THE ECOLOGY OF NITROGEN FIXATION ON SIGNY ISLAND, SOUTH ORKNEY ISLANDS

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ABSTRACT. Rates of biological nitrogen fixation were determined using the ^{15}N technique in a variety of habitats on Signy Island. The biomass of the principal nitrogen-fixing organisms, *Nostoc commune* and *Collema pulposum*, was also determined. It ranged from 28 mg. *Nostoc* – N $m.^{-2}$ and 13 mg. *Collema* – N $m.^{-2}$ in algae-rich sites to 4·5 mg. *Nostoc* – N $m.^{-2}$ and 2·9 mg. *Collema* – N $m.^{-2}$ in algae- and lichen-poor moraine areas. From these determinations it is estimated that *Nostoc* and *Collema* may together fix up to 2·1 mg. N $m.^{-2}$ $yr.^{-1}$ in the more favourable sites, which were bare, wet solifluction areas on gentle south-facing slopes influenced by basic rocks and supplied with melt water of pH 8 to 9 by large snow banks. Low temperatures and desiccation both severely limited nitrogen fixation. Evidence of nitrogen deficiency was found in *Nostoc*, in which the nitrogen content was sometimes as low as 0·5–0·6 per cent of the dry weight. Fixation of nitrogen by fresh-water phytoplankton or benthos was negligible compared with that in wet flushes and soils.

RECENT studies have established that biological nitrogen fixation occurs under Antarctic conditions (Fogg and Stewart, 1968). However, the amount of nitrogen fixed per unit area, its contribution to the environmental budget and the abundance of nitrogen-fixing organisms have not been determined hitherto. The present study, which was carried out on Signy Island, South Orkney Islands (lat. 60°43′S., long. 45°38′W.), over the whole of the snow-free period of the austral summer of 1966–67, had as its aim to measure the amount of nitrogen fixed per unit area and the biomass per unit area of nitrogen-fixing organisms in eight sites with widely differing physical and chemical properties.

METHODS

Lakes

Measurements of nitrogen fixation were carried out only in lake 2 which, being the largest lake on the island, had least chance of contamination of the lake phytoplankton with nitrogen-fixing organisms washed in from the surrounding land. Normal sampling from a boat was impossible during the summer of 1966–67 because of almost continual ice cover too thick for boating but too thin to walk on with safety. Representative samples in deeper parts of the lake from at least five widely separated sites well away from the water's edge were collected either by swimming or by wading into the lake as far as possible and using a Ruttner sampler on the end of a 3 m. long pole. The separate samples were then mixed and aliquots of the mixture used for the measurement of nitrogen fixation.

Nitrogen fixation was determined using a method of *in situ* incubation of the lake samples with $^{15}N_2$ tracer following the removal of atmospheric gases by sparging with a mixture of $O_2/CO_2/Ar$ (25 per cent O_2, 0·04 per cent CO_2, balance Ar). An all-glass apparatus was used and the tracer mixture contained 20 per cent $^{15}N_2$, 0·03 per cent CO_2, 20 per cent O_2, balance Ar. The apparatus was sterilized before each experiment (see Fogg and Horne, 1967; Horne and Fogg, 1970). After an *in situ* incubation period of 24 or 48 hr., 5 ml. of 98 per cent nitrogen-free sulphuric acid was used to terminate biological activity and the water sample reduced, by vacuum distillation at 25° C, to a small volume suitable for transport back to the United Kingdom. After Kjeldahl digestion, the ammonia was separated by steam distillation and the quantity present determined spectrophotometrically using Nessler's reagent. ^{15}N was measured using an AEI MS 20 isotope model mass spectrometer. Samples of lake water not exposed to $^{15}N_2$ but treated identically to the labelled samples in all other respects were used as biological controls of the ratio of $^{14}N/^{15}N$ (masses 28/29) for mass spectrometry. A correction factor of 1·5 was applied to the raw data to allow for the reduced partial pressure of nitrogen (20 per cent) used in the incubations (Stewart, 1967*a*).

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1

Semi-aquatic and terrestrial sites

Nitrogen fixation was determined using $^{15}N_2$ tracer and an *in situ* incubation technique (Fogg and Stewart, 1968). Samples of algae and lichens which were free from significant quantities of other organisms were chosen both at random and from selected algae- and lichen-rich places in each of seven major sites chosen for detailed study. Part of each sample was retained for species and heterocyst determination. Several samples were normally incubated, in separate dishes, in the same flask. Air was replaced by flushing with a gas mixture of $O_2/CO_2/Ar$ (20 per cent O_2, 0·03 per cent CO_2, balance Ar), the final pressure being approximately 0·8 atmospheres. $^{15}N_2$ (96 atom per cent excess) was then introduced to restore atmospheric pressure with a $^{15}N_2$ partial pressure of 0·2 atmospheres. A side arm containing Pardee buffer was used to maintain CO_2 tension at 0·03–0·08 per cent. The temperature inside a dummy flask and that of the vegetation and ground nearby were measured as often as possible. At the end of the incubation period, usually 24 or 48 hr., the samples were placed in separate screw-top bottles and biological activity terminated with 5 ml. of nitrogen-free H_2SO_4. After this they were treated in a similar fashion to the boiled-down lake samples.

Samples of probable nitrogen-fixing algae and lichen not exposed to $^{15}N_2$ were used as biological controls for the $^{14}N/^{15}N$ ratio (mass 28/29). Samples of non-fixing species, such as the alga *Prasiola crispa*, were also exposed to $^{15}N_2$ as controls. This served to test for the presence of nitrogen-fixing organisms such as bacteria which may have been generally distributed over the vegetation.

The raw ^{15}N data were corrected to give the actual *in situ* rates of fixation by multiplying by 1·5 to allow for the reduced partial pressure of $^{15}N_2$ in the same way as for the lake samples. In addition, an approximate correction was made for the difference in temperature between inside and outside of the incubation flask, assuming a Q_{10} value of 6 using the expression:

$$\log R_{t_o} = \log R_{t_i} - 0{\cdot}078\,(t_i - t_o),$$

where t_i and t_o are the mean temperatures inside and outside the flask, R_{t_i} the observed rate of nitrogen fixation and R_{t_o} that *in situ* (Fogg and Stewart, 1968).

Estimation of nitrogen-fixing biomass

The common growth forms of *Nostoc commune* and *Collema pulposum* are brown-black colonies easily visible to the naked eye (Fig. 3). Colonies greater than 1 mm. in diameter were collected from random quadrats on sites 1–4 and 6, and were separated from other algae and debris by washing whilst observing with a binocular microscope. Microscopic examination of soils after the removal of the visible colonies failed to reveal the presence of a significant number of isolated *Nostoc* filaments. It was only possible to collect samples at temperatures above 0° C, because at temperatures below this the frozen algal colonies became brittle and difficult to remove from the surface debris. The locally abundant green alga *Prasiola crispa*, which has a similar gross morphology to *Nostoc*, was collected in a similar manner.

