basin (Junk 1984, 1985). Although large standing crops of macrophytes and periphyton have been documented (Junk 1970; Junk & Howard-Williams 1984; Engle & Melack 1990), the nutrient sources required to support these standing crops are known for very few sites. In Lake Calado in the central Amazon region near Manaus, nutrient inputs and losses have been quantified in detail. Nitrogen is supplied to Lake Calado via local watershed runoff, water exchange with the nutrient-rich Amazon River, and rainfall (Lesack 1988; Lesack & Melack 1991). On an annual basis, the combined losses of nitrogen as export to the river and burial in sediment are larger than the measured inputs (Fisher et al. 1990). Denitrification and nitrogen fixation are processes which potentially affect the nitrogen budget and, as yet, have not been quantified at the floodplain scale for Lake Calado. Because of the numerous oxic/anoxic interfaces provided by flooded soils and aquatic macro-

phytes, denitrification is likely to be important on the Amazon floodplain, although Melack & Fisher (1988) were unable to detect N_2O accumulation in a limited number of measurements. Nitrogen fixation, which was also not considered in Fisher et al.'s (1990) N budget of the Lake Calado floodplain, is the focus of the research presented here.

Nitrogen fixation can be a significant source of nitrogen in wetlands (Bowden 1987; Howard-Williams 1985; Howarth et al. 1988a). A regional budget for the Amazon, based on limited data, implies that nitrogen fixation should be an important process in the Amazon system as well (Salati & Vose 1984). An cursory examination of the various components of the Central Amazonian system showed that floodplain soils and periphyton on aquatic macrophytes were potential sites of nitrogen fixation (Salati et al. 1982). In a more rigorous study, Melack & Fisher (1988) found significant acetylene reduction in samples of periphyton growing on floating aquatic macrophytes in Lake Calado, although they were unable to detect any acetylene reduction activity or $^{15}N–N_2$ fixation in the plankton from the open water. Since a significant portion of the floodplain may be covered with floating emergent macrophytes during the flood portion of the hydrograph (Junk 1985; Fisher & Moline 1992), and since these macrophytes are often heavily colonized by periphyton (Engle & Melack 1990), periphyton could be an important source of nitrogen to the floodplain via nitrogen fixation. The research reported here was undertaken to quantify nitrogen fixation by periphyton associated with floating macrophytes in Lake Calado. Additional information on the nitrogen fixation of plankton and periphyton associated with the flooded forest environment is also reported. Nitrogen fixation of seasonally flooded soils was not measured. This research is part of a larger study of the relative importance of periphyton and plankton in organic matter production and nutrient cycling on the Amazon's floodplain.

Methods

Community and study site description

Lake Calado is a dendritic floodplain lake permanently connected to the Amazon river throughout the year via a narrow, permanent channel (Fig. 1). The water in Lake Calado is a seasonally varying mixture of low-conductivity (~10 $\mu S \cdot cm^{-1}$) stream water from local catchments and high conductivity (~90 $\mu S \cdot cm^{-1}$) river water which episodically floods into the lake. These episodic invasions provide 32% of the known N and 50% of the known P inputs to the floodplain (Lesack 1988; Lesack 1993; Lesack & Melack 1991; Fisher et al. 1990). Invasion of river water onto the floodplain may occur through the permanent channel to the river, through high-water channels connecting Lake Calado to a neighboring lake, or when the levee separating the river from the floodplain is inundated (see Fig. 1).

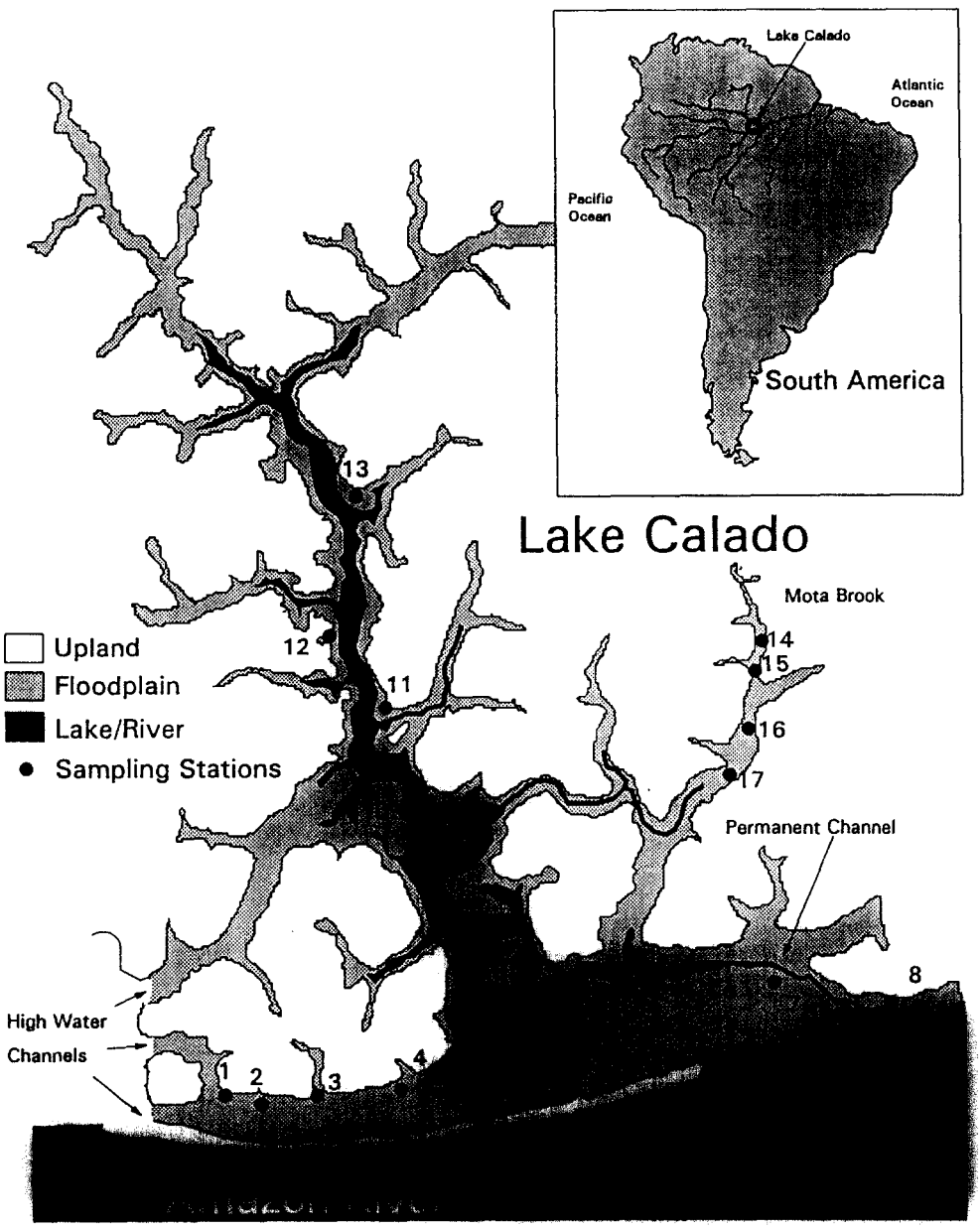

Fig. 1. Map of the Lake Calado floodplain on the north bank of the Amazon River (= Solimões). The black area is the permanent lake or river; the shaded area represents the land which is annually inundated and which is then covered by open water, floating meadows, or flooded forests; the white areas are uplands not usually subject to flooding. The floating meadows develop primarily on the inundated soils in the southern basin of the lake and in smaller embayments along the N–S axis of the lake. Numbered stations are those from which periphyton samples were collected for nitrogen fixation assays.

