Proc 8th Int Coral Reef Sym 1:803–808. 1997

CONTRIBUTION OF N_2 FIXATION TO N PRIMARY PRODUCTIVITY OF THE LAGOON OF TIKEHAU

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ABSTRACT

In atoll lagoons, a large part of the nutrients required for lagoon primary production are supplied by mineralization process and input from oceanic waters. However, major lagoonal primary producers are cyanobacteria, some of which are nitrogen-fixing. Rates of acetylene reduction and $^{15}N_2$ fixation were measured in Tikehau lagoon between 1991 and 1994. Nitrogenase activity was observed in all cyanobacterial communities: sediments, emerged mats, kopara mats, emerged beach sand, limestone surface, beach rock and the water column. Nitrogen fixation was much more important during the daylight. N fixation for the whole lagoon contributes in averages between 0.2% (without plankton contribution) and 13% (with the calculated plankton N fixation rate) of the nitrogen requiremenst to support the gross primary production.

INTRODUCTION

Ever since the earliest observations, coral reefs have posed a problem for the supply of nitrogen and phosphorus (Darwin 1844; Odum and Odum 1955 , Wiebe *et al* 1975). Typically coral reefs are found in tropical oceanic waters characterized by low nutrient concentrations and low plankton biomass (oligotrophic waters) and low diversity and biomass in contrast to the coral reefs which have some of the highest diversities and productivities of any ecosystem known. A common type is the coral atoll found in the Pacific Ocean with a large extended rim (up to 50 km diameter) surrounding a deep lagoon. Most studies of atolls have focused on the productivity and nutrient relations of shallow coral assemblages on the rim (D'Elia and Wiebe 1990; Larkum et *al* 1988, Shashar et *al*. 1994). However, in terms of area and volume, the lagoon may often be the most significant component of the system. As a result our investigation has been concerned with the supply of nutrients especially nitrogen to the major primary producers in the lagoon.

The supply of nitrogen to primary producers has been investigated in many studies (D'Elia and Wiebe 1990; Larkum *et al*, 1988; Shashar *et al* 1994, Capone *et al* 1992). Nitrogen can be supplied by at least three processes a) remineralisation of existing stocks b) input from outside (such as advection) and c)nitrogen fixation by nitrogen-fixing organisms of which cyanobacteria are the most common. In the present study, we have investigated nitrogen fixation in the benthic and planktonic compartments of Tikehau lagoon and associated communities.

MATERIALS AND METHODS

Site areas

The present study was carried out at Tikehau, which is situated in the north-west of the Tuamotu archipelago. Its geomorphological characteristics make it a suitable model of a mid-size open atoll.

Tikehau is almost circular (Fig. 1): its widest diameter (NE-SW axis) is nearly 28 km. The reef rim is ca 78 km long and has a width - taken between the algal ridge and the edge of the lagoon - ranging from less than 300 to 1300 m (Intes 1984). The lagoon has an area of 400 km^2; of which 91 % has a depth greater than 15 m. The average depth is 25 m (Lenhardt 1987). The 25 km^2 of islands are intersected by reef-flat spillways (hoa) which link the lagoon and the ocean; one of these, at the western end, forms a 200 m wide and 4 m deep passage. Except in the passage, currents in spillways generally flow into the lagoon at low velocity. The average replacement time for waters in the lagoon is estimated to be 176 days (Lenhardt 1988).

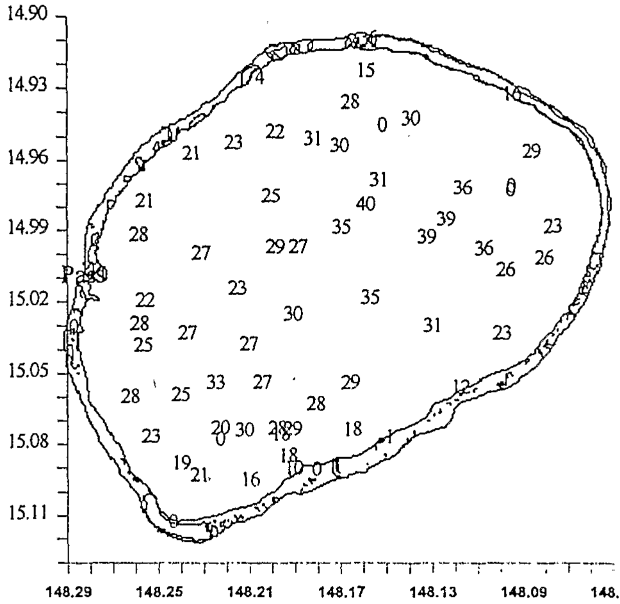

Fig. 1: Position and depth of experimental stations

Irradiance is high and 17 % of the light energy measured at the surface reaches 25 m (Charpy and Charpy-Roubaud 1990b).

