IN SITU DETERMINATIONS OF BIOLOGICAL NITROGEN FIXATION IN ANTARCTICA

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ABSTRACT. Nitrogen fixation has been demonstrated *in situ* on Signy Island, South Orkney Islands, by means of a ^{15}N tracer technique. Fixation was associated with the blue-green alga *Nostoc commune* and the lichens *Collema pulposum* and *Stereocaulon* sp., both of which contain *Nostoc* as a phycobiont. In *Stereocaulon* fixation was confined to the cephalodia, which contain the *Nostoc*. All three organisms are common on Signy Island. Fixation was found to be most active in areas subject to the influence of the basic rocks, amphibolite and marble. Although appreciable fixation occurs at temperatures in the vicinity of 0°C, the rate increases rapidly with rise in temperature and it is probable that the bulk of fixation is accomplished in brief periods when the micro-environment reaches temperatures of 10°C or more.

SUPPLY of available nitrogen may often be an important factor limiting the biological productivity of Antarctic habitats. Thus Holdgate, Allen and Chambers (1967) have shown that nitrogen is the only major nutrient likely to limit plant growth on Signy Island. Fixation of the free nitrogen of the atmosphere may therefore be of considerable ecological importance in the Antarctic.

Symbiotic nitrogen-fixing angiosperms are absent from Antarctica. Free-living bacteria belonging to a variety of different genera are now known to be capable of nitrogen fixation (Stewart, 1966) and species belonging to some of these, such as *Azotobacter*, *Achromobacter* and *Bacillus*, as well as yeasts of the genus *Rhodotorula* which may also fix nitrogen, have been reported from the Antarctic (Sieburth, 1965). However, little is known of the abundance or distribution of these forms in the Antarctic and no isolates from this region seem to have been tested for fixation. On the other hand, heterocystous blue-green algae likely to fix nitrogen are known to be both widespread and locally abundant in Antarctica (Holm-Hansen, 1964; Hirano, 1965). Of particular interest is the finding by Holm-Hansen (1963) that of 130 samples, collected from a variety of habitats in the McMurdo Sound area and inoculated into medium free from combined nitrogen, about half showed growth of *Nostoc commune*. Unialgal, but not bacteria-free, cultures of this alga were obtained and shown to be capable of nitrogen fixation using ^{15}N as tracer. Hérisset (1952) has previously demonstrated nitrogen fixation in an axenic culture of this species isolated from a French soil. There is thus good evidence that at least one nitrogen-fixing species is of general occurrence in the maritime Antarctic but it remains to be determined what rates of fixation are actually achieved in the various habitats.

During a visit to Signy Island, South Orkney Islands, in January to March 1966, one of us (G.E.F.) used a ^{15}N technique developed by the other (W.D.P.S.) to make *in situ* determinations of rates of nitrogen fixation. The results, reported here, show that appreciable rates of fixation are achieved under Antarctic conditions.

METHODS

The basic method was to expose a sample of vegetation or soil to an atmosphere enriched with ^{15}N under conditions otherwise as near as possible to those in the natural environment. After a specified exposure period, the ^{15}N-labelling of the test sample and of the gas phase was determined and by knowing the total N present per sample the rate of nitrogen fixation was calculated. Full details are given by Stewart (1967). The weight of the apparatus and ancillary equipment used in the field, including a hand vacuum pump and a small gas cylinder, was 21 kg.

Samples, of area 0·95 cm.^{2} except where otherwise stated, were generally taken with a cork borer. Acrocarpous mosses and fruticose lichens were sampled by picking a tuft representing an equivalent area. All samples were taken in sets of six, three being used for nitrogen determinations and three air-dried and kept for later identification of the species. Because of the necessity to conserve ^{15}N, three different sets of triplicate samples were usually exposed

