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Nitrogen fixation by *Nostoc* colonies in terrestrial environments of Aldabra Atoll, Indian Ocean

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The rates of acetylene reduction were compared *in situ* for six different types of terrestrial and semi-aquatic *Nostoc* colonies on Aldabra as an indication of their rates of nitrogen fixation. The rates per unit chlorophyll *a* were all rather similar during standard assays in mid- to late morning, with a mean rate of C_2H_4 production for all experiments of 0·0388 nM C_2H_4 μg chl a^{-1} min^{-1}. The rates for *Nostoc* colonies were at least ten times those for mature cushions of *Tolypothrix byssoidea*. Marked variation in rates of C_2H_4 production occurred throughout the day, with the peak rates occurring in early afternoon. Three of the types of *Nostoc* were re-wetted in the laboratory one year after they had been dried. All showed high rates of C_2H_4 production after a lag of up to one day. The lag was much shorter in a population of *N. commune* freshly collected in England. It is suggested that colonies of *Nostoc* are especially suitable for comparative studies of nitrogen fixation in different parts of the world, in view of the ease with which viable colonies can be stored for later extraction of chlorophyll or laboratory experiments.

Introduction

Species of the genus *Nostoc* are among the most widely distributed of any blue-green alga, and many, including obvious macroscopic forms such as *N. commune* Vaucher (Whitton & Sinclair, 1975) and *N. pruniforme* Ag. (Mollenhauer, 1970), are probably cosmopolitan. Many are known to fix atmospheric nitrogen (Stewart, 1973), and in view of the presence of heterocysts, presumably they can all do so. Baas Becking (1951) emphasized the importance of *N. commune* on coral atolls in the Pacific, as it plays a pioneer role on every atoll, and is a dominant in a certain phase of vegetational succession. Rates of nitrogen fixation for terrestrial growths on atolls have not been reported, but rates 'comparable with those found in managed agriculture' were reported for a marine *Nostoc* on Eniwetok (Enewetak) Atoll by Mague & Holm-Hansen (1975).

At Aldabra Atoll, conspicuous growths of *N. commune* occur in small depressions in the limestone platin (Whitton, 1971), and four other species forming macroscopic colonies, *N. carneum* Ag., *N. piscinale* Kütz., *N. pruniforme* and *N. sphaericum* Vaucher, occur in other types of habitat (Donaldson & Whitton, 1977b). The present study reports *in situ* and laboratory studies on several forms of *Nostoc* from Aldabra, together with a brief comparison with *Tolypothrix byssoidea* (Berk.) Kirchner, the most widespread terrestrial alga on the atoll.

A range of papers dealing with many aspects of Aldabra (9°24′S, 46°20′E) are included in Westoll & Stoddart (1971). More recent data are also available about the climate (Stoddart & Mole, 1977), chemistry of freshwater pools (Donaldson & Whitton, 1977a; Whitton & Potts, 1977) and on the terrestrial and freshwater algae (Donaldson & Whitton, 1977b). The present studies were all carried out in the wet season (see Table 2). The length of the dry season varies from year to year, but colonies of *Nostoc* probably sometimes remain for about 3 months with insufficient water to become fully re-wetted.

Materials and methods

Nitrogen fixation was estimated using the acetylene reduction assay technique. The rates of acetylene reduction were expressed in relation to chlorophyll *a*, and also in some cases, to unit area of community. An initial attempt to relate rates to the total N content of colonies was later discarded. There was large variation within one population in total N, when related to volume, dry weight or chlorophyll *a* content of colonies. (It seems probable that this was due to the presence of non-algal N, such as animal

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excreta.) Studies were also included on materials which were dried, returned to the laboratory in Durham, and then re-wetted.

Algal colonies

Representative samples were taken from situations where a particular algal community was well developed. The various types of *Nostoc* used for experiments are described below. They are listed in an order which reflects the extent to which their habitats hold water after rain. All except *N. sphaericum* were taken from ↑le Picard (West Island); *N. sphaericum* was taken from Grande Terre (South Island), in pool CC 11 (Donaldson & Whitton, 1975) near Bassin Flamant.

