Limitations and potentials for biological nitrogen fixation in the tropics

J. Dobereiner, R. H. Burris and A. Hollaender, eds. Plenum Press, New York

Stewart, W.P.D., Sampaio, M. J. Isichei, A. O. and Sylvester-Bradley, R. 1977. Nitrogen fixation by soil algae of temperate and tropical soils. Pages 41 - 63

~~E 2 (c) (ii)~~
E 2 (b) (i).

NITROGEN FIXATION BY SOIL ALGAE OF TEMPERATE AND TROPICAL SOILS

W.D.P. Stewart, M.J. Sampaio, A.O. Isichei
and R. Sylvester-Bradley*

Department of Biological Sciences
University of Dundee, Scotland
*Instituto Nacional de Pesquisas da Amazonia
Manaus, Amazonas, Brazil

INTRODUCTION

Blue-green algae are common components of the microbial flora of the soil in many parts of the world (7,8,18,30). In the tropics most attention has been paid to their role in rice paddy soils where, free-living (26,27) and in symbiotic association with the water-fern *Azolla* (3,19,21,36) they contribute substantial amounts of nitrogen to the ecosystem. In this paper we present information on the occurrence, activity, and factors affecting soil algae from tropical savanna regions of Nigeria and from the Amazon region of Brazil. The findings are compared with observations made on algae from temperate soils in Scotland. These studies complement ones from tropical (e.g. 22,26,27,37) and temperate (e.g. 6,9,12,28) regions.

THE STUDY AREAS AND THE OCCURRENCE OF POTENTIAL NITROGEN-FIXING ALGAE

Heterocystous, non-heterocystous and unicellular blue-green algae are now known to fix N_2 (see 35). Of these the heterocystous forms which invariably fix N_2 under aerobic and anaerobic conditions are ecologically the most important (see 18, 26,30) although the non-heterocystous strains which fix N_2 under anaerobic conditions only (23,24,29,33,34) may also be important in environments such as marine salt marshes (31).

The Scottish samples provide good examples from a temperate maritime climate and the sites chosen for study are shown in Fig. 1. At each sampling site of 10 km^2, the following soil classes were selected, if present: arable soils, bogland, coniferous woodland, deciduous woodland, fresh-water marshes, grassland, heathland, river banks, rock outcrops, marine rocky shores, marine sand-dunes and salt marshes. Samples were collected using a sterile cork-borer, or sterile scalpel, transferred to sterile containers and returned to the laboratory. They were then moistened with nitrogen-free medium (2) and incubated for 2 - 4 weeks in petri dishes at 3000 lux and 25°C. Table 1 shows the algae which predominated in the various samples at the end of this period. *Nostoc commune* was the most common heterocystous alga present, with *Calothrix* dominating in the rocky shore samples. *Oscillatoria tenuis* was the most common non-heterocystous form and in maritime marshy areas *Nodularia spumigena* and *Gloeocapsa* were particularly common.

Fig. 1. Map of Scotland showing the major sampling areas (●).

TABLE 1

The dominant algae detected in algal samples from the various habitats in Scotland after incubation of samples in nitrogen-free medium, at 25°C and 3000 lux for 2 - 4 weeks.

Habitat	Dominant algal genera
Arable land	*Nostoc, Anabaena, Cylindrospermum, Oscillatoria*
Acid bogland	*Oscillatoria, Anabaena, Lyngbya, Nostoc*
Coniferous woodland	*Oscillatoria*
Deciduous woodland	*Nostoc*
Freshwater marsh	*Oscillatoria, Phormidium, Nostoc, Anabaena*
Permanent grassland	*Nostoc, Oscillatoria, Lyngbya, Aulosira, Anabaena, Phormidium*
Heathland	*Nostoc*
Riverbanks	*Oscillatoria, Nostoc, Phormidium*
Rock outcrops	*Anabaena, Anabaenopsis, Nostoc, Phormidium, Oscillatoria, Lyngbya*
Marine rocky shores	*Calothrix*
Sand dunes	*Nostoc, Anabaena, Lyngbya, Oscillatoria*
Salt marshes	*Phormidium, Oscillatoria, Lyngbya, Anabaena, Nostoc, Nodularia, Gloeocapsa*

Studies on Nigerian algae were concerned with those which occurred as soil crusts (Plates 1 and 2). Fifty sampling sites were chosen and the major ones are shown in Fig. 2. These cover the 5 main types of savanna found in the country (derived savanna, Southern Guinea savanna, Northern Guinea savanna, Sudan savanna which covers about two-thirds of all savanna land in Nigeria, and Sahel savanna) as well as the predominantly forested areas of the south-west of the country. There is a decrease in rainfall from the south-west where the rainy season lasts for about 11 months, to the north-east where the rainy season lasts only for about three months and where the soils are extremely dry for most of the

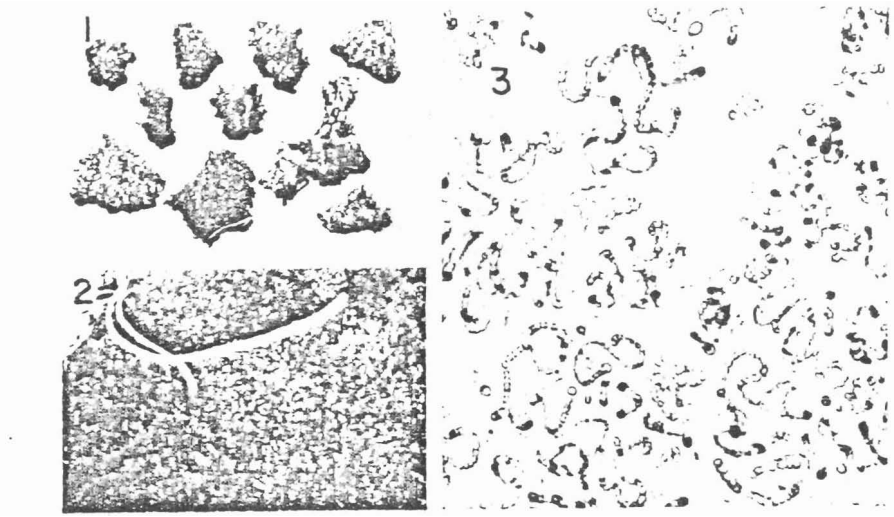


Plate 1. Soil crusts from Nigeria (x 0.18).