Two sampling methods were used: a series of transects from the sea to the crest of the ridge in sites 1 and 3, and a set of random samples in sites 1, 2, 3 and 6. The samples collected were analysed by the following methods:

i. *Dry weight* of washed samples was determined by drying to constant weight at 105° C.
ii. *Weight of ash* was found after the determination of dry weight; samples were ashed at 550° C.
iii. *Organic nitrogen* was determined on washed samples using either a standard Kjeldahl analysis or a Dumas combustion method using a Coleman 29a automatic nitrogen analyser.
iv. *pH of free water* was determined using a Beckmann pH meter. It was necessary to transport the samples to the laboratory for these readings, but care was taken to avoid long time delays and temperature changes.
v. *Carbon fixation* was measured in the lakes using standard techniques given by Strickland and Parsons (1965). The samples were not incubated *in situ* but at lake temperature at the surface of a large glass tank filled with water, at the British Antarctic Survey station. Incubations were carried out for 6 hr. from noon local time. Particulate matter was

collected by filtration through millipore HA filters which were stored in CO_2-free air prior to radioactive assay. Extra-cellular products were estimated using the method of Watt (1966). Radioactive assay was carried out on a Tracerlab SC 50-B automatic windowless gas-flow counter, and the data were processed using an IBM 1130 computer.

vi. *Pigments* were extracted in 90 per cent acetone using the methods of Strickland and Parsons (1965) and optical densities determined with a Unicam SP 600 spectrophotometer. The quantities of pigment present were calculated using the equations recommended by the SCOR/UNESCO working group 17 (1966).

Sites

Fresh-water lakes

Lake 2 has been described by Heywood (1967, 1968). Apart from the main inflows from lake 5 to the south and from snow-melt seepage, there are two other possible main sources of combined nitrogen: seal excreta in nearby wallows and excreta from birds. There is also some influence of sea spray on the lake but this is unlikely to be of significance to the nitrogen budget.

The lake is oligotrophic with a sparse phytoplankton crop, although some terrestrial algae, especially "snow algae", were washed or blown into the water. At depths down to at least 1 m. there is a carpet of benthic algae, mainly *Phormidium* spp.

Semi-aquatic and terrestrial sites

Previous work by Fogg and Stewart (1968) had indicated that the most productive areas for nitrogen fixation were the small, temporary run-off streams, wet flushes and solifluction tongues where *Nostoc commune* is abundant. Seven adjacent areas (sites 1–7), differing both physically and chemically, and 0·8–3·4 ha. in area, all containing *Nostoc* and delineated by convenient natural boundaries, were chosen for detailed studies (Table I). All the sites chosen were situated in the north-east of the island in an area bounded by Elephant Flats in the south, Stygian Cove in the north, the sea and central ice cap to the east and west, respectively (Fig. 1; Heywood, 1967).

Measurements of nitrogen fixation were made in sites 1–7 and quantitative estimates of the biomass of *Nostoc commune*, *Collema pulposum* and *Prasiola crispa* made in sites 1, 2, 3, 4 and 6.

Results

Nitrogen and carbon fixation in fresh-water lakes

During the summer period 1966–67 it was not possible to carry out more than two sets of measurements because lake 2 became comparatively ice-free for only a few days.

The results obtained are presented in Table II and they show that nitrogen fixation was low and just detectable at the $P = 0{\cdot}05$ significance level. In the light, rates of fixation of 0·009–0·010 per cent day^{-1} (22 March 1967) and 0·008 per cent day^{-1} (29 March 1967) were recorded, whilst a single dark fixation rate of 0·032 per cent day^{-1} was observed on 29 March 1967. The concentration of organic nitrogen in the lake was high for such an unproductive lake, 290 μg. N l.$^{-1}$ and 330 μg. N l.$^{-1}$ being present on 22 and 29 March 1967, respectively. This may be due to organic pollution from nearby seal wallows (Heywood, 1967).

Examination of samples collected at the same time as those for ^{15}N experiments showed the phytoplankton consisted mainly of green coccoid algae and no blue-green algae species bearing heterocysts. The phytoplankton standing crop measured on 29 March 1967 was low, there being only 3·2 mg. Chl. *a.* m.$^{-3}$. Primary production measured between 12.00 and 18.00 hr. on the same day was also low, 0·26 mg. carbon m.$^{-3}$ hr.$^{-1}$ being recorded, of which 0·06 mg. carbon was in the extracellular fraction.

Nitrogen fixation in semi-aquatic and terrestrial areas

The results obtained for *in situ* nitrogen fixation in each of the contrasting sites 1–7 are given in Table III. The positions of these sites on the island are indicated in Fig. 1. Nitrogen fixation,

TABLE I. BRIEF DESCRIPTION OF SITES 1–7 ON SIGNY ISLAND

Site	*Figs. in text*	*Situation*	*Area* (ha.)	*Slope and aspect*	*Surface water conditions*	*Main water source*	*Description of surface*	*Presence of marble or amphibolite*
1	2 and 3	West end of Water-pipe Beach slope	2·4	Gentle South-east	Waterlogged on west; drier on east	Snow melt from upper snow bank	Extensive moss cover with solifluction tongues	Boulders present
2	2	Slight hump in centre of Waterpipe Beach slope	0·8	Gentle South-east	Mainly dry	1. Early season melt from upper snow bank 2. Late season none	Mossy patches with much bare ground	Few boulders present
3	2	East end of Water-pipe Beach slope	1·5	Gentle South-east	Wet	Snow melt from upper snow bank	Moss patches with solifluction tongues and runnels	Not present
4	4	West of Mirounga Flats	1·6	Gentle South-east	Very wet	Snow melt from several snow fields	Extensive moss cover with runnels	Marble ridge overlooking site
5	—	Hillside at south-east end of Three Lakes Valley	n.d.	Steep North-west	A. Dry B. Wet flush	A. None B. Seepage	A. Bare rocks and earth with moss patches B. Wet flushes at base of hill	Present
6	5	South end of Three Lakes Valley	3·4	Gentle North	Very dry; few wet flushes	Snow melt as seepage	Almost bare moraine; large and small boulders	Not present
7	—	Marble Knolls, north of Elephant Flats	n.d.	Various	Mainly dry	Little snow melt	Moss cushions and bare marble slabs	Abundant

n.d. Not determined.

TABLE II. NITROGEN FIXATION IN LAKE 2,* SIGNY ISLAND

Light regime	*Atom per cent excess over biological standard*	*Nitrogen fixed* (μg. $l.^{-1}$ day^{-1} $\times$ 10^3)	*Date 1967*
Light	0·009	32	22 March to 24 March
Light	0·010	42	
Light	0·004	—	29 March to 31 March
Light	0·014	20	
Dark	0·064	114	

* The lake temperature was +0·6° C on both occasions.