A. *N. commune* Vaucher var. *flagelliforme* (Berk. *et* Curtis) Born. *et* Flah.: early in the wet season this form grows directly over shaded sand, but later also occurs connecting leaves of sedges and grasses.
B. Material intermediate between **A** and **C**: grows over coarse sand later in the wet season.
C. *N. commune*, resembling original description of species (Fig. 1A).
D. Small, firm, verrucose colonies, always including much *Phormidium jenkelianum* G. Schmid. These colonies grow in shallow depressions receiving about the same amount of wetting as **C**, but in an area with especially high phosphate levels. The association between the two species appears to be close, since the relative proportions are quite

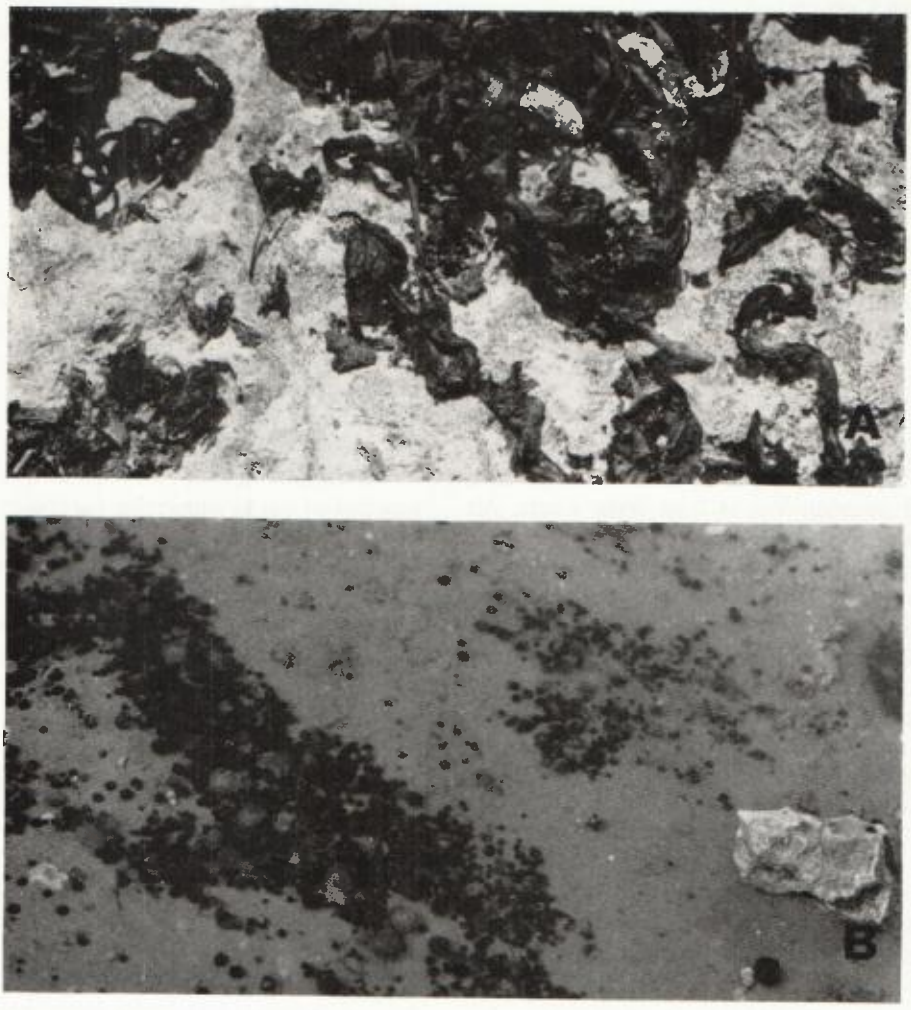

FIG. 1. (a) Type *Nostoc commune* in partially dried state overlying limestone platin with thin crust of *Tolypothrix byssoidea;* (b) *N. sphaericum* colonies in shallow pool.

TABLE 4. Comparison of rates found in present study for *in situ* nitrogen fixation in the light with those of other authors. For purposes of conversion of data to standard format, it is assumed that the rate of C_2H_4 reduction is 3 × rate of N_2 fixation (Hardy, Burns & Holsten, 1973; Mague & Holm-Hansen, 1975), chl *a* = 1% d.wt, N = 6% d.wt and protein = 37·5% d.wt. Data of Jones (1977) are included for comparison with data in Table 2.