Plate 2. Soil crust from Nigeria showing a gelatinous blue-green algal mat (x 0.9).

Plate 3. Light micrograph of a *Nostoc* sp from a Nigerian soil crust (x 270).

year (see 15). The diversity of soil types in Nigeria is great but most are ferruginous ferratites and, apart from areas where there are iron pans, the soil can usually support good algal crusts when moisture is available. Large areas of savanna are burnt annually and blue-green algae develop prominently in open areas after the start of the rainy season (see 15). They are least abundant in the dry Sahel.

On direct microscopical examination of the soil crusts, it was found that all were dominated by one algal genus *Scytonema*, together with small quantities of non-heterocystous Oscillatoriaceae. and occasionally with species of *Tolypothrix* and/or *Nostoc* (Plate 3). The *Scytonema* (Plates 4-6) shows typical false branching, numerous heterocysts and frequently brown, blackish, or ochre, thick mucilaginous sheaths (see e.g. Plate 6). The presence of one dominant alga only in Nigerian soil crusts contrasts with our findings for Scottish soils where species diversity is greater and where *Nostoc* is usually the dominant heterocystous alga.

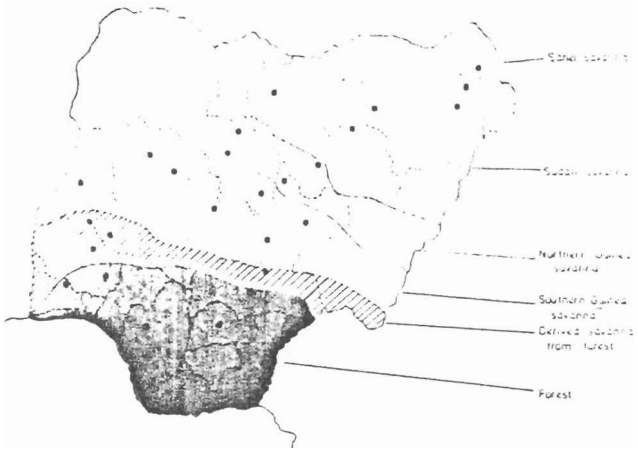
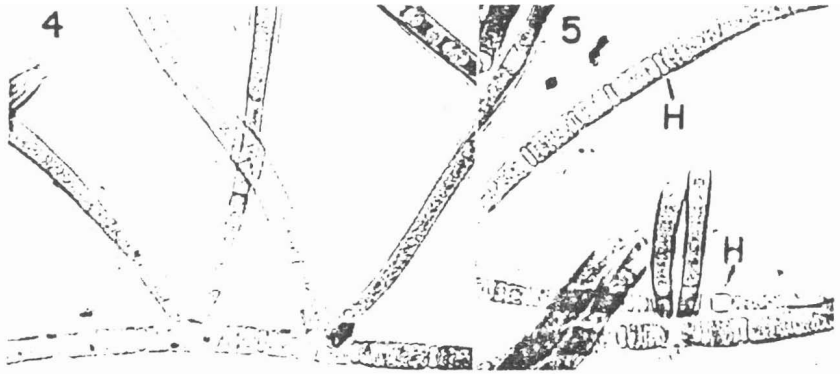
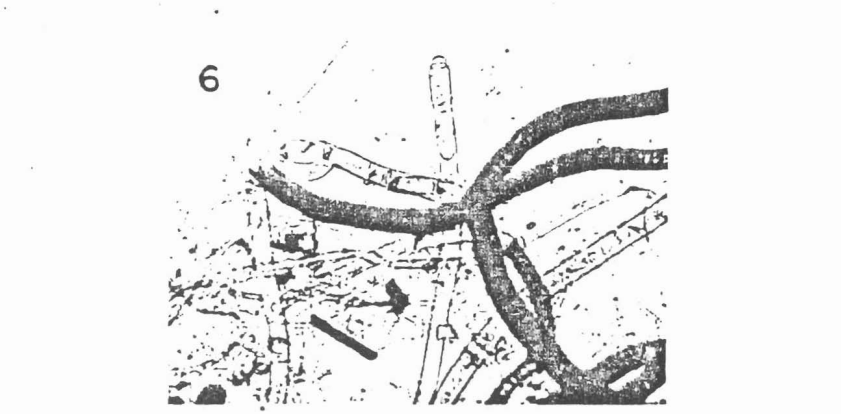


Fig. 2. Map of Nigeria showing the major sampling areas (●) and the different savanna zones.





Plates 4-6. Light micrographs of a *Scytonema* sp from a Nigeria soil crust. Note false branching, heterocysts (H) and blackish sheaths of certain filaments (x335).

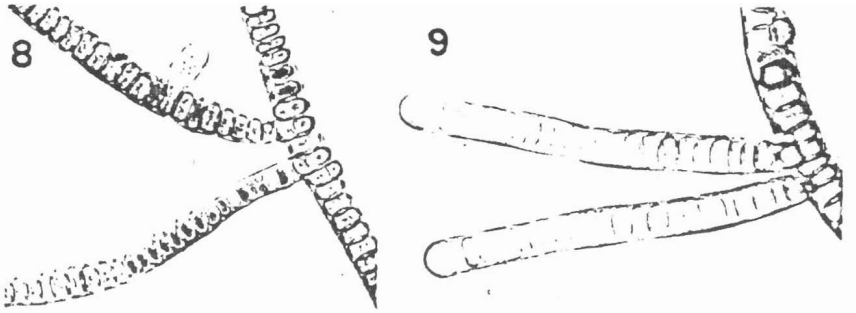


Plate 7. Colonies of *Stigonema panniforme* from Brazil (x 0.45).

Plates 8-9. Light micrographs of *Stigonema panniforme*. Note true branching (x 270).

