Nitrogen Fixation in the Algal Mats on Marion Island

R.L. Croome*

Department of Botany,
University of the Orange Free State,
Bloemfontein.

Introduction

In certain environments, for example in the Antarctic, the fixation of atmospheric nitrogen by blue-green algae may play an important part in soil fertility and thus in plant growth (*Holm-Hansen*, 1963; *Fogg & Stewart*, 1968; *Horne*, 1972; *Schofield & Ahmadjian*, 1972; see also *Schell & Alexander* 1972).

During the 1965-66 expedition to Marion Island it was found that the surface of many of the mires is covered by a thick gelatinous layer of Cyanophytes (*van Zinderen Bakker Sr.*, 1971). The work detailed here is an investigation of this mat, with particular emphasis placed on the determination of fixation of atmospheric nitrogen by the Cyanophytes (blue-green algae) present.

General Description of the Algal Mat

The algal mat was found to be present in the wetter mire areas and in some parts extended as deep as 8,0 cm. On its surface was a dry "crust" less than 1,0 mm thick. Beneath this there was a green layer about 1,5 cm thick and below this the mat had a brownish colour. The most abundant blue-green species present has been tentatively identified as *Stigonema ocellatum* (Dillw.) Thuret. Other tentative identifications are *Hapalosiphon aureus* (West & West) and *Tolypothrix sp.* These three species occurred in the top "crust" and in the green layer in which *Anabaena sp.* and several colonial green algae also occurred. The brown layer of the mat contained the same species, but all cells were dead. So the mat is homogeneous in species content except for the presence of colonial green algae in the 1,5 cm green layer. The hard layer on top consists of dried-out cells and the brown layer of dead cells. No succession is evident.

Generally speaking, the algal mat occurs in only the wettest areas. It is restricted to the centres of small basins in which a lake has not developed, in "run-ways" through which water drains but does so insufficiently to form a creek, and in small isolated patches which gather sufficient water. It therefore occurs in the areas most treacherous for walking and one easily sinks through the surface layer of the mire where the algae are present. In the "run-ways" and isolated spots it is associated with *Agrostis magellanica* and it is probable that the algae in these areas are subject to desiccation in dry times. In the wetter areas the mass is associated with mosses or itself forms a complete ground cover. It also occurs on *Agrostis* slopes below hills where there is a sufficiently constant seepage of water onto the surface, e.g. at Stoney Ridge.

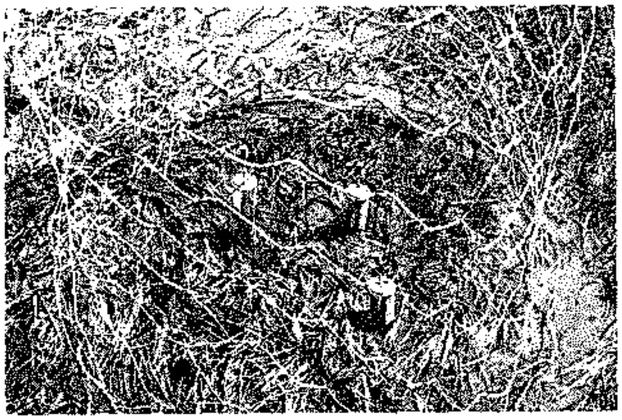
Towards the upper limit of the mire complexes small runnels develop through which water flows as small creeks. These contain a light-green, extremely copious mucilage in which the blue-green alga *Nostoc commune* occurs. This alga has been shown to be important in nitrogen fixation in sub-Antarctic and Antarctic regions (*Fogg & Stewart* 1968, *Holm-Hansen* 1963). The alga was not seen, however, in the algal mats on the mires.

Materials and Methods

Algae from the mire areas were cultured on the island and brought back to Bloemfontein. Both agar and liquid media were used. A liquid medium which was free of combined nitrogen was used to culture nitrogen-fixing Cyanophytes.

In the mires of Marion Island nitrogen fixation by the blue-green algae was assayed by the acetylene reduction method of *Hardy et al.* (1968). Algal samples were incubated *in situ* in 50 ml glass syringes (Ultra-Asept brand) in a gas mixture of acetylene and air (20% C_2H_2 — 80% air). The gas mixture of acetylene and air used in the incubation syringes was made in the field, the acetylene being carried under pressure in a flask. The acetylene had been scrubbed with concentrated sulphuric acid, as described by *Hardy et al.* (1968). Samples of gas were taken after one hour and after four hours. Three incubation syringes can be seen in Plate 1. (The wire netting is to discourage the Brown Skua, *Stercorarius skua*, from taking the rubber stoppers sealing the needle ends). The gas samples taken were kept in mercury-sealed phials and the gas mixture was analysed by gas chromatography on return to South Africa. It was assumed that if three moles of acetylene were reduced then this would represent fixation of one mole of

*Present Address: Department of Botany, University of Tasmania, Hobart, Tasmania.



nitrogen gas. The amount of organic nitrogen in the algal samples was assayed by steam distillation after Kjeldahl digestion.