Alga	Reference	Location		Experiment	Quoted rate	Rate, converted to standard format (μg N μg chl a^{-1} min^{-1})
Nostoc commune	this paper	Aldabra	9°S 46°E	terrestrial: afternoon peak	0·171 nM C_2H_4 μg chl a^{-1} min^{-1}	0·016
Nostoc (various)	this paper	Aldabra	9°S 46°E	terrestrial: morning assays (mean)	0·0388 nM C_2H_4 μg chl a^{-1} min^{-1}	0·0036
Nostoc	Mague & Holm-Hansen, 1975	Eniwetok	10°N 162°E	marine: range	2·83–5·00 nM C_2H_4 mg N^{-1} min^{-1}	0·0016–0·0028
Nostoc	Jones, 1977	Pretoria	26°S 28°E	terrestrial: afternoon peak	19·2 mg C_2H_4 m^{-2} h^{-1} (= 1·14 nM C_2H_4 cm^{-1} min^{-1})	
Nostoc	Bunt *et al.*, 1970	off Florida	*c.*26°N 80°W	laboratory culture assayed in sea	11·5 nM C_2H_4 mg protein $^{-1}$ h^{-1}	0·00067
Nostoc	Horne, 1975	California	39°N 123°W	stream (site 3), clear-day: mid morning	*c.*1·6 nM C_2H_4 mg d.wt $^{-1}$ h^{-1}	0·00025
N. commune	Fogg & Stewart, 1968	Signy Is.	60°S 45°W		2·3 μg N mg d.wt $^{-1}$ d^{-1}	0·000159
N. commune	Horne, 1972	Signy Is.	60°S 45°W	phytoplankton	0·05 μg N 3·6 μg chl a^{-1} day^{-1}	0·00000965
N. commune	Alexander, 1975	Devon Is.	approx. 75°N 90°W	no details	6200–53000 nM C_2H_4 g d.wt h^{-1}	0·00096–0·0082

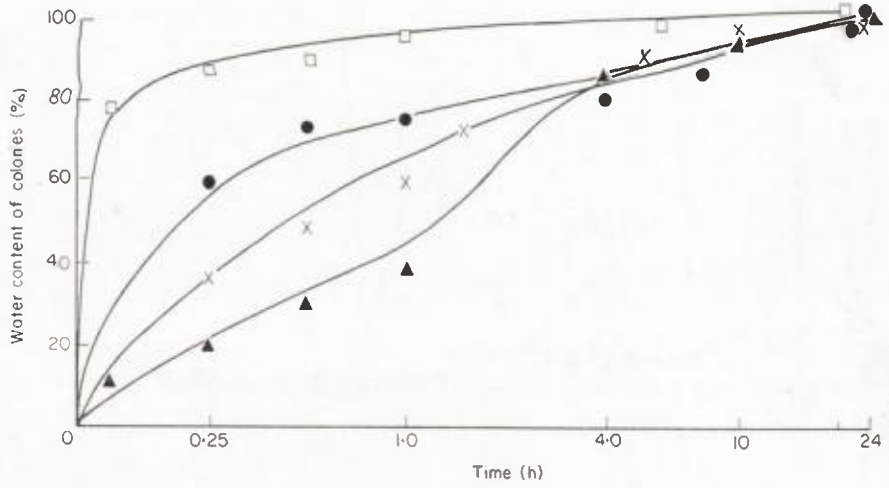

FIG. 3. Time course of water uptake by dry *Nostoc* colonies. □ *N. commune* (Aldabra); ▲ *Nostoc-Phormidium;* × *N. sphaericum;* ● *N. commune* (Tarn Moor).

TABLE 3. C_2H_4 production by *Nostoc* 1–2 days after re-wetting (see Fig. 4).

Type	Rate (nM C_2H_4 μg chl a^{-1} min^{-1})
Nostoc commune (Aldabra)	0·0531
Nostoc – Phormidium	0·0143
N. sphaericum	0·0744
N. commune (Tarn Moor)	0·0661

(Fig. 2a; Donaldson & Whitton, 1977a), a series of laboratory experiments was carried out to establish the influence of temperature on rates of C_2H_4 production by the various materials. As the variability in rates of C_2H_4 production by the colonies was quite high, only results which were demonstrated with a range of experiments are summarized here.

(i) When Aldabra *N. commune* was first incubated for prolonged periods at 32°C, and then incubated at a range of temperatures, the optimum rate of C_2H_4 production lay in the range 31–38°C, and fell to about 20% maximum rates at 22° and 44°C. The temperature of pre-incubation had a detectable effect on the rates of C_2H_4 production, and prior incubation at lower temperatures (20°C, 25°C), in particular, led to significant increases ($P < 0{\cdot}01$) in rates of C_2H_4 production at these temperatures. The maximum temperature at which detectable rates of C_2H_4 production were found was 46°C, whatever the previous incubation temperature.