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per flask. The gas mixture used to displace air from the apparatus contained 88 per cent argon and 12 per cent oxygen. The ^{15}N-enriched nitrogen (usually containing 96 per cent but sometimes 33 per cent atom per cent excess ^{15}N) was admitted to give a final partial pressure of 0·2 atmospheres. Pardee buffer was used to maintain 0·03–0·08 per cent of carbon dioxide within the flask. No particular difficulties were encountered in setting up the apparatus, even at temperatures a few degrees below 0° C, a low temperature grease, Apiezon H (Associated Electrical Industries Ltd.) being used for greasing ground glass joints. When set up, the exposure flask was clamped in position with the samples at ground level. Exposures were made for 24, or sometimes up to 96, hours. Temperature differences inside a dummy flask and outside on the surface of the substratum or among the vegetation cover were compared using mercury thermometers. Readings were made as frequently as possible. Sometimes this could only be once a day but, taken in conjunction with the routine meteorological observations made at the scientific station, these readings give an adequate picture of the temperature regime.

At the end of the exposure period, samples were placed individually in screw-topped bottles containing 2 ml. of 1 per cent mercuric chloride solution. On return to England each sample was assayed for total nitrogen and ^{15}N enrichment as described by Stewart (1967), using an Associated Electrical Industries Ltd. type MS3 mass spectrometer for the ^{15}N assay.

The corrections which must be applied to the raw data to obtain estimates of actual rates of fixation *in situ* have been discussed by Stewart (1967). In accordance with his findings, the effect of the lower partial pressure of nitrogen in the flasks has been allowed for by multiplying the observed rate by a factor of 1·47. Temperature differences inside and outside the flask are also likely to have been important. These rarely exceeded 3° C; lower temperatures inside, observed under overcast and windy conditions, being as frequent as higher temperatures inside, which occurred in sunshine (Table I). Nevertheless, some correction should be made for these differences. Fogg and Than-Tun (1960) found values of Q_{10} for nitrogen fixation of up to 5 in cultures of *Anabaena cylindrica*, and for natural populations of *Calothrix* and *Nostoc*, Stewart (1967) reported rates of increase up to 15° C corresponding to a Q_{10} value of about 6. The effect of temperature on *in situ* fixation on Signy Island is at least as great as this (Fig. 2) and a Q_{10} value of 6 has been assumed in making an approximate correction according to 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*.

Sites

The sites chosen for determinations were selected to include various types of plant community, substratum and exposure, but attention was concentrated on those having organisms likely to fix nitrogen. The positions of these sites are shown in Fig. 1 and other details are as follows:

Site 1. Amphibolite flush: receiving drainage (pH 6·9) from an amphibolite outcrop above; facing north-west; mixed bryophyte and lichen community with *Brachythecium subplicatum* and *Dicranum aciphyllum* predominating, and *Nostoc commune* and *Collema pulposum* abundant.

Site 2. Stone stripe area, Moraine Valley: strong solifluction, facing north-west; *Nostoc commune* the only visible growth on the mud between stone stripes.

Site 3. Stone stripe area, Waterpipe Beach: strong solifluction, many marble boulders; facing south-east; *Nostoc commune* abundant on mud between stone stripes.

Site 4. Wet ground, below Jane Peak col: moist area between pools near stream on west side of a rocky knoll at the mouth of the valley; *Cephaloziella varians* abundant.

Site 5. Dry slope, Gourlay Peninsula: on a sharp boundary between quartz-mica-schist (with *Usnea fasciata* and *Andreaea regularis*) and marble (with *Grimmia antarctica* and *Collema pulposum*); facing south.

Site 6. Dry slope, above the British Antarctic Survey scientific station: a quartz-mica-schist area facing north; *Dicranum aciphyllum* abundant.

Site 7. Factory Cove: Deschampsia antarctica turf on north-facing slope near sea.

TABLE I. RESULTS OF *IN SITU* DETERMINATION OF NITROGEN FIXATION ON SIGNY ISLAND, SOUTH ORKNEY ISLANDS

(The flask and ground temperatures are the maxima and minima observed and not necessarily the actual maxima and minima. Each value for atom per cent excess ^{15}N is the mean of triplicate determinations on separate samples; standard deviations of the mean are given. The estimated rates of nitrogen fixation have been corrected for differences in pN_2 and temperature as explained in the text.)