During the early filling phase (January–April), small invasions of river water into the lake via the permanent channel are common (1–3 per month). Such invasions are generally short lived (< 3 days) and influence only the regions adjacent to the permanent channel. Larger invasions lasting 1–3 weeks and extending well into the southern basin of the lake occur less frequently (0–3 per year). A large invasion occurred in Lake Calado in January 1990 and a similar event has been described in detail for nearby Lake Januaca (Fisher & Parsley 1979). Later in the filling stage (April–July) water may flow into Lake Calado from high-water channels to the west as recently described by Engle & Melack (1993). Finally, during years with very high floods, the levee separating the river from the floodplain may be inundated. Such an event took place during this study period (June–August, 1989).

Like other Amazonian floodplain lakes, Lake Calado is a continuously changing mosaic of flooded forest, "meadows" of floating emergent macrophytes, and open, lake-like areas in which the plankton is the dominant community (Fig. 2). The relative proportions of these communities fluctuate through the year in response to the hydrograph of the river. At the study site, the filling phase of the annual hydrographic cycle usually begins in January and continues through June. During this time, the flood waters rise at rates of 1–3 meters per month with a total elevation change of 10–12 meters. Maximum high water occurs during June and July. This is followed by a rapid drainage phase in August and September, and the low-water phase of October–December completes the annual cycle.

The leaves and branches of the trees in flooded forest trees provide abundant surfaces for periphyton growth during the flood phase. As the flood waters rise, the shrubs and trees bordering the lake and inflowing streams are inundated for months at a time (Prance 1979).

The grasses that form the floating meadows begin as rooted terrestrial plants but develop floating growth forms during the filling phase. By the high water period the macrophytes form extensive floating rafts of vegetation, which are left stranded on trees or exposed soils as the flood waters recede during the drainage phase (Junk & Howard-Williams 1984). Emergent stems of the macrophytes form a leaf canopy above the surface of the water; below the water surface, aquatic root clusters develop at each node along the submersed stem (see Fig. 2). The aquatic root clusters, stems, and decaying leaves form a tangled mat just below the water surface, and the top 10–20 cm receives light penetrating through the leaf canopy and is well colonized by a diverse epiphyte assemblage including green algae and cyanobacteria. The lower portion is severely shaded and contains few algae (Doyle 1991).

Most floating meadows develop in areas which receive periodic influxes of water (and nutrients) from the adjacent Amazon River, such as the southern basin of Lake Calado (see Fig. 1). In Lake Calado smaller and less dense pockets of macrophyte meadows also grow in protected coves farther from the river. During the study period floating meadows occupied an average of

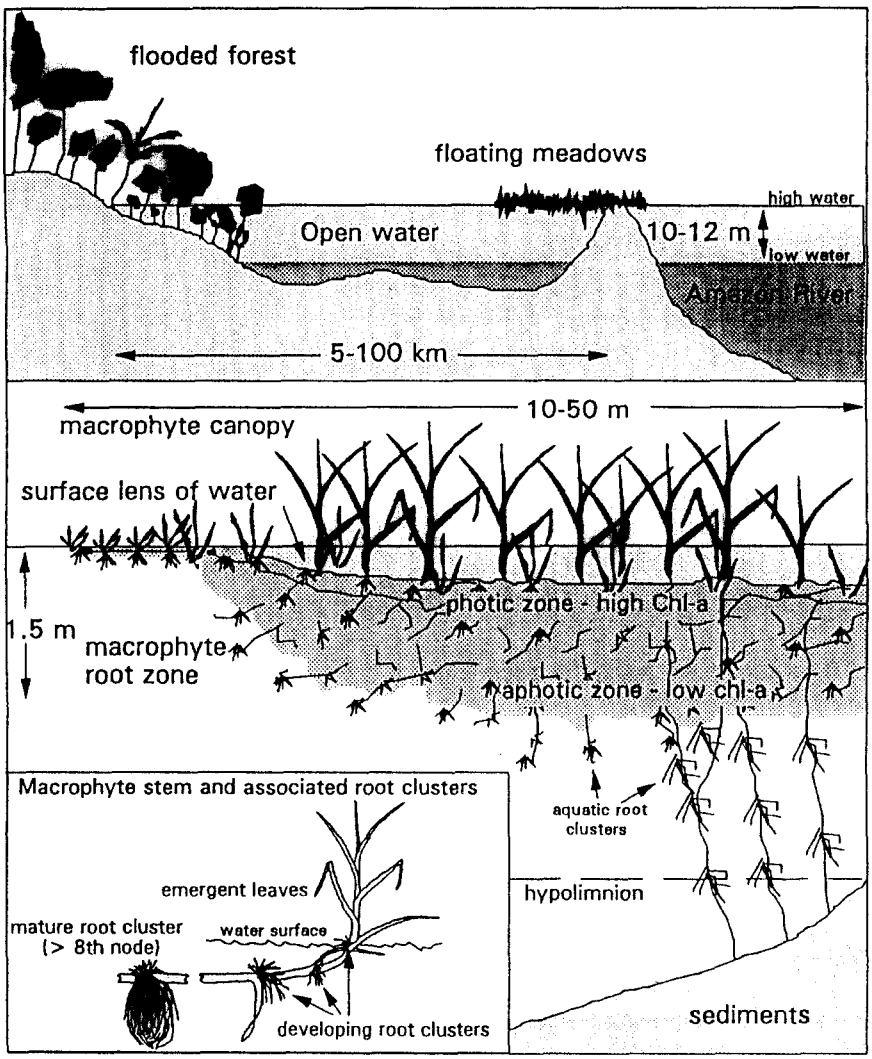

Fig. 2. TOP: Cross-section of the floodplain at Lake Calado showing the location of the floating meadows, flooded forests, and open water. BOTTOM: Vertical and horizontal structure of mature floating meadows on Lake Calado. Vertically the meadow consists of an emergent canopy and a submersed root zone. Within the root zone only the top few cm are within the photic zone and capable of supporting autotrophic algal populations. Horizontally, *Echinochloa polystachya* grows rooted in shallow areas near shore, while *Paspalum repens* grows over deeper water and is no longer rooted. INSERT: Details of a single macrophyte stem showing the emergent stem and aquatic root clusters.

0.4 km^2 within the 3.0 km^2 southern basin of the lake (Doyle 1991; Fisher & Moline 1992).

The plankton occur primarily in the open, lake-like areas of the Amazon's floodplain. The water column is usually stratified when water depth exceeds 5 m; the hypolimnion is anoxic, while the epilimnion is usually subsaturated in oxygen (MacIntyre & Melack 1984, 1988). Significant photosynthetic and zooplankton activity is restricted to the epilimnion (Fisher et al. 1983; Melack & Fisher 1983).

Acetylene-reduction technique

Nitrogen fixation measurements were made primarily using the acetylene reduction technique calibrated and supplemented by measurements of ^{15}N–N_2 incorporation. Acetylene reduction assays of periphyton samples were performed in 50 ml Erlenmeyer flasks or screw-cap vials fitted with rubber septa. In either assay flask, the vapor phase was 47% of the total volume. Acetylene, freshly generated from calcium carbide, was injected into the vapor phase to yield a concentration of 0.12–0.14 ml acetylene per ml of water (5.0–5.8 mM). The protocol was shown experimentally to saturate acetylene reduction without inhibition, to have no significant lag time before the onset of ethylene generation, and, under constant light, to be linear during the incubation (Doyle 1991).

The assays were made either on the periphyton attached to the aquatic roots of the floating macrophytes or on periphyton growing on submersed leaves or stems (see Fig. 2). Intact periphyton samples were gently clipped along with a portion of the macrophyte tissue upon which the periphyton was growing and enclosed while still underwater. Most of the periphyton samples were taken from the lighted layer of the mats, but occasionally samples from the lower, aphotic portion of the mat were also assayed.