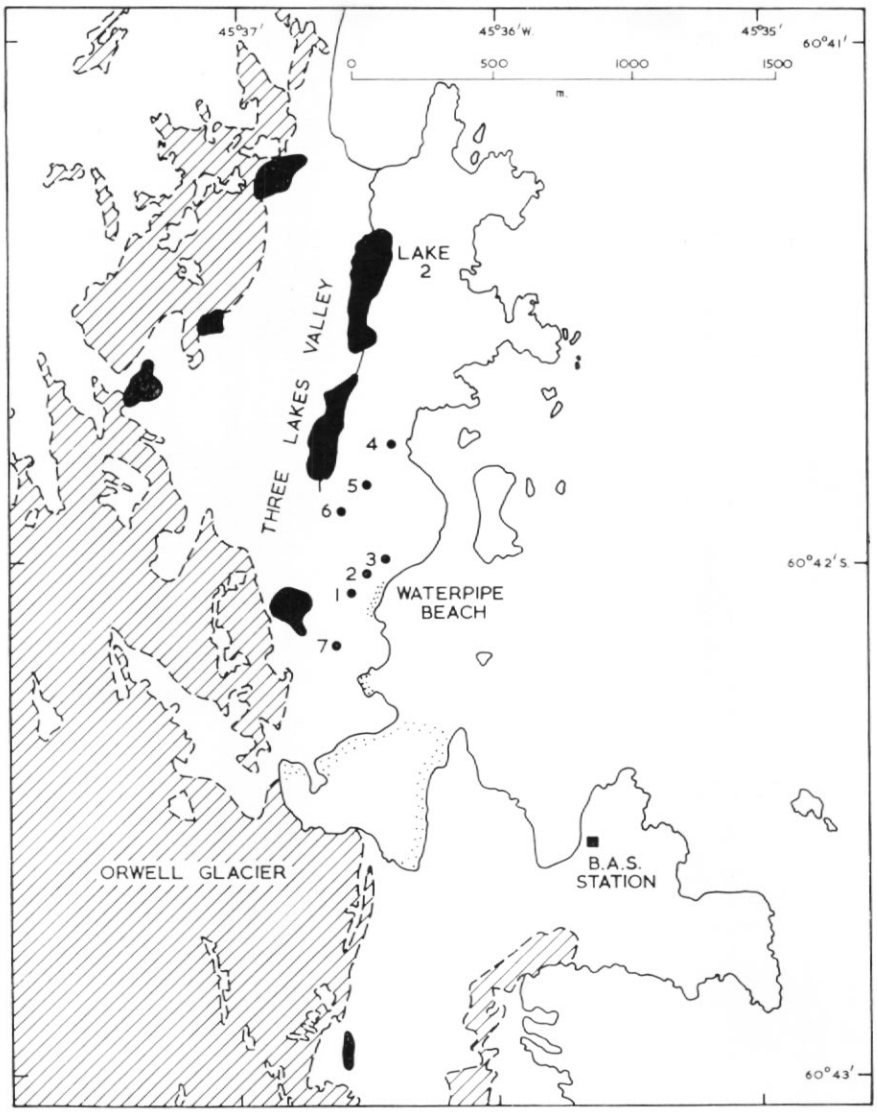

Fig. 1. Sketch map of the area studied on Signy Island, showing the positions of sites 1–7 and lake 2. The dotted lines indicate permanent ice/snow fields; black areas are lakes.

rates of which varied from 0·013 per cent day^{-1} to 0·439 per cent day^{-1}, was associated only with *Nostoc commune*, found either as a free-living organism or as a phycobiont, mainly with the lichen *Collema pulposum*.

Site 1 (Figs. 2 and 3). This was the main site studied and it contained *N. commune*, which was locally abundant, a mean quantity of 25·4 mg. *Nostoc*-N m.$^{-2}$ and a maximum of 60·7 mg. m.$^{-2}$ being recorded. The alga was most often found on the bare tongues of moraine extruded by solifluction from amongst the general moss or rock cover. The solifluction tongues were covered by a slow-moving film of water of variable depth, on which occurred gelatinous clumps of *Nostoc* ranging in diameter from a few millimetres to several centimetres. Lesser concentrations of *Nostoc* were found amongst the moss. *Collema pulposum* occurred less commonly than

TABLE III. NITROGEN FIXATION IN SEMI-AQUATIC AND TERRESTRIAL SITES

Site	Date 1967	Time	Temperature (°C)			Direct sunshine (per cent of daylight hours)	Principal components of sample	Nitrogen fixed		
			Screen	Algal/ground	Flask			(atom per cent excess ^{15}N)	(per cent* day^{-1})	(μg. N $m.^{-2}$ day^{-1})
1	20 February	12.00	+5	+10	+11·5	19	1. *Nostoc commune*	0·1282	0·162	41·2
		15.00	+3	+ 6	+ 6		2. Moss stems growing amongst *Nostoc*	−0·0005	0	—
	21 February	14.00	0	+ 3	+ 3	0				
	28 February	14.00	+1	—	+ 7	0	*Nostoc commune*	0·2263	0·439	113
	1 March	14.00	+2	—	+10	21				
	7 March	15.15	+2	+ 5	+ 5	0	*Nostoc commune*	0·0541	0·116	29·4
1–3	13 March	12.00	−1	+ 3·5	+ 5	15				
		15.00	−1·2	0	0		1. *N. commune* (site 1)	0·1128	0·090	22·5
	14 March	15.15	—	+ 3	+ 2	4	2. Moss (site 1)	0	0	—
		17.15		+ 2	+ 1		3. *N. commune* (site 2)	0·0167	0·014	3·78
	15 March	14.45	—	+ 6	+10	45	4. *N. commune* (site 3)	0·0054	0	—
		15.00	—	+ 5·5	+ 9					
4	28 February	15.15	+1	+ 7	—	0	1. *N. commune*	0·0722	0·116	†
							2. *Collema pulposum*	0·0454	0·074	†
	1 March	15.00	+2	+ 9·5	—	21	3. *Phormidium* spp.	−0·0010	—	—
							4. Moss	0·0011	—	—
5	3 March	15.00	+1	+11·5	+11·5	14	1. *Collema*—dry hillside	−0·0046	0	—
		17.00	+4	+ 5	+ 5		2. *Collema*—wet flush	0·0029	0	—
							3. *Nostoc*—wet flush	0	0	—
	4 March	15.30	—	+ 7	+ 7	0	4. *Stereocaulon*	−0·0041	0	—
							5. Moss	−0·0005	0	—
	13 March	12.30	−1	—	—	15	1. *Collema*—wet flush	0·0260	0·023	†
	14 March	15.00	—	+ 1·5	—	4	2. *Nostoc*—wet flush	0·0478	0·040	†
	15 March	15.00	—	+ 6	—	45	3. *Stereocaulon*—dry hillside	−0·0019	0	—
6	28 February	15.00	+1	+ 7	—	0	1. *Collema*	0·0215	0·038	1·1
	1 March	15.50	+2	+14	—	21	2. Moss	−0·0039	0	—
	13 March	12.30	−1	—	—	15	1. *Collema*	0·0157	0·014	0·41
	14 March	15.00	—	+ 1·5	—	4	2. *Nostoc*	0·0196	0·017	0·76
	15 March	15.00	—	+ 6	—	45				
7	1 March	16.00	+2	+ 9	—	21	1. *Nostoc*—dry	0·0138	0·027	†
	2 March	11.00	—	+ 7	—		2. *Nostoc*—wet	0·0592	0·114	†
		16.00	—	+ 1	—	14	3. Moss	0·0000	0	—
‡	22 February	14.15	+0·5	+ 3	+ 3	17	1. *Peltigera* (?) and *Nostoc*	0·0373	0·013	—
	25 February	12.00	+4	+ 7	+12	62				
	28 February	12.00	—	+ 7	—	0	2. Moss growing amongst *Peltigera* and *Nostoc*	0·0433	0·016	—