Studies on Brazilian algae were concerned with one alga *Stigonema parviforme*. In parts of the Amazon region of Brazil near Manaus this alga develops in the open "Campina" areas among the rain forest. It is most abundant in the rainy season when it extensively covers the bare soil, but during the dry season it is less abundant, developing over the soil surface as blackish granules, a few mm in diameter (Plate 7). Light micrographs of the alga (Plates 8-9) show the true branching of this alga, which is a very distinctive member of the Stigonematales.

TESTS FOR NITROGENASE ACTIVITY

Tests for nitrogenase activity were carried out using the acetylene reduction assay (22). Studies on Scottish soils were carried out *in situ* and also by returning soil samples to the laboratory, incubating them in nitrogen-free medium for 4 weeks, and then re-assaying them for nitrogenase activity. The data, summarised in Table 2, show that light-dependent aerobic nitrogenase activity was detected in every major habitat type tested. However the % of each type which showed activity *in situ* ranged from only 3% in heathland to 80% in moist rock outcrops where blue-green algae were abundant. The mean rates of *in situ* acetylene reduction by all samples tested were low (< 2 nmoles C_2H_4 $cm^{-2}h^{-1}$) except on rock outcrops where the mean activity was 15.7 nmoles C_2H_4 $cm^{-2}h^{-1}$. After incubation in the laboratory for 4 weeks, a much higher percentage of samples showed activity with a maximum of 12,240 moles C_2H_4 $cm^{-2}h^{-1}$ being obtained with rock outcrop samples. Activity was also high in samples from permanent grassland.

The possible importance of nitrogenase activity by soil crust algae from Nigeria is summarised in Table 3. The samples were collected dry in the field, returned to the laboratory, moistened and then rates of C_2H_2 reduction were obtained under otherwise simulated field conditions. Every soil crust tested showed nitrogenase activity, with the rates being highest in crusts taken from the open areas in the forest (mean 11.2 nmoles C_2H_4 $cm^{-2}h^{-1}$) and minimal in crusts from the Sahel savanna (2.0 nmoles C_2H_4 cm^{-2} h^{-1}). On average these values are considerably higher than those found in Scottish soils, although the average rates obtained *in situ* on the rock outcrops in Scotland are higher than those of any of the Nigerian soil crust samples. Tests were also carried out in which the soil crusts were moistened with nitrogen-free culture medium and then maintained in the laboratory at 25°C and 3000 lux for 2 - 4 weeks before assaying for nitrogenase activity. Very much higher rates of nitrogenase activity were then obtained with a maximum value of 76.4 nmoles C_2H_4 $cm^{-2}h^{-1}$ being obtained in forest soil crust samples.

TABLE 2

Acetylene reduction rates by samples from various habitats in Scotland incubated *in situ* and in the laboratory

Soil type	No. of samples taken	% fixing *in situ*	% fixing after incubation for 4 weeks in the laboratory	Mean rates of C_2H_2 reduction *in situ* (nmoles $C_2H_4 cm^{-2}h^{-1}$)	Maximum rates of C_2H_2 reduction occurring in the laboratory (nmoles $C_2H_4 cm^{-2}h^{-1}$)
Arable	48	6	54	0.02	41
Bogland	8	12	12	0.3	8
Coniferous forest	39	8	8	0.2	292
Deciduous woodland	32	18	28	0.5	765
Freshwater marshes	30	35	68	0.5	387
Permanent grassland	54	22	45	1.2	3627
Heathland	33	3	3	0.3	342
River banks	48	31	55	1.1	1503
Rock outcrops	12	60	76	15.7	12240
Marine rocky shores	8	50	50	3.6	299
Sand dunes	18	22	72	1.3	*
Salt marshes	5	80	80	1.6	279

*not tested. The *in situ* tests were carried out during the months of May - September.

TABLE 3

Acetylene reduction by algal crust samples from various habitats in Nigeria

Area	Mean rate of C_2H_2 reduction (nmoles $cm^{-2}h^{-1}$)	Maximum rate of C_2H_2 reduction (nmoles $cm^{-2}h^{-1}$)
Sahel savanna	2.0	10.0
Sudan savanna	6.6	25.2
Northern Guinea savanna	5.7	29.7
Southern Guinea savanna	9.2	23.7
Derived savanna	10.6	24.5
Forest	11.2	76.4

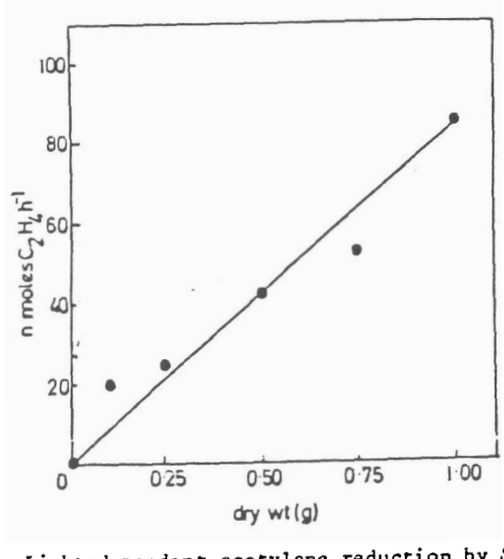


Fig. 3. Light-dependent acetylene reduction by *Stigonema panniforme* at 25°C and 3000 lux. The acetylene reduction assay period was 60 min.

Tests for nitrogenase activity by *Stigonema* were carried out in the laboratory by weighing out different quantities of algae collected during the dry season, wetting these and then testing for light-dependent nitrogenase activity 24 h later. As Fig. 3 shows nitrogenase activity increases with increase in algal biomass indicating that the alga is a N_2-fixing species. No data were obtained on the quantitative significance of this alga in the field.