The periphyton samples were returned to the lab and transferred to assay flasks containing unfiltered water from the collection site. After being sealed, the flasks were injected with acetylene and swirled gently for several minutes to produce an equilibrium distribution of acetylene while incurring as little damage as possible to the structure of the periphyton community. Control assays were made routinely with only unfiltered water from the collection site to correct for both planktonic acetylene reduction and ethylene initially present. Separate assays of periphyton were occasionally made without acetylene ($n = 8$), but no endogenous ethylene production was detected. The flasks were incubated in the lake in floating surface racks for 3–5 h, usually between 1300 and 1700 hours, at ambient temperatures of 28–32 °C. To approximate the natural light climate within the mats, the samples collected from the upper portions of the mat were covered by either a neutral density screen (56% light transmitted) or by a layer of macrophyte leaves and stems (30–60% light transmitted); samples from the lower, aphotic zone of the mat were wrapped in aluminum foil to ensure complete darkness during the incubation. The

incident photosynthetically active radiation (PAR, 400–700 nm) was measured during all experiments at 1 minute intervals with a flat LiCor PAR sensor connected to a datalogger (Omnidata Easylogger).

At the end of the incubation period, the flasks were shaken vigorously. 100 µl samples were taken from the gas phase and injected directly into a Carle AGC 100 gas chromatograph equipped with a flame ionization detector and a stainless steel column packed with molecular sieve 5-A. The column temperature was 70 °C; helium was the carrier gas and ethylene standards were used to calibrate the instrument daily. Calculation of ethylene production rates took into account the solubility of ethylene (Flett et al. 1976). If subsequent samples were to be taken from the same flask, it was not shaken vigorously in order not to disrupt the structure of the periphyton community. Instead, the flask was swirled gently for 45 seconds. Comparison of this procedure with vigorous shaking showed 93–97% recovery of the ethylene. After completion of the assay, the periphyton was manually stripped from the macrophyte substratum and subsampled for algal biomass as chlorophyll-a (Doyle 1991). Detection limits were 0.03 µmol C_2H_4 mg Chl-$a^{-1}\cdot h^{-1}$ (~0.01 µmol N_2–N·mg Chl-$a^{-1}\cdot h^{-1}$).

Acetylene-reduction assays for planktonic nitrogen fixation were performed in 50 ml glass syringes (Flett et al. 1976). Water samples were collected from various depths in a horizontal Van Dorn sampler and transported to the field lab within 20 minutes. The syringes were rinsed with water from the collection site and then filled to 30 ml. Five ml of freshly generated acetylene was drawn into the syringe, which was then sealed and shaken for 15 s. The samples were incubated in surface racks under neutral density screens to adjust the incident light to approximate the light climate at the collection station and depth. After 2–5 h incubations, fresh air was drawn into the syringe as necessary to make a final vapor phase of 15 ml. The syringe was sealed and shaken vigorously. 100 µl subsamples of the vapor phase were collected and analyzed immediately with no storage or additional transfers of the gas. Heat-killed sterile controls were also analyzed.

^{15}N techniques

^{15}N–N_2 was used for two purposes: 1) to determine the proper conversion factor between acetylene reduction and N_2–N fixation (Graham et al. 1980; Peterson & Burris 1976), and 2) to supplement the plankton nitrogen fixation assays during periods when the gas chromatograph was unavailable.

Periphyton ^{15}N–N_2 fixation assays were performed in 50 ml glass syringes under a shade screen (56% incident light, 7 h incubations). 40 ml of GF/F filtered lake water was placed into each syringe along with 20 ml of air. The syringes were shaken vigorously, the syringe plungers removed, and a sample of periphyton placed into each syringe. The headspace was expelled from the syringe and the total volume of water was adjusted to 35 ml. Two or three ml of 97 atom % ^{15}N–N_2 were injected into each syringe gently mixed for

1 minute. The ^{15}N atom % in the N_2 pool within the syringe was always about 80% and was estimated for each assay from the volume of ^{15}N–N_2 injected and the solubility of N_2 in equilibrium with air at the incubation temperature. Occasional samples were collected for ^{15}N–N analysis, which confirmed the validity of these assumptions.

Planktonic ^{15}N–N_2 fixation assays were performed in 1 liter glass bottles with teflon-lined stoppers drilled and fitted with butyl rubber septa. The larger sample volume was required to provide sufficient seston nitrogen for analysis on a mass spectrometer. Five ml of 97 atom % ^{15}N–N_2 was injected into each bottle and shaken for an initial atom % ^{15}N–N_2 of ~25%. The plankton samples were incubated in surface racks under neutral density screens to match the light climate at the collection station and depth. Incubation times ranged from 7 to 24 h and the resulting detection limits ranged from 0.3–1.0 μmol N_2–N·mg Chl-a^{-1}·h^{-1}.

After incubation, the plankton or stripped periphyton slurries were filtered onto GF/F glass fiber filters. The filters were dried and later combusted with a micro-Dumas technique (Fiedler & Proksch 1975) for analysis in an AEI MS-10S mass spectrometer yielding estimates of both μmol N and the atom % ^{15}N in each sample.

Results

Calibration of acetylene reduction assays

The mean (± SD, n = 4) calibration factor for the acetylene reduction assay was 3.4 ± 0.7 moles C_2H_2 reduced per mole N_2–N fixed under illuminated conditions (Table 1). This calibration factor exceeds the theoretical ratio often assumed in converting acetylene reduction rates to rates of N_2–N fixation, but falls between the empirical conversion factors reported by Peterson & Burris (1976) and Graham et al. (1980) for planktonic cyanobacteria (2.1 and 3.75 respectively). Possible reasons for the discrepancy between theoretical and empirical ratios are discussed by Graham et al. (1980) and Howarth et al. (1988a). Two potential reasons applicable to the data presented here are (1) excretion of ^{15}N–NH_4 or ^{15}N–DON, neither of which would be detected during isotopic analysis of the particulate fraction, and (2) competition between hydrogen and nitrogen for necessary reductant and energy resources (Pearl 1982). Conversion from acetylene reduction to nitrogen fixation rates were made for the entire study utilizing the reported average conversion factor.

Influence of light on rates of nitrogen fixation

The average rate of periphyton nitrogen fixation (acetylene reduction) in the light was compared to the rate in the dark. After 2–3 h incubations in the light,

Table 1. Acetylene reduction and parallel ^{15}N–N_2 fixation under illuminated conditions by intact periphyton communities from Lake Calado.[a]

Date (1989)	N_2–N fixation	C_2H_2 reduction	mol C_2H_2; mol N_2–N
	μmol·mg Chl-a^{-1}·h^{-1}		
16 May[b]	2.20 ± 0.84 (4)	6.74 ± 2.42 (4)	3.1 ± 1.6
16 May	2.62 ± 0.92 (4)	8.69 ± 1.64 (4)	3.3 ± 1.3
16 May	2.74 ± 0.96 (4)	10.31 ± 0.40 (4)	3.8 ± 1.3
06 Aug	2.56 ± 1.12 (9)	8.23 ± 1.77 (9)	3.2 ± 1.6
		Mean ratio =	3.4 ± 0.7

[a] Mean ± SD, with the number of replicates in parenthesis. SD was propagated from the first two columns to the last column following Bevington (1969).
[b] On May 16 samples were collected from three different sites.

the assay flasks were darkened with aluminum foil. The assays were continued until for an additional 1.5–2 h following darkening to estimate dark fixation rates. These experiments showed that the dark rate corresponded to 26% of the average light rate (SD = 12.5, n = 20).

The rate of nitrogen fixation under various light levels was also determined once for both periphyton and plankton. For the periphyton experiment, three macrophyte root clusters coated with periphyton were collected and five subsamples were taken from each and randomly assigned to one of five light levels. For the plankton experiment, a larger water sample was collected and triplicate subsamples were randomly assigned to each of the five light levels. All samples were pre-incubated under experimental conditions for 1.5 h prior to addition of acetylene, and the incubations were made from about 1200 to 1430 hours on a cloudless day. Both periphyton and plankton nitrogen fixation was strongly light-dependent and was saturated at light level between 500–900 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 3). Since a maximum of only 20–60% (mean = 28%) of the incident light penetrates the grass canopy, the daily maximum light levels for periphyton within the mat are generally 400–700 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Doyle 1991). The data in Fig. 3 indicate that periphyton nitrogen fixation may become light saturated during the noon period, but that rates are generally light dependent for most the day. Likewise, due to the high light attenuation of the stained waters characteristic of the Amazon floodplain, nitrogen fixation within the plankton was also usually light dependent.