Site	Period of exposure and date	Maximum and minimum temperature (°C)			$(t_i - t_o)$	Sunshine during exposure (hr.)	Principal components of sample	Atom per cent excess ^{15}N	Nitrogen fixed (μg./mg. N/day)
		Flask	Ground	Air					
1	24 hr. from 12.25 19.1.66	15·4 2·2	11·1 2·2	3·8 0·0	+1·56	1·9	a. *Collema pulposum* b. Peat c. *Psora* sp.	0·080±0·011 0·000±0·001 −0·002±0·001	1·02 — —
1	24 hr. from 12.15 24.1.66	4·0 0·2	4·2 0·2	1·1 −1·4	+0·48	0·0	a. *Collema pulposum* b. *Drepanocladus uncinatus* c. *Psora* sp.	0·021±0·001 0·007±0·002 0·005±0·001	0·31 0·08 0·11
1	48 hr. from 12.15 29.1.66	10·2 2·1	9·9 1·7	5·7 0·2	+1·50	11·8	a. *Collema pulposum* b. *Nostoc commune* c. Peat	0·072±0·002 0·131±0·003 0·038±0·002	0·45 0·81 0·24
1	24 hr. from 12.40 5.2.66	5·1 1·3	6·5 1·7	5·1 −0·2	−0·90	0·0	a. *Collema pulposum* b. *Lyngbya* sp. c. *Bryum imperfectum* (?)+ a little *Collema pulposum*	0·019±0·001 0·000±0·000 0·011±0·003	0·36 — 0·02
1	24 hr. from 12.15 11.2.66	4·2 1·0	2·6 1·5	3·0 −1·2	+0·20	0·0	a. *Collema pulposum* b. *Nostoc commune* c. *Stereocaulon* sp.	0·019±0·002 0·042±0·001 0·025±0·002	0·30 0·65 0·40
1	24 hr. from 12.30 20.2.66	0·9 −1·0	0·1 −0·1	0·5 −4·1	−0·20	0·0	a. *Collema pulposum* b. *Nostoc commune* c. *Stereocaulon* sp.	0·003±0·000 0·003±0·000 0·000±0·000	0·05 0·05 —
1	48 hr. from 11.55 1.3.66	10·1 1·8	7·1 1·0	2·1 −1·5	+1·45	4·1	a. *Collema pulposum* b. *Nostoc commune* c. *Stereocaulon* sp.	0·040±0·002 0·061±0·002 0·009±0·002	0·25 0·37 0·06
2	24 hr. from 12.10 21.1.66	10·0 0·5	12·0 1·3	1·7 −0·7	−1·76	1·5	a. *Nostoc commune* b. Mud and silt	0·017±0·001 0·003±0·001	0·36 —
3	48 hr. from 14.45 2.2.66	11·5 6·8	9·5 4·4	7·0 1·7	+0·98	1·8	a. *Nostoc commune*, old b. Silt+young *Nostoc commune* c. Silt d. *Bryum imperfectum* (?)	0·094±0·004 0·011±0·002 0·003±0·001 0·000±0·001	0·65 0·08 — —
4	48 hr. from 15.50 2.2.66	9·6 3·0	9·2 4·0	7·0 1·7	−0·70	1·8	a. *Dichothrix* sp. b. *Cephaloziella varians*	0·008±0·007 0·001±0·000	— —
5	96 hr. from 15.50 1.2.66	13·6 2·4	10·3 3·7	7·8 −0·9	+0·80	3·7	a. Soil over schist b. Soil over marble c. *Collema pulposum* from marble	−0·001±0·000 0·015±0·001 0·000±0·000	— 0·05 —
5	72 hr. from 16.20 5.2.66	1·8 −0·2	2·8 0·6	4·7 −4·3	−0·90	0·5	a. *Collema pulposum* from marble b. *Grimmia antarctica* from marble c. *Andreaea regularis* from schist d. *Usnea fasciata* from schist	0·055±0·001 0·003±0·001 −0·001±0·000 0·000±0·001	0·35 — — —
6	96 hr. from 12.10 15.2.66	10·2 −1·2	11·6 0·3	5·5 −3·2	−0·36	2·0	a. Peaty dead moss+immature *Stereocaulon* sp. b. *Stereocaulon* sp. c. *Dicranum aciphyllum*	0·000±0·001 0·000±0·000 −0·001±0·001	— — —
7	48 hr. from 10.00 1.2.66	7·9 1·0	10·9 1·5	2·1 −1·5	−1·08	5·4	a. *Deschampsia antarctica* turf b. Soil+*Oscillatoria* sp. c. Soil+various bryophytes, lichens and *Nostoc commune*	0·000±0·001 0·001±0·000 0·026±0·002	— — 0·26