ENVIRONMENTAL FACTORS AFFECTING NITROGENASE ACTIVITY BY ALGAE

In natural ecosystems N_2-fixing algae are subjected to a variety of extreme and often rapidly fluctuating environmental conditions (18,32). Among the environmental parameters which are important are pH, temperature, light, moisture, and nutrients other than nitrogen. The effect of variation in these on the nitrogenase activity of the various soil algae are presented below.

pH

Blue-green algae are characteristic of neutral and slightly alkaline soils; they are less common in acid soils and are rarely active in pure culture at pH levels below 5. Data on the effect of pH on algae from Scotland, Nigeria and Brazil are presented in Fig. 4. It is seen that all three types have a wide pH tolerance with an optimum near pH 8 and with good activity occurring at pH 10. Nitrogenase activity by the Scottish samples decreases markedly below pH 6. The tropical algae, on the other hand, show good activity even at pH 4. Such algae must possess an efficient pH buffering mechanism because *in vitro* the nitrogenase enzyme of cyanophytes is very susceptible to pH change outside the range 7.0 - 7.5 (10). The capacity of tropical algae to fix N_2 under acid conditions may be a factor contributing to their ecological success in many areas.

Temperature

Most blue-green algae grown in laboratory culture show temperature optima near 32.5 - 35°C, although exceptions occur (see 32), and field populations may show very different temperature optima. Data on the effect of temperature on soil cores from Scotland, soil crusts from Nigeria and *Stigonema* from the Amazon region are presented in Fig. 5.

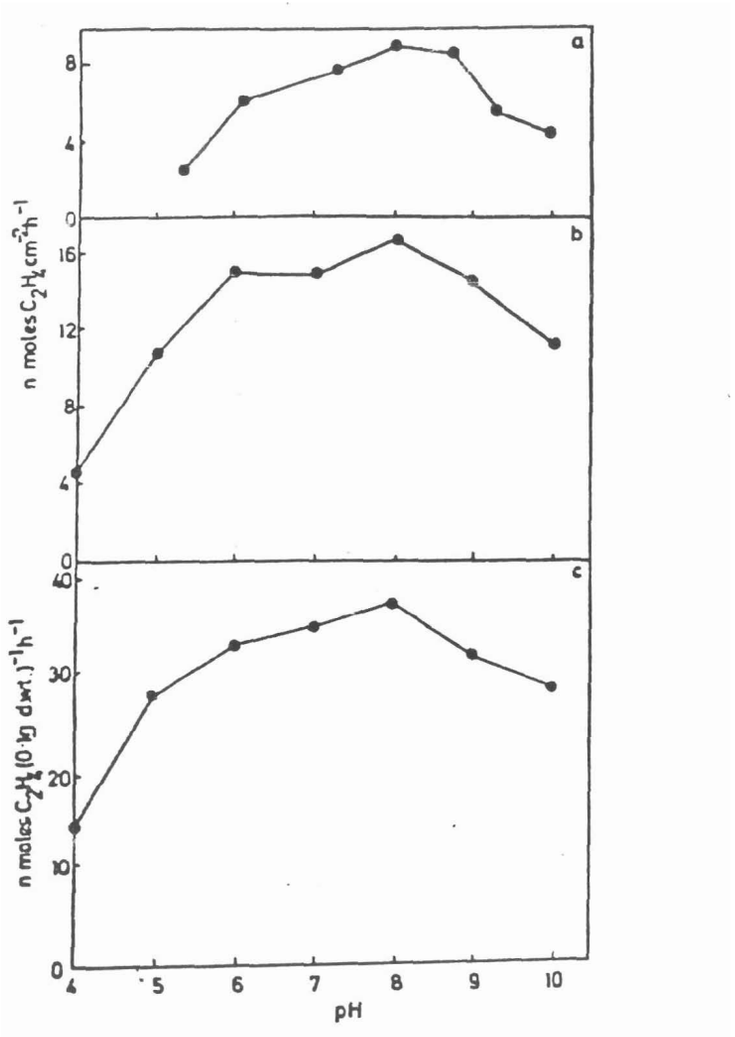


Fig. 4. The effect of pH on acetylene reduction by (a) a Scottish soil *Anabaena*, (b) *Scytonema* crusts and (c) *Stigonema panniforme*. The samples were incubated at the various pH levels for 24 h prior to the 60 min acetylene reduction assay. The pH of the medium did not change from the values given by more than 0.2 pH units during the experimental period.

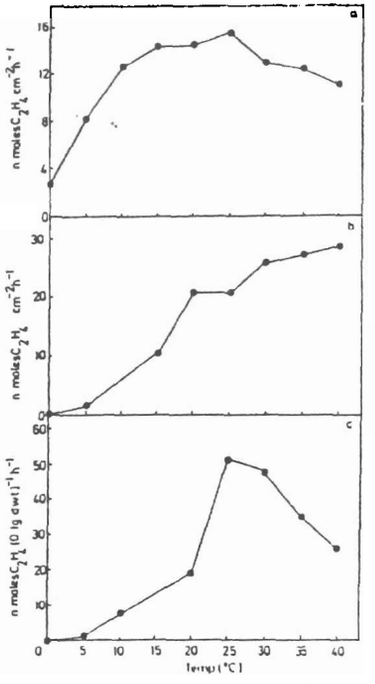


Fig. 5. The effect of temperature on acetylene reduction by (a) Scottish soil cores dominated by *Nostoc* and *Cylindrospermum*, (b) *Scytonema* crusts from Nigeria, and (c) *Stigonema panniforme* from Brazil.

The algae show rather different responses to temperature. Thus, while the Scottish samples reduced acetylene at 0°C, the tropical forms showed little activity even at 5°C. Activity by the Scottish samples, however, was still high at 40°C, although they had a temperature optimum of 15 - 25°C. The Nigerian samples, surprisingly, showed increased nitrogenase activity with increase in temperature to 40°C, while activity of *Stigonema* declined steeply above 30°C. The Scottish algae showed the greatest response to temperature increases from 0 - 10°C, with the corresponding ranges for Nigerian and Brazilian samples being 15 - 20°C and 20 - 25°C respectively. There is thus a general direct correlation between the temperature responses of the algae and the temperatures of the habitats from which they were collected, although the Scottish algae were able to reduce acetylene at temperatures which would seldom, if ever, be experienced in Scotland, except possibly in

certain localised soil niches for short periods in summer. Alexander (1), for example, reported how in the Arctic, heat absorption by dark coloured mosses in summer may result in high temperatures and high rates of N_2-fixation then. The difference in response of the Nigerian and Brazilian algae to high temperatures is, however, unexpected.

