Nitrogen fixation in a desert stream ecosystem

NANCY B. GRIMM* & KEVIN C. PETRONE
Department of Zoology, Box 871501 Arizona State University, Tempe, AZ 85287-1501;
*Correspondence: Department of Zoology, Box 871501, Arizona State University, Tempe, AZ 85287-1501. Phone: (602) 965-4735; Fax (602) 965-2519; e-mail: nbgrimm@asu.edu

Abstract. Few measurements of nitrogen fixation exist for streams. Desert streams are warm, well lighted, and often support abundant cyanobacterial populations; thus N_2 fixation may be significant in these N-poor ecosystems. N_2 fixation was measured in situ by acetylene reduction for two patch types (*Anabaena* mat and an epilithic assemblage). Patch-specific rates were high compared with published values (maximum 775 μg N_2 [83 μmol C_2H_4] mg chl a^{-1} h^{-1} or 51 mg N_2 [5.4 mmol C_2H_4] m^{-2} h^{-1}). Daytime fixation was higher than nighttime fixation, and temperature, light and inorganic N concentration explained 52% of variance in hourly rates over all dates. Diel input-output budgets were constructed on five dates when cyanobacteria were present in the stream. Diel N_2 fixation rates were measured for comparison with reach-scale diel nitrogen retention, to assess the importance of this vector to N economy of the stream. Fixation accounted for up to 85% of net N flux to the benthos, but its importance varied seasonally. Finally, we applied biomass-specific fixation rates to 1992 and 1993 biomass data to obtain seasonal and annual N_2 fixation estimates. Cyanobacteria were absent or rare during winter and spring, thus most of the annual N_2 fixation occurred during summer and autumn. Annual rates of nitrogen fixation for 1992 and 1993 (8.0 g/m^2 and 12.5 g/m^2) were very high compared to other streams, and moderately high compared to other ecosystems. Like other phenomena in this disturbance-prone ecosystem, nitrogen fixation is strongly influenced by the number and temporal distribution of flood events.

Introduction

Nitrogen fixation is a process of great importance to ecosystem nitrogen (N) balance, particularly where fixed forms of this element are in short supply. Under conditions conducive to growth of N_2-fixing organisms, inputs via this pathway may account for a significant fraction of the N entering and eventually cycling within ecosystems. Nitrogen fixation can, therefore, alleviate N limitation, leading Vitousek & Howarth (1991) to ask why N limitation should be prevalent in aquatic and terrestrial ecosystems. One possible answer is that N limitation and N_2 fixation are segregated in space and time as a consequence of ecosystem heterogeneity. Furthermore, constraints or controls on the process of N_2 fixation change with scale. We define constraints as factors whose variation at larger scales set limits to a process, and controls as factors whose variation at the scale of interest affect process rates. Improved knowl-

edge of the factors promoting N_2 fixation at several scales in a variety of N-limited ecosystems will contribute much to understanding of this important process.

Nitrogen is potentially the limiting nutrient in many freshwater ecosystems of western North America (Grimm & Fisher 1986a, 1986b; Elser et al. 1990). In streams of the desert Southwest in particular, supply of phosphorus (P) is ample because waters drain soils of recent volcanic origin that are rich in mineral P. Light is seldom limiting, as stream channels shaped by very large flash floods are wide relative to stream width at baseflow, and shading by riparian vegetation is minimal. Molar nitrogen to phosphorus ratio (i.e. inorganic nitrogen:soluble reactive phosphorus) in streamwater usually is <16, indicating potential N limitation (Grimm & Fisher 1986a). Bioenrichment experiments conducted in a typical southwestern stream, Sycamore Creek, Arizona, indicated that N is limiting to algal growth whereas P is neither a primary nor a secondary limiting factor (Grimm & Fisher 1986b). Availability of N is extremely variable both spatially and temporally. In Sycamore Creek, N is abundant immediately following floods that bring nitrate-laden water from the terrestrial system, but N concentration declines rapidly thereafter as floodwaters recede and succession occurs (Fisher et al. 1982; Grimm 1994). Under baseflow conditions, nitrogen supply is high at points of hyporheic or groundwater discharge (Valett et al. 1994) and concentration declines downstream from these points (Grimm et al. 1981; Grimm 1994).

Although desert streams are not unique in being N-limited, the combination of this characteristic with high light, temperature, and P, slow currents, and long, flood-free growing seasons contrasts with most streams in which N_2 fixation has been examined. These conditions are favorable to growth of heterocystous cyanobacteria (Carr & Whitton 1982; Bergmann & Welch 1990; Horne & Carmiggelt 1975), and indeed heterocystous cyanobacteria such as *Anabaena* sp. and *Calothrix* sp. are important late-successional components of the periphyton in Sycamore Creek (Fisher et al. 1982; Peterson & Grimm 1992). Horne & Carmiggelt (1975), who measured N_2 fixation in a California stream, suggested that few streams exhibit conditions suitable for high rates of N_2 fixation because most are either light- or P-limited. Here we report rates of nitrogen fixation for a stream that does exhibit such conditions.

Our objectives were to identify factors causing temporal and spatial variation in cyanobacterial abundance and N_2 fixation rates, and to determine the contribution of nitrogen fixation to the nitrogen budget of a desert stream. We measured N_2 fixation using the acetylene reduction method on a patch-specific basis, and calculated whole ecosystem rates from these measurements, based upon abundance of cyanobacteria. We examined variation (in fixation rates

and/or cyanobacterial abundance) at several temporal scales (hourly, diel, seasonal, and annual) over spatial scales ranging from the patch (periphyton assemblages on rocks, algal/cyanobacterial mats) to reach (100–300 m lengths of stream) and whole-stream (10-km mainstem section) scales. To determine the importance of N_2 fixation as an input of new N to the ecosystem, we calculated whole-system rates (reach scale) for comparison with input-output budgets for inorganic N, annual algal N demand, and published rates for other ecosystems. Based upon work by others and our knowledge of the physiology of cyanobacteria, we predicted the contribution of N_2 fixation would be especially pronounced during times (or at places) of low inorganic nitrogen availability, high light and temperature, and high cyanobacterial biomass.

Study site

This study was conducted in Sycamore Creek (Maricopa County, Arizona), a spatially intermittent stream located in the northern Sonoran Desert in central Arizona (33°45' N, 111°30' W). The stream drains a 505 km^2 catchment of steep terrain from mountain conifer forest to lowland desertscrub. Annual precipitation ranges from 510 mm at 1040 m elevation to 390 mm at 510 m elevation, and exhibits winter and late summer peaks. Intense, localized summer storms result in flash floods that obliterate stream biota and alter channel morphology. Winter storms occur over broader areas and may last longer, but can cause similar changes in biotic and geomorphic structure of the stream. Flood-free intervals may be long (≥ 3 months), thus high standing crops of both primary producers and consumers can develop during succession following spates.

The primary study reach in Sycamore Creek was a 120-m run at 700 m elevation. Baseline monitoring reaches, 3 and 8 km upstream from the study reach at 720 and 770 m elevation, were similar in most physicochemical respects to the N_2-fixation study reach. We used data on periphyton cover and biomass from these reaches to estimate annual reach-scale N_2 fixation (see Methods). Stream substrata in all three reaches were primarily fine gravels, with some larger boulders in the active channel beyond the wetted perimeter. Riparian vegetation, dominated by willow (*Salix goodingii*), ash (*Fraxinus arizonensis*), and sycamore (*Platanus wrighti*) was separated from the wetted stream by broad expanses (2–5 m) of unvegetated gravel bars, thus shading was minimal. During summer and fall low-flow periods, water depth ranged from 5 to 10 cm, discharge was from 0.03 to 0.08 m^3/s, and stream width was from 2 to 5 m; these values are typical of mid-elevation, perennial sections of Sycamore Creek. During spates, instantaneous discharge may increase

several orders of magnitude; the largest spate in 1992–1993, on 8 January 1993, exceeded 250 m^3/s (USGS 1995).

Methods

Nitrogen fixation methods

We used the acetylene reduction assay to estimate N_2 fixation in the field (Stewart et al. 1967; Hardy et al. 1968). Recirculating plexiglas chambers (volume = 3.8 L, test area = 220 cm^2) containing natural substrata were used for all measurements. Chambers were connected via hoses to submersible pumps that passed water over the test substrata at a velocity of 5–8 cm/s. Chambers were set on the stream bottom under water to maintain temperatures near ambient; however, the pumps did heat the water by 1–2.5 °C over ambient water temperatures during the incubation. Substrata and associated cyanobacterial/algal assemblages were placed in the test compartment, then acetylene-filled balloons were introduced into the mixing compartment and the chamber was sealed. Acetylene was generated by reaction of water and calcium carbide. A syringe needle was inserted through the sampling septum to pop the balloon, which simultaneously mixed the acetylene into the water and created a headspace. Headspace volume was controlled by filling balloons to a predetermined size; vapor phase volume averaged ≈300 mL (sufficient acetylene to saturate nitrogenase; Flett et al. 1976) compared with an average aqueous volume of ≈3,000 mL. Water temperature in each chamber was recorded at the beginning and end of the incubation, and all sediment and algal material was collected for determination of biomass (chlorophyll *a* and ash-free dry mass, AFDM) using methods described below.

Duplicate gas samples were collected into 3-mL evacuated tubes within ten minutes of sealing the chambers (initial) and again after 2 h of incubation (final) using a double-ended needle inserted through the sampling septum. Prior to sampling, chambers were gently shaken to homogenize the gas and ensure equilibrium partitioning between gaseous and aqueous phases. Pilot studies indicated that increase in ethylene concentration was linear for approximately four hours; we used a 2-hour incubation. We also checked for background ethylene production (cyanobacteria incubated without acetylene) and impurities in the acetylene (acetylene incubated without cyanobacteria) in pilot experiments; ethylene was never detected under these conditions. Ethylene was measured using a Varian 3300 gas chromatograph equipped with a flame ionization detector and Poropak R columns (oven temperature 80 °C) with a N_2 carrier gas flow rate of 80 mL/min.

Ethylene production was calculated as change in concentration in the chamber headspace times headspace volume divided by incubation time. Headspace concentration was adjusted for ethylene dissolved in the aqueous phase (Flett et al. 1976) by using the bunsen partitioning coefficient at the incubation temperature (mean of initial and final temperature). To convert ethylene production values to N_2 fixation, a molar ratio of ethylene produced to N_2 fixed of 3 was assumed. We did not perform ^{15}N calibrations, and use this conversion factor to maximize comparability with other studies (e.g., cited in Howarth et al. 1988a), although it is probably a reasonable estimate for benthic cyanobacterial mats (Howarth et al. 1988a). Nitrogen fixation was expressed both on an areal (divided by test area) and biomass-specific (divided by chlorophyll a) basis.