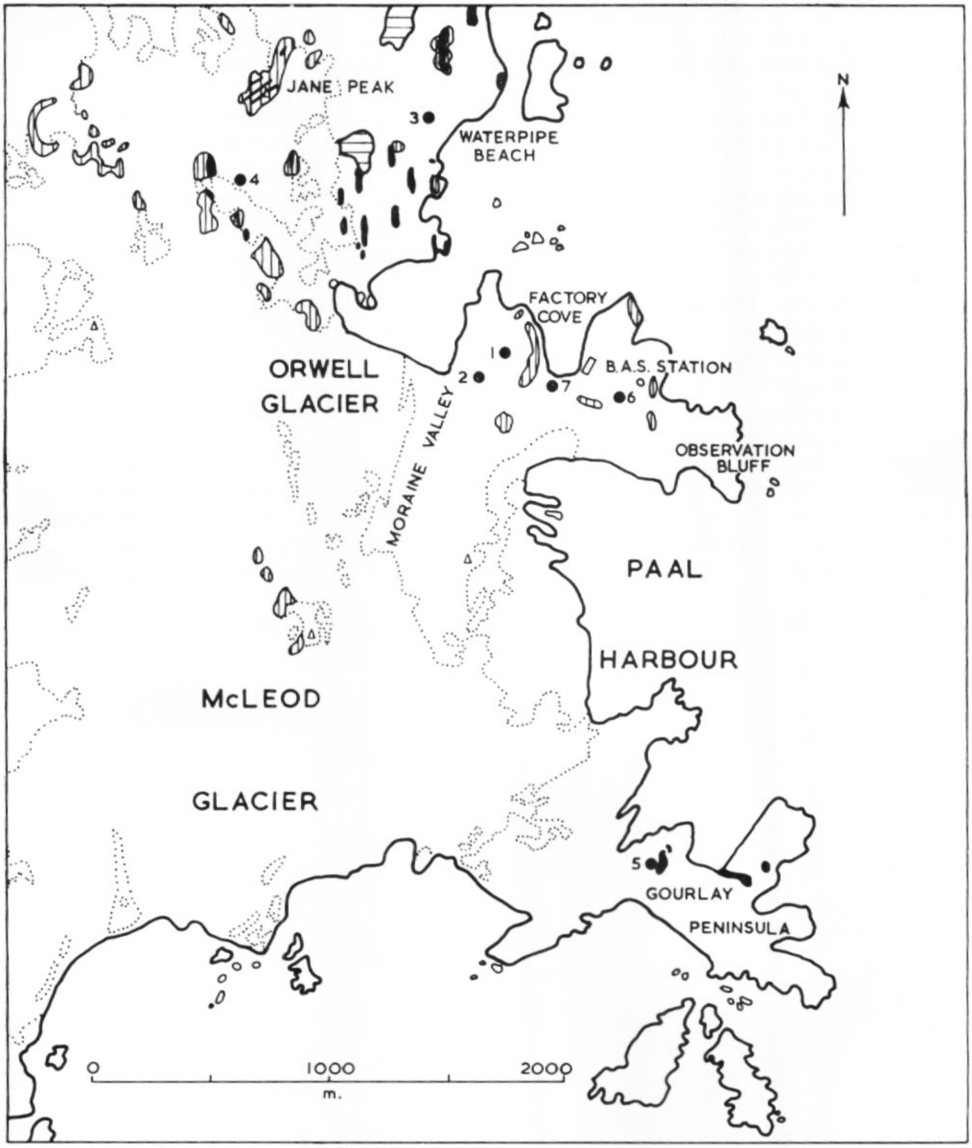

Fig. 1. Sketch map of the south-east part of Signy Island, South Orkney Islands, showing the sites at which determinations of nitrogen fixation were made. The dotted line represents the approximate limit of permanent ice and semi-permanent snow fields. The approximate position of outcrops of amphibolite are marked by vertically hatched areas and those of marble by solid black.