Nitrogen fixation in the periphyton of the floating meadows

Nitrogen fixation of the periphyton community growing in the floating meadows was measured between March 1989 and June 1990. This study period encompasses the complete annual hydrographic cycle (filling, high-water, drainage, and low-water) for 1989 and the filling and high-water phases of 1990 (Table 2). Periphyton samples were assayed approximately monthly

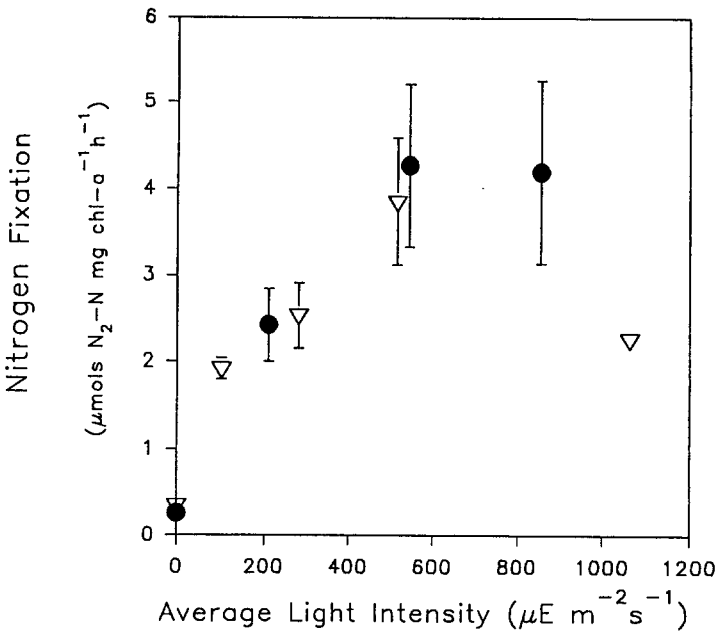

Fig. 3. Influence of average photosynthetically active radiation (PAR, 400–700 nm) during the incubation period on the rates of nitrogen fixation by periphyton (closed circles) and plankton (open triangles). Symbols are the mean ± SD, n = 3. Data from September, 1989.

Table 2. Summary of nitrogen fixation of periphyton associated with the floating meadows on the Lake Calado floodplain in relation to hydrologic stage and presence of riverine NO_3^-. Detection limits of assay ~0.01 μmol N_2–N·mg Chl-a^{-1}·h^{-1}.

Hydrologic stage (months sampled)	Riverine NO_3^- present on floodplain?	μmol N_2–N·mg Chl-a^{-1}·h^{-1}				# of assays
		mean	SD	min	max	
Filling 1989 (Mar, Apr, May)	no	3.38	3.10	0.00	14.68	77
High 1989 (July)	yes	0.62	0.68	0.06	2.68	35
Drainage 1989 (Aug, Sept)	no	3.18	2.27	0.28	12.48	65
Low 1989–1990 (Dec, Jan)	yes	0.01	0.02	0.00	0.08	38
Filling 1990 (Mar, Apr)	yes	0.38	0.72	0.00	4.01	61
High 1990 (June)	no	0.79	0.88	0.00	2.79	17

during the growth period of the macrophytes (Jan–Sept). Most (~65%) of the samples were collected from site 5, which is located in the center of the southern basin of the lake (Fig. 1). Various other sites within the lake's southern basin (sites 1–8) or along the major north-south axis of the lake (sites 9–13, Fig. 1) were also sampled to characterize the variability of nitrogen fixation across the lake.

The average periphyton nitrogen fixation rate from each sampling location on Lake Calado was estimated from 2–8 measurements on each sampling date. To estimate the errors associated with such small numbers of replicates, we made a more intensive study of site 5 one time. A single periphyton sample was taken from each of 24 root clusters and assayed for nitrogen fixation. The coefficient of variation for these samples was 53%. Although the variability may change through time, in this experiment the mean rate from any 3 randomly selected root clusters always came within ±50% and on average (n = 10) came within ± 30% of the grand mean of all 24 assayed root clusters.

Periphyton nitrogen fixation within the floating meadows was limited to the upper, lighted regions of the mat. Nitrogen fixation was high in the surface zone (range = 0.16–6.98 μmol N_2–N·mg Chl-a^{-1}·h^{-1}) but undetectable in the lower portion of the mats.

The seasonal pattern of periphyton nitrogen fixation at Lake Calado was closely related to nitrate concentration (Table 2, Figs. 4 and 5). The rate of periphyton nitrogen fixation was low during June–July 1989 and again in January–May 1990. During both of these periods riverine NO_3^- was present on the floodplain. In June–July 1989 river water was present in Lake Calado due to an unusually high flood phase which overran the levee separating the lake from the river (see Figs. 1 and 4). In January 1990 there was a prolonged invasion of river water into the lake as the river rose rapidly at the beginning of the filling phase (Fig. 4). River water from this invasion flooded the entire lake, raising the NO_3^- concentration and depressing nitrogen fixation. Collectively, these data indicate a strong depression of nitrogen fixation by measurable quantities of NO_3^- (Fig. 5).

An example of the effect of NO_3^- on nitrogen fixation is shown in Fig. 6. A gradient of NO_3^- concentration existed on the floodplain on 21 April 1989, and was reflected by a similar, but inverse gradient of nitrogen fixation.

The frequency distribution of biomass-specific rates of nitrogen fixation for 1989 and 1990 illustrate strong interannual variability of nitrogen fixation on the Amazon floodplain (Fig. 7). In 1989 there were extended periods when the floodplain was free of riverine NO_3^- influence and high rates of nitrogen fixation were common. In 1990 the floodplain was subject to a prolonged invasion of riverine water and the nitrogen fixation rates were much lower.

Nitrogen fixation in the periphyton of the flooded forest

During the drainage phase (August 1989), nitrogen fixation was measured on periphyton growing on leaves of submersed trees within the flooded forest.

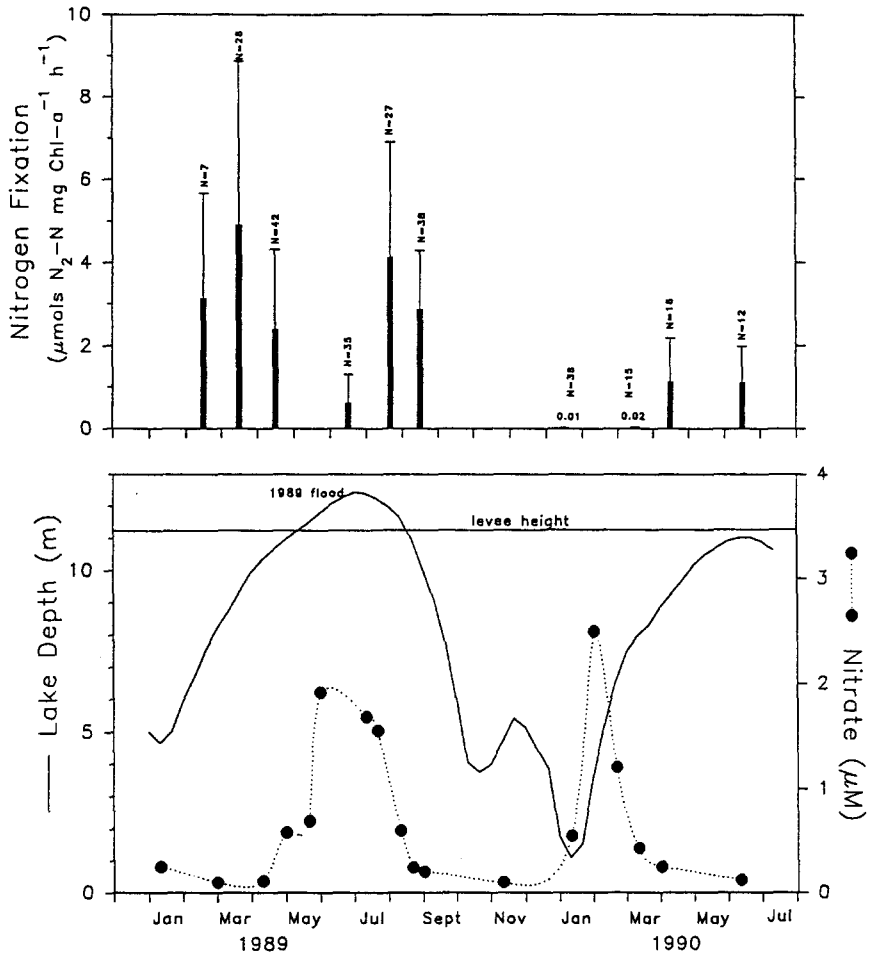

Fig. 4. Seasonal pattern of nitrogen fixation (top panel, mean of entire lake ±SD, *n* given above each bar), nitrate concentration at station 5 (closed circles lower panel), and maximum water depth (solid line lower panel) on the Lake Calado floodplain during the study period.