In all, twenty-one incubations were completed on Marion Island. Two mire areas were selected. Six incubations were conducted in Study Area One (the first area chosen for intensive investigation of mineral cycling), nine in a mire situated immediately south of "ZS2M1" (the old radio shack), and six incubations were done in old seal wallows at Trypot Beach. The wallows were of the elephant seal *Mirounga leonina*.

Soil water was taken from as near to the surface as possible. The water draining into a hole about 15 cm deep was collected. Ammonium and nitrate were determined colorimetrically after steam distillation (*Golterman,* 1969). Soils were air-dried at room temperature (4-5°C) on Marion Island. Returned to South Africa, the soils were passed through a 2 mm sieve. After extraction with potassium chloride, available nitrogen was determined by steam distillation after *Black* (1965).

Results and Discussion

The work was done almost exclusively in the mire areas of the north-eastern coastal plain. The mat was found to cover less than one per cent of the total mire area. Most commonly it was about 1 cm thick but did extend occasionally to a depth of 8 cm. For fifteen samples taken the mean wet weight/m² surface area of mat was found to be 12,3 kg, mean dry weight 0,5 kg, giving a weight loss during drying of 96%.

The concentrations of ammonium nitrogen and nitrate nitrogen might be expected to be higher below the algal mat. However, analyses made of soil water gave no definite results concerning the addition of nitrogen to the mires by blue-green algae. No firm conclusions can therefore be drawn, but it appears that the algal mat has little effect on the concentration of nitrogen in the ground water in this mire. Soil analyses also gave inconclusive results concerning the addition of nitrogen to the soils below algal mats.

Algal Culturing

Two Cyanophytes were successfully cultured in nitrogen-free medium. These were *Tolypothrix sp.* and *Calothrix sp.* Both these genera have previously been shown to fix nitrogen (*Stewart,* 1966).

The growth form of *Tolypothrix sp.* in culture was interesting. On Marion Island the species usually had one, or occasionally two, heterocysts wherever a branching of the filament occurred, as may be seen from the preserved material shown in Plate 2. In culture the species had up to five heterocysts at a branching and as many as nine heterocysts in a row in the main filament. This may be seen in Plates 3 and 4 from material in culture at Bloemfontein.

Although a few other smaller algae were present in the nitrogen-free cultures of *Tolypothrix sp.* and *Calothrix sp.*, it is probable that these were using nitrogen fixed by the Cyanophytes. This indirect evidence indicates that these two algae may contribute towards the fixation of nitrogen in the mire areas of Marion Island.

The most common alga of the mires, tentatively identified as *Stigonema ocellatum,* proved impossible to isolate as a pure culture. On the basis of field observations, however, it is felt that this species is the most important with regard to the fixation of atmospheric nitrogen on Marion Island.

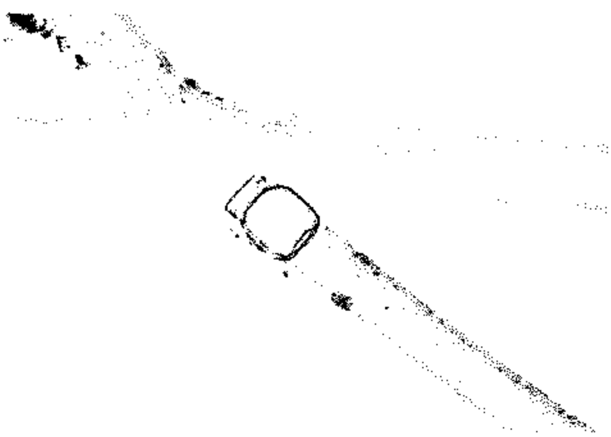


Plate 2. Tolypothrix sp. *Preserved material from Marion Island showing single heterocyst at point of branching.*

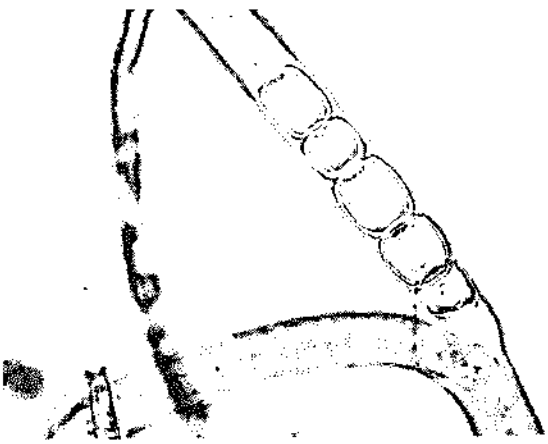


Plate 3. Tolypothrix sp. *Material from culture showing several heterocysts at point of branching.*

Table 1

Location, date, temperature, light conditions and nitrogen fixation of blue-green algae incubated in mires. Some green algae were included.