Results

In situ determinations

The results of the *in situ* determinations are given in Table I. On the usually accepted basis that an excess of 0·015 atom per cent above the proportion of ^{15}N in unexposed material is positive evidence, fixation has been demonstrated in 16 out of a total of 42 samples. In most of these instances values well in excess of 0·015 atom per cent ^{15}N were obtained. In agreement with the conclusions of Holm-Hansen (1963), nitrogen fixation was associated with the presence of *Nostoc commune*, this species being the principal component in six of the samples yielding positive results. In another seven *Collema pulposum* was the main constituent. It was to be expected that this lichen should fix nitrogen since the phycobiont is *N. commune* and the related species *C. granosum* has been shown to possess the property (Bond and Scott, 1955). A negative result obtained with *C. pulposum* at site 5 on 1.2.66 is probably attributable to the material being particularly dry at the time of sampling. In one clear instance fixation occurred in a sample of *Stereocaulon* sp. Although its principal phycobiont is a green alga, this lichen possesses cephalodia containing *N. commune* and, as will be shown below, nitrogen fixation is associated with these structures. In only two samples showing definite nitrogen fixation (peat from site 1 (29.1.66) and soil from over marble on site 5 (1.2.66)) was *Nostoc* (or lichens containing it) not obvious to the naked eye but microscopic observation showed it to be present nevertheless. The results for the heterocystous blue-green alga, *Dichothrix* sp., are equivocal. The samples were far from uniform and of the three exposed two showed no enrichment, whereas the third had 0·022 atom per cent excess ^{15}N. Neither of the two samples containing the non-heterocystous blue-green algae, *Oscillatoria* sp. and *Lyngbya* sp., as dominants showed any evidence of nitrogen fixation, nor did any of the bryophytes or lichens having chlorophycean phycobionts.

In terms of nitrogen fixed per unit amount of combined nitrogen per unit time, *Nostoc commune* stands out as the most active of the organisms encountered. As is seen from Fig. 2, comparisons made on a number of different occasions at site 1 consistently showed it to be about twice as active as *Collema pulposum*. *Stereocaulon* appeared on the whole to be less active than *Collema*. These activities run parallel with the content of *Nostoc* cells in the material.

It will also be seen from Fig. 2 that the rate of fixation at site 1 shows some relation to