Patch-specific nitrogen fixation

We measured N_2 fixation on 18 dates in the following months: July, August, September, October, and November 1992 and April, June, July, and September 1993. Nitrogen fixation was measured for the following patch types: epilithic periphyton, often but not always dominated by *Calothrix* sp.; *Anabaena* sp. mats; *Nostoc* sp. colonies; mixed flocculent material, often containing significant quantities of mixed cyanobacteria; and *Cladophora glomerata* mats with mixed cyanobacteria. To obtain samples for incubations, we used a variety of methods. We placed unglazed ceramic tiles and mesh trays filled with clean gravel into the stream for various incubation periods (1–6 weeks) to permit colonization by different patch types. We filled chamber test compartments with small cobbles for the epilithic assemblage. We incubated individual *Nostoc* sp. colonies picked from rocks or gravel. Finally, we cored periphyton mats (especially *Anabaena* sp.) with a rectangular mesh tray and transported these samples to chambers using a rectangular plastic plate. In all cases, material was collected after incubations for determination of biomass and confirmation of species identity.

To determine how various environmental factors influenced patch-specific rates of N_2 fixation, we measured light (photosynthetically active radiation, or PAR), temperature, dissolved inorganic N (DIN) concentration, and soluble reactive P (SRP) concentration at the time of incubation. PAR was measured with a LiCor quantum sensor, temperature using a hand-held thermometer, and water samples for chemical analyses were collected from the stream in triplicate, acid-cleaned polyethylene bottles. Laboratory analyses, completed within 24 h on filtered (GF/F glass fiber filters, ≈ 0.7 μm pore size) samples, included the molybdate-antimony method for SRP (Murphy & Riley 1962), the phenol-hypochlorite method for ammonium (Solorzano 1969), and the cadmium-copper reduction method for nitrate (Wood et al. 1967).

Ammonium-N and nitrate-N concentrations were summed to obtain DIN concentration. We analyzed patch-specific N_2 fixation data using stepwise multiple regression. Inorganic nitrogen, SRP, light, and biomass-specific N_2 fixation were ln-transformed to achieve normal distributions. All statistical analyses were done with SYSTAT.

Diel fixation and nitrogen budgets

Inorganic N budgets were constructed for five 24-hour periods in summer and fall 1992–1993 when cyanobacteria were present in the stream. Study dates were 15–16 July, 15–16 September, 22–23 October, and 19–20 November 1992 and 23–24 September 1993. Triplicate water samples were collected approximately every 4 h at the top and bottom of the 120-m reach, and analyzed for SRP and nitrate- and ammonium-N as described previously. Dissolved oxygen was measured at the same times using a modified Winkler method, and water temperature and PAR were recorded frequently (at least every 4 h). We measured discharge once during midmorning of the first two 1992 and the 1993 sampling dates as the product of cross-sectional area and velocity. In October and November 1992, discharge was measured 4 times over the 24-h period using a short-term chloride injection (Stream Solute Workshop 1990). Total DIN import and export were calculated as the 24-h integral of the product of discharge and up- and downstream concentrations, respectively. Dissolved inorganic N retention was calculated as the difference between total DIN import and total DIN export divided by stream area.

Nitrogen fixation was measured for the two most abundant N-fixer-dominated assemblages in the stream at the time of each diel study (in most cases, *Anabaena* sp. mat and the *Calothrix*-dominated epilithic assemblage). Daily nitrogen fixation estimates for each assemblage were calculated from 2-4 incubations per date, including both day and night incubations.

On each study date except for 15–16 July and 22–23 September, we estimated relative cover of cyanobacteria and other algae on at least 10 (usually 20) transects. Relative cover was not measured on the remaining two dates, but a rough estimate of cyanobacterial and epilithic patch coverage was recorded based on visual inspection of the reach. A daily N_2 fixation rate was calculated for the reach by multiplying patch-specific daily estimates by relative cover, for comparison with DIN import, export, and retention.

Distribution and abundance of cyanobacteria

Distribution of cyanobacteria at two spatial scales was determined in a one-time survey. The smaller scale is the reach scale, defined by discrete, 100–300 m lengths of stream that can be characterized as runs, riffles, or pools based on

substratum and morphometry (Grimm & Fisher 1992). Runs are distinguished from riffles and pools by their finer, more uniform substrate and shallower depths, respectively. Percentage cover of 2 cyanobacterial patches (*Anabaena* and *Nostoc*) was recorded in June 1991 at 3 positions (top, middle, bottom) of 23 runs along 10 km of the stream. Three permanent springs occur along this transect, associated with decreases in valley width at the larger, "section" scale (ca. 2–4 km length; Grimm & Fisher 1992). Data were analyzed using analysis of covariance, with position as the factor and distance from perennial springs as the covariate, to test the hypothesis that cyanobacterial abundance is inversely related to nitrogen availability. At the reach scale (i.e., the 23 runs), we predicted higher per cent cover at nutrient-poor downwelling zones (bottom of runs) than at upwelling zones (top of runs) where N-rich water emerges from subsurface (hyporheic) flow (Valett et al. 1994). At the section scale (i.e., the 3 springs), we predicted increased per cent cover with distance from permanent springs, because such waters may be linked with deeper groundwater that carries high N concentrations.

Temporal changes in relative cover of cyanobacteria and other algal patches were measured during 1992 and 1993 in baseline monitoring runs at 720 and 770 m elevation. Width of each patch type along 5 fixed transects was recorded every two or three weeks, except after flash floods when sampling frequency was increased. Five samples of epilithic periphyton were collected by coring and biomass measured as chlorophyll *a* (methanol extraction; Tett et al. 1975) and AFDM (mass loss on ignition at 550 °C after 48 h drying at 60 °C). Biomass for other patch types, including cyanobacteria, was taken as the mean chlorophyll *a* and AFDM measured using the same methods between 1985 and 1994 on >50 samples of each patch type; thus temporal variation primarily reflects changes in relative cover. Mean values for the two reaches were used in all subsequent calculations of seasonal and annual N_2 fixation rates.

Estimation of seasonal pattern and annual rate of N_2 fixation

A continuous record of relative cover of different periphyton assemblages (including cyanobacteria) at baseline monitoring sites on Sycamore Creek, coupled with measurements of biomass-specific N_2 fixation for each assemblage at the study reach, permitted a preliminary estimate of whole-system N_2 fixation for a representative stream reach. This estimate will be sensitive to variation in cyanobacterial cover, thus we do not claim it is the actual rate of fixation for the specific study reach. Mean biomass-specific N_2 fixation rates of two predominant patch types (*Anabaena* sp. mat and *Calothrix*-dominated epilithic assemblage) were applied to abundance data from the monitoring sites for the years 1992 and 1993 to estimate whole-system N_2

Table 1. Results of stepwise multiple regression analysis relating biomass-specific ethylene production to environmental variables measured during incubations (all patch types and dates combined, $n = 158$). Units of independent variables: temperature – °C; PAR – mE cm^{-2} s^{-1}; DIN – μg/L. All variables except temperature were ln-transformed prior to analysis.

Variable	Partial correlation	Coefficient	Multiple r^2
Constant	–	−0.841	–
Temperature	0.686	0.153	0.470
ln (PAR)	0.493	0.365	0.498
ln (DIN)	−0.362	−0.015	0.518
ln (SRP)	n.s.[1]	–	–

[1] n.s. = Not significant.

fixation rates. Rates for the epilithic assemblages were assumed to be zero when cyanobacterial mats were absent from the system. Although this may result in an underestimate of total ecosystem N$_2$ fixation rates, relative abundance of fixers within the epilithic assemblage in prior years was low during winter and spring, especially following winter flooding, when cyanobacterial mats were absent (C.G. Peterson, Loyola University of Chicago, personal communication). Day and night rates were calculated separately from mean patch- and chlorophyll-specific rates, and then summed, accounting for day length changes.

Results

Patch-specific nitrogen fixation

At the scale of the algal patch, a wide range of N$_2$ fixation rates was observed, from 0 to 775 mg N$_2$ (0 to 83 μmol C$_2$H$_4$) mg chl a^{-1} h^{-1}, and from 0 to 51 mg N$_2$ (0 to >5,400 μmol C$_2$H$_4$) m^{-2} h^{-1} (assuming a C$_2$H$_4$ to N$_2$ ratio of 3). Nitrogenase activity was detectable at night but was much lower than during the day (Figure 1). At low light (<200 μE cm^{-2} s^{-1}), rates were not significantly different than those measured in darkness, and overall morning and midday rates were not significantly different. Biomass-specific N$_2$ fixation was positively correlated with temperature and light and negatively correlated with DIN (Figure 2). These three variables explained 52% of the variance in the 158 measurements (Table 1).

Major patch types that exhibited acetylene reduction activity were the epilithic assemblage on rock and gravel substrata (dominated by *Calothrix* sp. when cyanobacteria were abundant) and a cyanobacterial mat (mainly *Anabaena* sp.), although biomass-specific rates of up to 200 μg mg^{-1} h^{-1}
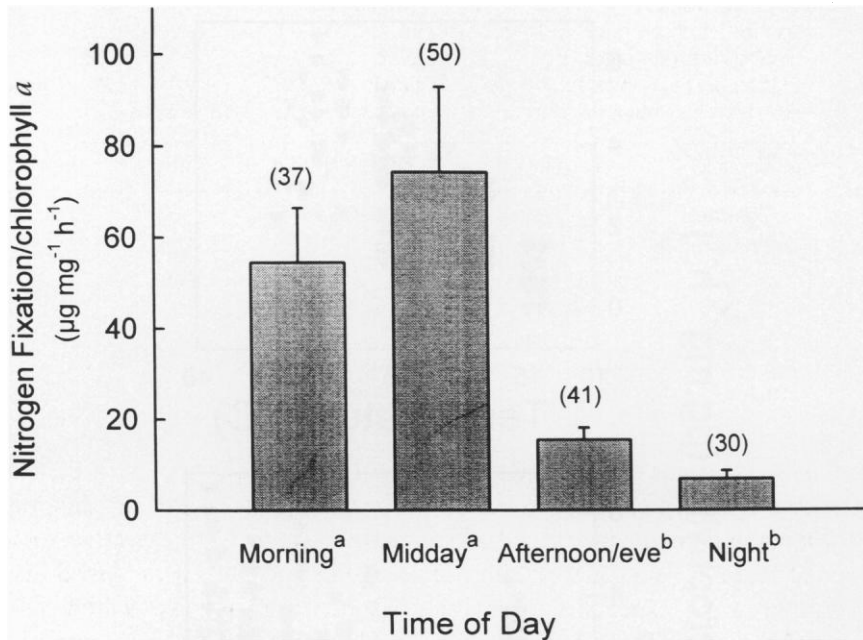
Figure 1. Variation in biomass-specific N_2 fixation rate with time of incubation (morning = 0700–1100; midday = 1100–1500; afternoon = 1500–1900; night = 1900–0600). Significant differences among means are denoted by different superscripts on x-axis labels.