* This quantity is defined for example as μg. N fixed per day per 100 μg. algal-N present.
† Values for fixation per unit area could not be calculated accurately due to sparsity of nitrogen-fixing biomass.
‡ Knife Point near the British Antarctic Survey station.

Fig. 2. Sites 1–3 viewed from the south-east above Waterpipe Beach. The many solifluction flushes of site 1 are visible as grey patches in the right foreground near the snow bank.

Fig. 3. Wet flush on site 1 caused by solifluction. A marble boulder is surrounded by abundant *Nostoc commune* (dark glossy blobs) in shallow water.

Nostoc, the average quantity present being 13 mg. *Collema*-N m.$^{-2}$, although 62·5 mg. m.$^{-2}$ was recorded at one point.

Rates of nitrogen fixation for *Nostoc* on site 1 were the highest found on the island, with rates of 0·162 per cent day^{-1} (41·2 μg. N m.$^{-2}$ day^{-1}) on 20 February 1967, soon after the start of the snow melt on this south-east-facing slope. Shortly after this, on 28 February, a maximum rate of nitrogen fixation of 0·439 per cent day^{-1} (113 μg. N m.$^{-2}$ day^{-1}) was recorded.

TABLE IV. RELATIONSHIP OF pH OF SURFACE WATERS IN WET FLUSHES TO *Nostoc commune* ON VARIOUS SITES ON SIGNY ISLAND

Site	*Position*	*Presence of Nostoc commune*	*pH of flush water*	*Date of measurement 1967*
1	Upper flush	+	9·0	2 March
	Centre flush	−	7·5	
	Bottom flush	+	8·7	
3	Upper flush	−	7·6	3 March
	Centre flush a	−	7·0	
	Centre flush b	+	7·6	
	Centre flush c	+	6·4	
4	*Nostoc* pool	+	6·7	7 March
	Collema runnel	+	6·6	
	Prasiola pool	−	6·4	
	Runnel	−	6·5	
	Phormidium runnel	−	6·7	
6	Main stream near lake	−	6·0	6 March
	Stony flush	+	7·6	
	Moss flush	+	8·4	
Spindrift Rocks on west coast	*Nostoc* flushes	+	6·7	9 March
	Stream in moss	−	6·1	
	Nostoc flush	+	7·0	
	Bare *Collema* flush	+	6·7	
	Moss bank	−	4·2	
Moraine east of Jane Peak	Stream flowing into lake 6	−	6·7	9 March
Slope on west of island above Foca Point	*Collema* flush	+	5·6	14 March
	Collema runnel	+	5·8	
	Stream	−	6·5	

As can be seen in Table IV, the wet nitrogen-fixing areas of this site were alkaline with pH values of 8·7–9·0, while the non-fixing areas had a lower pH of 7·5.

Site 2 (Fig. 2). Here, nitrogen fixation attributable to *Nostoc* was low, a rate of 0·014 per cent day^{-1} being recorded in mid-March. In terms of fixation per unit area, however, fixation was relatively high (3·8 μg. N m.$^{-2}$ day^{-1}) due to the abundance of *Nostoc* on this site (26·8 mg. *Nostoc*-N m.$^{-2}$). Site 2 was on the eastern border of site 1 and had relatively good drainage, which kept the algae in a desiccated state for most of the snow-free season, with the result that nitrogen fixation was much reduced.

Nostoc and Collema occurred frequently on the lower barer ground, but they became less abundant towards the top of the slope where more moss cover occurred.

Site 3 (Fig. 2). No significant nitrogen fixation was found in this site, although there were considerable quantities of potential nitrogen-fixing organisms (28 mg. *Nostoc*-N m.$^{-2}$ and approximately 7 mg. *Collema*-N m.$^{-2}$). However, the *Nostoc* present on this site immediately east of site 2 was unusual in the low heterocyst frequency. As heterocysts are probably the site of nitrogen fixation, this may explain the lack of fixation (Stewart and others, 1969). Also, the *Nostoc* occurred in much larger, more gelatinous clumps than on other sites. The alga was

found in waterlogged situations such as small puddles and runnels where the pH was 6·4–7·6, and was almost absent elsewhere.

Site 4 (Fig. 4). Fixation rates of 0·116 per cent day^{-1} for *Nostoc* and 0·074 per cent day^{-1} for *Collema* were recorded in early March in this very wet site which contained little *Nostoc* amidst the almost complete cover of moss. There were a few shallow semi-permanent water channels, on the beds of which were luxuriant growths of *Phormidium* spp., with *Nostoc* and *Collema* only locally common. The few small pools present occasionally contained *Nostoc* but more usually *Prasiola crispa*. Because of the small amounts and irregular distribution of nitrogen-fixing species, no estimate of fixation per unit area was attempted. It is of interest that the pH of the water was low, 6·6–6·7 being recorded for water containing *Nostoc* and/or *Collema*.

Site 5. Site 5A on the steep upper slope of a hill overlooking Three Lakes Valley was dry and exposed, and consisted of mats of moss with pieces of marble, amongst which were infrequent *Collema* colonies. Site B at the foot of the hill included numerous wet flushes, some of which contained *Nostoc* and *Collema*. No fixation was recorded for *Nostoc* or *Collema* in experiments carried out at sites 5A or B on 3 March 1967; but on 13 March the amounts of nitrogen fixed in the wet site 5B were 0·040 per cent day^{-1} for *Nostoc* and 0·023 per cent day^{-1} for *Collema*. The sparsity of the *Nostoc*/*Collema* cover precluded any estimation of fixation on a unit area basis.

Site 6 (Fig. 5). The site was a north-facing, almost bare moraine sloping gently down to a moss-covered area at the southern end of Three Lakes Valley. Its upper edge, dividing it from sites 1–3, was partially covered with moss but this merged with the bare scree and a snow bank. A small stable boulder scree showed distinct solifluction stripes with damp flushes containing the only vegetation in this area. 4·5 mg. N m.$^{-2}$ was recorded as the *Nostoc* biomass and approximately 3 mg. N m.$^{-2}$ for the biomass of *Collema*.