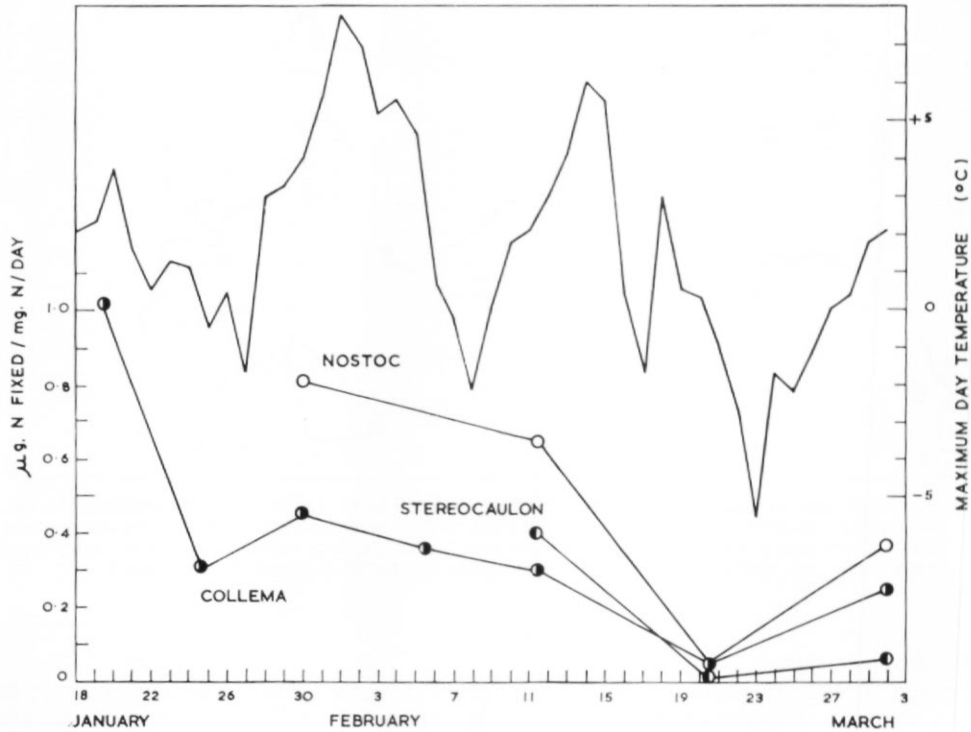

Fig. 2. Nitrogen fixation by *Nostoc commune* (○), *Collema pulposum* (◑) and *Stereocaulon* sp. (◐) at site 1 (the amphibolite flush), January to March 1966. The upper curve shows the variation in maximum daytime temperature.

temperature. This was examined further by plotting the rate of fixation by *C. pulposum* as a function of the maximum temperature observed at the ground surface (Fig. 3). This shows a distinct increase in rate of fixation with increase in temperature, the points being reasonably consistent with the value of 6 assumed for the Q_{10} in making corrections for temperature differences. Another physical factor which probably had a considerable effect which was not, however, investigated was the degree of hydration of the material.

The base status of the substratum is clearly of particular importance in determining whether fixation occurs or not. The three nitrogen-fixing organisms were mainly to be found on marble or amphibolite or on substrata receiving drainage from outcrops of these rocks (see Fig. 1). Soil in a *Deschampsia antarctica* community (site 7) also supported a growth of *Nostoc* and gave a positive result for nitrogen fixation. The analyses of Holdgate, Allen and Chambers (1967) show such soils to have about twice the calcium content of montane schist soils, samples from which showed no trace of nitrogen fixation. The two experiments carried out at site 5 show particularly clearly the effect of substratum on nitrogen fixation for no evidence of fixation was obtained with samples from schist soils, under conditions which allowed substantial fixation by samples taken from the contiguous marble area.

Laboratory determinations

To supplement studies made in the field some determinations were also carried out in the laboratory using freshly collected samples which might have included nitrogen-fixing organisms. The results are given in Table II. Since these determinations were made at relatively