Light

Many blue-green algae are obligate photoautotrophs receiving their necessary energy from photophosphorylation, some grow photoheterotrophically at low light intensities which do not support photoautotrophic growth, and others grow slowly in the dark (see 32). Data obtained with *Stigonema panniforme* show the type of result most usually obtained (Fig. 6). It is seen that on incubating this alga in the dark, there is a rapid decline in nitrogenase activity, compared with the light, with activity ceasing after 20 h in the dark. In soil samples from Scotland a rather similar pattern was observed, although some samples showed a low rate of dark nitrogenase activity for at least 24 h.

Data were also obtained on the response of the tropical algae to increase in light intensity. It was found that both photosynthesis (O_2 evolution) and nitrogenase activity increased with increase in light intensity up to 80,000 lux probably because the dark-pigmented sheaths of these algae served as a light screen. This was likely to be the case particularly with the *Stigonema* which occurred on light coloured "Campina" soils. Data on the relative light transmission through algal mats of different thickness were then obtained by filtering different amounts of *Stigonema* onto Whatman GFC filters, clearing the filters in Cedar Wood oil and then measuring transmission of light of 665nm (the absorption maximum of chlorophyll *a*). As Fig. 7 shows, with increase in algal biomass there is, as expected, a decrease in light transmission with an algal layer less than 2 mm thick reducing light transmittance by approximately 80%. Thus in nature it is likely that these tropical algae adapt to the pertaining light intensity by varying their pigmentation and/or by aggregating together to cause self-shading, as Castenholz noted with some filamentous hot spring algae (5).

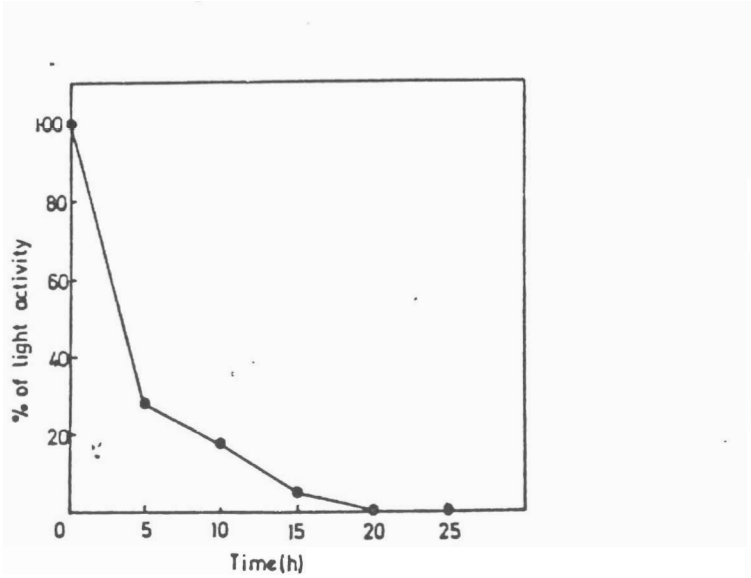


Fig. 6. Nitrogenase activity in the dark as % of nitrogenase activity in the light by *Stigonema panniforme*. The alga had been growing previously at 3000 lux and 25°C before being placed in the dark at 0 time.

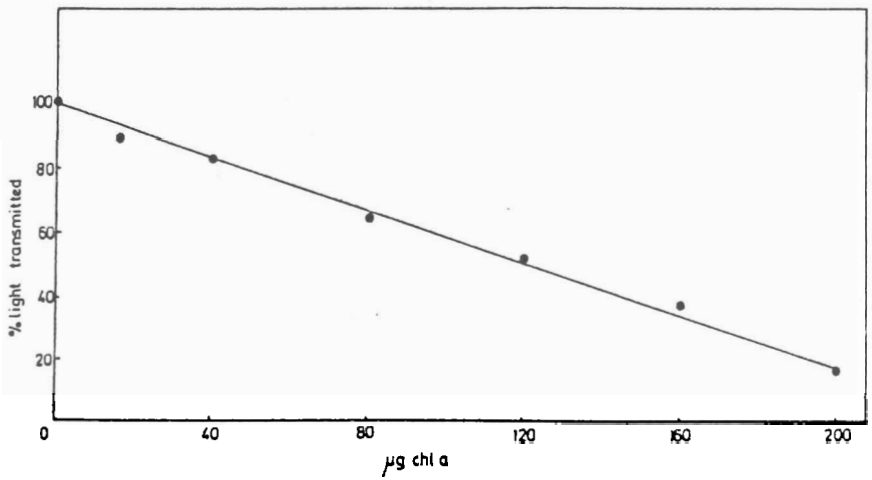


Fig. 7. Relative transmittance of 665 nm wavelength light through *Stigonema panniforme* mats. A value of 200 µg chl *a* corresponds approximately to an algal layer 2 mm thick. Light was supplied by a 100 watt incandescent bulb placed 15 cm from the algal mat.

Moisture

Desiccation or lack of moisture is a major factor affecting algal growth and nitrogenase activity by blue-green algae in temperate (12,13,25), tropical (26,37) and polar regions (7,14). However blue-green algae with their gel-like protoplasm and thick mucilaginous sheaths are able to absorb water extremely quickly when it is available and lose it much more slowly. Thus as Fig. 8 shows when air-dried *Stigonema panniforme* clumps are moistened they absorb several times their dry weight of water within 60 sec and take several hours to lose that absorbed water even on subsequent incubation at a relative humidity of 40%. Similar results were obtained with algae from Nigerian and Scottish soils.