Mineral nutrient concentrations were lower inside the lagoon than outside (Charpy-Roubaud *et al.* 1990).

Phytoplankton biomass was low (0.2 mg Chlorophyll m^{-3}, Charpy and Charpy-Roubaud (1991) and dominated by picoplankton. Microscopic observations performed during the southern summer showed a large number of cyanobacteria (Blanchot *et al.* 1989). Average phytoplankton production (integrated up to 25 m) was estimated to 0.44 g C m^{-2} d^{-1} (Charpy-Roubaud *et al.* 1989).

The lagoon bottom is formed of a fine to very fine calcareous sand. Extensive areas are covered with reddish patches of Cyanophyceae. In places, the lagoon brown bottom is colonized by *Halophila* seagrass beds. Macroalgae are very sparse. Average benthic primary production in sandy areas was estimated to 0.25 g C m^{-2} d^{-1} (Charpy-Roubaud 1988).

The upper zone (0 to 2 m) of pinnacles is colonized by algae (*Halimeda, Pocockiella, Caulerpa, Liagora ceranoides*, algal turfs). The windward zone is mainly colonized by large colonies of *Porites lobata* and *Millepora platyphylla* and the leeward zone by *Acropora variabilis, A. hyacinthus* and *A. hemprichii*. In the mid zone (2 to 6 m), algae (*Halimeda, Caulerpa*) compete with corals (*Montipora, Astropora, Psammocora, Porites, Platygyra, Pavona*) for sites. The lower zone (6 to 15 m) is occupied by coral patches of *Montipora verrucosa, Stylocoeniella, Platygyra daedalea* and bushes of *Acropora formosa, Stylophora pistillata* and *Favia favus*, growing on a detrital or gentle sandy slope (Faure and Laboute 1984, Harmelin-Vivien 1985).

Community Classification of substrata

Soft bottom sediments were classified into four groups (Table 1) according to the visual density of cyanobacteria communities. Sand 3 is made of filamentous cyanobacteria (*Lyngbya*).

Table 1: Description of cyanobacterial communities of Tikehau atoll lagoon

Communities	Organisms
Sand 0	Diatoms, Dinoflagellate, no apparent cyanobacteria
Sand 1	*Spirulina, Oscillatoria, Anabaena, Dinoflagellate*
Sand 2	Same species as Sand 1 but more dense
Sand 3	*Lyngbya* associations
Exposed mats	*Scytonema, Schizothrix*
Kopara mats	*Phormidium, Oscillatoria, Lyngbya, Johannes baptista*
Exposed beach sand	*Schizothrix*
Limestone surfaces	*Calothrix, Oscillatoria, Pleurocapsa*
Beach Rock	*Calothrix*
Water column	*Synechococcus sp.*

Exposed mats cover large areas of the intertidal zone (motu). These mats begin at the lagoon level, but a large part are above the intertidal limit.

Kopara mats are located in ponds isolated from the lagoon by land. Description of these ponds appears in Defarge *et al.* (1994).

Cyanobacterial communities are also found on the beach fringing the lagoon to a depth of 2cm in the sediment. These communities are submerged only during exceptional increases of lagoon water level.

Beach rock occurs at the water edge in some parts of the lagoon, especially near the reef flat spillways (hoa).

N_2 fixation
Nitrogen fixation was estimated using the acetylene reduction/ethylene production method (Stewart *et al.* 1967, Hardy *et al.* 1968, Larkum *et al.* 1988).

Benthic communities
Incubations were carried out in 500 ml glass jars with spring-loading perspex lids with a rubber injection port. Rock chippings or other substrata from the sampling site were immersed in 150 ml sea-water. Experiments were started by the removal of 50 ml air and the injection of 50 ml of C_2H_2 followed by swirling for several minutes. Bottles were incubated on a submerged platform. Wind and wave action caused gentle stirring. The exposed surface area of rock samples was measured according to Larkum *et al* (1988). For sediment, the surface area of the section of the glass jar was taken as the exposed area.

