T. Rosswall (ed). 1980
Nitrogen Cycling in West African Ecosystems

NITROGEN FIXATION BY BLUE-GREEN ALGAL SOIL CRUSTS IN NIGERIAN SAVANNA*

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Abstract

Blue-green algae, many of which are known to be nitrogen fixers, occur on the surface of the soil as crusts. Crusts are masses of algal filaments that grow on top of each other. These blue-green algal crusts were collected from all of the savanna zones of Nigeria in order to estimate the quantitative role they may play in the nitrogen economy of savanna ecosystems. Algae of the genus *Scytonema*, which are nitrogen fixers, were dominant in all the crust samples collected.

Using the acetylene reduction assay, it was found that the crust samples fixed nitrogen 24 h after rewetting and were affected by pH, temperature, light and moisture variations.

If sufficient light were available for near maximum photosynthesis, with an algal cover of the soil surface of about 30 % and mean to maximum-fixation during 70 % of the rainy season of 180 days of 10-hour day-length, from 3.3 to 9.2 kg ha^{-1} yr^{-1} of nitrogen would be fixed. This amount would replace much of the nitrogen lost from the grass standing crop as a result of annual burning of the savanna.

Introduction

For the past three years a study has been carried out to investigate the stocks and flows of nitrogen in some chosen savanna ecosystems in Nigeria. The study involves evaluating input, cycling and output of nitrogen in these ecosystems.

Blue-green algae are known to fix nitrogen (see for example Fogg *et al.*, 1973) and are therefore considered as sources of nitrogen input. Most attention has been paid to their role in rice paddies where – free-living (Singh, 1961, 1972) and in symbiotic association with the water fern, *Azolla* (Moore, 1969) – they contribute substantial amounts of nitrogen to the ecosystem. The blue-green algae are also common components of the microbial flora of the soil in many parts of the world.

In Nigeria, blue-green algae also occurs as crusts 1 to 5 mm thick on the surface of soil that is exposed and not too sandy. These crusts are composed of intertwining filaments of blue-green algae which usually dry up in the dry season and begin to grow again with the coming of the rains or when wetted. They are very resistant to drought and quite resistant to fire.

* Part of this material, in different form, was presented at the Symposium on "The potentials for nitrogen fixation in the tropics", Rio de Janeiro, Brazil, 18–25 July, 1977: "Nitrogen fixation by soil algae of temperate and tropical soils' '. (Stewart, W.D.P., Sampaio, M.J., Isichei, A.O. & Sylvester-Bradley, R.).

No work on surface crust blue-green algae has been reported from Nigeria or other tropical regions, excepting the preliminary report at the Brazilian symposium which includes some of the present work (Stewart *et al.*, 1977). Jones (1977) has investigated the effects of environmental factors on *Nostoc* mats in southern Africa, a sub-tropical-Mediterranean region. This study is concerned with finding out whether surface crust blue-green algae in Nigerian savanna fix nitrogen, and elucidating the environmental factors that affect their fixing ability. It must, however, be emphasized that the present work is of a preliminary nature and is concerned with only the general aspects of nitrogen fixation by the blue-green algal crusts of this specific region and the factors that may affect their quantitative contribution of nitrogen to the savanna ecosystem.

Materials and methods

Crust samples were collected from all the savanna zones and from some parts of the forest and sub-montane zones of Nigeria (Fig. 1). The samples were collected by scooping the crusts from the soil surface with a spoon. The crusts were sealed in envelopes and notes made of the area and the nature of the soil. The samples were later air dried. Each sampling was divided into two portions: one packed for later study of the effects of environmental factors at the laboratories of Professor W.D.P. Stewart in Dundee, and the other sent to Professor K. Anagnostides of Athens University for culturing and identification. The cover was estimated by laying random quadrats of a string grid and recording the number of grid intersections above the algal crusts (Isichei, in preparation).