The amounts of nitrogen fixed were 0·038 per cent day^{-1} on 28 February 1967 and 0·014 per cent day^{-1} on 13 March for *Collema*, and 0·017 per cent day^{-1} for *Nostoc* on 13–15 March. This corresponds to values of nitrogen fixation of 1·1 and 0·41 μg. N m.$^{-2}$ day^{-1} for *Collema* and 0·76 μg. N m.$^{-2}$ day^{-1} for *Nostoc*.

The pH of the wet flush was 8·4 where *Nostoc* and *Collema* were common but lower elsewhere (Table IV).

Site 7. This was an area of marble knolls, generally marble slabs with some moss cover, and contained a few small hollows with abundant growth of *Nostoc* but no *Collema*. A relatively high fixation rate of 0·114 per cent day^{-1} was recorded on 1 March 1967, but fixation was low in terms of fixation per unit area due to low algal abundance. The quantity of nitrogen fixed in a season was also low on this site because algal desiccation occurred quickly in this well-drained area. Table III shows the much lower rates of nitrogen fixation given by algae in a naturally dry state, even in an atmosphere of 100 per cent relative humidity. On site 7, *in situ* nitrogen fixation by naturally dry *Nostoc* was 24 per cent of that fixed by wet *Nostoc*.

Other variables: Temperatures observed during active nitrogen fixation varied in range from −1·2° C to +5° C (air temperatures) and from 0° C to 14° C (algal/ground temperatures) (Table III). The highest algal temperatures occurred on the north-facing sites 5A and 6, and the greatest range of temperatures in the dry sites such as 5A. The results presented in Table III show that there was little relation between meteorological (screen) air temperature and algal temperature. Continuously recording thermometers were not used and thus insufficient readings of algal temperature were taken to enable the precise relationship of temperature and *in situ* nitrogen fixation to be ascertained. In general, *in situ* fixation occurred when the algal temperature remained above +1° C for considerable periods, a conclusion in agreement with the earlier study of Fogg and Stewart (1968).

Direct sunshine appeared to have little effect on nitrogen fixation in these sites (Table III). In the drier sites, direct sunshine produced desiccation, and in the wet sites temperature increases due to absorption of solar radiation were mitigated by the cold surrounding water.

The pH of several of the wet flushes where nitrogen fixation occurred is given in Table IV. These measurements were possible only on sites 1–4 and 6, and an area on the west coast of the island where there was standing water. It can be seen that, although *Nostoc* was present and fixed nitrogen at a pH as low as 6·6, rates were low, and high fixation rates were associated with pH 8·7–9·0.

TABLE V. BIOMASS OF POTENTIAL NITROGEN-FIXING ALGAE AND LICHENS

Site	Species	*Biomass as organic nitrogen*			*Biomass as dry or ash-free dry weight*				*Nitrogen as a percentage of:*	
		N (mg. $m.^{-2}$)	*Number of samples collected*	*Number of samples containing Nostoc/Collema*	*Dry weight* (g. $m.^{-2}$)	*Ash-free dry weight* (g. $m.^{-2}$)	*Number of samples collected*	*Number of samples containing Nostoc/Collema*	*Dry weight*	*Ash-free dry weight*
1	*Nostoc*	25·4	10	9	4·19	2·50	70	55	0·61	1·02
	Collema	13·2	10	6	0·48	0·26	69	19	2·8	5·1
2	*Nostoc*	26·8	10	10	n.d.	n.d.	—	—	—	—
	Collema	11·4	10	8	n.d.	n.d.	—	—	—	—
3	*Nostoc*	28·0	10	7	5·78	4·69	14	9	0·48	0·60
	Collema	7·0	10	1	0·51	0·31	14	1	1·4	2·3
6	*Nostoc*	4·5	10	4	n.d.	n.d.	—	—	—	—
	Collema	2·9	10	1	n.d.	n.d.	—	—	—	—

n.d. Not determined.

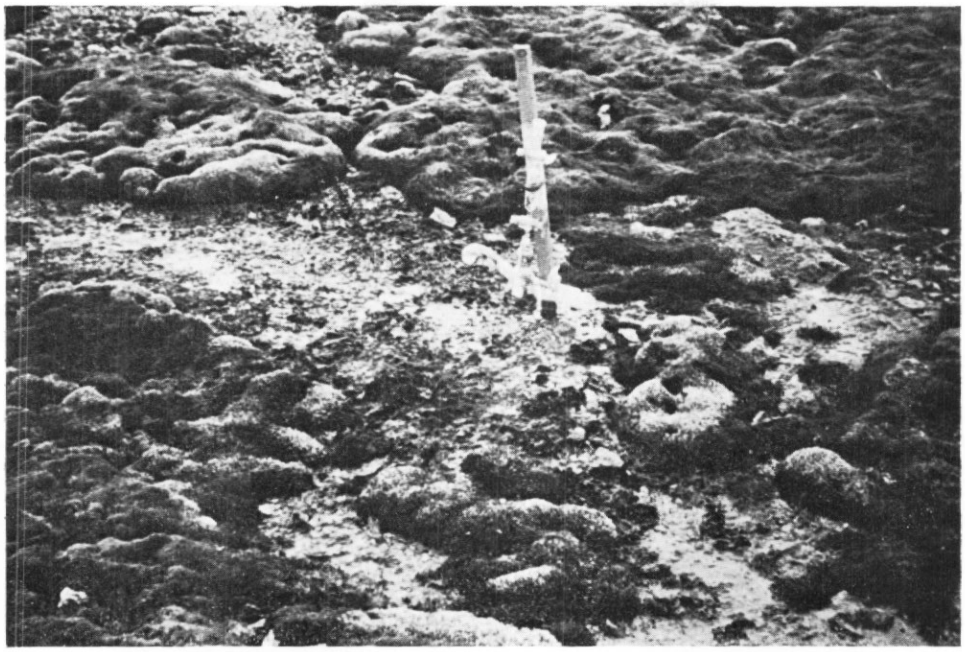

Fig. 4. Wet runnel on site 4 showing experimental flask. *Nostoc* and *Collema* are visible in the lower part of the runnel.

Fig. 5. A view of site 6 on the moraine at the south end of Three Lakes Valley. A flush is present at the right centre of the photograph, as indicated by a short pole. Very little vegetation is present.

Amounts of nitrogen-fixing biomass

Considerable quantities of algal/lichen nitrogen were found in sites 1–3 which contained many bare, wet solifluction tongues rich in *Nostoc*. Smaller amounts of *Nostoc* were also found amongst mosses growing near these flushes. Values of 25·4–28·0 mg. N m.$^{-2}$ were found for *Nostoc commune* and 7–13·2 mg. N m.$^{-2}$ for *Collema pulposum* in these sites.

It is of interest to note that *Prasiola crispa* was found in significant amounts on only two occasions in sites 1–7. Out of ten random quadrats in each site, *Prasiola* was found in single quadrats in sites 1 and 2, in small amounts in sites 3 and 4, but it was never observed in the drier sites 5, 6 or 7.