Planktonic community
Incubations were carried out in April and May 1993 in 1200 ml polycarbonate bottles with a rubber injection port. The volume of sea water incubated was 1000 ml. Experiments were started by removal of 50 ml air and injection of 50 ml of C_2H_2. Plankton was incubated at the surface (0.5m) during both the day and night to observe the influence of light. *Synechococcus* abundance was recorded.

C_4H_4 analysis
Gas samples were taken from the incubation jars after swirling for several minutes. Ethylene production was assayed using a portable gas chromatograph with a stainless steel column (Poropak T) and a sensitive thermal conductivity detector for C_4H_4 detection. Fifty to 500 µl of gas was injected and the C_4H_4 peak was followed on a strip-chart recorder. The system was calibrated daily with known dilutions of C_4H_4.

Determination of the C_2H_2 : N_2 ratio
Calibration of the acetylene reduction method was made in 1995 using benthic communities. Cyanobacteria were incubated *in situ* for 24 h in a culture chamber with a light energy of 50 µE m^{-2} h^{-1}. After the C_2H_4 measurements, gaseous N_2 was removed from sample flasks by flushing the slurry with 100% He (Seitzinger and Garber 1987). Fifty ml of He were then removed and replaced by 50 ml of $^{15}N_2$. The benthic cyanobacteria were then re-incubated for 24 h in the same conditions as before with 0.2 atm of 98% atom % $^{15}N_2$. At the end of the incubation, gas samples were taken using 4 ml evacuated tubes and the cyanobacteria were immediately frozen. The incubation water and organisms were digested by Kjeldahl procedures and the ammonia was steam-distilled into boric acid and titrated with dilute HCl as described by Bergersen (1980). Micro-diffusion techniques were used for recovery of the dissolved inorganic and organic nitrogen (Slawyk and Raimbault 1995). ^{15}N measurements were made on a mass-spectrometer at the COM of Marseilles.

Benthic community biomass
Fifty eight stations were randomly selected in the lagoon. At each station, five 0.25 m^2 quadrates divided into 25 10cm x 10cm sections were positioned on the soft bottom. Community surface areas were calculated as cm^2 of organisms per m^2 of sediment. The limestone surface area of pinnacles and beach rock was estimated by multiplying the pinnacle projected area (2% of the lagoon surface) by 3 and the projected area of beach rock (0.7%) by 1.5 following Larkum *et al.* (1988).

Phytoplankton biomass
Tikehau lagoon was sampled during seven expeditions between 1991 and 1993. Six stations were occupied within the lagoon. Twenty five ml of water were filtered immediately onto 0.2 µm Nuclepore filters. Cell numbers were determined using a Zeiss standard epifluorescence microscope equipped with blue excitation filters. Thirty fields or at least 200 cells were counted (Blanchot *et al.* 1989).

RESULTS

Acetylene reduction rates were measured as described in Materials and Methods for all the major benthic communities found in Tikehau lagoon, for intertidal and exposed communities and for planktonic communities.

Acetylene reduction rates in soft bottom benthic communities of Tikehau lagoon

Four sand communities are given in Table 2. From these data the average daily reduction rate was estimated (as given in the last column for the average depth of the lagoon 25m). The major nitrogen fixers appeared to be cyanobacteria.

Table 2: Mean of acetylene reduction rates measured in sand habitats during daylight and during the night (nmol cm^{-2} h^{-1}), percentage of reduction at 25m depth (%25m) and daily average reduction rate at 25m depth (DARR25) in µmol m^{-2} day^{-1}

	Daylight	Night	%25m	DARR25m
Sand 0	0.33±0.03	0.03±0.01	100	43.2
Sand 1	0.52±0.23	0.32±0.06	100	100.8
Sand 2	1.30±0.97	0.74±0.14	100	244.8
Sand 3	0.99±0.36	0.16±0.02	49	67.6

Acetylene reduction rates of intertidal and exposed communities

In the case of Kopara ponds, some sulfur bacteria are present (Defarge *et al.* 1994)) in addition to oxygenic anobacteria. However, these communities had quite low etylene reduction rates (Table 3).

o communities of hard substrates are given in Table

Table 3: Mean ± 1 SE acetylene reduction rates neasured during daylight and during the night(nmol cm^{-2} h^{-1}) and daily average reduction rates (DARR) in μmol m^{-2} day^{-1} by exposed beach sand (EBS), exposed mats (EM) and Kopara mats (KM).