(ii) The maximum temperature at which detectable

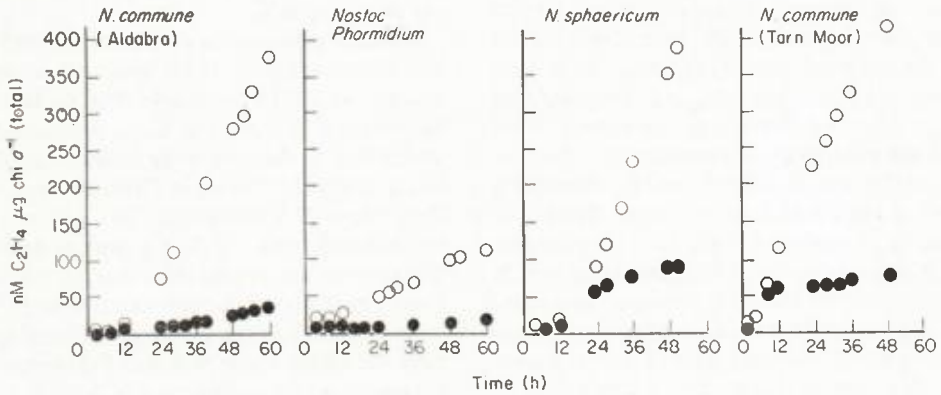

FIG. 4. Time course of C_2H_4 production when dry *Nostoc* colonies are rewetted. Aldabra colonies dried for one year, then incubated at 32°C; Tarn Moor colonies dried for one week, then incubated at 20°C. ○ light; ● dark.

contains (in mg l^{-1}): Na, 91; K, 56; Mg, 19·4; Ca, 18·0; Mn, 0·5; Fe (as EDTA chelate), 4; Co, 0·01; Cu, 0·02; Zn, 0·05; PO_4-P, 55·9; Cl, 153; SO_4-S, 25·9.

Analysis of gases

The samples stored in vacutainers were analysed for acetylene and ethylene, using a Varian aerograph series 1200 gas chromatograph.

Chlorophyll a

Chlorophyll *a* was extracted from the colonies used in field assays after return to Durham. The colonies were first incubated overnight in medium (see below) at 32°C in the light (3000 lx), and then incubated with 95% methanol in 30 ml McCartney bottles for 15 min at 70°C in the dark. Both pre-treatment and the use of methanol (as opposed to acetone) proved essential for complete extraction of the chlorophyll. Extracts were cleared using pressure filtration through glass fibre discs, and the absorbance then measured at 665 nm before and after acidification with HCl. Chlorophyll *a* was calculated using the formula given by Marker (1972), but with a constant derived from a different 'acid' factor; a mean value of 1·85 for the 'acid' factor has been found by us for a wide range of field and laboratory blue-green algae, and this has been used in all the present calculations. (For *Nostoc commune*, the actual value obtained was $1{\cdot}86 \pm 0{\cdot}054$, $n = 200$.) Critical comparison of data from various sources must await a thorough study of the methods used for estimating absolute amounts of chlorophyll *a*. For any given chlorophyll extract, the lower the 'acid' factor used in making calculations, the greater the amount indicated for the chlorophyll *a*, and hence the smaller the rate indicated for C_2H_4 produced per unit chlorophyll *a*.

Laboratory experiments on wetting, drying and re-wetting colonies of type *N. commune* indicated that this procedure had no detectable effect on the levels of chlorophyll *a* extracted (Table 1) or on the ratio of chlorophyll *a* to phaeophytin *a*. The pigment extracts of *Tolypothrix byssoidea* included the brown sheath pigment. As absorbance of this at 665 nm is low, it has been ignored in making estimates of chlorophyll *a*. (Detailed study of variation within this species would, however, probably necessitate the separation of brown pigment from chlorophyll *a*.)

TABLE 1. Effect of drying and re-wetting on amount of chlorophyll *a* extracted from *Nostoc commune*. The twenty colonies chosen all appeared similar visually (range of wet weights among all colonies, 0·344–0·526 g).

	Colonies wetted	Colonies wetted, dried and re-wetted
$\frac{\text{Chl } a}{\text{Wet wt}}$	$1{\cdot}89 \times 10^{-4} \pm 0{\cdot}48 \times 10^{-4}$	$2{\cdot}04 \times 10^{-4} \pm 0{\cdot}57 \times 10^{-4}$

Results

Three main types of field experiment on rates of acetylene reduction were carried out: time course in individual serum bottles in the light; incubation of various communities *in situ* for 1 or 2 h, usually commencing at 1000 h; changes in one particular population during the day. The results of the time course studies indicated that the periods of 1 or 2 h used for the standard assay were suitable. Marked changes in rate during this period of incubation are apparently due mostly to changes in light intensity and/or temperature, rather than anything that might have been caused by incubation in limited volume, such as CO_2 deficiency. It is however not possible to assess the effects of raised temperatures in those cases where the temperature inside the bottle was slightly higher than the ambient temperature (Table 2). The results of the incubations for 1 or 2 h periods are shown in Table 2, and those of the changes taking place during the day in Fig. 2. Estimates of rates of acetylene reduction per unit area are also included in Table 2 for those instances where it was considered that the biomass per unit area in the serum bottles corresponded closely to that in the adjacent natural community.