Distribution of nitrogen-fixing organisms relative to their immediate physical surroundings

The results of biomass determinations are summarized in Table V. Records of the detailed data for individual quadrats have been deposited with the British Antarctic Survey Botany Section. These detailed results show only one clear correlation: that of *Nostoc* with bare wet flushes which are formed by recent solifluction. A slight zonation was apparent in the distribution of *Collema* with distance from the sea (or up the slope). More *Collema* was found in the upper drier parts of the site.

It was not possible on all occasions to distinguish between some of the forms of *Nostoc* and *Collema*. Immediately following the melt on sites 1–3 (2–9 February 1967) there was little difficulty in recognizing the well-developed *Collema* or the large older clumps of *Nostoc*. At this time there were large numbers of small balls of *Nostoc*, filaments of 2–30 cells being embedded in firm mucilage. By early March, however, small balls were less common and larger flatter clumps of *Nostoc* filaments embedded in comparatively little mucilage were abundant. These algal masses were penetrated to varying degrees by fungal hyphae, some to an insignificant extent but others to such a degree that the algal filaments had broken into small groups of 2–8 cells and fungal hyphae occupied most of the available space within the mucilage. It is possible that the small balls found early in the season were new growth of *Nostoc*, and that the fungal attack occurring later in the season was a stage in the formation of *Collema pulposum*. In estimating nitrogen-fixing biomass, only definite examples of *Collema* with distinct layers of algal and fungal cells were counted as lichen. All other samples were regarded as *Nostoc*, and thus it is possible that the biomass of *Collema* may have been underestimated relative to that of *Nostoc*.

Nitrogen content of nitrogen-fixing species

The nitrogen content of *Nostoc*, expressed as a percentage of dry weight, was low, 0·48, in site 3 and only slightly higher, 0·61, in site 1 (Table V). In contrast, the nitrogen content of *Collema* in site 3 was higher but still below average at 1·4 per cent, while that in site 1, 2·8 per cent was in the range normally found for lichens.

Discussion

Nitrogen fixation in the fresh-water lakes of Signy Island was negligible compared with that in the wet flushes, small runnels and puddles containing *Nostoc commune* and *Collema pulposum*. Nitrogen-fixing algae were absent from the plankton with the exception of *N. commune*, which was found infrequently and was probably washed in from the surrounding drainage area. Also during the austral summer 1966–67 conditions favourable for lake nitrogen fixation did not occur because the lake temperature never rose above +0·6° C and ice and snow cover persisted for most of the period. Conditions which are conducive to blue-green algal nitrogen fixation in temperate lakes have been discussed by Horne and Fogg (1970), who showed that it was directly correlated to concentrations of dissolved organic nitrogen. In lake 2, although dissolved organic nitrogen concentrations were high, the low ambient temperatures probably limited nitrogen fixation. This contrasts with the situation in shallow Arctic lakes (Table VI), which, although cold in winter, are often as warm as temperate lakes in summer and thus not comparable with the larger Signy Island lakes where temperatures greater than +4° C are rarely achieved. Nevertheless, the rates of fixation in lake 2 were of a similar magnitude to those found in Upper Jennifer Lake, Alaska (Dugdale and Guillard, 1966).

TABLE VI. NITROGEN FIXATION IN POLAR LAKES

Lake	*Estimated mean depth (z)* (m.)	*Estimated area (A)* (ha.)	*Temperature* (°C)		*Chlorophyll a* (μg. l.$^{-1}$)	*Probable major nitrogen-fixing algae*	*Nitrogen fixed* (μg. N l.$^{-1}$ day^{-1})	*Reference*
			Lake	*Sample incubation*				
Upper Jennifer Lake (Alaska)	10·4	44	6–12	6–12	5	*Anabaena* sp. (phytoplankton)	0·04 0·007	Dugdale and Guillard, 1966 Dugdale and Dugdale, 1961
Paul's Pond (Point Barrow; lat. 71°14′N., long. 156°38′W.)	0·10–0·15	0·03	5–8*	5–8*	—	*Nostoc* sp. (benthic)	3·34	Dugdale and Toetz, 1961
Bill's Pond (Alaska; lat. 71°19′N., long. 156°31′W.)	1	0·04	5–8*	5–8*	—	Phytoplankton	0·20	Dugdale and Toetz, 1961
Ikroavik Lake (lat. 71°13·5′N., long. 156°38′W.)	2·2	400	5–8*	5–8*	—	Phytoplankton	0·112	Dugdale and Toetz, 1961
Lake 2, Signy Island	2·0	4·1	0·6	0·6 (*in situ*)	3·6	*Nostoc commune* (?) (phytoplankton)	0·05	Present study
Lake 5, Signy Island	1–2	3·3	4–5·5	14–18·5 (laboratory)	—	*Phormidium* spp. (benthos)	0	Fogg and Stewart, 1968
Little Kitoi Lake (Alaska)	9–10	42	5–8*	5–8*	—	*Anabaena* sp. (?) (phytoplankton)	0·007	Dugdale and Dugdale, 1961
Smith Lake (Alaska; lat. 64°52′N., long. 146°52′W.)	2·0	1·6	15–20	15–20	0–130	*Anabaena flos-aquae* (phytoplankton)	1–3† (μg. N l.$^{-1}$ hr.$^{-1}$)	Billaud (Dugdale), 1967

* Estimated from unpublished data in personal communication from V. A. Alexander (Dugdale).
† Average ranges from two summers.

The annual rate of fixation by semi-aquatic and terrestrial algae as estimated from the findings for site 1, the most extensively studied, was 1·8 mg. N $m.^{-2}$ $yr.^{-1}$. Considering the short time when nitrogen fixation was possible, this was a high rate of fixation in such an inhospitable environment, but nevertheless it was much lower than annual rates of fixation by other free-living micro-organisms due to the relative sparsity of the algal cover. Stewart (1966), reviewing work by many authors, listed various blue-green algae having fixation rates of 3–90 kg. N $ha.^{-1}$ $yr.^{-1}$ (= 300–9,000 mg. $m.^{-2}$ $yr.^{-1}$), and 42–720 kg. N $ha.^{-1}$ $yr.^{-1}$ (= 4,200–72,000 mg. m^{-2} $yr.^{-1}$) for (possibly) free-living soil micro-organisms. However, many of these rates were derived from studies on crop yield or average nitrogen increases which were carried out without the benefit of modern isotopic tracer techniques and may not be true estimates of biological nitrogen fixation. In other cases, studies were made on small areas specially selected for their rich algal growth (Mayland and others, 1966). The limitations of extrapolating values of nitrogen fixation per few square centimetres to larger areas have been discussed (Stewart, 1967*a*) who pointed out the heterogeneity, in space and time, of blue-green algae in such areas as dune slacks. In this study the choice of large site areas, the use of $^{15}N_2$ to measure nitrogen fixation directly, and the method of random collection of samples ensure that values for nitrogen fixed are typical for the areas sampled.