Communities	Daylight	Night	DARR
EBS	1.03±0.29	0.25±0.14	153.6
EM	3.61±0.85	0.21±0.02	458.4
KM	0.27±0.03	0.09±0.02	43.2

Table 4: Mean ± 1 SE acetylene reduction rate (nmol cm^{-2} h^{-1}) and daily average reduction rate (DARR) in μmol m^{-2} day^{-1} by cyanobacterial communities on limestone surfaces (LS) and beach rocks (BR)

Communities	Daylight	Night	DARR
LS	1.83±0.26	0.26±0.08	251
BR	1.78±0.16	2.29±0.30	488

etylene reduction rates of planktonic communities of kehau lagoon

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$_2$/N fixation rate

$_2$ reduction and ^{15}N fixation rates in sand mmunities and exposed mats were compared. The results e shown in Table 6.

e spread of values varied from 2 to 8 moles fixed N_2 r moles of reduced C_2H_2. Values of the ratio from the terature are also quite variable (Seitzinger and rber 1987). Here, we have chosen a value of 3.0 to convert our C_2H_2 reduction rates to N fixation (cf. Larkum et al, 1988).

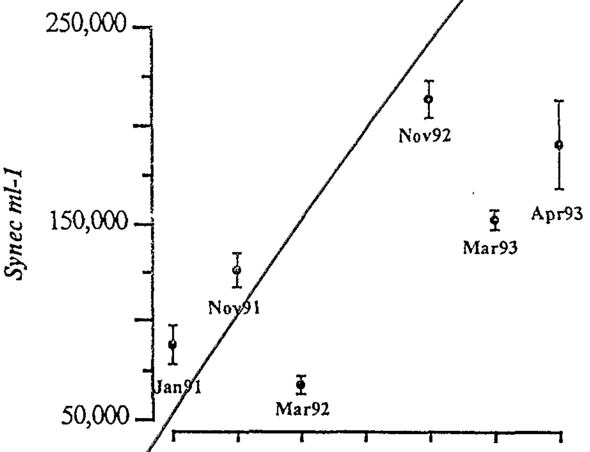

Fig. 2: Average ± 1 SE *Synechococcus* abundance in Tikehau lagoon between 1991 and 1994

Total benthic fixation for the lagoon

Table 2 to Table 4 show results for all the major benthic cyanobacterial communities resident in Tikehau lagoon. The contribution of each of those associations depends on the proportion of the total projected area of the lagoon of which they are a part. This value was calculated from a 58 station (randomly selected) survey of the lagoon. From these values, the mean value of acetylene reduction rate for each association and the ratio ARR : N_2 = 3, the total fixation (per day) can be calculated (Table 7 column 4).

The total benthic fixation including beach rocks and limestone surfaces amounts to 0.8 mg N m^{-2} day^{-1}.

DISCUSSION

Soft bottom communities covered 93% of the total lagoon surface area and their average N_2 fixation was 0.7 mg m^{-2} day^{-1}. This value is in the lower part of the range of N_2 fixation in coral reef sediments summarized in Table 8. The value given by Shashar *et al.* (1994) is very high compared with other assessments.

The mean N_2 fixation rate measured for limestone surfaces (2.3 mg m^{-2} day^{-1}) was higher than the values given by Larkum *et al.* (1988) (0.2-0.4 mg m^{-2} day^{-1}) but 3 orders of magnitude lower than the value given by Shashar et *al.* (1994) (93 mg m^{-2} day^{-1}).

Table 5: Measured acetylene reduction rates by plankton communities during daylight and during the night, and over 24 hours (24h). Daily average reduction rates per m^2 of lagoon (DARR25) are integrated over a 25m depth using the average *Synechococcus* abundance.

Daylight	Night	24h	DARR25
nmol cell^{-1} h^{-1}	nmol cell^{-1} h^{-1}	nmol cell^{-1} day^{-1}	μmol m^{-2} day^{-1}
6.69 10^{-8}±0.01 10^{-8}	4.47 10^{-8}±0.15 10^{-8}	1.32 10^{-6}	5121

Table 6: Molar ratios (R) of reduction of acetylene and nitrogen.

Community	Atom % ^{15}N excess	Total N (mg)	nmol N_2 h^{-1}	nmol C_2H_4 h^{-1}	R
Sand 1	0.004	28.63	1.58	4.62	2.92
Sand 2	0.005	63.786	2.53	4.77	1.89
Sand 3	0.001	32.89	0.45	3.87	8.54
Exposed mats	0.030	47.48	19.56	38.20	1.95

cyanobacteria. However, these communities had quite low acetylene reduction rates (Table 3).

Two communities of hard substrates are given in Table 4.