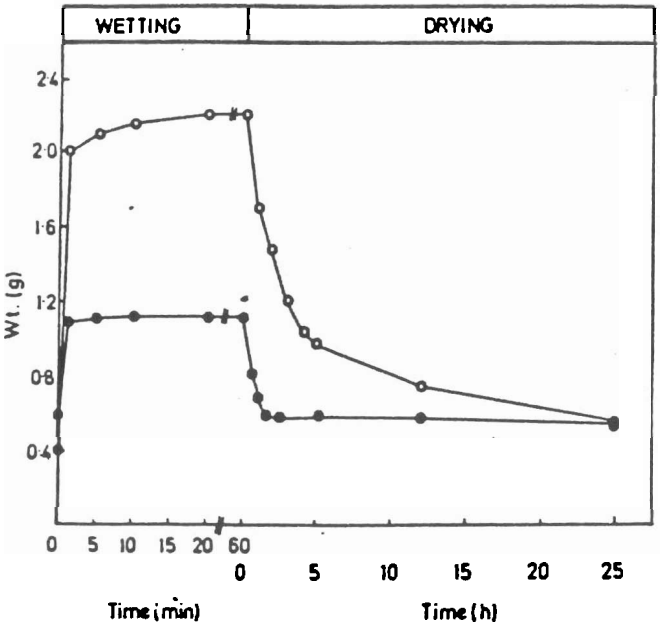


Fig. 8. Uptake and subsequent loss of water by *Scytonema* from Nigeria (●) and by *Stigonema panniforme* from Brazil (O). The samples were initially placed at a relative humidity of 40% until they equilibrated, they were then placed in water at 0 time and uptake of water measured by weighing of surface-dried samples at intervals thereafter. After 60 min they were removed from the water and the rate of water loss at a relative humidity of 40% monitored. The temperature throughout was 25°C.

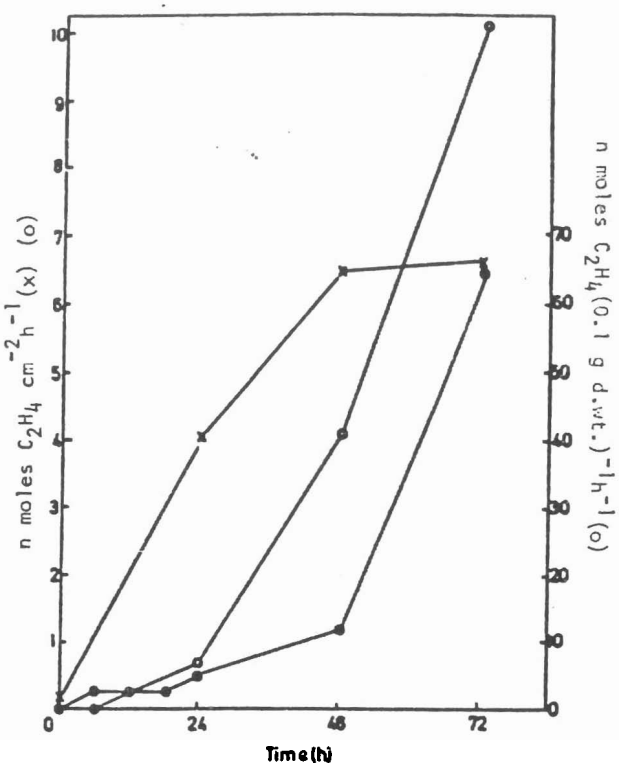


Fig. 9. Time course of acetylene reduction after rewetting air-dried samples of algae (*Nostoc* and *Anabaena*) from Scottish soils (X), *Scytonema* from Nigeria (O) and *Stigonema panniforme* from Brazil (●). The temperature was 25°C.

The results in Fig. 9 show further than on re-wetting air-dried and inactive algae, light-dependent nitrogenase activity restarts within 24 h even in the case of Nigerian and Brazilian samples which had been kept dry for several months before remoistening. Another factor which may be important, and which has been seldom considered in relation to moisture supply is the relative humidity of the atmosphere. Thus as Fig. 10 shows, Scottish soil algae retained under a relative humidity of 97.5% sustain an active nitrogenase when other factors are non-limiting, whereas algae exposed to lower relative humidities (87% and 75%) lose their activity within 60 h. **Activity recovers just as quickly as it is** lost however when the algae are subsequently returned to a high

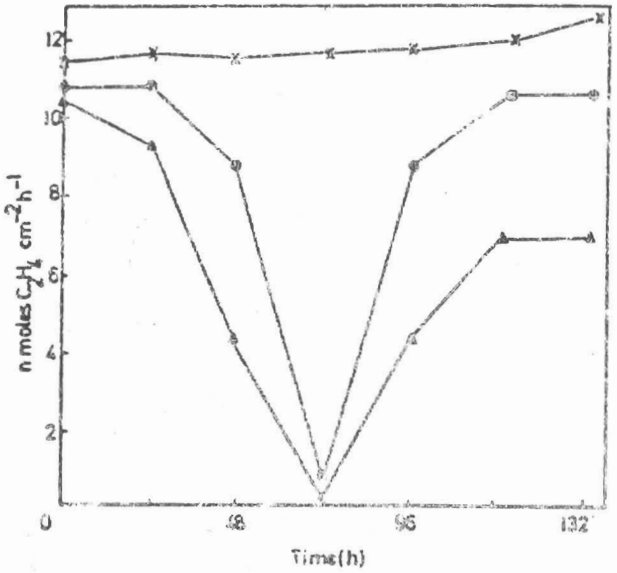


Fig. 10. The effect of different relative humidities on acetylene reduction by soil algae (*Nostoc* and *Anabaena*) from Scotland. The algae were incubated at the different relative humidities shown (✕ , 97.5; ● , 87.0 ▲ , 75.0) for 72 h, after which all samples were placed at a relative humidity of 97.5.

relative humidity. In natural ecosystems nitrogenase activity can be governed by soil moisture and/or the relative humidity of the atmosphere, and this is of importance in relation to the time at which field assays for nitrogenase activity are carried out. For example in studies on soil algae from Morocco we have found (W.D.P. Stewart and H.W. Pearson, unpublished) that *Nostoc* colonies showed nitrogenase activity in early morning when the relative humidity and soil moisture were high, but not around noon when the algae had become desiccated.