Table 2. Limits (minimum-maximum) and mean (standard error in parentheses) hourly rates of biomass-specific, dark and light N_2 fixation for 2 patch types in Sycamore Creek, Arizona, 1992–1993.

Patch type	Dark		Light	
	Limits	Mean (se)	Limits	Mean (se)
		$(\mu g \ mg^{-1} \ h^{-1})$		
Epilithic	0–34	5.6 (1.0)	0–161	43.3 (6.6)
Cyanobacteria mat	1–77	21.0 (4.6)	2–775	85.6 (22.0)

were measured in mixed *Cladophora-Anabaena-Nostoc* mats. We compared daytime (PAR > 200 μE cm^{-2} s^{-1}) and nighttime (PAR < 200 μE cm^{-2} s^{-1}) N_2 fixation rates between the two assemblage types. Mean nighttime rates were higher in the mat compared with the epilithic assemblage (t-test on ln-transformed data, $p < 0.05$) but daytime rates were not significantly different ($p = 0.72$; Table 2).

Nitrogen fixation at the scale of the patch varied seasonally (Figure 3), assuming rates were very low or zero on winter and spring days when
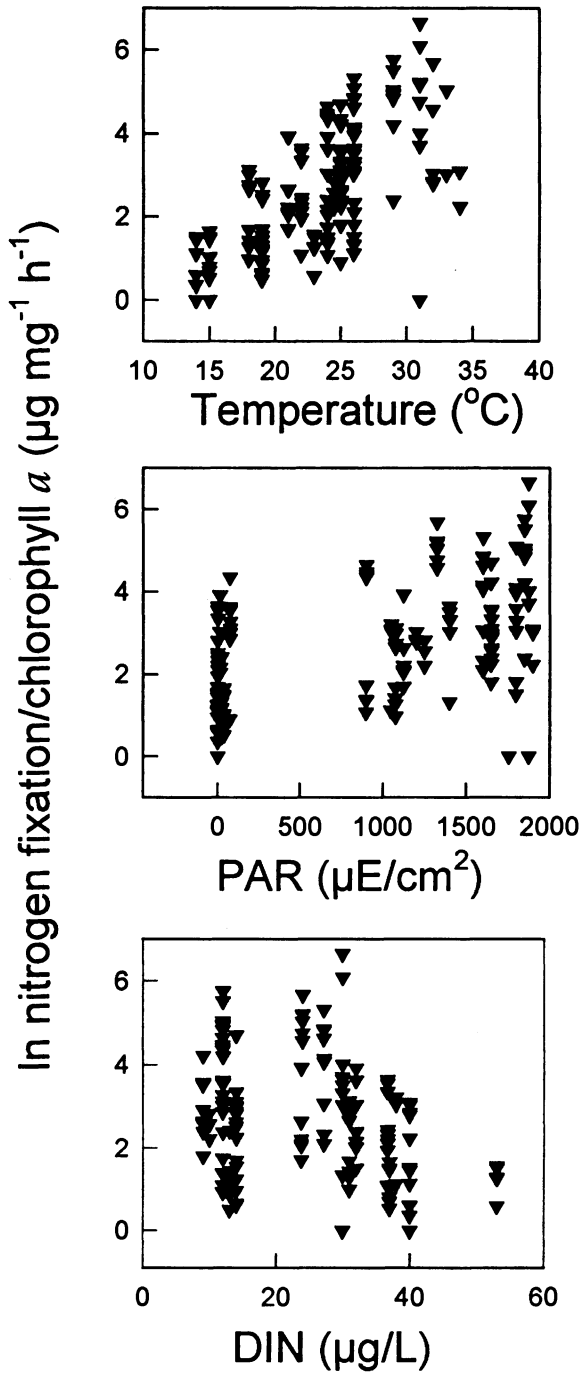
Figure 2. Relationship between natural log of biomass-specific N_2 fixation and temperature (upper), light (photosynthetically active radiation, PAR; middle), and dissolved inorganic nitrogen (DIN; lower) in Sycamore Creek, Arizona, 1992–1993 ($n = 158$).
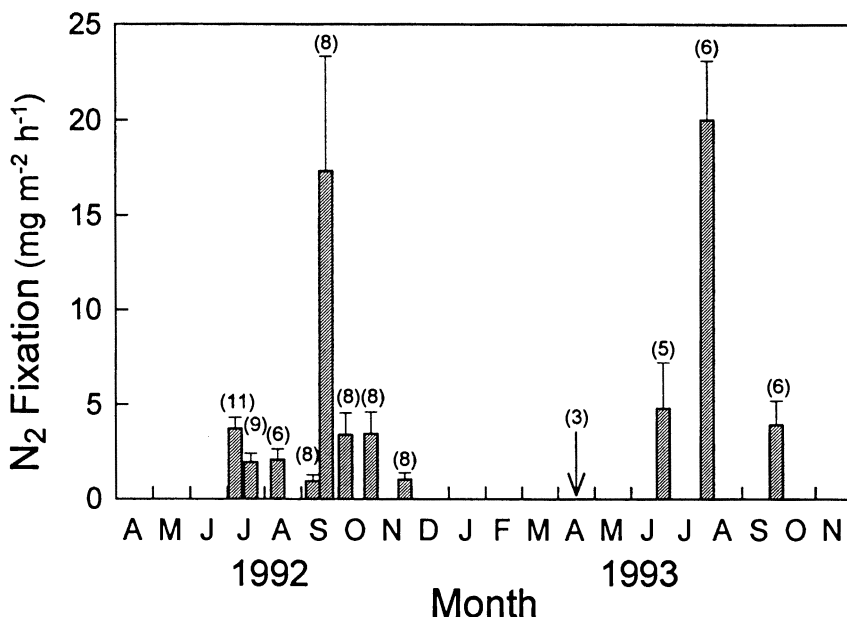
Figure 3. Hourly daytime rates of N_2 fixation for all sampling dates in Sycamore Creek, 1992–1993, all patch types combined ($n = 86$). Bars are means ± standard error; values in parentheses are sample sizes. Cases where measurements were made on two consecutive dates (1–2 Aug 1992, all diel dates) are combined in a single mean. Note arrow for April 1993 measurement, when no N_2 fixation was detected.

cyanobacterial mats were not present. Acetylene reduction assays were carried out on only one such date (midday on 30 April 1993); no nitrogenase activity was detected (arrow in Figure 3). In summer and autumn, mean hourly rates during daylight ranged from 0 to 261 μg N_2 mg chl a^{-1} and from 0 to >20 mg N_2 m^{-2}, and were highest in September 1992 and July 1993 (Figure 3). There was little variation in nighttime nitrogenase activity during the summer-autumn period.

Diel fixation and nitrogen budgets

Physical, chemical, and biologic properties of the stream reach varied among the five diel study dates (Table 3). Seasonal variation was reflected in declining maximum PAR and temperature from July to November 1992. Summer flash floods occurred in August of both 1992 and 1993, but the 1992 spate had a larger impact on periphyton. In 1993, minor floods were of insufficient magnitude to scour and transport algae.

Table 3. Physical, chemical, and biological features of the 120-m study reach, Sycamore Creek, Arizona, during five diel studies. NA = data not available.

Parameter	Diel study				
	15 July	15 September	22 October	19 November	23 September
Days since flood	106	23	70	98	31
Discharge (L/s)	41	30	27	63	88
Surface area (m^2)	444	460	460	486	588
Temperature (limits; °C)	23–28	23–30	17–23	14–19	19–26
PAR (maximum; μE cm^{-2} s^{-1})	2050	1800	900	1200	1650
Dissolved oxygen (limits; mg/L)	4–14	6–12	6–10	8–11	6–9
Ammonium-N (limits, μg/L)	7–25	5–25	6–19	0	0
Nitrate-N (limits, μg/L)	13–50	4–13	21–41	21–40	19–45
Soluble reactive P (limits, μg/L)	12–21	29–38	36–41	36–38	NA
DIN:SRP (atomic; mean)	5.0	1.5	2.5	1.9	NA
Per cent cover of: [1]					
Anabaena sp.	80	45	5	0	5
Epilithic assemblage	20	50	54	55	95
Filamentous Chlorophyta	–	5	0	5	–
Flocculent organic matter	–	0	41	40	–
%fixers in epilithic assemblage (mean of 3–4 counts)	29	57	9	18	NA
N_2 Fixation (limits, mg m^{-2} h^{-1})	0.11–4.9	0.14–51	0.10–7.3	0–2.7	0.08–18

[1] 15 July and 23 September values based on rough estimates of epilithic and cyanobacterial mats only, recorded at the site.

Table 4. Input-output budgets of inorganic N for a 120-m reach of Sycamore Creek, Arizona. Reach-scale N_2 fixation is included for comparison.

Date	Import	Export	Inputs to benthic zone	
			Retention (I-E)	N_2 fixation
			$(mg\ m^{-2}\ d^{-1})$	
July '92	421	237	184	35
Sept '92	149	107	42	227
Oct '92	249	196	53	48
Nov '92	174	134	40	8
Apr '93	–	–	–	0
Sept '93	444	399	45	38

Inorganic N concentration was very low in September 1992 and moderate on other dates (Table 3). Concentration exhibited strong diel variation and was usually higher at the top of the reach than at the bottom. Soluble reactive P varied less than DIN, and molar N:P was always low (<5), indicating potential N limitation. Oxygen varied dramatically through the day, suggesting high rates of metabolism. Waters were usually supersaturated with respect to dissolved oxygen in the afternoon (up to 210%).

Periphyton distribution was patchy, and patch structure varied among dates. *Anabaena* was very abundant in mid- to late-summer 1992, recovered quickly after the August spate, but then abruptly crashed in late September (Table 3). By the 22 October diel study date, 41% of the reach was covered by fine autochthonous detritus (flocculent organic matter in Table 3), in locations that had previously supported a thick cyanobacterial mat. Diazotrophs apparently also declined in the epilithic assemblage, from >57% of live cells in September to <10% 1 mo later (Table 3).

Diel rates of N_2 fixation for the 120-m stream reach varied among dates in magnitude and importance relative to other N inputs. Although we did not calculate DIN budgets in April 1993, we include this date for comparison with the diel budget studies because N_2 fixation was measured to be zero. Reach-scale N_2 fixation thus was from 0–227 mg m^{-2} d^{-1} on the six dates (Table 4). Mass balance (import-export) yielded a measure of net retention of hydrologic inorganic N inputs to the benthic community; i.e., the rate of inorganic N flux from the water to the benthic zone. On all diel study dates, this net retention was positive, varying from 40–184 mg N m^{-2} d^{-1} (Table 4). We evaluated the importance of N_2 fixation by expressing it as a percentage of all inputs to the benthic community (i.e., inputs = net retention + N_2 fixation; Figure 4). Nitrogen fixation accounted for up to 85% of these combined hydrologic and atmospheric net fluxes.