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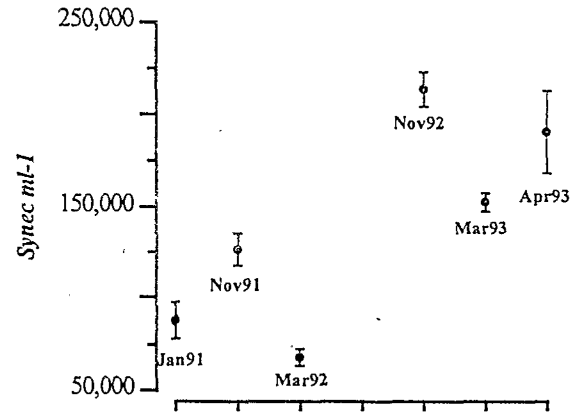
Acetylene reduction rates of planktonic communities of Tikehau lagoon
Monthly average abundances of *Synechococcus* varied between 60 10^3 (March 1992) and 220 10^3 cells ml^{-1} (November 1992) (Fig. 2). Pooling all the results, the average abundance of *Synechococcus* was 155 ± 7 10^3 cells ml^{-1}.

Acetylene reduction rates were measured on many occasions for the waters of Tikehau lagoon. The results are given in Table 5.

Using the average abundance of *Synechococcus* and integrating the average water column to give a projected surface area value, these results indicate a mean water column acetylene reduction rate of 5121 µmol m^{-2} day^{-1}.

C_2H_2/N fixation rate
C_2H_2 reduction and ^{15}N fixation rates in sand communities and exposed mats were compared. The results are shown in Table 6.

The spread of values varied from 2 to 8 moles fixed N_2 per moles of reduced C_2H_2. Values of the ratio from the literature are also quite variable (Seitzinger and Garber 1987). Here, we have chosen a value of 3.0 to convert our C_2H_2 reduction rates to N fixation (cf. Larkum *et al,* 1988).



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Exposed mats	0.030	47.48	19.56	38.20	1.95

The mean N_2 fixation rate measured for Beach Rock (4.6 mg m^{-2} day^{-1}) was in the range of values given by Burris (1976) in Lizard Island, Great Barrier Reef (1.9 - 8.4 mg m^{-2} day^{-1}) but again was higher than the values given by Larkum *et al.* (0.2-0.4 mg m^{-2} day^{-1}) and Shashar *et al.* (0.9 mg m^{-2} day^{-1}).

Table 7: Mean N_2 fixation mg N per m^2 day^{-1} by benthic lagoon cyanobacteria communities (2), percentages of lagoon surface area covered by cyanobacteria communities (3) and average N_2 fixation mg N per m^2 of lagoon day^{-1}(4). N_2 fixation was calculated using $C_2H_2:N_2 = 3$.

Communities (1)	(2)	(3)	(4)
Soft bottom			
Sand 0	0.40	43	0.17
Sand 1	0.93	39	0.37
Sand 2	2.29	3	0.06
Sand 3	0.63	8	0.05
Total		93	0.65
Exposed communities			
Exposed sands	1.44		
Exposed mats	4.27		
Kopara mats	0.40		
Hard surface			
Limestone surface	2.34	6	0.14
Beach rock	4.55	1	0.05
Total		7	0.19
Total benthos		100	0.84

The average rate (Table 5) observed for the phytoplankton community was very high (5121 µmol m^{-2} day^{-1}) compared with benthic cyanobacteria. However, this value is about 10 times lower than the only value recorded in the literature for *Synechococcus* : 100 10^{-8} nmol cell^{-1} h^{-1} (Huang and Chow 1986). The absolute abundances of *Synechococcus* reported for Tikehau lagoon have been reported for other areas, but there is no evidence for such high fixation rates. The C_2H_4 concentration observed at the end of the plankton incubation was very low and close to the detection limit of our Gas Chromatograph. We do not have a calibration factor to convert our C_2H_2 reduction rates to N fixation for the planktonic community. Therefore, the measured planktonic N fixation has to be considered cautiously.

Nitrogen fixation rate in emerged communities (1.4-4.3 mg N m^{-2} day^{-1}) were lower than the range (6-107 mg N m^{-2} day^{-1}) given by Pearl *et al.* (1993) for intertidal mats in Tomales Bay (California). In the case of Tikehau, emerged communities do not contribute greatly to the lagoon N budget. The tidal range inside the lagoon is normally less than 15 cm (Lenhardt, 1988) and the nitrogen fixed by this community could contribute to the lagoon only during occasional large elevation of the lagoon level or after heavy rainstorms.