My studies were carried out during the austral summer of 1966–67, which was unusual in the late persistence of snow cover and sea ice. The period for which the *Nostoc*-rich sites 1–3 were free from thick snow/ice cover began after a quick melt between 2 and 9 February 1967 and ended in mid-April. This period of only 60–70 days was considerably shorter than the more normal summer in the previous year. The average air temperature for May 1967 was −8·4° C and this, together with the short daylight period, reduces the likelihood that significant nitrogen fixation occurred outside February to April in sites 1–3 because of the low temperatures and low light intensities under the thick snow cover.

Where simultaneous experiments were carried out on several sites, it was possible to estimate the annual nitrogen-fixation rates from relatively few estimations by comparison with the well-studied area, site 1. These estimates are given in Table VII, which includes data from other workers for comparison. Nitrogen fixation by sites 4, 5 and 7, which have a sparse and irregular distribution of nitrogen-fixing organisms, could not be determined accurately from this study, but fixation rates were certainly much less than at the other sites.

TABLE VII. ESTIMATED ANNUAL NITROGEN FIXATION AND BIOMASS INCREASE DUE TO FIXATION FOR CONTRASTING AREAS OF SIGNY ISLAND AND SOME OTHER AREAS

Site	*Nitrogen fixed* (mg. N $m.^{-2}$ $yr.^{-1}$)	*Main fixing species*	*Annual increase in biomass* (per cent)	*Reference*
1	1·8 0·3	*N. commune* *C. pulposum*	7·1 2·3	Present study
2	0·3 0·09	*N. commune* *C. pulposum*	1·1 0·8	Present study
3	0 0	*N. commune* *C. pulposum*	0 0	Present study
6	0·08 0·03	*N. commune* *C. pulposum*	1·8 1·0	Present study
Intertidal rocky shore (United Kingdom)	2,500	*Calothrix scopulorum*	up to 41	Stewart, 1967*a*
Semi-arid soils (Arizona, U.S.A.)	40*	*Nostoc* *Scytonema* *Anabaena*	70†	Mayland and others, 1966

* Estimated indirectly and neglecting precipitation.
† Estimated under field-simulated conditions.

Although the sites on which the initial study was made in 1965–66 were not all re-investigated in 1966–67, the most productive site of the 1965–66 study—near Waterpipe Beach (site 3 of Fogg and Stewart (1968))—corresponds to sites 1–3 of the present study. Fogg and Stewart reported a rate of nitrogen fixation of 0·047 per cent day^{-1} in early February for old *Nostoc commune* in this area, whilst the present study gave rates of 0·162, 0·439, 0·116 and 0·090 per cent day^{-1} for mature *N. commune* in the Waterpipe Beach area between mid-February and mid-March. The 1965–66 melt in this area was much earlier than in 1966–67 and thus by February 1966 the area was probably drier and less suitable for high rates of nitrogen fixation. Allowing for this, the values given by Fogg and Stewart (1968) are similar to those found in 1966–67. This suggests that the detailed nitrogen productivity measurements given in this paper are reasonably close to typical annual figures for the island and that annual production of biologically fixed nitrogen shows no large annual variations.

The results presented in this study demonstrate that the two principal nitrogen-fixing organisms on Signy Island, *Nostoc commune* and *Collema pulposum*, contribute significant amounts of *new* nitrogen to the environment in certain areas. The absolute amounts are low compared with that contributed by blue-green algae in the littoral region of temperate seas (Stewart, 1967*a*), for example. It should be borne in mind, however, that in the immediate vicinity of *Nostoc*-rich solifluction flushes nitrogen fixation per unit area will be many times greater than the average values for whole areas and with consequent greater local effect on the nitrogen balance.

In terms of increase in nitrogen biomass, nitrogen fixation increased *Nostoc*-nitrogen by approximately 7 per cent yr.$^{-1}$ and *Collema*-nitrogen by 2·3 per cent in the most favourable area (site 1) in an algal growing season which in 1966–67 probably did not exceed 60 days. In site 2, less favourable for nitrogen fixation but containing similar amounts of *Nostoc* and *Collema*, nitrogen fixation accounted for only 1·1 per cent yr.$^{-1}$ increase in *Nostoc*-nitrogen and 0·8 per cent for *Collema*-nitrogen. In site 3, where there was a large amount of *Nostoc* and a moderate amount of *Collema*, N_2 fixation was insignificant in increasing plant nitrogen. It is of interest that in the almost bare moraine area (site 6) nitrogen fixation increased the nitrogen biomass by as much as 1·8 per cent yr.$^{-1}$ for *Nostoc* and 1 per cent for *Collema*. This is probably due to the sunny aspect of this site combined with good water supply in 1966–67. The spots where nitrogen-fixing organisms grew on this site were few in number but they were generally suitable for nitrogen fixation. Due to the instability of the moraine soil, however, nitrogen-fixing organisms were unable to build up a large biomass over several seasons.

Holdgate and others (1967) have shown available nitrogen may be the only major nutrient likely to limit plant growth on Signy Island, although Allen and Northover (1967) found the supply of principal nutrients was well in excess of the requirements of the island's flora. Heywood (1968) has shown high nitrate concentrations in the inflow streams of lakes 2 and 5, which flow through moss stands and *Nostoc*-rich flushes, while an inflow stream of lake 6 flowing over bare scree had a low nitrate concentration. Experiments carried out during the present study in areas to which Heywood referred show significant fixation of nitrogen by *Nostoc* and *Collema* which may at least partially account for the high nitrate concentrations in such streams. Similar phenomena have been reported for streams passing through nitrogen-fixing *Alnus* groves in Alaska (Goldman, 1960, 1961; Dugdale and Dugdale, 1961; Dugdale and others, 1966). The last-mentioned authors proposed that biologically fixed nitrogen gives rise, presumably indirectly, to ammonia which is relatively immobile in the soil but once nitrified becomes highly mobile and moves quickly to the streams as nitrate. It has been suggested that the increases in nitrate and phosphate concentrations after streams have passed through moss on Signy Island are due to the washing off of materials deposited there by nutrient-laden aerosols originating from quite distant seal wallows or bird colonies (Allen and Northover, 1967; Heywood, 1968). Samples taken on the same day by Heywood (1968) showed 0·89 p.p.m. nitrate in water flowing through moss, compared with 0·08 p.p.m. in an adjacent stream flowing over bare scree. Phosphates, however, showed only a very small increase from 0·001 p.p.m. for the stream on bare scree compared with 0·002 p.p.m. for the stream flowing through moss. As the aerosols are presumed to carry both nitrate and phosphate (Allen and Northover, 1967; Heywood, 1968), it is not proven that they constitute an important nutrient input in the areas studied in this paper, although nearer the wallows a different situation may occur.

However, Hutchinson and Viets (1969) have detected ecologically significant quantities of ammonia at distances of several kilometres from the cattle feedlots from which ammonia was volatilized. The ammonia loss from animal excreta, the possibility of its absorption on acid surfaces and subsequent nitrification could produce an overall increase in nitrate as well as larger increases in very acid peats or mosses.