IMPORT

NFIX

EXPORT

TOTAL INPUTS
- Hydrologic flux to benthic zone:
  □ Retention=import-export
- Atmospheric flux
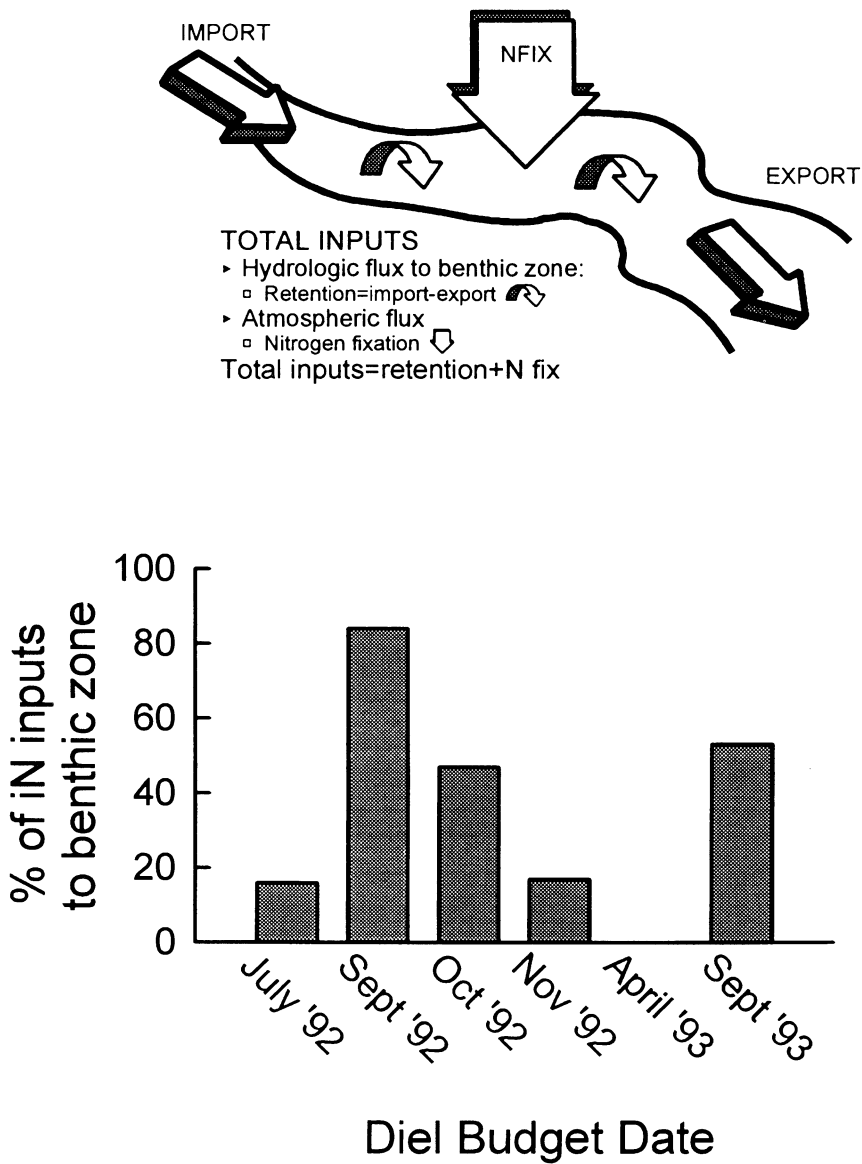 □ Nitrogen fixation
Total inputs=retention+N fix

Figure 4. Top: Nitrogen fixation represents an input of new nitrogen to the benthic zone. To determine the relative importance of this vector to the ecosystem in a manner that is independent of system size, rates are compared with hydrologic inputs to the benthic zone (difference between hydrological import and export = retention). Bottom: Nitrogen fixation expressed as a fraction of inputs to the benthic zone during 5 diel budget studies in Sycamore Creek, Arizona, 1992–1993. For comparison, April 1993 is included because nitrogen fixation was measured and found to be 0 on that date.

Table 5. Analysis of covariance for spatial variability in algal patch distribution in a 10-km stretch of Sycamore Creek, summer 1991. Dependent variables % cover of diatoms, *Nostoc* sp., *Anabaena* sp., and all other patch types were arcsin square root-transformed prior to analysis; vertical hydraulic gradient (VHG) was not transformed. Factors are horizontal and longitudinal position within individual runs; covariate is distance along stream from permanent springs (see Figure 5). Greater VHG indicates a greater tendency for water to move from the bed sediments to the water column. Significance levels indicated by asterisks (NS = not significant): * $p < 0.05$; *** $p < 0.001$.

Variable	Horizontal position (Edge = E or Center = C)	Logitudinal position	Distance along stream
% Diatoms	E > C*	NS	Decrease***
% *Nostoc*	NS	NS	Increase***
% *Anabaena*	NS	NS	Increase***
% All other	C > E*	NS	NS
VHG	NS	Top > bottom***	NS

Distribution and abundance of cyanobacteria

At the time of the spatial survey of cyanobacterial distribution (June 1991), *Anabaena* sp. and *Nostoc* sp. were the most common cyanobacteria. Neither patch type was significantly more abundant at the bottoms compared with the tops of runs, although the former sites exhibited significantly lower vertical hydraulic gradients, indicating downwelling (Table 5). At the section scale (2–4 km), however, there was a significant effect of the covariate, distance from springs, with lower cyanobacterial abundance immediately downstream from springs (Table 5, Figure 5).

The two years for which we examined temporal changes in cyanobacterial abundance relative to other periphyton patch types differed in the timing, frequency, and magnitude of spates. Moderate floods (10–50 m^3/s peak discharge) characterized winter-spring 1992, whereas a series of very large flash floods (three exceeding 100 m^3/s maximum discharge) occurred between 8 January and 29 March 1993. Discharge remained high through the spring of 1993, DIN flux was initially higher, and chlorophytes were more abundant than in 1992 (Figure 6). Cyanobacteria peaked at the same time in summer, but were not as abundant in 1993 as in 1992. Although summer storms produced flash floods in both years, two events in 1993 (27 August and 13 September) were relatively mild (<1 m^3/s peak discharge) and did not result in complete removal of periphyton, nor was there a measurable change in inorganic N flux (Figure 6). A pronounced peak in filamentous chlorophytes (primarily *Cladophora glomerata*) after the large (46 m^3/s peak discharge) flash flood of 23 August, 1992, was relatively muted in 1993.
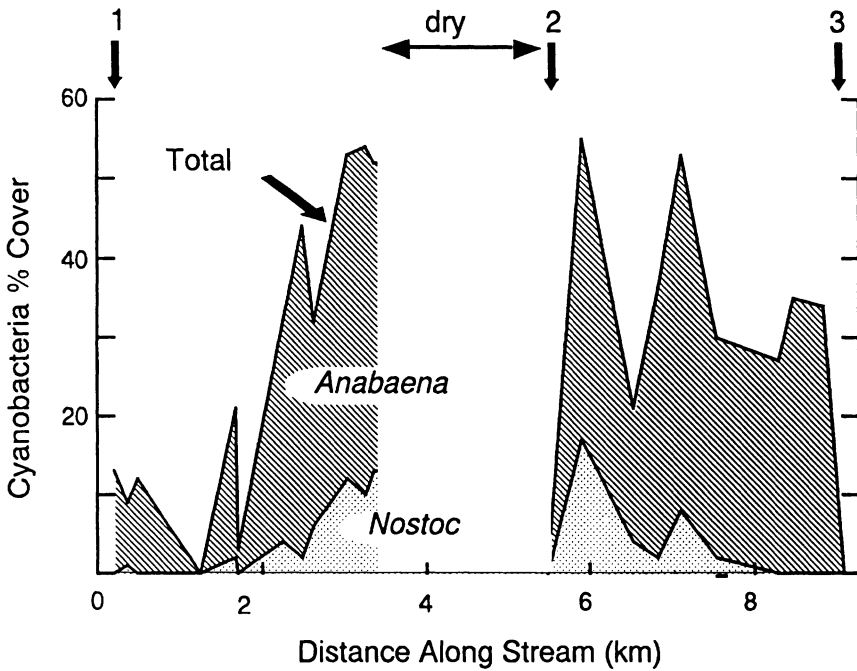
Figure 5. Abundance (% cover) of cyanobacteria patches (*Anabaena* sp. mats and *Nostoc* sp.) along 10 km of Sycamore Creek, Arizona, in June 1991. Arrows denote points where perennial springs (1–3) arise in the stream channel. No data were recorded for the dry segment between 3.3 and 5.3 km. The study reach is located at approximately 9.2 km and the two monitoring reaches at 0.9 km and 6.3 km along this transect.

Seasonal pattern and annual rate of N_2 fixation

Mean hourly rates of N_2 fixation for each patch type (Table 2) were applied to mean biomass (chlorophyll *a*) data and per cent cover for the baseline monitoring reaches on each sampling date in 1992–1993 (n = 45 dates). Using a simple formula for day length: 8 h daylight in winter (December–February), 12 h daylight in summer (June–August), and 10 h daylight in spring and autumn, we calculated daily N_2 fixation rates for each sampling date. These values, plotted against time (Figure 7), were integrated to generate annual estimates. Nitrogen fixation was restricted to summer and autumn, and decreased markedly after flash floods. Estimated annual N_2 fixation rates for 1992 and 1993 were 8.0 and 12.5 g/m^2, respectively.
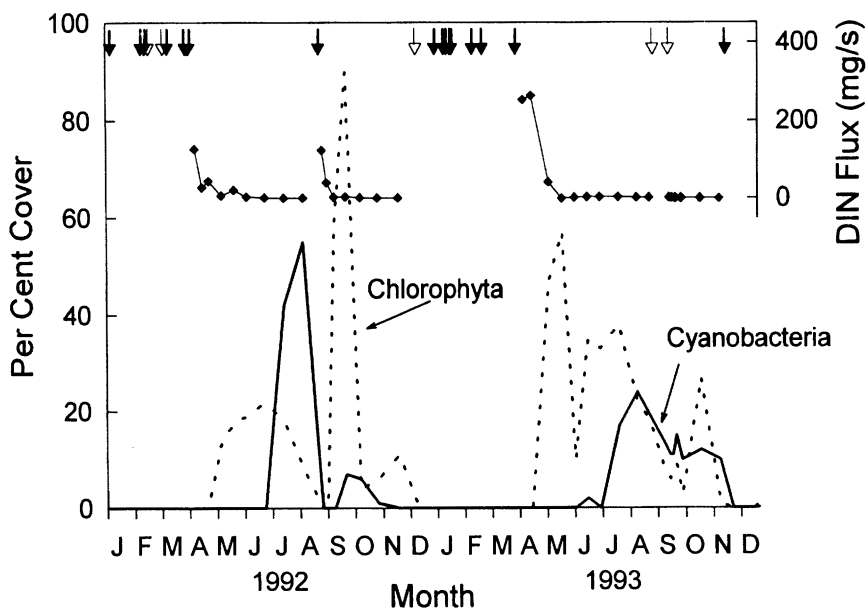
Figure 6. Temporal changes in DIN flux (DIN concentration × discharge), a measure of availability of DIN (♦), and the major macroalgal patches Chlorophyta (dotted line), and Cyanobacteria (solid line) during 1992 and 1993 in Sycamore Creek, Arizona. Vertical arrows denote key flash flood events; filled arrows indicate floods >10 m^3/s peak discharge. Peak discharge values (m^3/s) for winter-spring 1992 were 14, 68, 43, 3, 3, 49, 15, and 18; for summer 1992 – 46; for winter-spring 1993 – 0.73, 24, 294, 189, 74, 157, 70, 74, and 24; and for summer-fall 1993 – 0.3, 0.94, and 30.