Although both species and environment varied, the results of the assays summarized in Table 2 show that all the rates for *Nostoc* in the light were quite similar. If the results for all the experiments starting between 0930 and 1230 are pooled, then the rates range only from 0·0239 to 0·0567 nM C_2H_4 μg chl a^{-1} ($\bar{x} = 0{\cdot}0388$, s.d. $= \pm 0{\cdot}0123$). All the rates for *Nostoc* found during the standard morning assays are much higher than those found during the three assays on *Tolypothrix byssoidea* ($\bar{x} = 0{\cdot}00180$ nM C_2H_4 μg chl a min^{-1}).

The two studies on changes in rates during the day (Figs 2a, b) showed that much higher rates occurred in the early afternoon than in the morning, with a maximum rate for *Nostoc commune* of 0·170 nM C_2H_4 μg chl a^{-1} min^{-1}. The rates of fixation in the dark were relatively uniform throughout the day,

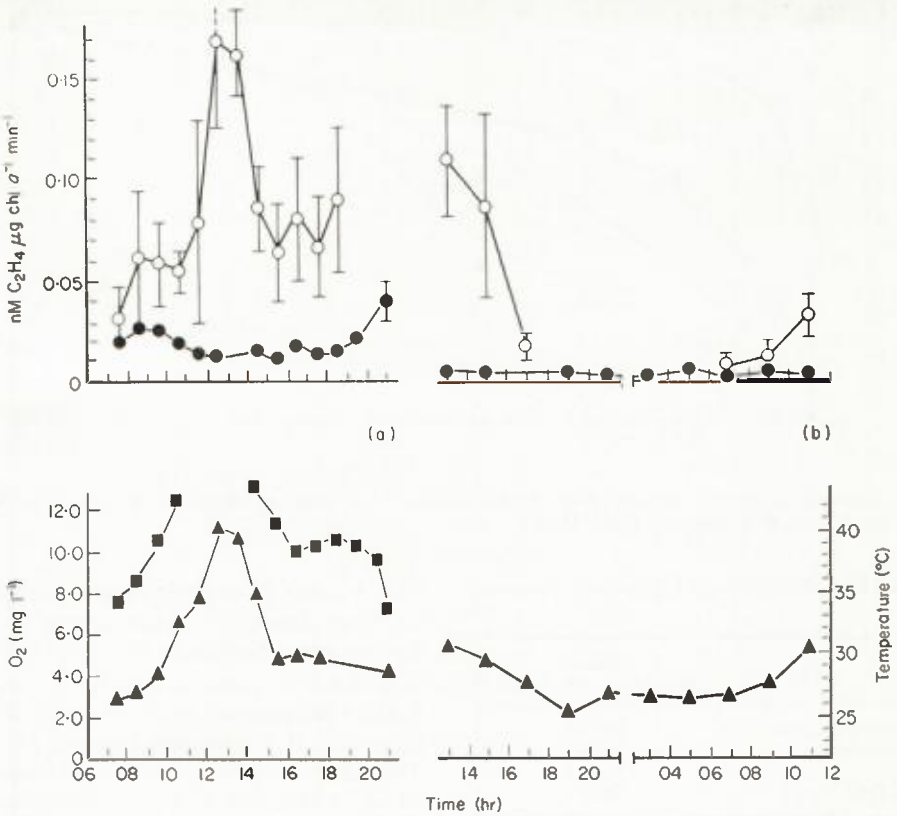

FIG. 2. Changes in rates of acetylene reduction during the day by (a) *Nostoc commune* and (b) *N. sphaericum*, together with changes in ambient temperature and (for a) dissolved oxygen. Rates are plotted for the mid-point of the period during which the assay was made. ○, light; ● dark; ■ dissolved oxygen; ▲ temperature. (Part of the dissolved oxygen curve omitted because the levels of supersaturation were above those for which instrument was calibrated; part of the night-time curve for B not shown because all rates were very low and similar to those at 2300 and 0300 h.)

but there was an increase in the rate of (dark) fixation by *N. commune* in the evening.