In view of the large number of proposed but quantitatively undetermined nitrogen sources in the Signy Island drainage basins, the precise role of *Nostoc commune* in the nitrogen budget cannot be fully assessed. It can be stated, however, that *Nostoc commune* and *Collema pulposum* which fixed up to 2·1 mg. N $m.^{-2}$ $yr.^{-1}$ of *new* nitrogen into the environment would have had a disproportionate effect on nearby plant growth if nitrogen was limited as suggested by Holdgate and others (1967).

As can be seen from Table V, the *Nostoc* but not the *Collema* from sites 1 and 3 is unusual in its very low nitrogen content expressed as a percentage of dry weight. This low percentage can be used as a measure of low total available nitrogen in the environment which includes NH_4^+, NO_2^-, amino-N and organic-N as well as the more commonly measured NO_3^- (Thomas and Krauss, 1955; Fitzgerald, 1969). The low percentage of nitrogen in this alga provides the evidence that nitrogen is a limiting nutrient in this area.

The cycling of nitrogen in the Antarctic environment has been little studied, but observed grazing on a colony of *N. commune* by what was probably a testate amoeba or a naked rhizopod suggests that animals may benefit from the nitrogen fixed. Although grazing was observed on only one occasion, the microscopically visible effects of presumably similar predation were not uncommon. Heal (1965), in a study of testate amoeba from Signy Island, has recorded *Corythion dubium*, *Phrynganella hemispherica*, *Trinema lineare* and *T. enchelys* from counts of samples from a soil similar to that which commonly supports growth of *N. commune* and/or *C. pulposum*, namely a granular marble soil with little or no vegetation, pH 6·0–8·5. He recorded many more species from other soil types on the island, most of which are adjacent to or amongst areas containing nitrogen-fixing organisms. Some testate amoeba can feed on a variety of algal species (Heal, 1964) either by direct ingestion or by pseudopodial secretion of extracellular enzymes, the products of whose activity can then be ingested. Almost inevitably some loss of originally algal cellular material will occur and this would then be available in the surrounding environment. Similar losses when microbivorous testate amoeba were present on a much decomposed cellulose film have been studied by Tribe (1961), who also noted nitrogenous excretion by testate amoeba (Tribe, 1964). Such releases of combined nitrogen would produce a much quicker turnover of nitrogen from N_2 to non-fixing organisms than would normal death and bacterial and fungal decay, a slow process at the low temperatures experienced in Antarctica.

It is known that nitrogen fixed by the phycobiont in some lichens is passed to the fungal partner (Scott, 1956; Millbank and Kershaw, 1969) and this presumably occurs in *C. pulposum*. Lichen growth and decay in Antarctic conditions is slow and release of nitrogenous material presumably also slow. In the areas studied on Signy Island, *N. commune* is more abundant than *C. pulposum* in terms of biomass and it is more likely to contribute nitrogen directly to the environment.

One likely method, apart from the grazing mentioned above, involves the extracellular release of nitrogenous material by *N. commune*. In a study of the extracellular products of a pure culture of the marine blue-green alga *Calothrix scopulorum*, Jones and Stewart (1969*a*, *b*) showed that large percentages of cellular nitrogen in various forms were excreted into the surrounding medium. Furthermore, they showed that the percentage excretion was higher when pH, temperature, light or ionic conditions were not optimum. This is important because in the natural environment non-optimum conditions are the rule rather than the exception. It has also been shown that nitrogenous extracellular products of blue-green algae can be assimilated by a variety of mosses, fungi, bacteria or other algae both in culture and *in situ* (Mayland and others, 1966; Stewart, 1967*b*; Jones and Stewart, 1969*b*). Moss growing among *Nostoc* on Signy Island became labelled with $^{15}N_2$ after an exposure period of 6 days but it was not possible to be totally certain that this was due to assimilation of algal extracellular products.

Apart from the length of the snow-free season, the most important influences on the rates of nitrogen fixation on Signy Island were temperature and presence of liquid water. During the 3 summer months (January to March) the mean air temperature was −0·5° C, but algal/ground

temperatures were often several degrees above air temperature (Table III). This was due to solar heating which occurred with wet algae despite the generally cloudy conditions, although the increases in temperature above ambient were much less than those recorded with moss cushions (Longton and Holdgate, 1967). Significant nitrogen fixation probably started when algal temperatures were greater than +1° C (Fogg and Stewart, 1968), so that for considerable parts of the summer day low air temperatures did not prevent the occurrence of some fixation. Fixation may be possible at temperatures below 0° C. It has been shown that nitrogen fixation monitored as acetylene reduction occurs at 0° C or below in the natural populations of the marine alga *C. scopulorum* (personal communication from R. Robinson). Furthermore, photosynthesis by lichens has been shown to occur at temperatures as low as −18° C (Ahmadjian, 1970). However, at prolonged temperatures less than 0° C, lack of liquid water probably limits fixation rather than low-temperature inactivation of the nitrogenase enzyme system.

Desiccation was of greater importance than temperature *per se*. The *Nostoc* on Signy Island in contact with water had a rubbery consistency and in this condition fixed much more nitrogen than when in a dry, friable form, even if this was in an atmosphere saturated with water vapour. This suggests that most of the *in situ* nitrogen fixation occurs when liquid water is present. However, precipitation on Signy Island is rather low (40 cm. $yr.^{-1}$) and falls mainly as snow or mist droplets rather than as rain. With the exception of a rare heavy rain shower and the brief period of the melt when the snow cover still provides water but does not prevent light penetration, most of the land was short of water even when temperatures were high enough to provide this in liquid form. Since almost all the water came from a frozen store, a favourable solar-warmed aspect was unlikely to co-exist for long with a supply of liquid water. In sites on the north-facing slopes, such as 5a and 6, higher temperature will have the effect of increasing the rate of fixation but reducing its duration, due to desiccation.

A consideration of the adjacent sites 1–3 (Tables III) shows that, although site 1 had an expected higher rate of fixation than either the drier site 2 or the less alkaline site 3, the amounts of *Nostoc*-nitrogen present in each site were almost identical, while those of *Collema*-nitrogen were not. This may be explained by the ubiquitous distribution of the algae, but not the lichen, in these sites. *Nostoc* was found in abundance on bare, wet solifluction tongues but was present in almost every sample taken from sites 1 and 3 (Table V), whilst *Collema* was not. This was almost certainly due to the distribution of algal fragments by the wind, a phenomenon often observed on careful study. *Collema*, being attached to the substrate, was not subject to wind disturbances to the same extent and its distribution may reflect the favourability of site 1 relative to sites 2 and 3 for nitrogen-fixing organisms.

The conclusions of Fogg and Stewart (1968), that nitrogen-fixing organisms occurred only where there was an influence of basic rock, were confirmed in this study. However, the pH of the surface waters seems to be the directly important factor.

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