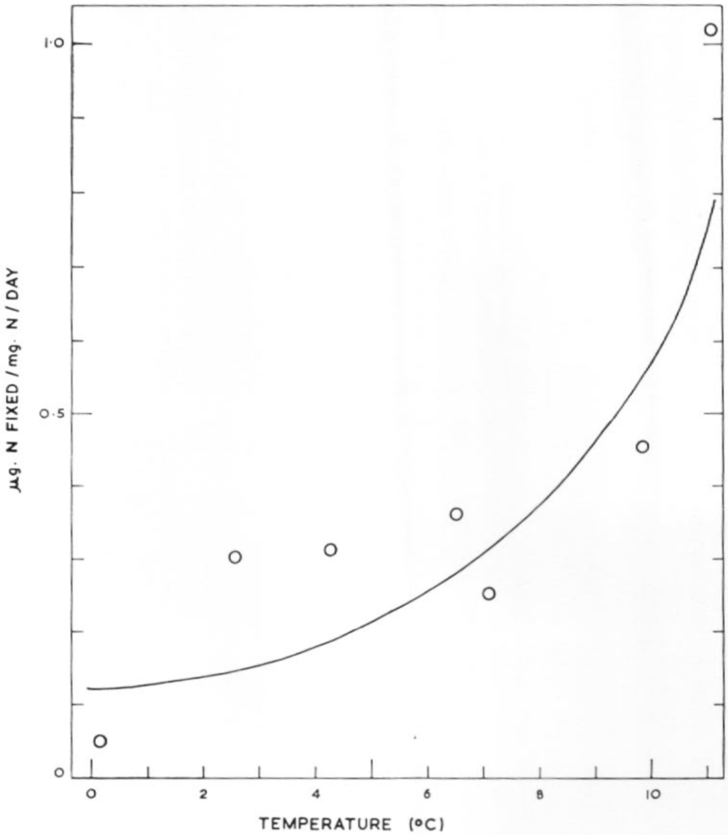

Fig. 3. Nitrogen fixation by *Collema pulposum* at site 1 (the amphibolite flush) in relation to maximum recorded ground-surface temperature. The curve has been drawn assuming a Q_{10} value of 6·0.

TABLE II. LABORATORY DETERMINATIONS OF NITROGEN FIXATION BY FRESHLY COLLECTED SAMPLES FROM SIGNY ISLAND, SOUTH ORKNEY ISLANDS

(Exposures were made in diffuse daylight at temperatures which varied between 14 and 18·5°C. Each value for atom per cent excess ^{15}N is the mean of triplicate determinations on separate samples except c.i, ii and iii on 16.2.66, which were in duplicate only; standard deviations of the means are given. The estimated rates of nitrogen fixation have been corrected for differences in pN_2.)

Date	*Duration* (hr.)	*Material*	*Atom per cent excess* ^{15}N	*Nitrogen fixed* (μg./mg. N/day)
4.2.66	24	a. Pieces of marble, with dense growth of *Hyella caespitosa*, area about 14·55 cm.^{2}	0·002±0·0015	—
		b. *Dichothrix* sp., area 6·9 cm.^{2}	0·001±0·001	—
8.2.66	24	a. Silt from lateral moraine of Orwell Glacier, area 3·53 cm.^{2}	0·005±0·001	0·08
		b. *Deschampsia antarctica* turf, area 3 cm.^{2}	0·001±0·001	—
		c. *Peltigera venosa* from marble, area 3 cm.^{2}	0·001±0·001	—
16.2.66	27	*Stereocaulon* sp., superficial area 2·27 cm.^{2}		
		a. From schist area	0·000±0·000	—
		b. From amphibolite area	0·011±0·0035	—
		c. From amphibolite area		
		i. Associated moss	0·000±0·000	—
		ii. Cephalodia	0·017±0·000	0·19
		iii. The rest of the lichen	0·001±0·001	—
18.2.66	24	*Phormidium* felt from lacustrine shelf of lake 2, samples of 0·95 cm.^{2} area	0·002±0·001	—
		Samples 0·95 cm.^{2} in area from site 1		
21.2.66	27	a. *Nostoc commune*	0·017±0·001	2·36
		b. *Collema pulposum*	0·001±0·000	—

high temperatures, it is possible that the activity of the organisms may have been impaired but this is not likely since the rate of nitrogen fixation of *Nostoc commune* at an average temperature of 16·25° C was found to be 2·36 μg./mg. N/day as compared with 2·53 μg./mg. N/day estimated on the basis of the *in situ* determination at site 1 on 29.1.66 assuming a Q_{10} value of 6.

Nitrogen fixation by *Nostoc commune* was thus confirmed. Other blue-green algae tested gave negative results. For *Hyella caespitosa*, a member of the Pleurocapsales, and the felt consisting of various *Phormidium* spp., which was examined because of its ubiquitous occurrence in fresh water, this was expected since these are non-heterocystous forms and the capacity for nitrogen fixation in the blue-green algae shows a correlation with the presence of heterocysts (Stewart, 1966). *Dichothrix* sp. has heterocysts but nevertheless gave no indication of fixation in these experiments, so that the positive result among those obtained *in situ* remains unconfirmed.

Again, nitrogen fixation was found only in those lichens with *Nostoc* as phycobiont. The negative result obtained for *Collema pulposum* may be related to desiccation. The negative result with *Peltigera venosa* is not surprising, because, although other members of this genus have been shown to fix nitrogen (see Stewart, 1966), this particular species contains a chlorophycean phycobiont.