Laboratory experiments were carried out on the effects of re-wetting dried *Nostoc* colonies about one year after they had originally been collected on Aldabra. Recently collected *N. commune* from Tarn Moor, England (see Materials and methods) was included as a control. Three types of colonies from Aldabra were used, type *N. commune* (C), *Nostoc–Phormidium* (D) and *N. sphaericum* (F). When dry, colonies of all three were hard and brittle, but within a few minutes of medium being added, they became soft and pliable and had clearly increased in size. It was visually obvious that this response was more rapid in (Aldabra) *N. commune* than in *Nostoc–Phormidium* or *N. sphaericum*. This effect was shown clearly in time course studies (Fig. 3), in which *N. commune* reached 80% saturation in 5 min, whereas the other two types took longer than 1 h to reach this level. Uptake of water by Tarn Moor *N. commune* was also slower than that of Aldabra *N. commune* even when experiments on the former were carried out at 32°C.

All four populations of *Nostoc* showed more or less linear rates of C_2H_4 production during the second day of 2–3 day assays (Fig. 4). However, the behaviour with respect to C_2H_4 production did not correspond to that shown by moisture uptake. There was a much shorter lag in C_2H_4 production by the Tarn Moor *N. commune* (at 20°C) than for any of the Aldabra types (at 32°C), and in contrast with the water uptake results there was no indication that the lag in C_2H_4 production was shorter for Aldabra *N. commune* than for the other Aldabra types. The rates during the linear phases of C_2H_4 production by the four types are summarized in Table 3.

As colonies of *Nostoc* on Aldabra may be subject to marked changes in temperature during the day

rates were found was also 46°C for both the *Nostoc–Phormidium* and the *N. sphaericum* colonies.

(iii) The maximum temperature at which detectable rates were found for Tarn Moor *N. commune* was 38°C. This alga showed quite similar rates of C_2H_4 production over a broad temperature range (22–34°C), and showed no obvious optimum under the conditions of culture used, which included prior incubation at temperatures from 20–32°C.

Discussion

Nostoc communities studied on Aldabra been fully wetted showed high rates of acetylene reduction. There was no obvious indication of differences in rates of reduction in the light between the various types of colony when incubated during the morning. In view of the range of forms and habitats which were sampled, the rates are all remarkably similar. The rates for *Nostoc* are all much higher than those for mature cushions of *Tolypothrix byssoidea*, although the difference is less marked when expressed per unit area rather than per unit chlorophyll. A comparison similar to that between *Nostoc* and mature *Tolypothrix byssoidea* can also be made for the intertidal zone of the lagoon on Aldabra ıere populations of several species such as *Rivularia* sp. have much higher rates than mature mats of *Scytonema* sp. when expressed per unit chlorophyll, but where the difference is less marked when expressed per unit area (Potts & Whitton, 1977).

There was considerable variation during the morning assays in the rates of reduction in the dark as compared with those in the light, but in general the rates of dark reduction, as a percentage of light reduction, were lower for *Nostoc* than found in the survey of intertidal communities. However, it is evident from Fig. 2 that rates of reduction in the light vary so markedly with time of day that it is difficult to make meaningful comparisons without taking measurements throughout the day. The rates of dark reduction found for *N. sphaericum* (Fig. 2b) were very low; it seems likely that this was due to the experiment being carried out several days after the colonies were first collected, when the cellular levels of photosynthate were low. The rise in rate found after dusk (Fig. 2a) is similar to the rise found by Horne (1975) in a stream *Nostoc* from sunny sites just prior to the onset of darkness. As time course experiments were not carried out in the dark within single serum bottles, it is uncertain whether there would be a similar rise in rates over several hours if colonies were transferred to the dark earlier in the day, or whether this effect is apparent only at the end of the daylight period when stored products are at their highest level.

The rates per unit chlorophyll recorded for some of the terrestrial *Nostoc* populations from Aldabra are among the highest recorded in the literature for *in situ* studies of blue-green algae. Comparisons are made difficult by the variety of methods used by different authors and sometimes also lack of information about the time of day, but a few observations on *Nostoc* are summarized in Table 4. In general the rates get less the further away from the tropics, but the data of Alexander (1975) for Devon Is. (Canadian Arctic) indicate a maximum rate almost as high as the maximum recorded for Aldabra. The mean rate found for the Aldabra terrestrial *Nostoc* populations during the morning assays is similar to the maximum recorded for any intertidal communities of the lagoon on Aldabra (Potts & Whitton, 1977), and also to the maximum found for a marine *Nostoc* on Eniwetok Atoll (Mague & Holm-Hansen, 1975). It is not possible to make a direct comparison of rates per unit chlorophyll with that for *Nostoc* in sub-tropical grassland reported by Jones (1977). Rates per unit area can however be compared (Table 2 against Table 4). The afternoon peak rate reported by Jones is about 40% that recorded during the morning for Aldabra *Nostoc* type B, the form probably most similar to that studied by Jones.