In connexion with the effect of moisture, it is of interest to note with soil crust algae from Nigeria, that on wetting them after a period of dryness, there is an immediate release of extracellular nitrogen, prior to the restart of nitrogenase activity. This production of extracellular nitrogen on change from one set of environmental conditions to another is similar in some respects to the findings of Jones and Stewart (16,17) for the marine *Calothrix scopulorum*.

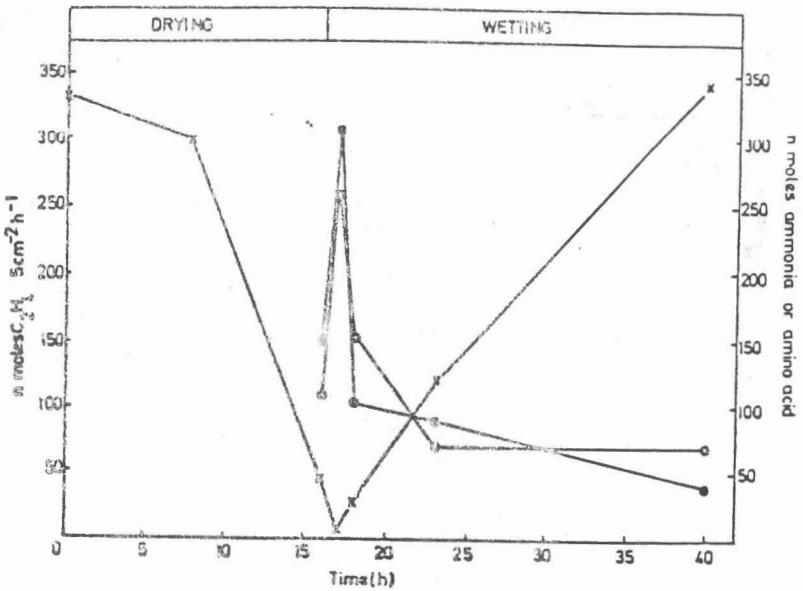


Fig. 11. The production of soluble nitrogen when dried *Scytonema* crusts from Nigeria are moistened ✕ , acetylene reduction; ○ , soluble amino acids; ● , soluble ammonia (see 34 for methods). The experiment was carried out at 25°C.

Molybdenum

In tests on Scottish soils, data were also obtained on the responses of soil cores to molybdenum, an essential component of the nitrogenase enzyme (4). It was found that approximately one-quarter of the soils tested showed an increase in light-dependent nitrogenase activity when Mo concentrations as low as 0.1 p.p.m. were provided. In all cases 0.5 p.p.m. of added Mo was found to be saturating. Typical data are presented in Fig. 12. These data resemble those obtained by Wolfe (38) who found that the optimum molybdenum concentrations for N_2-fixation by *Anabaena cylindrica* was 0.2 p.p.m.

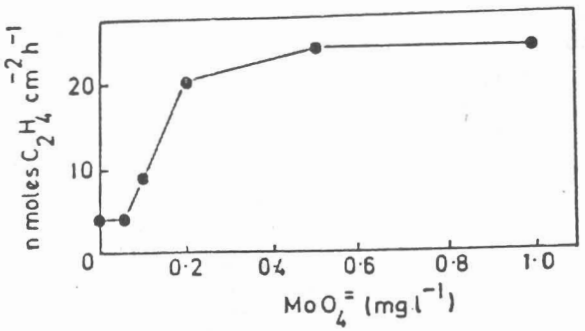


Fig. 12. The response of Scottish soil cores (dominated by *Anabaena* and *Nostoc*) to added molybdenum as Na_2MoO_4.

QUANTITATIVE SIGNIFICANCE OF N_2-FIXATION BY THE SOIL ALGAE

The quantitative significance of nitrogen fixation in any ecosystem depends on the total requirement of that ecosystem for nitrogen and on the relative contribution of biological nitrogen fixation, relative to other sources of nitrogen input. In tropical soils, for example, which are often characteristically poor in nitrogen, the input of a few kg nitrogen ha^{-1} ann^{-1} may be as important to the maintenance of the ecosystem, as are the additions of very much higher quantities of nitrogen by legumes in intensively cultivated agricultural land.

The data presented in this paper obtained using the C_2H_2 reduction assay, over short periods of time, provide information on whether or not nitrogenase activity is likely to occur in any habitat, but quantitative extrapolations of the data obtained to amounts of nitrogen fixed must be treated with extreme caution. Nevertheless it is important to obtain some indication of the order of magnitude of fixation in particular areas, and the following extrapolations attempt to do that. In general it is clear from our studies on Scottish soils that the overall input of biological nitrogen fixation by blue-green

algae is very low, and assuming a 100-day period of fixation, each with activity occurring for a 16 h period and assuming a 3 : 1 ratio for the rates of C_2H_2 reduction : N_2 reduction then average rates of fixation calculated are of the order of 1 - 2 kg N ha^{-1} ann^{-1}. These values are very much lower than the values attributed to algae in the Broadbalk wilderness by Day *et al.* (6).

The data for Nigerian savanna soils are, on the whole, appreciably higher and assuming a 12 h period of fixation per day, a 3 : 1 ratio, as above, and 250 days of activity per year in the wet south-west of the country this could account for an annual input of combined nitrogen of approximately 3 g N $m^{-2}ann^{-1}$. In the dry north-east of the country, on the other hand, the input by algal crusts is probably around 0.3 g N $m^{-2}ann^{-1}$. According to Nye and Greenland (20) there is annual input in the soil-plant system of the tall-grass savanna of Nigeria of about 38 kg N $ha^{-1}ann^{-1}$ and in this connexion input of nitrogen from these crusts may be of considerable importance.

ACKNOWLEDGEMENTS

B.J. Harbott assisted in the study of the Scottish soil algae, which was carried out as part of the U.K. contribution to the International Biological Programme. We thank Gail Alexander for assistance with other aspects of the work.