We can compare the N_2 fixation rate by cyanobacterial communities with the nitrogen requirement for their primary production estimated using the Redfield C:N ratio of 6.6.

Gross primary production of soft bottom communities was estimated to be 250 mg C m^{-2} day^{-1} (Charpy-Roubaud, 1988). The measured N_2 fixation by these communities (0.65 mg N m^{-2} day^{-1}) would contribute only 0.8% of their nitrogen requirements.

Recently, planktonic primary production in Tikehau was estimated to be 700 mg C m^{-2} day^{-1} (Charpy 1996); therefore, N_2 fixation by *Synechococcus* (44 mg m^{-2} day^{-1}) would contribute 19% of their nitrogen requirements. This percentage seems very high and reflects the large error in estimating the planktonic N fixation rate.

Primary production of coral reef pinnacles was estimated to be 4000 mg C m^{-2} day^{-1} (Charpy and Charpy-Roubaud, 1996). Therefore, N_2 fixation by limestone surface communities (2.3 mg m^{-2} day^{-1}) would contribute only 0.2 % of their nitrogen requirements.

For the whole lagoon, we estimate that nitrogen fixation contributes between 0.2% (without plankton contribution) and 13% (with the plankton contribution) of the nitrogen requirement of the gross production (1 g C m^{-2} day^{-1}). Thus the phytoplankton component is very important. Because of the very large biomass of the phytoplankton only a small rate of nitrogen fixation contributes a very significant proportion of the total fixation. Clearly further work on the phytoplanktonic nitrogen fixation is needed to fully confirm those preliminary results. Larkum et al (1988) have calculated mass-balance estimates which show that nitrogen fixation provides between 9 and 14% of the

Table 8: Reported rates of nitrogen fixation in coral reef sediments. (In part from Capone *et al.* 1992). GBR = Great Barrier Reef.

Place	Methods	mg m^{-2} day^{-1}	Source
Barbados	C_2H_2 red; $^{15}N_2$	1.8	Patriquin and Knoweles (1975)
Kanehoe Bay, Hawaii	C_2H_2 red	3.4	Hanson and Gunderson (1976)
GBR (Central)	C_2H_2 red	1.1-8.5	Wilkinson *et al.* (1984)
Same sites (muds)	C_2H_2 red	4.4-8.6	Corredor and Capone (1985)
Same sites (sands)	C_2H_2 red	0.3-2.4	Corredor and Capone (1985)
GBR (One Tree)	C_2H_2 red; $^{15}N_2$	0.2-0.5	Larkum *et al.* (1988)
Bermuda (muds)	C_2H_2 red	2.0-8.1	O'Neil and Capone (1989)
Bermuda (sands)	C_2H_2 red	0.3-7.7	O'Neil and Capone (1989)
Hydrolab., St. Croix	C_2H_2 red	4.0	King *et al.* (1990)
Australia	C_2H_2 red; $^{15}N_2$	2.7-6.7	O'Donohue *et al.* (1991)
GBR (19°S, 140°E)	C_2H_2 red	3.4-8.7	Capone *et al.* (1992)
Eilat (Red Sea)	C_2H_2 red	32.8	Shashar *et al.* (1994)
Tikehau (Sand 0)	C_2H_2 red; $^{15}N_2$	0.4	This study
Tikehau (Sand 1)	C_2H_2 red; $^{15}N_2$	0.9	This study
Tikehau (Sand 2)	C_2H_2 red; $^{15}N_2$	2.3	This study
Tikehau (Sand 3)	C_2H_2 red; $^{15}N_2$	0.6	This study
Tikehau (average)	C_2H_2 red; $^{15}N_2$	0.7	This study

annual nitrogen requirement on One Tree Reef (GBR, Australia).

CONCLUSIONS

In Tikehau, benthic nitrogen fixation supply less than 1% of benthic primary production N requirements. These results despite the fact that several communities exhibit high nitrogen fixation rates which are comparable with rates observed elsewhere. It is due mainly to the large size of the water body in the lagoon which is comparatively deep. As a result a small rate of nitrogen fixation by the phytoplankton (mainly *Synechococcus*) contributes most of the fixation for the lagoon, which may be as much as 15% of the total requirement.

ACKNOWLEDGMENTS

This work was carried out in French Polynesia in the framework of the ORSTOM CYEL program and the PNRCO program (France). We thanks for their help and advice L. Lemasson, T. Lecampion and P. Raimbault.

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