It is evident that, at least from terrestrial or semi-aquatic environments, colonies of *Nostoc* may be dried, and re-wetted for use in laboratory experiments long after they were originally collected. The observation that Aldabra *N. commune* initially took up water more rapidly than the other forms corresponds to general observations made on the atoll: the more transitory is the period of wetting for a particular form of *Nostoc*, the more rapidly does it take up water. The lag of up to one day in acetylene reduction shown by the Aldabra colonies assayed in Durham was almost certainly a consequence of long storage. Colonies of *N. commune*, in particular, are subject in the field to frequent cycles of wetting and drying, and the one field experiment showed that about half the probable maximum rate of acetylene reduction occurred after re-wetting for 1 h. In the laboratory assays, the rates after 1 day were of the same order as those found in the field during the morning assays. The results are however not

strictly comparable due to the differing environmental conditions, there being a much lower light intensity and much higher nutrient levels in the laboratory assays.

The facts that at least some forms of *Nostoc* are cosmopolitan, that colonies of *Nostoc* are often free of any other algae, and that these colonies may be dried down for subsequent extraction of chlorophyll and laboratory experiments, all make this alga especially useful for comparative observations on rates of nitrogen fixation in different environments and in different parts of the world. It is suggested that when field studies of nitrogen fixation are carried out in different regions, local populations of *N. commune* should as far as possible always be included as a control, and that samples of this alga should be returned to the laboratory for subsequent comparisons under standard conditions. At the same time it is important to establish just how much variation in rates exists between apparently healthy populations at one site. It seems possible that *Nostoc* colonies are usually associated with rates of fixation that are high for a particular climatic region, and that communities with lower rates of fixation are usually dominated by other species of blue-green algae.

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TABLE 2. Rates of C_2H_4 production by *Nostoc* and *Tolypothrix byssoidea* colonies. For explanation of colony types, numbers of replicates and other experimental details, see Materials and methods. L = light, d = dark

Species	Morphology of colonies	Date	Period of Time incubation (h)		Environmental	Mean temperature (°C)				μg chl *a* cm^{-2}		nM C_2H_4 min^{-1} cm^{-2}		nM C_2H_4		μg chl *a*$^{-1}$ min^{-1}	
						Air or water	Ambient					L	D	L		D	
										$\bar{x}$	*s.d.*	$\bar{x}$	$\bar{x}$	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*
Nostoc commune	C	14.12.72	1000	1	open platin: full light	A				24·7	8·21	0·7830		0·0317	0·0170		
Nostoc commune	E	28.3.73	1000	2	shallow pool (W10)	W	33·8	33·8	33·8					0·0239	0·0097	0·0012	
Nostoc commune	E	29.3.73	1000	1	shallow pool (W10)	W	31·7	31·7	31·7	20·4	3·61	1·133	0·3879	0·0555	0·0102	0·0190	
Nostoc commune	E	1.6.73	1000	2	removed from W10 to open platin	A	34·8	34·8	33·8	84·2	15·80	1·980	1·517	0·0235	0·0089	0·0180	0·0064
Nostoc commune	C: old expanded	1.6.73	1000	2	open platin: full light	A	36·0	37·0	36·0	106	6·07	5·618	0·5507	0·0530	0·0154	0·0052	0·0031
Nostoc commune	C: quite dry before wetting	14.5.75	0900	1	rewetted for 1 h	A								0·0084	0·00074	0·00296	0·00049
Nostoc commune	C	15.5.75	0900	1	same community as 14.5.75	A								0·016	0·00185	0·0006	0·00049
N. commune var. *flagelliforme*	A	12.12.72	1000	1	coconut grove, ground	A	28·0					0·3651		0·0567	0·0067		
flagelliforme	A	28.3.73	0930	2	coconut grove, ground	A	28·3							0·0435	0·0092	0·0001	
flagelliforme	A	28.3.73	1130	2	coconut over *Fimbristylis*	A	33·8							0·0239	0·0092		
flagelliforme	B	1.6.73	1020	2	coconut over ground	A	30·5	30·5	30·5	61·3	21·45	2·879	0·4900	0·0470	0·0115	0·0080	0·0023
N. commune/ *Phormidium*	D	2.6.73	1100	2	open platin: full light	A	35·8	35·4	33·6	281	67·52	9·993	1·011	0·0356	0·0047	0·0036	0·0016
N. sphaericum	F	29.4.73	1000	2	from pool, incubated by Aldabra lab.	W	29·6	30·0	30·0	50·4	6·38	1·638	0·0605	0·0325	0·0132	0·0012	0·0010
Tolypothrix byssoidea		28.3.73	1030	2	pavé	A	34·8							0·00130	0·00160	0·00010	
byssoidea		1·6.73	1000	2	open platin	A	36·0	36·0	36·0	383	55·13	1·409	0·0972	0·00368	0·00133	0·00237	0·00220
byssoidea		7.1.75	1000	1	pavé: 100% cloud	A				77·4	29·2	0·0379	0·0185	0·00049	0·00049	0·00024	0·00012