REFERENCES

1. Alexander, V.A. (1975). In: Nitrogen Fixation by Free-Living Micro-organisms (ed. Stewart, W.D.P.) pp. 175-188. Cambridge University Press, Cambridge.
2. Allen, M.B. and Arnon, D.I. (1955). Pl. Physiol. Lancaster, 30, 366.
3. Becking, J.H. (1975). In: Proc. 1st Int. Symp. of Nitrogen Fixation (eds. Newton, W.E. and Nyman, C.J.) Vol. 2. pp. 556-580. Washington State University Press, Pullman, Washington, D.C.
4. Burns, R.C. and Hardy, R.W.F. (1975). Nitrogen Fixation in Bacteria and Higher Plants. Springer-Verlag, Berlin. 189 pp.
5. Castenholz, R.W. (1968). J. Phycol. 4, 132.
6. Day, J., Harris, D., Dart, P.J. and van Berkum, P. (1975). In: Nitrogen Fixation by Free-Living Micro-organisms (ed. Stewart, W.D.P.) pp. 71-84. Cambridge University Press, Cambridge.

7. Fogg, G.E. and Stewart, W.D.P. (1968). Br. Antarct. Surv. Bull., 15, 39.

8. Fogg, G.E., Stewart, W.D.P., Fay, P. and Walsby, A.E. (1973). The Blue-green Algae. 459 pp. Academic Press, London and New York.

9. Granhall, U. (1975). In: Nitrogen Fixation by Free-Living Micro-organisms (ed. Stewart, W.D.P.) pp. 189-198. Cambridge University Press, Cambridge.

10. Haystead, A. and Stewart, W.D.P. (1972). Arch. Microbiol. 82, 325.

11. Henriksson, E. (1971). Pl. Soil Sp. Vol., 415.

12. Henriksson, E., Henriksson, L.E. and Da Silva, E.J. (1975). In: Nitrogen Fixation by Free-Living Micro-organisms (ed. Stewart, W.D.P.) pp. 199-206. Cambridge University Press, Cambridge.

13. Hitch, C.J.B. and Stewart, W.D.P. (1973). New Phytol. 72, 509.

14. Horne, A.J. (1972). Br. Antarct. Surv. Bull., 27, 1.

15. Jones, M.J. and Wild, A. (1975). Tech. Comm. No. 55, Commonwealth Bureau of Soils, Harpenden.

16. Jones, K. and Stewart, W.D.P. (1969*a*). J. Mar. Biol. Ass. U.K., 49, 475.

17. Jones, K. and Stewart, W.D.P. (1969*b*). J. Mar. Biol. Ass. U.K., 49, 701.

18. Magee, T. (1977). In: A Treatise on Dinitrogen Fixation Section IV. Agronomy and Ecology (eds. Hardy, R.W.F. and Gibson, A.H.) Wiley Interscience, New York.

19. Moore, A.W. (1969). Bot. Rev., 35, 17.

20. Nye, P.H. and Greenland, D.J. (1960). Tech. Comm. No. 51, Commonwealth Bureau of Soils, Harpenden.

21. Peters, G.A. (1975). In: Proc 1st Int. Symp. on Nitrogen Fixation (eds. Newton, W.E. and Nyman, C.J.) Vol. 2, pp. 592-610. Washington State University Press, Washington, D.C.

22. Renaut, J., Sasson, A., Pearson, H.W. and Stewart, W.D.P. (1975). In: Nitrogen Fixation by Free-Living Micro-organisms (ed. Stewart, W.D.P.) pp. 229-246. Cambridge University Press, Cambridge.

23. Rippka, R., Neilson, A., Kunisawa, R. and Cohen-Bazire, G. (1971). Arch. Mikrobiol., 76, 341.

24. Rippka, R. and Waterbury, J.B. (1977). FEBS Letts. (submitted).

25. Shtina, E.A. (1972). In: Taxonomy and Biology of Blue-Green Algae (ed. Desikachary, T.V.) pp. 294-295. University of Madras, Madras.

26. Singh, R.N. (1961). Role of Blue-green Algae in Nitrogen Economy of Indian Agriculture, 175 pp. Indian Council of Agricultural Research, New Delhi.

27. Singh, R.N. (1972). Physiology and Biochemistry of Nitrogen Fixation by Blue-green Algae. Final Technical Report, 1967-1972, 66 pp. Banaras Hindu University, Varanasi-5, India.

28. Stewart, W.D.P. (1967). Ann. Bot. N.S., 31, 385.

29. Stewart, W.D.P. (1971). Pl. Soil Sp. Vol., 377.

30. Stewart, W.D.P. (1973). A. Rev. Microbiol. 27, 283.

31. Stewart, W.D.P. (1975). In: Proc. 9th Europ. mar. Biol. Symp. (ed. Barnes, H.) pp. 637-660. Aberdeen University Press, Aberdeen.

32. Stewart, W.D.P. (1977). In: A Treatise on Dinitrogen Fixation, Section III Biology (eds. Hardy, R.W.F. and Silver, W.S.) pp. 63-123. Wiley Interscience, New York.

33. Stewart, W.D.P. and Lex, M. (1970). Arch. Mikrobiol. 73, 250

34. Stewart, W.D.P., Rowell, P. and Apte, S.K. In: Proc. 2nd International Symposium on Nitrogen Fixation (ed. Rodriguez-Barrueco, C.) Academic Press, London (in press).

35. Stewart, W.D.P., Rowell, P., Codd, G.A. and Apte, S.K. (1977). In: Proc. 4th Internat. Congress Photosynthesis, Reading, U.K. (in press).

36. Thuyet, T.O. and Tuan, D.T. (1973). In: Vietnamese Studies, Agricultural Problems, Vol. 4, pp. 119-127.

37. Venkataraman, G.S. (1975). In: Nitrogen Fixation by Free-Living Micro-organisms (ed. Stewart, W.D.P.) pp. 207-218. Cambridge University Press, Cambridge.

38. Wolfe, M. (1954). Ann. Bot. 18, 299.