Periphyton samples were collected from trees and shrubs immediately bordering the stream bed at four sites along the course of inflowing water from Mota Brook (sites 14–17, Fig. 1). Triplicate leaf samples with associated periphyton were collected at each site from the best lighted, most heavily colonized portions along the stream channel and probably reflect the best conditions within the flooded forest for autotrophic nitrogen fixation. The data show an increasing trend in the downstream direction with values of 6.76 ± 1.60, 39.06 ± 10.54, 37.04 ± 10.66, and 58.18 ± 22.78 μmol N_2–N·m^{-2} leaf area·h^{-1} for sites 14–17, respectively. Detection limits were approximately 0.2 μmol N_2–N·m^{-2} leaf area·h^{-1}. Although the NO_3^- concentration at all sites was below detection limits at the time of sample collection, this transect

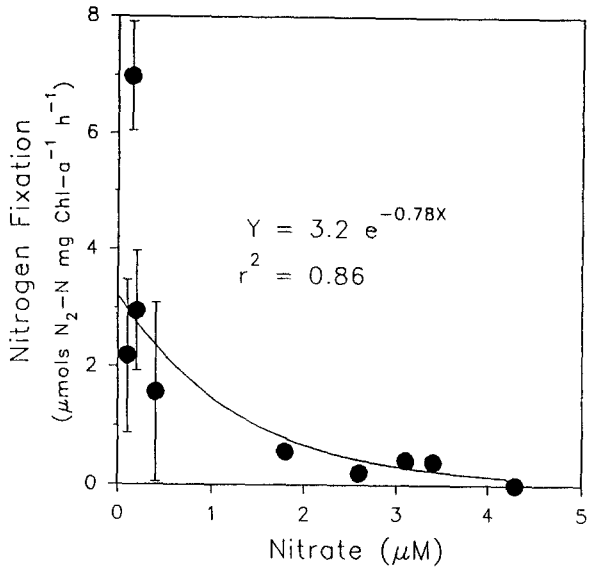

Fig. 5. Influence of ambient nitrate concentration on observed rates of nitrogen fixation in the periphyton community of the floating meadows (mean ±SD).

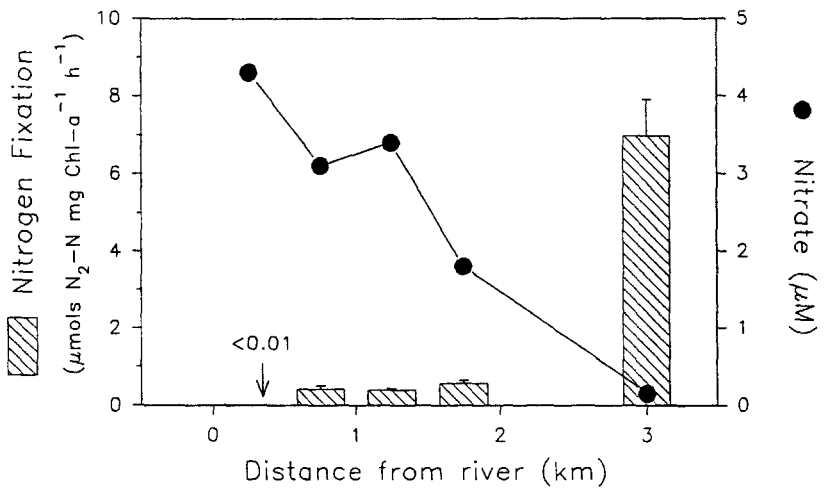

Fig. 6. Observed nitrate concentration (closed circles) and periphyton nitrogen fixation rate (bars, mean ± SD, $n = 3$) along a transect from the river to the lake (stations 1–5 respectively).

represents a continuum of exposure to NO_3^-. The stream water at site 14 (upstream site) often had NO_3^- concentrations of 5–10 μM, while the concentrations at site 17 were generally below detection limits (Williams 1992; Alves 1993).

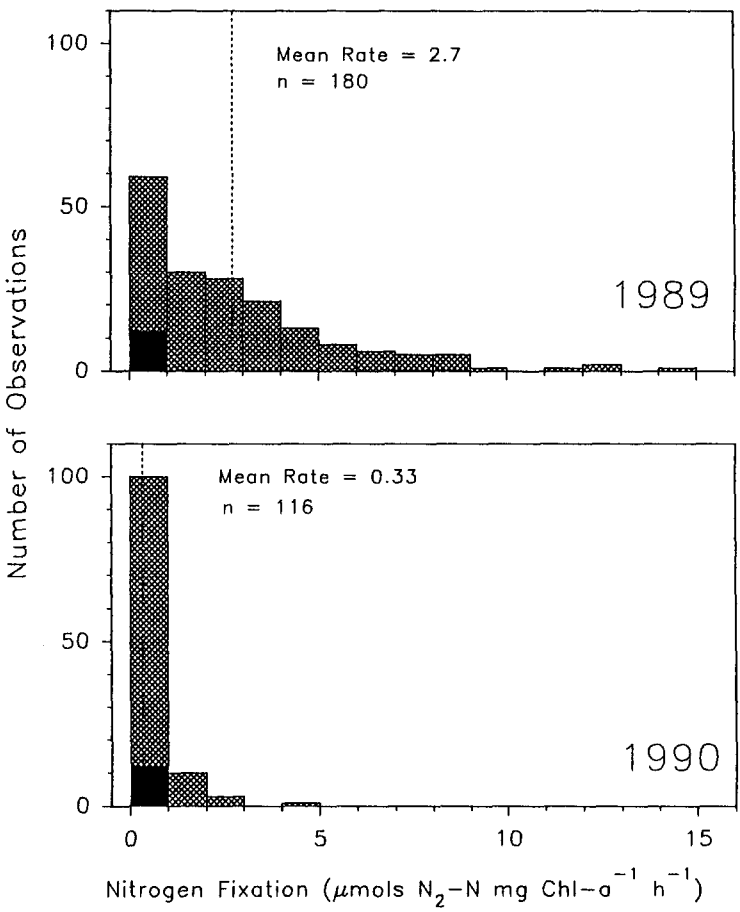

Fig. 7. Frequency distribution and mean rate of periphyton nitrogen fixation in 1989 (top panel) and 1990 (bottom panel). Black section of bars represent the nitrogen fixation assays which were below detection limits.

Planktonic nitrogen fixation

Phytoplankton assays show strong dependence on incubation light intensity (Fig. 3). The maximum volumetric rate under optimum light conditions was about 30 nmol N_2–N·l^{-1}·h^{-1} (4.4 μmol N_2–N·mg chl-a^{-1}·h^{-1}), a value which falls within the range reported for Lake Valencia, Venezuela (Levine & Lewis 1985). Planktonic nitrogen fixation, like that of the periphyton, showed strong seasonal variability. Nitrogen fixation was high during the high-water and drainage phases of 1989, but rates were below detection limits during the remainder of the study (Table 3).

Table 3. Plankton nitrogen fixation rates in Lake Calado during 1989 and 1990 (mean ± SD).