Discussion

On a patch-specific basis, nitrogen fixation rates in Sycamore Creek are among the highest reported in the literature, rivaled only by marine cyanobacterial mats (Table 6). The ratio of ethylene production to N_2 fixation that we used (C_2H_4:N_2 = 3) is reasonable for benthic cyanobacteria (Howarth et al. 1988a), but even if the ratio varies by a factor of 2 (reported ratios for benthic cyanobacteria range from ~ 2 to ~ 6), nitrogen fixation in *Anabaena* mats and in epilithic communities dominated by *Calothrix* is still very high. Few measurements have been made for freshwater periphyton communities; rates reported here are 3–10 times higher than the two (river and lake) previous reports.

Despite these high rates, N_2 fixation varied dramatically, even at the scale of the patch. Some of this variation is related to seasonal and diel changes in temperature, light, and availability of inorganic N. Within days, seasons, and years, N_2 fixation rates and abundance of cyanobacteria were greatest when

Table 6. Daily rates of patch-specific N_2 fixation for periphyton and cyanobacterial mats in various aquatic ecosystems characterized by patchy distributions of N_2 fixers. Rates are per unit patch area, and thus are not scalable to whole ecosystem rates. Conversions from ethylene production values are made assuming 3 moles of ethylene are produced for each mole N_2 fixed.

| Patch type – ecosystem (location)	N_2 fixation $(\text{mg } N_2 \text{ m}^{-2} \text{ d}^{-1})$	Reference	Notes
Cyanobacterial mat – Salt marsh (MA, USA)	7.1	Van Raalte et al. 1974	Hourly rate × 12
Nostoc – Seasonal river (CA, USA)	11	Horne & Carmiggelt 1975	Maximum rate
Lyngbya mat – Coastal (Gt. Britain)	13	Jones 1992	(Mean daytime rate × 12) + (82% of mean daytime rate × 12)
Periphyton – Arctic lake (NWT, Canada)	14	Bergmann & Welch 1990	Mean hourly rate × 20 (summer, max rate)
Cyanobacterial mat – Coastal (NC, USA)	20	Bebout et al. 1987	Mean of diel hourly rates × 24
Cyanobacterial mat – Coastal arctic (NWT, Canada)	21	Chapin et al. 1991	Hourly rate × 20 (summer)
Cyanobacterial mat – Salt marsh (MA, USA)	12–24	Carpenter et al. 1978	Hourly rate × 12; summer
Epilithic assemblage – Desert stream (AZ, USA)	42	This study	Mean of mean day and night rates × 24
Cyanobacterial mat – Coastal (CA, USA)	6–79	Joye & Paerl 1994	Seasonal range
Mixed cyanobacteria – Desert stream (AZ, USA)	83	This study	Mean of mean day and night rates × 24
Calothrix mat, Coastal (Gt. Britain)	120	Jones 1992	(Mean daytime rate × 12) + (18% of mean daytime rate × 12)
Anabaena – Desert stream (AZ, USA)	144	This study	Mean of mean day and night rates × 24
Calothrix, Tropical coral reef (Marshall Is.)	191	Wiebe et al. 1975	Mean of mean day and night rates × 24
Cyanobacterial mat – Tropical coral reef (HI, USA)	208	Hanson & Gunderson 1977 (cited in Howarth et al. 1988a)	Polluted
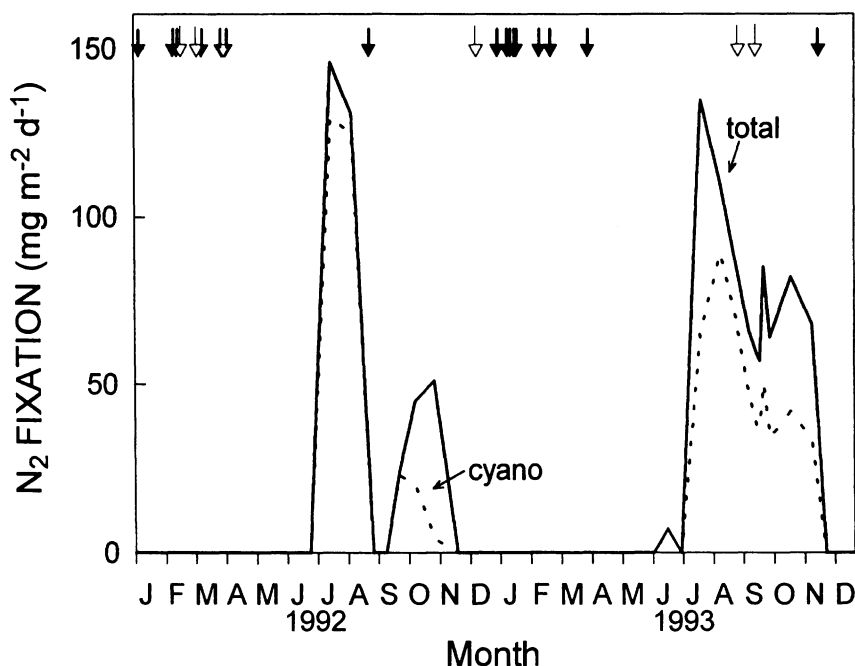
Figure 7. Estimated seasonal pattern of N_2 fixation in Sycamore Creek, Arizona, during 1992 and 1993. Dotted line indicates N_2 fixation rate for the cyanobacterial mat (*Anabaena*); remainder of total is attributable to the epilithic assemblage. Vertical arrows as in Figure 6.

light and temperature were high and inorganic nitrogen concentration, and molar N:P, were low. This conforms with findings from many other aquatic environments in which cyanobacteria are the major N_2 fixers (reviewed in Howarth et al. 1988a, 1988b). It is difficult, however, to discern which factor exerted the strongest influence since light, temperature, and DIN (inversely) tended to covary. For example, the diel temperature range was as great as 10–12°C, and high temperature corresponded with high light and low inorganic N concentration. Seasonally, highest rates were measured in late summer/early autumn when light was high and inorganic N concentration low. However, such conditions can also be found in early summer when cyanobacteria are rare, so these factors are not sufficient to explain the low springtime rates. Late summer/early fall does tend to have higher maximum daytime water temperature (to 32–34 °C) because higher humidity associated with the summer monsoon reduces evaporative cooling.

Light obviously is essential for high rates of N_2 fixation, owing to the high energy requirement to generate necessary reducing power. The major diazotrophs of Sycamore Creek are heterocystous cyanobacteria, thus we did not expect, nor did we find, higher rates of fixation at night. This diel pattern

has been reported for many marine mats dominated by *Microcoleus, Lyngbya*, and other non-heterocystous taxa (Jones 1992; Bebout et al. 1987, 1993) that must shield nitrogenase from oxygen produced during photosynthesis. Horne (1975), on the other hand, reported an unusual diel pattern for *Nostoc* in a small California stream, consisting of a midday peak followed by a late afternoon depression, and then an increase through the night. He attributed the afternoon reduction in rates to competition with photorespiration for reductant. Such a pattern was not evident in Sycamore Creek. Although there was usually some dark fixation, rates were always lower than daytime rates.

Inorganic nitrogen concentration is often low in Sycamore Creek, but it also is highly variable. Flash floods usually result in a pulse of high DIN (Grimm & Fisher 1992), which may explain why cyanobacteria appear much later in succession. Patch-specific N_2 fixation was inversely correlated with DIN (Table 1), but addition of this variable increased r^2 of the multiple regression only slightly, and no clear pattern of DIN concentration explained seasonal variation in rates. Among diel budgets, however, fixation was the major source of nitrogen to the stream ecosystem in September 1992, when other N inputs (due to a combination of low concentration and low flow) were minor. Because DIN concentration varies so much both temporally and spatially, a complete understanding of the importance of this factor to cyanobacterial abundance and N_2 fixation would benefit from an experimental approach.

Much study has been devoted to the idea that cyanobacterial blooms are caused by low N:P loading (Flett et al. 1980; Howarth et al. 1988b; Smith 1990; Hendzel et al. 1994) since it was originally proposed by Schindler (1977). In Sycamore Creek, molar N:P is nearly always very low (<5), and SRP concentration rarely drops below 20 μg/L. SRP concentration also is much less variable than that of nitrate (coefficients of variation over 18 y 106% and 237%, respectively; unpublished data), suggesting that dynamics of this nutrient are controlled by physicochemical processes rather than biotic uptake (Grimm & Fisher 1986a). Thus, it is unlikely that variations in P availability have any effect on fixers or fixation rates. Nitrogen fixation may be limited by the availability of micronutrients during some times of year (Howarth et al. 1988b; Marino et al. 1990); N_2-fixers may require up to 10 times the Fe used by non-fixers (Reuter & Petersen 1987). The role of Fe and other micronutrients in controlling abundance and activity of fixers in Sycamore Creek is unknown, but worthy of investigation.