Location	Date 1972	Temperature of mire surface °C	Light conditions	Nitrogen fixed µg N/mg algal N/3h
Study area one	17/2	13–15	Sunny	1,82
Study area one	17/2	13–15	Sunny	0,53
Study area one	26/2	11–14	Scattered cloud	3,33
Study area one	28/2	14–19	Sunny	2,27
Study area one	4/3	10–12	Scattered cloud	0,38
Study area one	28/3	13–16	Overcast	0,30
South of ZS2M1	23/2	16–17	Overcast	Nil
South of ZS2M1	23/2	16–17	Overcast	Nil
South of ZS2M1	25/2	8–15	Sunny	Nil
South of ZS2M1	26/2	11–14	Overcast	Nil
South of ZS2M1	28/2	14–20	Sunny	1,85
South of ZS2M1	4/3	10–12	Scattered cloud	Nil
South of ZS2M1	28/3	13–16	Overcast	Nil
South of ZS2M1	29/3	15–	Overcast	Nil
South of ZS2M1	31/1	14–15	Overcast	Nil

Measurement of Nitrogen Fixation

Of the six incubations in Study Area One previously referred to, all showed nitrogen fixation; of the nine behind "ZS2M1" only one showed fixation; and the six incubations behind Trypot Beach showed no nitrogen fixation in the wallows of *Mirounga leonina*. The experimental results are shown in Table 1.

In Study Area One as much as 3,33 µgN/mg algal N was found to be fixed in three hours. At "ZS2M1" only one sample showed fixation - on a very bright sunny day when the temperature reached 20°C (on the other days the temperature did not exceed 17°C). For Study Area One the mean fixation was 1,73 µgN/mg algal N/3 h. *Fogg & Stewart* (1968), in similar work on Signy Island, found that the amount of nitrogen fixed was much lower - for example, in samples of which the principal component was *Nostoc commune*, a known nitrogen fixer, the amount of nitrogen fixed averaged 0,48 µg N/mg algal N per day. However, they did note that 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. There is some evidence in Table 1 that fixation rates on Marion Island may be positively correlated with temperature and radiation. The figures given in Table 1 may be compared with those of *Stewart et al.* (1968). Their results (for pure cultures of algae) have been converted from moles C_2H_4/mg protein/minute to µg N/mg algal N/3 h and are given in Table 2.

As might be expected, fixation is much greater in culture, but the results show that significant amounts of nitrogen are fixed in the mires of Marion Island.

Although care had been taken in preparing the gas, it was subsequently found in South Africa that the acetylene was contaminated with ethylene and that the level of ethylene was between 300 and 440 parts per million in the syringe at the beginning of an incubation. This could have inhibited the conversion of acetylene to ethylene by the algae. It is therefore surprising that the results showed that significant fixation occurred on the island. In view of this it is very probable that significant amounts of fixed nitrogen are added to the mire ecosystem by the algal mats.

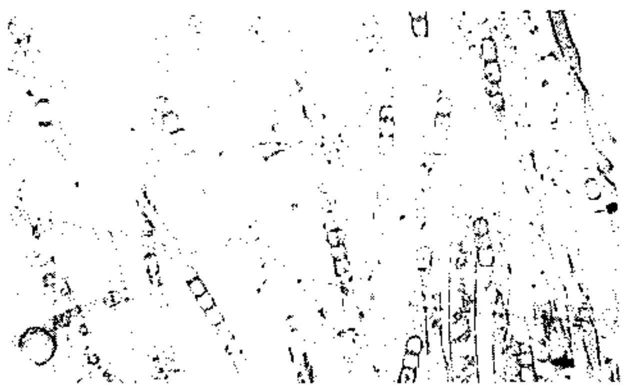


Plate 4. Tolypothrix sp. *General view of culture showing numerous heterocysts.*

Table 2

Nitrogen fixation by cultured algae. The fixation rates have been calculated from data of *Stewart et al.* (1968)

Species	Nitrogen fixed µg N/mg algal N/3 h
Anabaena cylindrica	19,9
Anabaena flos-aquae	49,3
Mastigocladus laminosus	10,5
Nostoc entophytum	9,4
Nostoc muscorum	38,8
Nostoc sp.	4,2
Tolypothrix tenius	18,9
Anacystis nidulans	—

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