The results for *Stereocaulon* sp. show clearly that nitrogen fixation is a property of the cephalodia. From Table I it will be seen that samples of this lichen from the amphibolite flush fixed nitrogen, whereas one from a schist area did not. Examination of samples collected from various places on the island showed that those from amphibolite areas always possessed cephalodia, whereas those from schist areas generally lacked them. *Stereocaulon* was never found growing on marble. In the experiment, the results of which are reported in Table II, a sample of the lichen from a schist area, which totally lacked cephalodia, showed no trace of nitrogen fixation, whereas the same species from the amphibolite flush (site 1), with abundant cephalodia, gave a positive result. A duplicate sample from site 1 was dissected into its components. Neither the associated moss nor the lichen minus its cephalodia showed fixation but the cephalodia showed a significant degree of labelling. Thus fixation is confined to the cephalodia and within the period of the experiment there was no detectable translocation of products of fixation to the rest of the lichen thallus.

The result obtained for moraine silt is of interest since this material is rich in mineral nutrients except combined nitrogen (Holdgate, Allen and Chambers, 1967) and may have 10^6 bacteria/cm.^{3} (personal communication from J. H. Baker). Further examination of nitrogen fixation on recent moraines is clearly desirable.

Discussion

The results show that appreciable nitrogen fixation occurs in a variety of terrestrial habitats on Signy Island. This is associated with the presence of the blue-green alga *Nostoc* either in free-living form or in symbiotic association in the lichens *Collema* and *Stereocaulon*. Examination of a variety of materials has shown that other nitrogen-fixing organisms, if present, are of minor importance. A photosynthetic nitrogen-fixer such as *Nostoc* is obviously at an advantage in habitats where low temperatures prevail and labile organic matter is generally in short supply.

Nostoc, *Collema* and *Stereocaulon* with cephalodia are almost entirely confined to areas subject to the influence of the basic rocks, amphibolite and marble, and nitrogen fixation is correspondingly restricted to such areas. This preference of *Nostoc* spp. for neutral or alkaline conditions is well known both from field observations and laboratory studies (Fogg, 1956). The nitrate concentration in these basic habitats is generally about four times higher than in more acid but otherwise similar situations (Holdgate, Allen and Chambers, 1967) and this may well be related to the occurrence of nitrogen fixation.

The impression given by physiological studies on blue-green algae, such as those of Fogg and Than-Tun (1960), is that nitrogen fixation is greatly reduced at low temperatures. In this study perceptible fixation was observed at temperatures which cannot have exceeded 4·2° C (site 1, 11.2.66; Table I). Nevertheless, the temperature coefficient is high and it is evident that the bulk of nitrogen fixation on Signy Island is accomplished in brief intervals as micro-habitats reach temperatures of 10° C or above during periods of insolation. It is to be suspected that desiccation is often an important factor limiting fixation when temperature conditions are suitable. The efficiency of fixation in terms of μg. N fixed/mg. nitrogen/day varies within the same range as that recorded for *Calothrix* on the Scottish coast during the period October to April (Stewart, 1967).

Nostoc, *Collema* and *Stereocaulon* are common on Signy Island and locally abundant so that their contribution of fixed nitrogen may be of appreciable ecological significance. It would be premature to attempt a quantitative estimate of this contribution on the basis of the data contained in this paper but it is hoped that further studies on the distribution of these organisms will enable this to be done.

Acknowledgements

This work was undertaken as part of the United Kingdom contribution to the International Biological Programme. Our thanks are due to the following: Sir Vivian Fuchs and the British Antarctic Survey for enabling one of us (G.E.F.) to visit Signy Island; to Westfield College for the necessary leave of absence; to the Royal Society for a travel grant; to members of the British Antarctic Survey for assistance in many different ways; to Dr. C. H. Gimingham, Mr. R. I. L. Smith and Mr. D. C. Lindsay for help with identification of bryophytes and lichens; to Mr. T. Lymn for technical assistance and to the Science Research Council for grants given in support of work on nitrogen fixation at Westfield College.

MS. received 10 October 1967

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