Additional unexplained variation in patch-specific N_2 fixation may also be due to changes in community structure; i.e., seasonal or successional changes in the abundance of fixers within patches. For example, the large decrease in patch-specific fixation rate between September and October 1992 was

mirrored by a large reduction in fixer biovolume in the epilithic community (Table 2). This observation suggests that phenomena operating at the microscale of a single rock community, such as competition between cyanobacteria and other epilithic taxa (primarily diatoms), influence a larger-scale collective property (patch-specific N_2 fixation). Further, epilithic communities of Sycamore Creek, although often dominated by *Calothrix*, contain other fixers such as *Schizothrix, Amphithrix, Rivularia, Nostoc,* and, notably, epithemiacean diatoms that contain endosymbiotic fixers (*Epithemia sorex, E. turgida, Rhopalodia gibba, R. gibberula*). Even in the absence of competition, variable patch-specific fixation rates may result from physiological differences among taxa within the periphyton community. N_2-fixing taxa undoubtedly differ in their abilities to use dinitrogen, just as there are well-documented interspecific differences in abilities of phytoplankton species to acquire nutrients in fixed form (Tilman et al. 1982; Tilman et al. 1986). Physiological differences in N_2 fixation efficiencies and interspecific competition between fixers and non-fixers are topics deserving attention, and may help to explain spatial and temporal variation in N_2 fixation at the patch-specific scale.

Spatial and temporal distribution of cyanobacteria

The spatial distribution of cyanobacteria was predicted by spatial variation in DIN availability only at certain scales. In our survey of algal distribution, we predicted lower abundance of nitrogen fixers at the tops of runs, where N-enriched water emerges from the hyporheic zone. This prediction was not supported by data collected at the reach scale, rather, we found that variation in cyanobacterial abundance was only predictable at a larger scale (Figure 5, Table 5). Springs are points where deep groundwater discharges into the surface stream, and are restricted to areas that are kilometers apart (i.e., large scale). Springs may represent a more consistent N input than do upwelling zones at the tops of runs (occurring at a medium scale). Furthermore, the expected medium-scale pattern may be masked by finer-scale growth responses of algae to localized nutrient inputs. A survey of algal distribution within a single reach conducted a few weeks after the more extensive survey of June 1991 revealed that, while the reach was dominated by cyanobacteria, local points of emergence of subsurface water (from within gravel bars) had higher nitrate-N concentrations and supported luxuriant growth of filamentous green algae.

Temporal distribution of cyanobacteria reflected seasonal and successional variation in environmental factors. Cyanobacteria were most abundant in late summer and autumn, but were strongly affected by summer flash floods. During this season, light and temperature were high and DIN concentra-

tion and discharge were low; these are conditions that favor cyanobacterial growth. Among diel study dates, abundance of *Anabaena* and percentage of fixers in the epilithic assemblage were highest under this combination of conditions. Duncan & Blinn (1989) reported seasonal peaks in cyanobacterial abundance in an Arizonan canyon stream associated with maximum light and temperature, but did not find any correlations with chemical variables. In lakes, blooms of cyanobacteria often are associated with summertime high temperature and light, but only under conditions of increased P loading (Flett et al. 1980; Hendzel et al. 1994).

The answer to the question, "what limits N_2 fixers?", depends on scale. Among years, the distribution of flash floods appears to dictate temporal patterns of chlorophyte vs. cyanobacterial abundance. Within years, seasonal variation suggests that a combination of factors, rather than their values, is critical (i.e., low DIN and high light are insufficient without high temperature). Within-season (i.e., successional scale) variation is often characterized by spectacular accumulations followed by abrupt crashes of cyanobacteria (especially *Anabaena*). An intriguing possibility is that, as mats develop and become very thick, trichomes in deeper layers become isolated from flowing water. They may then experience micronutrient or P limitation or lowered dissolved oxygen concentration. If hypoxia or anoxia develops within mats, these can become ideal sites for denitrification to occur (e.g., Joye & Paerl 1994), resulting in loss of N_2 (or N_2O). Preliminary measurements indicate considerable denitrifying enzyme activity within algal and cyanobacterial mats in Sycamore Creek (unpublished data). Local anoxia in mats can make the assemblage more susceptible to consumption or export due to sloughing of dead cells. Finally, at the diel time scale, localized conditions within mats also are critical to competitive outcomes among taxa. Large diel changes in physicochemical factors, notably oxygen, may bring about brief, unfavorable episodes both for fixers and non-fixers.

Role of nitrogen fixation in desert stream ecosystems

The contribution of nitrogen fixation to this desert stream ecosystem varied seasonally in concert with variation in physicochemical factors and abundance of N_2 fixers. As a percentage of total DIN inputs (import + N_2 fixation), N_2 fixation ranged from 0 to 61% (calculated from data in Table 4). This measure, however, is sensitive to the size of the reach studied because increasing reach length increases the surface area available for benthic processes (such as N_2 fixation) while import remains constant (see discussion in Cummins et al. 1983). For this reason this measure can only be used for comparison among dates given a constant reach length. Instead, we calculated N_2 fixation as a percentage of inputs to the benthic zone (retention + N_2 fixation; Figure 4).

By this measure, the contribution of N_2 fixation ranged from 0 to 85% of inputs to the benthic zone, thus the Sycamore Creek ecosystem can be highly dependent upon N_2 fixation as a source of N. Parallel measures of the N_2 fixation contribution to the N budget in marine and fresh waters are as high as our estimate only in Pyramid Lake, Nevada, and a few eutrophic lakes (Table 2 in Howarth et al. 1988a).

The role of N_2 fixation is seasonally restricted in Sycamore Creek, with extended periods when N_2 fixation is negligible. Because annual element budgets are dominated by import and export during flash floods (see Fisher et al. 1982), we are unable to assess directly the contribution of N_2 fixation on an annual basis. Indeed, year-to-year variation is likely to be high because of variable disturbance regimes (Grimm & Fisher 1989).

Our preliminary estimates of annual N_2 fixation for 1992 and 1993 put this ecosystem among those with the highest reported annual rates of N_2 fixation (Figure 8). Annual N_2 fixation of 8.0 and 12.5 g/m^2 is exceeded by reported rates for a coral reef, tropical forest, and alder woodland, and is close to rates in eutrophic lakes and rice fields. All of the N_2 fixation occurs during summer and fall months, whereas N demand by autotrophs can be high in late spring and early summer as well. To estimate the potential contribution of N_2 fixation to annual N demand, we used published daily rates of gross primary production (P_G) and assumed a photosynthetic quotient of 1 and a C:N of 6.625 (Redfield 1958). Daily P_G in 1981 ranged from 2.74–12.25 g O_2 m^{-2} (Grimm 1987), suggesting an annual N demand of 66–295 g m^{-2} (stoichiometric conversion $\times$ 365 d/y); thus N_2 fixation can supply as little as 2.7% and as much as 19% of N demand by autotrophs on an annual basis.

Clarifying the importance of specific processes to whole ecosystem function depends on spatial and temporal scale (O'Neill et al. 1986; Levin 1992; Pickett et al. 1994). In this study, our approach has been to examine N_2 fixation at the patch scale and whole reach scale over annual, seasonal, and diel time periods (Table 7). Among years, the processes that influence temporal distribution of cyanobacteria (primarily disturbance regime and seasonal changes in physicochemical factors), coupled with dynamics of N_2 fixation on a patch-specific basis, interact to yield year-to-year variation in the importance of N_2 fixation to the annual N economy of the ecosystem. Within years, the absence of N_2-fixing organisms at certain times of year means that the importance of atmospheric inputs (via N_2 fixation) is negligible at those times. During summer-fall successional sequences, changes in cyanobacterial patches (and hence, N_2 fixation) are not simply controlled by seasonal changes in large-scale physicochemical variables, but may be sensitive to phenological changes in internal dynamics of the mat. And finally, within assemblages dominated by N_2 fixers, rates of N_2 fixation are controlled by a suite of physicochemical
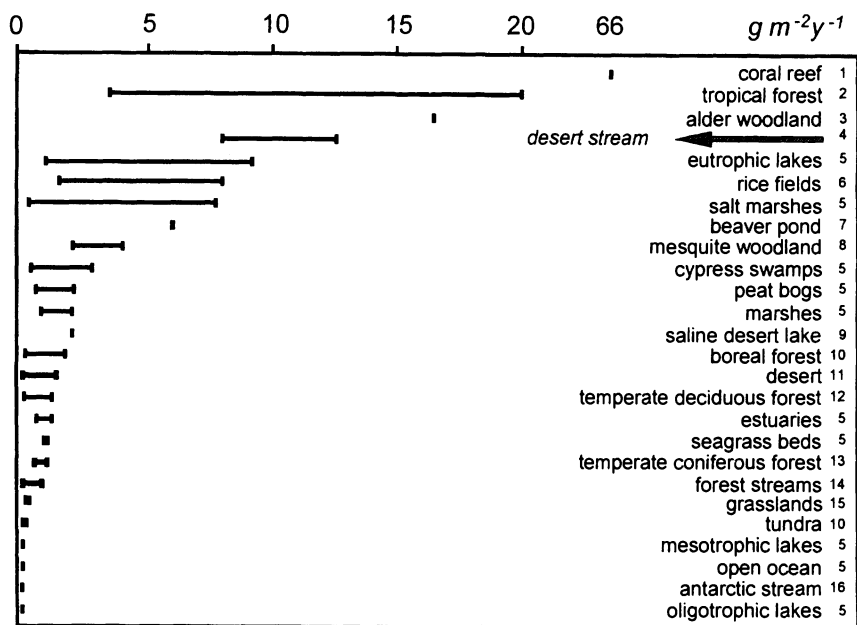
Figure 8. Reported range of whole-ecosystem, annual N_2 fixation rates in a variety of ecosystems. Key to references (footnote numbers): 1 – Wiebe et al. 1975; 2 – Herrera & Jordan 1981; 3 – Klingensmith & Van Cleve 1993; 4 – this study; 5 – Howarth et al. 1988a; 6 – Bisoyi & Singh 1988 (values are per growing season); 7 – Naiman & Melillo 1984; 8 – Jarrell & Virginia 1990; Diagne & Baker 1984 (estimate is for average plantation density); 9 – Horne & Galat 1985; 10 – Van Cleve & Alexander 1981; 11 – Rychert et al. 1978; 12 – Melillo 1981; 13 – Gosz 1981; 14 – Triska et al. 1984 (0.06–0.76), Naiman & Melillo 1984 (<0.1), Leland & Carter 1985 (control; hourly rate × 12 h × 200 d = <0.001); 15 – Horne & Carmiggelt 1975; 16 – Woodmansee et al. 1981; 17 – Horne 1972.

factors. The process can dominate diel N balance when fixers are abundant in the ecosystem.