Dates	Sites	Method	Incubation light level (percent incident light)	N-fixation rate (μmol N_2–N·mg Chl-$a^{-1}\cdot h^{-1}$)
07/05/89	11	C_2H_4	100%	0.97 ± 0.04
09/15/89	11	C_2H_4	0, 14, 35, 56, & 100%	2.73 ± 1.14
	5	C_2H_4	0, 14, 35, 56, & 100%	2.55 ± 0.55
12/29/89	11	$^{15}N_2$–N	0, 14, 35, 56, & 100%	ND[1]
12/30/89	5			
01/01/90	8			
02/08/90	11, 5	C_2H_4	56%	ND[2]
03/08/90	8			
04/11/90	8			
04/17/90	5	$^{15}N_2$–N	0, 14, 35, 56, & 100%	ND[3]

ND = not detectable.
1 = 10 h incubation, detection limits of ca. 8 nmol N_2-N·$l^{-1}\cdot h^{-1}$.
2 = 5 h incubation, detection limits of ca. 1 nmol N_2-N·$l^{-1}\cdot h^{-1}$.
1 = 24 h incubation, detection limits of ca. 3 nmol N_2-N·$l^{-1}\cdot h^{-1}$.

Discussion

Light dependence and organisms responsible for nitrogen fixation

The light dependence of nitrogen fixation in both the periphyton and the plankton (Fig. 3) implies that algae are responsible for nitrogen fixation. This conclusion is reinforced by microscopic observation of heterocystous cyanobacteria in the periphyton and plankton during the study period, and the absence of measurable periphyton nitrogen fixation in the deeper, aphotic layers of the macrophyte mats.

Although the rates of nitrogen fixation were light dependent, significant fixation also took place in the dark (26% of light rate) and this could be interpreted to mean that some of the nitrogen fixation was being done by heterotrophic organisms. The complex and highly dynamic microenvironment with the macrophyte root clusters produces many oxic/anoxic interfaces (Doyle 1991) which could be used by heterotrophic nitrogen fixers. However, dark rates of nitrogen fixation can be high in pure cultures of cyanobacteria (Carpenter et al. 1978), and numerous studies of natural populations have shown significant dark nitrogen fixation in systems where the principal nitrogen fixers were considered to be autotrophic (Table 4).

Light dependence of nitrogen fixation is often reported for autotrophic nitrogen fixers (Corkran & Wickstrom 1987; Penhale & Capone 1981) or for heterotrophic organisms symbiotic on plants (Head & Carpenter 1975). The

Table 4. Comparison of reported values for nitrogen fixation in the light and dark by photosynthetic nitrogen fixing organisms. The reported values are the average dark fixation as a percent of fixation in the light. Where available, the number of assays (n) and standard deviation of the mean (SD) are also presented.

Observed mean dark fixation rate (% of light rate)	SD	n	Reference
87	58	5	Gotto et al., 1981
63	37	21	Potts and Whitton, 1977
44	50	4	Hanson, 1977
40	20	11	Goering and Parker, 1972
33	NR	NR	Horne, 1975
26	**13**	**20**	**This study**
14	NR	6	Capone et al., 1979
12	7	6	Capone and Taylor, 1980
12	NR	"many"	Corkran and Wickstrom, 1987

NR = Not reported.

strong light dependence found in this study is similar to that reported by Penhale & Capone (1981) for a *Microdictyon*-epiphyte complex. However, in that study nitrogen fixation was maximal at lower light intensities (200–300 $\mu E \cdot m^{-2} \cdot s^{-1}$) than in the present study (500–900 $\mu E \cdot m^{-2} \cdot s^{-1}$).

Spatial and temporal variability of nitrogen fixation

Nitrogen fixation in Lake Calado was variable in both time (Fig. 4) and space (Fig. 6). Nitrogen fixation rates decreased rapidly with increasing levels of NO_3^- (Fig. 5). At NO_3^- concentrations above 4 μM there was no detectable nitrogen fixation. Similar inhibition of nitrogen fixation by the availability of an alternative nitrogen source has been reported for numerous other microbial assemblages. For example, Head & Carpenter (1975) found an exponential decrease in nitrogen fixation with increasing levels of total dissolved nitrogen ($NO_3^- + NH_4^+$), with no detectable nitrogen fixation when total dissolved nitrogen concentrations were above 10 μM. van Raalte et al. (1974) found that nitrogen fixing bacteria of coastal salt marshes of Massachusetts utilized alternative nitrogen sources instead of dissolved N_2 when such alternative sources were available. Capone & Taylor (1980) report decreasing acetylene reduction activity in the rhizosphere of marine seagrasses with experimental additions of NO_3^-. Finally, Dieberg & Brezonik (1981) found that in natural *Cypress* domes, nitrogen fixation accounted for 14% of the observed increase in nitrogen content of decomposing leaves, while in sewage-enriched domes nitrogen fixation was lower and accounted for only 1% of the increase in nitrogen.

Plankton nitrogen fixation showed temporal variability similar to that of the periphyton. Nitrogen fixation was detectable in the plankton only during the late high-water (July) and drainage phases (August–September) of 1989.

Similar results in temporal variability have been reported for the Orinoco floodplain where planktonic acetylene reduction was maximal during the drainage phase and early low-water phases, when river was absent from the floodplain (Hamilton & Lewis 1987).

A potential reason that the July–September period was the only time of measurable plankton nitrogen fixation may be that this is the time of greatest nitrogen stress on the plankton during the annual hydrograph. During the low water phase (Oct–Jan, maximum water depth of 4 m) the lake is well mixed, and nutrients from the sediments may be available to the plankton. During the filling phase (Jan–May) the plankton on the floodplain receives periodic pulses of nutrient-rich river water from short-term invasion events that may serve to alleviate nitrogen stress. Consequently, the high-water and drainage phases of the hydrograph may be the only times that the plankton does not receive substantial allochthonous nitrogen inputs.

This hypothesis is partially supported by data from Lake Calado and from the Orinoco floodplain. Setaro & Melack (1984) conducted nutrient limitation studies on the plankton of Lake Calado and found both nitrogen and phosphorus deficiency throughout most of the year, but with greater phosphorus deficiency during the filling phase and nitrogen deficiency during the drainage phase. Also, Morrissey & Fisher (1988) report that epilimnetic turnover of NH_4^+ in Lake Calado was rapid (< 1 h) at all times of the year except during periods of high turbidity (river water invasions or wind events at low water). Hamilton & Lewis (1987) report that on the Orinoco floodplain, heterocystous cyanobacteria populations were low during the filling and high-water phases of the annual hydrograph when nitrate and ammonium nitrogen concentrations were highest. Populations rose dramatically during the drainage and low-water phases, when dissolved nitrogen concentrations decreased. However, even then, populations declined in response to periods of high turbidity caused by re-suspension of sediments.

Scaling of nitrogen fixation rates on the Amazon floodplain

It is not simple to extrapolate rates measured on small, enclosed microalgal samples to estimate annual rates for the entire floodplain. We have attempted to integrate over the variability of depth, space, and time to provide such an estimate. These extrapolations have been made separately for each of the three microalgal communities as described below in order to estimate N inputs to the floodplain via microalgal nitrogen fixation.

Periphyton of the floating meadows. Nitrogen entering the floodplain at Lake Calado via periphyton nitrogen fixation in the floating meadows was calculated for each month (Table 5). Propagation of errors was computed for all estimates according to Bevington (1969). Missing parameters were estimated from the data of previous or following months (see parenthesis in Table 5).

Spatial extrapolations for the floating meadow periphyton were made based

Table 5. Biomass and nitrogen fixation rates of periphyton within the floating meadows on the Lake Calado floodplain for each month between January 1989 and June 1990.[a] Macrophyte area for the floodplain computed by multiplying the data from Fisher and Moline (1992) by 1.25 to account for floating meadows within the Lake Calado floodplain excluded from their aerial surveys of the south basin of the lake.