similar in various colonies inspected. An estimate of the contribution of the *Nostoc* to the total biomass was made by comparing the relative volumes of the *Nostoc* and the *Phormidium*. This was done by measuring filament lengths and average cell width (and hence cross-sectional area) on homogenized material viewed in a haemocytometer cell. *Nostoc* was found to contribute about 70% of the total cell volume. (In order to compare acetylene reduction rates of the *Nostoc* in these colonies with that of the other *Nostoc* colonies, the assumptions are made that the chlorophyll content per unit cell volume is similar for both *Nostoc* and *Phormidium*, and that this *Phormidium* is not reducing acetylene.)

E. Round colonies intermediate between **C** and **F**, associated with pools that are more permanent than those where **C** occurs.

F. *N. sphaericum* Vaucher (Fig. 1B), grows in pools that sometimes hold water for many days.

Cores of *Tolypothrix byssoidea* (Berk.) Kirchner were taken from old cushions of this species overlying limestone pavé. Colonies of *Nostoc commune* from England were used as a comparison in some laboratory experiments. These were taken from the edge of a small, intermittent, calcareous flush at Tarn Moor (Sunbiggin), Cumbria, England. The vicinity of this flush has been described in detail by Holdgate (1955).

Field experiments

The following general assay procedure was used. Colonies were taken which had already been moistened naturally for at least some hours. An amount of alga was taken which gave about the same biomass per unit area inside the bottle as outside it. One ml of rain water was included in each 7 ml serum bottle. The bottles were then sealed and incubated *in situ* for 15 min prior to the addition of acetylene. One ml gas (East African Oxygen Ltd) was injected ($pC_2H_2 = 0{\cdot}17$ kN m^{-2}), 1 ml gas removed to equalize the pressure, and the bottles then incubated for a further 1 or 2 h. 'Dark' controls were obtained by wrapping bottles in foil. Four replicates were used for most assays, but where no standard deviation is shown in Table 2, only 1–2 bottles were used. All the standard experiments except one (Table 2) were carried out on sunny days, with cloud cover never exceeding 50% other than for transitory periods. Three or four temperature measurements were taken at intervals during the experiment in replicate serum bottles including alga; the mean values are given in Table 2. An indication of the ambient environment (air or water) was obtained with a thermometer in an open serum bottle filled with water. Dissolved oxygen measurements were made using a Lakeland Instruments Co. meter with a Mackereth electrode.

At the end of the experimental period, gas samples were removed with multiple-sample vacutainer needles and stored in non-sterile, non-silicone coated, 5 ml draw vacutainers (Potts & Whitton, 1977). The algal colonies were dried carefully under low light and sealed in polythene bags for subsequent extraction of chlorophyll *a*.

Laboratory experiments

Experiments were carried out after return to Durham with various dried materials similar to those used for field assays. Details of the materials are included with the results. Assays in the light were carried out with continuous illumination (warm white fluorescent source, 3000 lx). Experiments on the influence of temperature were carried out on an aluminium block with a temperature gradient from 56° to 20°C, and with a light intensity of 5000 lx. Laboratory acetylene reduction assays were carried out in 250 ml conical flasks with 25 ml medium, $pC_2H_2 = 0{\cdot}17$ kN m^{-2}, and usually three colonies; the flasks were sealed with Suba-seal closures (manufacturer: Freeman, Spaincross, Barnsley). Gas samples were collected by a method similar to that used in the field.

The measurement of water uptake by a dry colony was carried out by immersing the colony in medium and then taking it out again at various intervals for weighing. Excess water was removed by absorbent paper. At the end of the experiment the colony was dried at 105°C to obtain the dry weight. The percentage water content at a particular time is given by:

$$\frac{W_t - W_d}{W_s - W_d} \times 100$$

where W_t = wet weight at time t
W_d = dry weight
W_s = final wet weight

Culture medium

The culture medium was one modified from that of Allen & Arnon (1955), which has proved successful in growing many algae from Aldabra. This medium