Patterns of N_2 fixation, as well as controls and constraints on those patterns, thus show a high degree of temporal variability and scale dependence. But whatever the pattern, results from this study enforce the view that streams are highly open ecosystems with respect to nitrogen. In considering this defining characteristic of streams, emphasis has been placed on their large and variable hydrologic fluxes (e.g., Fisher & Likens 1973; Cummins et al. 1983; Triska et al. 1984; for a treatment of streams as a "case study" in N budgets, see Sprent 1987). In comparison, area-specific processes and fluxes (e.g., photosynthesis) often can seem dwarfed by this large throughput. In the case of desert streams, hydrologic flux on an annual basis is extreme, since >99% of the water output occurs during <<1% of the time. Across multiple events, storage of N in the surface stream system probably does not change

Table 7. Patterns, controls (factors influencing rate), and constraints (variation at larger scales that sets limits on rate) on nitrogen fixation at three temporal scales in a desert stream ecosystem. Controls at any given scale also include those listed for lower scales.

Time scale	Pattern	Controls	Constraints
Diel	Daytime peak	Light, temperature, DIN	Cyanobacterial biomass at patch scale
Successional (i.e., between floods)/seasonal	Increase with time since flood; restricted to autumn	Cyanobacterial biomass at reach scale; internal mat dynamics at patch scale	Seasonal variation in temperature, DIN
Annual	Higher if summer floods absent or minor	Seasonal distribution of cyanobacteria	Flood regime

(although it may increase in the riparian zone), as all of the N accumulated between events ultimately finds its way to downstream systems (reservoirs, large rivers, and groundwater). Between events there is very little fixed N input from the surrounding terrestrial environment; in fact, exchange with the atmosphere via N_2 fixation at times is the primary source of fixed N. The extent to which desert streams are sources of N in the context of the aridland landscape, by virtue of their high N_2 fixation rates, will depend on the balance of gaseous inputs and outputs, i.e., nitrogen fixation and denitrification. In this sense, budgets for any stream that are based only on hydrologic inputs and outputs may be misleading. Therefore, lotic ecologists should consider the potential importance of gaseous fluxes to the nitrogen budgets of these open ecosystems.

Acknowledgements

References

Bebout BM, Fitzpatrick MW & Paerl HW (1993) Identification of the sources of energy for nitrogen fixation and physiological characterization of nitrogen-fixing members of a marine microbial mat community. Appl. Environ. Microbiol. 59: 1495–1503

Bebout BM, Paerl HW, Crocker KM & Prufert LE (1987) Diel interactions of oxygenic photosynthesis and N_2 fixation (acetylene reduction) in a marine microbial mat community. Appl. Environ. Microbiol. 53: 2353–2362

Bergmann MA & Welch HE (1990) Nitrogen fixation by epilithic periphyton in small arctic lakes in response to experimental nitrogen and phosphorus fertilization. Can. J. Fish. Aquat. Sci. 47: 1545–1550

Bisoyi RN & Singh PK (1988) Effect of seasonal changes on cyanobacterial production and nitrogen-yield. Microb. Ecol. 16: 149–154

Carpenter EJ, Van Raalte CD & Valiela I (1978) Nitrogen fixation by algae in a Massachusetts salt marsh. Limnol. Oceanogr. 23: 318–327

Carr NB & Whitton BA (1982) The Biology of Cyanobacteria. Blackwell Scientific, Oxford

Chapin DM, Bliss LC & Bledsoe LJ (1991) Environmental regulation of nitrogen fixation in a high arctic lowland ecosystem. Can. J. Bot. 69: 2744–2755

Cummins KW, Sedell JR, Swanson FJ, Minshall GW, Fisher SG, Cushing CE, Peterson RC & Vannote RL (1983) Organic matter budgets for stream ecosystems: Problems in their evaluation. In: Barnes JR & Minshall GW (Eds) Stream Ecology: Application and Testing of General Ecological Theory (pp 299–353). Plenum Press, New York

Diagne O & Baker DD (1994) Quantification of symbiotic N fixation by *Prosopis juliflora* (Swartz) D.C using ^{15}N-isotope dilution methodology. Soil. Biol. Biochem. 26: 1709–1710

Duncan SW & Blinn DW (1989) Importance of physical variables on the seasonal dynamics of epilithic algae in a highly shaded canyon stream. J. Phycol. 25: 455–461

Elser JJ, Marzolf ER & Goldman CR (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. Can. J. Fish. Aquat. Sci. 47: 1468–1477

Fisher SG, Gray LJ, Grimm NB & Busch DE (1982) Temporal succession in a desert stream ecosystem following flash flooding. Ecol. Monogr. 52: 93–110

Fisher SG & Likens GE (1973) Energy flow in Bear Brook, New Hampshire: An integrative approach to stream ecosystem metabolism. Ecol. Monogr. 43: 421–439

Flett RJ, Hamilton RD & Campbell NER (1976) Aquatic acetylene-reduction techniques: Solutions to several problems. Can. J. Microbiol. 22: 43–51

Flett RJ, Schindler DW, Hamilton RD & Campbell NER (1980) Nitrogen fixation in Canadian Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 37: 494–505

Gosz JR (1981) Nitrogen cycling in coniferous forest ecosystems. In: Rosswall T & Clark FE (Eds) Terrestrial Nitrogen Cycles 33 (pp 405–426). Ecol. Bull.

Grimm NB (1987) Nitrogen dynamics during succession in a desert stream. Ecology 68: 1157–1170

Grimm, NB (1994) Disturbance, succession, and ecosystem processes in streams: A case study from the desert. In: Giller PS, Hildrew AJ & Raffaeli DG (Eds) Aquatic Ecology: Scale, Pattern and Process (pp 93–112). Blackwell Scientific, Oxford

Grimm NB & Fisher SG (1986a) Nitrogen limitation potential of Arizona streams and rivers. J. Ariz.-Nev. Acad. Sci. 21: 31–43

Grimm NB & Fisher SG (1986b) Nitrogen limitation in a Sonoran Desert stream. J. No. Amer. Benthol. Soc. 5: 2–15

Grimm NB & Fisher SG (1989) Stability of a desert stream ecosystems to disturbance by flash floods. J. No. Amer. Benthol. Soc. 8:

Grimm NB & Fisher SG (1992) Responses of arid land streams to changing climate. In: Firth P & Fisher SG (Eds) Global Climate Change and Freshwater Ecosystems (pp 211–233). Springer-Verlag, New York

Grimm NB, Fisher SG & Minckley WL (1981) Nitrogen and phosphorus dynamics in hot desert streams of Southwestern USA. Hydrobiologia 83: 303–312

Hanson, RB & Gundersen K (1977) Relationship between nitrogen fixation (acetylene reduction) and the C:N ratio in a polluted coral reef ecosystem, Kaneohe Bay, Hawaii. Est. Coastal Mar. Sci. 5: 437–444

Hardy RWF, Holsten RD, Jackson EK & Burns RC (1968) The acetylene-ethylene assay for N_2 fixation: Laboratory and field evaluation. Plant Physiol. 43: 1185–1207

Hendzel LL, Hecky RE & Findlay DL (1994) Recent changes in N_2-fixation in Lake 227 in response to reduction of the N:P loading ratio. Can. J. Fish. Aqu. Sci. 51: 2247–2253

Hererra R & Jordan CF (1981) Nitrogen cycle in a tropical Amazonian rain forest: The caatinga of low mineral nutrient status. In: Rosswall T & Clark FE (Eds) Terrestrial Nitrogen Cycles 33 (pp 493–506). Ecol. Bull.

Horne AJ (1975) Algal nitrogen fixation in California streams: Diel cycles and nocturnal fixation. Freshwat. Biol. 5: 471–477

Horne, AJ (1972) The ecology of nitrogen fixation on Signy Island, South Orkney Island. Brit. Antarctic Surv. Bull. 27: 1–18

Horne AJ & Carmiggelt WW (1975) Algal nitrogen fixation in California streams: seasonal cycles. Freshwat. Biol. 5: 461–470

60

Horne AJ & Galat DL (1985) Nitrogen fixation in an oligtrophic, saline desert lake: Pyramid Lake, Nevada. Limnol. Oceanogr. 30: 1229–1239

Howarth RW, Marino R & Cole JJ (1988b) Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. Limnol. Oceanogr. 33: 688–702

Howarth RW, Marino R, Lane J & Cole JJ (1988a) Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. Limnol. Oceanogr. 33: 669–687

Jarrell WM & Virginia RA (1990) Soil cation accumulation in a mesquite woodland: Sustained production and long-term estimates of water use and nitrogen fixation. J. Arid Environ. 18: 51–58

Jones K (1992) Diurnal nitrogen fixation in tropical marine cyanobacteria: A comparison between adjacent communities of non-heterocystous *Lyngbya* sp. and heterocystous *Calothrix* sp. Brit. Phycol. J. 27: 107–118

Joye SB & Paerl HW (1994) Nitrogen cycling in microbial mats: Rates and patterns of denitrification and nitrogen fixation. Mar. Biol. 119: 285–295

Klingensmith KM & Van Cleve K (1993) Denitrification and nitrogen fixation in floodplain successional soils along the Tanana River, interior Alaska. Can. J. For. Res. 23: 956–963

Leland HV & Carter JL (1985) Effects of copper on production of periphyton, nitrogen fixation and processing of leaf litter in a Sierra Nevada, California, stream. Freshwat. Biol. 15: 155–173

Levin SA (1992) The problem of pattern and scale in ecology. Ecology 73: 1943–1967

Marino R, Howarth RW, Shamess J & Prepas E (1990) Molybdenum and sulfate as controls on the abundance of nitrogen-fixing cyanobacteria in saline lakes in Alberta. Limnol. Oceanogr. 35: 245–259

Melillo JM (1981) Nitrogen cycling in deciduous forests. In: Rosswall T & Clark FE (Eds). Terrestrial nitrogen cycles. Ecol. Bull. 33: 427–442

Murphy J & Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27: 31–36

Naiman RJ & Melillo JM (1984) Nitrogen budget of a subarctic stream altered by beaver (*Castor canadensis*). Oecologia 62: 150–155

O'Neill RW, DeAngelis DJ, Waide J & Allen TH (1986) A hierarchical concept of ecosystems. Monographs in Population Biology, Vol. 23. Princeton University Press, Princeton

Peterson CG & Grimm NB (1992) Temporal variation in enrichment effects during periphyton succession in a nitrogen-limited desert stream ecosystem. J. No. Amer. Benthol. Soc. 11: 20–36

Pickett ST, Kolasa J & Jones CG (1994) Ecological Understanding. Academic Press, New York

Redfield AC (1958) The biological control of chemical factors in the environment. Amer. Sci. 46: 205–221

Rueter JG & Peterson RR (1987) Macronutrient effects on cyanobacterial growth and physiology. New Zeal. J. Mar. Freshwat. Res. 21: 435–445

Rychert R, Skujins J, Sorenson D & Porcella D (1978) Nitrogen fixation by lichens and free-living microorganisms in deserts. In: West NE & Skujins JJ (Eds) Nitrogen in Desert Ecosystems (pp 20–30). Dowden, Hutchinson, & Ross, Stroudsburg

Schindler DW (1977) Evolution of phosphorus limitation in lakes. Science 195: 260–262

Smith VH (1990) Nitrogen, phosphorus, and nitrogen fixation in lacustrine and estuarine ecosystems. Limnol. Oceanogr. 35: 1852–1859

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799–801

Sprent J (1987) Ecology of the Nitrogen Cycle. Cambridge University Press.