	Biomass (mg Chl-a·m^{-2} floating meadows)	Nitrogen fixation rate (μmol N_2–N mg Chl-a^{-1}·d^{-1})	(mmol N_2–N m^{-2}·d^{-1})	Macrophyte area (km^2)	N input to floodplain (10^3 mol N·month^{-1})
1989					
Jan	5 ± 1 (24)	[45][b]	0.23	1.06	7.6
Feb	4 ± 1 (21)	[45][b]	0.19	0.60	3.3
Mar	66 ± 9 (30)	45 ± 39 (7)	2.97 ± 2.61	0.48	44.2 ± 39.1
Apr	72 ± 10 (24)	79 ± 58 (28)	5.65 ± 4.25	[0.46][f]	78.0 ± 59.2
May	66 ± 11 (30)	40 ± 26 (36)	2.72 ± 1.77	0.44	37.1 ± 24.4
Jun	29 ± 3 (30)	[22][c]	0.63	0.63	11.9
Jul	17 ± 2 (30)	9 ± 10 (35)	0.15 ± 0.17	0.46	2.1 ± 1.9
Aug	44 ± 4 (30)	59 ± 41 (27)	2.62 ± 1.82	0.50	40.6 ± 28.5
Sept	[44][d]	41 ± 21 (24)	1.82	[0.16][f]	8.7
			1989 input to floodplain = 233,500 mols		
1990					
Jan	[8][d]	0 ± 0 (38)	0.00	1.23	0.0
Feb	8 ± 1 (23)	[0][c]	0.00	[0.79][f]	0.0
Mar	38 ± 5 (32)	0 ± 0 (15)	0.00	0.35	0.0
Apr	36 ± 5 (20)	7 ± 12 (46)	0.25 ± 0.43	[0.38][f]	2.9 ± 4.9
May	[33][d]	[12][c]	0.39	[0.38][f]	4.6
Jun	31 ± 4 (28)	17 ± 12 (12)	0.53 ± 0.38	0.40	6.4 ± 4.6
Jul	40 ± 4 (24)	[17][c]	0.68	0.41	8.6
Aug	38 ± 4 (12)	[17][c]	0.65	[0.41][f]	8.3
Sept	[38][d]	[17][c]	0.65	[0.14][f]	2.7
			1990 input to floodplain = 33,500 mols		

Notes:

[a] Reported values are means ± SD (n). SD of column 3 was propagated from columns 1 and 2, and the SD of the final column was propagated from columns 1, 2, and 4 following Bevington (1969). Error associated with macrophyte area assumed to be ± 10% of mean (Fisher & Moline 1992).

[b] Nitrogen fixation data is not available for January and February 1989. It was assumed that the biomass specific rates were the same as those measured in March 1989.

[c] Nitrogen fixation data is not available for this month. It was assumed that the biomass specific rate was the average of the previous and following month.

[d] Periphyton biomass data is not available for all months. Biomass data has been estimated from previous and/or following months.

[e] Nitrogen fixation data is not available for July–September 1990. It was assumed that the rates were the same as June 1990.

[f] Macrophyte areal distribution is not available for all months. Where possible the areal distribution has been estimated as the mean of previous and following months. September values are estimated to be 1/3 of August values.

on fixation per unit microalgal biomass (as chlorophyll-a). The average periphyton biomass within the floating meadow community was computed each month from 24–30 measurements made on the Lake Calado floodplain (Doyle 1991). The total area of the floating meadows and open water in the southern basin of the lake was determined approximately monthly during the study period from aerial video images (Fisher & Moline 1992). The areal macrophyte coverage reported by Fisher & Moline (1992) for the southern basin of Lake Calado has been increased by 25% to account for pockets of floating meadows along the lake axis.

Samples were usually incubated for 3–5 h during the afternoon. This method precludes the use of a numerical model incorporating light variations such as that proposed by Levine & Lewis (1987) to estimate daily rates of nitrogen fixation. However, because samples were subject to most of the daily range of irradiance intensities common in situ during the incubation period, the measured rates were interpreted as a daytime average. It was assumed that nitrogen fixation proceeded at the measured daytime rate for 12 hours and at 26% of that rate during 12 hours of darkness, as described above.

The average rate of nitrogen fixation was computed for each month as the average of all available measurements for the month (Table 5). Because of the high spatial heterogeneity (see Fig. 6 as example), the confidence intervals around the mean are large (typically 60–75% of the mean).

The average daily rate of nitrogen fixation was similar to the maximum summer rates of many studies from temperate latitudes, and greater than that reported for a similar community of microalgae associated with floating macrophytes in southern Brazil (Table 6). Although high rates of nitrogen fixation within cyanobacterial mats are common, these communities are sometimes of little importance to the nitrogen budget of an ecosystem due to the limited area (Howarth et al. 1988a). The periphyton associated with the floating macrophytes of the Amazon floodplain appear to be unusual in that the community is both widely distributed and supports high rates of nitrogen fixation. Howard-Williams et al. (1989) also reported high rates of nitrogen fixation by cyanobacterial populations associated with floating macrophytes in a floodplain lake from southern Brazil.

Annual nitrogen fixation per m^2 of floating meadow surface area was 6.5 times larger in 1989 than in 1990 (Table 5). This difference was primarily due to changes in the biomass-specific rate of nitrogen fixation (Fig. 7) which was related to nitrogen availability. In 1989 the nitrogen fixation was high until the high water period (June) when river water flowed directly into the lake over the flooded levee (Fig. 4). During the drainage phase of 1989 (August–September) the rates increased again as the NO_3^--rich river water was pushed out of the lake by local watershed drainage. In 1990 the rates of nitrogen fixation were quite low during the early filling phase (January–March) as a result of the large invasion of NO_3^--rich river water at the beginning of the flood season. In 1990 the maximum flood level was not high enough to overrun the levee and nutrient concentrations in the lake were

Table 6. Comparison of nitrogen fixation rates from this study to those of other systems with autotrophic nitrogen fixation.

	Reference No. and location of study	Community type	Annual N_2–N fixation (mmol $N \cdot m^{-2} \cdot yr^{-1}$)	Summer N_2–N fixation (μmol $N \cdot m^{-2} \cdot d^{-1}$)
1	Massachusetts marsh	blue-green algal mat	163	700–1400
2	Texas intertidal	blue-green algal mat	290	1380
3	Massachusetts bog	blue-green algal mat	71[a]	~400
4	English salt marsh	blue-green algal mat	1250	–
5	Indian Ocean intertidal	blue-green algal mat	876[b]	2,400
6	Australian coral reef	blue-green algal mat	–	~4,000
		(reef average)	57–114	~350
7	Bahamas *Thalassia* beds	periphytic algae	57[c]	80–230
8	Georgia *Spartina*	periphytic algae	14[d]	~55
9	Chesapeake Bay	periphytic algae	36[c]	160
10	Lake Tahoe	epilithic algae	7–36	200–500
11	Brazil macrophytes	periphytic algae	–	357
	this study, 1989	periphytic algae	438	1890
	this study, 1990	periphytic algae	67	350

Notes:
[a] Presence of both autotrophic and heterotrophic organisms.
[b] Average hourly rate, assume 12 $h \cdot d^{-1}$, 365 $d \cdot yr^{-1}$.
[c] Average daily rate from 2 sampling sites, assume 365 $d \cdot yr^{-1}$.
[d] Control plots, assume 10 $hr \cdot day^{-1}$.
[e] Assumes 12 $h \cdot d^{-1}$, 200 $d \cdot yr^{-1}$.
References:
1) Carpenter et al. 1978. 2) Gotto et al. 1981. 3) Chapman and Hemond 1982.
4) Jones 1974. 5) Potts and Whitton 1977. 6) Larkum et al. 1988.
7) Capone et al. 1979. 8) Hanson 1977. 9) Lipschults et al. 1978.
10) Reuter et al. 1983. 11) Howard-Williams et al. 1989.

low during the high water phase (June–July) and nitrogen fixation rates were again higher. Data are not available for the drainage phase of 1990.