Stewart WDP, Fitzgerald GP & Burris RH (1967) In situ studies on nitrogen fixation using the acetylene reduction technique. Proc. Na. Acad. Sci. 58: 2071–2078

Stream Solute Workshop (1990) Concepts and methods for assessing solute dynamics in stream ecosystems. J. No. Amer. Benthol. Soc. 9: 95–119

Tett P, Kelly MG & Hornberger G M (1975) A method for the spectrophotometic measurement of chlorophyll *a* and pheophytin *a* in benthic microalgae. Limnol. Oceanogr. 20: 887–896

Tilman D, Kiesling R, Sterner R, Kilham SS & Johnson FA (1986) Green, bluegreen and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Arch. Hydrobiol. 106: 473–485

Tilman D, Kilham SS & Kilham P (1982) Phytoplankton community ecology: the role of limiting nutrients. Ann. Rev. Ecol. Sys. 13: 349–372

Triska FJ, Sedell JR, Cromack K Jr, Gregory SV & McCorison FM (1984) Nitrogen budget for a small coniferous forest stream. Ecol. Monogr. 54: 119–140

Valett HM, Fisher SG, Grimm NB & Camill P (1994) Vertical hydrologic exchange and ecological stability of a desert stream ecosystem. Ecology 75: 548–560

Van Cleve K & Alexander V (1981) nitrogen cycling in tundra and boreal ecosystems. In: Rosswall T & Clark FE (Eds) Terrestrial Nitrogen Cycles 33 (pp 375–404). Ecol. Bull.

Van Raalte CD, Valiela I, Carpenter EJ & Teal JM (1974) Inhibition of nitrogen fixation in salt marshes measured by acetylene reduction. Estuar. Coast. Mar. Sci. 2: 301–305

Vitousek PM & Howarth RW (1991) Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13: 87–115

Wiebe WJ, Johannes RE & Webb KL (1975) Nitrogen fixation in a coral reef community. Science 188: 257–259

Wood ED, Armstrong FAJ & Richards FA (1967) Determination of nitrate in seawater by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. UK 47: 23–31

Woodmansee RG, Vallis I & Mott JJ (1981) Grassland nitrogen. In: Rosswall T & Clark FE (Eds) Terrestrial Nitrogen Cycles 33 (pp 443—462). Ecol. Bull.

LINKED CITATIONS

- Page 1 of 4 -

References

Nitrogen Fixation by Algae in a Massachusetts Salt Marsh
Edward J. Carpenter; Charlene D. van Raalte; Ivan Valiela
Limnology and Oceanography, Vol. 23, No. 2. (Mar., 1978), pp. 318-327.
Stable URL:
http://links.jstor.org/sici?sici=0024-3590%28197803%2923%3A2%3C318%3ANFBAIA%3E2.0.CO%3B2-K

Temporal Succession in a Desert Stream Ecosystem Following Flash Flooding
Stuart G. Fisher; Lawrence J. Gray; Nancy B. Grimm; David E. Busch
Ecological Monographs, Vol. 52, No. 1. (Mar., 1982), pp. 93-110.
Stable URL:
http://links.jstor.org/sici?sici=0012-9615%28198203%2952%3A1%3C93%3ATSIADS%3E2.0.CO%3B2-6

Energy Flow in Bear Brook, New Hampshire: An Integrative Approach to Stream Ecosystem Metabolism
Stuart G. Fisher; Gene E. Likens
Ecological Monographs, Vol. 43, No. 4. (Autumn, 1973), pp. 421-439.
Stable URL:
http://links.jstor.org/sici?sici=0012-9615%28197323%2943%3A4%3C421%3AEFIBBN%3E2.0.CO%3B2-2

Nitrogen Dynamics During Succession in a Desert Stream
Nancy B. Grimm
Ecology, Vol. 68, No. 5. (Oct., 1987), pp. 1157-1170.
Stable URL:
http://links.jstor.org/sici?sici=0012-9658%28198710%2968%3A5%3C1157%3ANDDSIA%3E2.0.CO%3B2-C

Nitrogen Fixation in an Oligotrophic, Saline Desert Lake: Pyramid Lake, Nevada

A. J. Horne; D. L. Galat
Limnology and Oceanography, Vol. 30, No. 6. (Nov., 1985), pp. 1229-1239.
Stable URL:

http://links.jstor.org/sici?sici=0024-3590%28198511%2930%3A6%3C1229%3ANFIAOS%3E2.0.CO%3B2-D

Nitrogen Fixation in Freshwater, Estuarine, and Marine Ecosystems. 2. Biogeochemical Controls

Robert W. Howarth; Roxanne Marino; Jonathan J. Cole
Limnology and Oceanography, Vol. 33, No. 4, Part 2: Comparative Ecology of Freshwater and Marine Ecosystems. (Jul., 1988), pp. 688-701.
Stable URL:

http://links.jstor.org/sici?sici=0024-3590%28198807%2933%3A4%3C688%3ANFIFEA%3E2.0.CO%3B2-Y

Nitrogen Fixation in Freshwater, Estuarine, and Marine Ecosystems. 1. Rates and Importance

Robert W. Howarth; Roxanne Marino; Judith Lane; Jonathan J. Cole
Limnology and Oceanography, Vol. 33, No. 4, Part 2: Comparative Ecology of Freshwater and Marine Ecosystems. (Jul., 1988), pp. 669-687.
Stable URL:

http://links.jstor.org/sici?sici=0024-3590%28198807%2933%3A4%3C669%3ANFIFEA%3E2.0.CO%3B2-2

The Problem of Pattern and Scale in Ecology: The Robert H. MacArthur Award Lecture

Simon A. Levin
Ecology, Vol. 73, No. 6. (Dec., 1992), pp. 1943-1967.
Stable URL:

http://links.jstor.org/sici?sici=0012-9658%28199212%2973%3A6%3C1943%3ATPOPAS%3E2.0.CO%3B2-6

Molybdenum and Sulfate as Controls on the Abundance of Nitrogen-Fixing Cyanobacteria in Saline Lakes in Alberta

Roxanne Marino; Robert W. Howarth; Jennifer Shamess; Ellie Prepas
Limnology and Oceanography, Vol. 35, No. 2. (Mar., 1990), pp. 245-259.
Stable URL:

http://links.jstor.org/sici?sici=0024-3590%28199003%2935%3A2%3C245%3AMASACO%3E2.0.CO%3B2-W

Evolution of Phosphorus Limitation in Lakes
D. W. Schindler
Science, New Series, Vol. 195, No. 4275. (Jan. 21, 1977), pp. 260-262.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819770121%293%3A195%3A4275%3C260%3AEOPLIL%3E2.0.CO%3B2-N

Nitrogen, Phosphorus, and Nitrogen Fixation in Lacustrine and Estuarine Ecosystems
Val H. Smith
Limnology and Oceanography, Vol. 35, No. 8. (Dec., 1990), pp. 1852-1859.
Stable URL:
http://links.jstor.org/sici?sici=0024-3590%28199012%2935%3A8%3C1852%3ANPANFI%3E2.0.CO%3B2-0

Determination of Ammonia in Natural Waters by the Phenolhypochlorite Method
Lucia Solorzano
Limnology and Oceanography, Vol. 14, No. 5. (Sep., 1969), pp. 799-801.
Stable URL:
http://links.jstor.org/sici?sici=0024-3590%28196909%2914%3A5%3C799%3ADOAINW%3E2.0.CO%3B2-7

In situ Studies on N 2 Fixation Using the Acetylene Reduction Technique
W. D. P. Stewart; G. P. Fitzgerald; R. H. Burris
Proceedings of the National Academy of Sciences of the United States of America, Vol. 58, No. 5.
(Nov. 15, 1967), pp. 2071-2078.
Stable URL:
http://links.jstor.org/sici?sici=0027-8424%2819671115%2958%3A5%3C2071%3AISSONF%3E2.0.CO%3B2-X

**A Method for the Spectrophotometric Measurement of Chlorophyll a and Pheophytin a in
Benthic Microalgae**
Paul Tett; Mahlon G. Kelly; George M. Hornberger
Limnology and Oceanography, Vol. 20, No. 5. (Sep., 1975), pp. 887-896.
Stable URL:
http://links.jstor.org/sici?sici=0024-3590%28197509%2920%3A5%3C887%3AAMFTSM%3E2.0.CO%3B2-J

Phytoplankton Community Ecology: The Role of Limiting Nutrients
David Tilman; Susan S. Kilham; Peter Kilham
Annual Review of Ecology and Systematics, Vol. 13. (1982), pp. 349-372.
Stable URL:
http://links.jstor.org/sici?sici=0066-4162%281982%2913%3C349%3APCETRO%3E2.0.CO%3B2-6

LINKED CITATIONS
- Page 4 of 4 -

Nitrogen Budget for a Small Coniferous Forest Stream

Frank J. Triska; James R. Sedell; Kermit Cromack, Jr.; Stan V. Gregory; F. Michael McCorison
Ecological Monographs, Vol. 54, No. 1. (Mar., 1984), pp. 119-140.
Stable URL:

http://links.jstor.org/sici?sici=0012-9615%28198403%2954%3A1%3C119%3ANBFASC%3E2.0.CO%3B2-M

Vertical Hydrologic Exchange and Ecological Stability of a Desert Stream Ecosystem

H. Maurice Valett; Stuart G. Fisher; Nancy B. Grimm; Philip Camill
Ecology, Vol. 75, No. 2. (Mar., 1994), pp. 548-560.
Stable URL:

http://links.jstor.org/sici?sici=0012-9658%28199403%2975%3A2%3C548%3AVHEAES%3E2.0.CO%3B2-1

Nitrogen Limitation on Land and in the Sea: How Can It Occur?

Peter M. Vitousek; Robert W. Howarth
Biogeochemistry, Vol. 13, No. 2. (1991), pp. 87-115.
Stable URL:

http://links.jstor.org/sici?sici=0168-2563%281991%2913%3A2%3C87%3ANLOLAI%3E2.0.CO%3B2-E

Nitrogen Fixation in a Coral Reef Community

W. J. Wiebe; R. E. Johannes; K. L. Webb
Science, New Series, Vol. 188, No. 4185. (Apr. 18, 1975), pp. 257-259.
Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819750418%293%3A188%3A4185%3C257%3ANFIACR%3E2.0.CO%3B2-W