Plankton. Areal rates of nitrogen fixation by plankton were estimated by integration of the depth profiles of September 15, 1989 (Fig. 3; chlorophyll-a = 8 μg/l). The measured rates at each depth were assumed to represent an average for the 12 hours of daylight and to then continue at the measured dark rate at night. The areal plankton nitrogen fixation rate for this date ranged from 0.5–0.8 mmol N_2–$N \cdot m^{-2} \cdot d^{-1}$.

Although plankton nitrogen fixation was of limited duration, the high rates measured and the large area occupied by this community resulted in a substantial estimate of plankton nitrogen fixation. Assuming that 0.5 mmol N_2–$N \cdot m^{-2} \cdot d^{-1}$ was representative of the plankton nitrogen fixation rate for July–September 1989, and assuming an average area of 2.0 km^2 of open water on the floodplain of Lake Calado (Fisher & Moline 1992), the total nitrogen fixation in Lake Calado via plankton during these months was 90,000 mol

N_2–N, or 40% of the annual nitrogen fixation by periphyton associated with the floating meadows (Table 5). On an areal basis, the annual planktonic nitrogen fixation rate was about 45 mmol N_2–N·m^{-2}·y^{-1} (6.3 kg·ha^{-1}·y^{-1}). This rate falls in the lower portion of the range reported for eutrophic lakes of the world (Howarth et al. 1988a), and is about half of that estimated for the Orinoco floodplain (Hamilton & Lewis 1987), and Lake Valencia, Venezuela (Levine & Lewis 1985).

Periphyton of flooded forest. Annual nitrogen fixation by periphyton within the flooded forest is uncertain. Nitrogen fixation in this community was measured only once during the entire study period, and the areal extent of this community on the floodplain was not measured. However, for a preliminary estimate of the potential importance of nitrogen fixation in this community, the maximal areal extent of the flooded forest was estimated as 1.5 km^2, based in part on the bathymetric data of Lesack (1988) for the floodplain at Lake Calado. Because there is no flooded forest at low water, the average area during the duration of the flood phase is estimated as half the maximal area, or 0.75 km^2. Assuming the nitrogen fixation rate was 0.2 mmol N_2–N·m^2 flooded forest·d^{-1} (actual data range = 6–60 μmol N_2–N·m^2 leaf area·h^{-1}) for 9 months, the annual nitrogen input to the floodplain from the flooded forest periphyton was ~40,500 mols N_2–N. This is a probable upper limit for autotrophic nitrogen fixation because the samples were collected from high-light environments within the flooded forests and because it assumes the rates were constant during the entire 9-month flood phase. This estimate corresponds to 50% of the estimated inputs from the plankton and 20% of that estimated for the periphyton of the floating meadows and certainly warrants more detailed investigation as a potentially significant source of nitrogen to the floodplain.

Importance of nitrogen fixation on the Amazon floodplain at Lake Calado

In 1984–1985, nitrogen inputs to Lake Calado were dominated by surface runoff and river inflow (Lesack 1988). The measured nitrogen fixation rates by periphyton and plankton on the floodplain during 1989–1990 correspond to only about 8% of the 1984–1985 inputs. Even considering that the macrophytes, which serve as the major substratum for nitrogen fixing periphyton, were only 30–50% of areal coverage seen in previous years (personal observation) nitrogen fixation appears to be a small (5–10%) portion of the combined nitrogen inputs to the floodplain.

Biological nitrogen fixation measured in the periphyton and plankton is insufficient to balance the excess of losses over inputs to the floodplain at Lake Calado (Table 7). During 1984–1985 the combined measured inputs were 3.07×10^6 moles N y^{-1}, while the total losses were 4.80×10^6 moles N y^{-1} (Fisher et al. 1990). Nitrogen fixation of periphyton and plankton during 1989–1990 is estimated at 0.26×10^6 moles N y^{-1} and contributes only ~15%

towards balancing the measured nitrogen deficit on the floodplain. This finding underscores the conclusion of Howarth et al. (1988b) that nitrogen fixation is generally of minor importance in the nitrogen economy of "open" wetlands which receive large allochthonous nutrient inputs. Instead, the importance of nitrogen fixation seems to be that of a relatively slow biological correction to N:P imbalances in the ecosystem (Schindler 1977; Howarth et al. 1988b; Howarth & Marino 1990; Smith 1984).

Although nitrogen fixation does not appear to be a major component of the annual nitrogen budget, it may be important to the overall trophic structure of the floodplain. Newly fixed nitrogen at least periodically provides a substantial portion of the nitrogen requirements of the periphyton and plankton, the two principal microalgal communities on the floodplain. These microalgal communities serve as the base of the food chain for numerous commercially important fish species (Araujo-Lima 1986; Goulding 1980; Goulding & Carvalho 1982). Based on measurements of gross photosynthesis and C:N ratios for the periphyton community during the same study period of 1989 (Doyle 1991), nitrogen fixation contributed about 11% of the photosynthetic nitrogen requirements of the periphyton. Likewise, during the high-water and drainage phase of 1989 the observed planktonic nitrogen fixation rates (10–30 nmol N_2–$N \cdot l^{-1} \cdot h^{-1}$) corresponded to a 2–6 week turnover time of the plankton standing crop (~5 μmol $N \cdot l^{-1}$).

Nitrogen fixation rates appear to have changed significantly in the last 10 years. Melack & Fisher (1988) reported no detectable nitrogen fixation in the plankton during any time in 1980–1981 and the rates for periphyton are less than 1% of those reported here. Because the data base of Melack & Fisher is relatively small (n = 13) and because their samples had to be stored for months before chromatographic analysis, it is likely that those data underestimated the true rates. Nonetheless, the extremely large differences suggest that the rates have significantly increased. Since nitrogen fixation rates are strongly related to lake trophic status and N:P nutrient loading ratios (Howarth et al. 1988b, Howarth & Marino 1990), these data may indicate that a major change has occurred in the ecology of the floodplain at Lake Calado during the last 8–10 years.

The apparent changes in the nitrogen fixation rates may be related to the observed increase in deforestation in the drainage basin. In 1984–1985, much of the Calado watershed was still forested. During this study period (1989–1990) more than half of the forests had been cleared and burned to make room for shifting agriculture and cattle pasture (personal observation from aerial surveys).

Comparison of data from 1984–1985 (Lesack 1993) with data from 1989–1990 (Williams 1992) provides details of the effects of forest removal on the water chemistry of local watershed inputs to Lake Calado. In 1984–1985 the atomic N:P ratio of all inputs was 32:1 (Lesack 1993), twice the generally accepted composition ratio of microalgae, indicating a surplus of N relative to P in the inputs. In 1989–1990, following partial deforesta-

tion of the watershed of Mota Brook, the TN yield from the stream nearly doubled but the TP yield increased by a factor of 7 (Williams 1992). Since local watershed inputs are an important source of nitrogen and phosphorus to the lake (see Table 7), such dramatic changes in water chemistry of the inflowing streams following deforestation may well be altering the trophic status of the lake and the N:P ratios of the inputs may be substantially lower than in 1984–1985. Further studies of nitrogen fixation are clearly needed to resolve whether the high areal rates reported here are typical of the Amazonian floodplain in general, or only of areas which have recently been disturbed by deforestation.

Table 7. Importance of biological nitrogen fixation to the nitrogen budget of Lake Calado. The original data for N losses and inputs other than nitrogen fixation are from 1984–1985 (Lesack 1988) and are summarized by Fisher et al. 1990.

Nitrogen inputs to floodplain	(10^6 mols $N \cdot y^{-1}$)	Percent of N input
Rainfall	0.24	7.2
Surface runoff	1.29	38.7
Groundwater exchange	0.26	7.5
Inflow from adjacent lakes	0.25	7.5
Exchange with the Amazon River	1.03	30.9
Nitrogen fixation		
Periphyton of floating meadows (mean of 1989 and 1990)	0.13	3.9
plankton in open water	0.09	2.7
periphyton of flooded forest	0.04	1.2
Total reported N inputs	3.33	100.0
Total reported N losses	−4.80	144

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