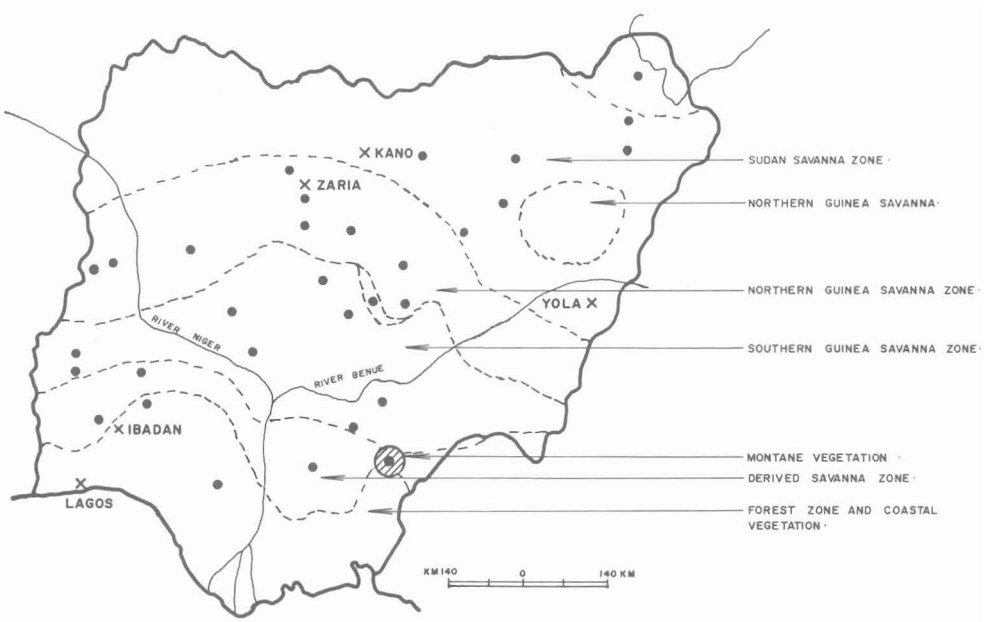

Figure 1. Nigeria: Dots indicate areas where algal crust samples were collected.

Before the effects of environmental factors were studied, the samples were wetted with a nitrogen-free medium, 'BG–11' (Stanier *et al.*, 1971) and incubated at 3000 lux and 27°C. The wetted samples were first tested for nitrogenase activity using the acetylene reduction assay (Renaut *et al.*, 1975). Since crust effectiveness in N-fixation will be expressed per unit area, tests were carried out using 1 cm^2 circular crusts sampled by using a metal corer 1 cm^2 in area to cut out samples from the crust collections.

The incubated samples were used to test the effects of the following environmental variables: (1) moisture; (2) pH; (3) light intensity; (4) temperature. The first reaction to moisture of the dry crusts would be water uptake. This would be manifested by increase in weight on immersing the crusts in water and decrease in weight when the immersed sample is exposed to air. The crusts were therefore immersed in water, brought out immediately and the weight monitored continuously by suspending them from a hand spring balance ('Pesola', Switzerland, 5 g). This was repeated for several crust samples.

Relative humidity is a measure of available moisture in the atmosphere, to which the crusts are exposed. Incubated samples were subjected to different relative humidities at room temperature (20°C). The different humidities (RH) were achieved by use of saturated salt and sugar solutions (Winston & Bates, 1960), as shown below:

Solution	Approximate RH at 20°C	Solution	Approximate RH at 20°C
Water	100.0 %	Sodium chloride	77.5 %
Potassium sulphate	97.5 %	Glucose	55.0 %
Potassium sodium tartarate	87.0 %	Air in the laboratory	40.0 %
		Silica gel	20.0 %

When these solutions are kept in closed containers for some time, the air above them is assumed to have the stated relative humidities. The solutions were placed in tightly closed bottles and crust samples were put into the bottles in open 5 cm^3 vials. The bottles were left closed for at least 24 h before acetylene reduction assay was carried out. The level of acetylene reduction in each sample was tested immediately before each experiment. After 24 h the level of acetylene reduction was expressed as a proportion of the original value.

To test the effect of pH, the crust samples were kept at pH levels of 4 to 10 for 24 h. The pH buffers used were prepared from nitrogen-free universal buffer (Teorell & Stenhagen, 1938). The samples were incubated for acetylene reduction assay for 60 minutes. The pH buffers were fresh so that there was no change in pH of greater than 0.2 pH units during the experimental period.

Tests for acetylene reduction by a particular sample were carried out at various light intensities. Light intensity was varied by moving the crust sample away from a projector light source. At each point the light intensity was measured using a light meter (Corning-EEL Lightmaster 18/335b). The range of intensity used was from 200 to 34,000 lux. Each sample was left at each light intensity for an hour before being subjected to a 60-minute acetylene reduction assay.

Temperature effects were tested by putting a sample in a closed vial and leaving it in a water bath at the required temperature for one hour before assay.

Results

It was found that all crust samples were dominated by *Scytonema myochrous* (Dillw.) Ag. ex Born. et Flah., which has well developed heterocysts. Small quantities of non-heterocystous *Oscillatoriaceae* and occasional *Tolypothrix* and/or *Nostoc* species were also found. It can thus be stated that the crusts from all Nigerian locations contain mostly potentially nitrogen-fixing blue-green algae. The mean values of acetylene reduced are given in Table 1.

The crusts were found to absorb water very fast (to double their weight in a few minutes, Fig. 2) but to lose it slowly. Dry crusts start reducing acetylene within 24 h of wetting, and activity increases exponentially after this time until at least 72 h (Fig. 3).

Table 2 illustrates the effect of various relative humidities on acetylene reduction. The maximal reduction occurs at a relative humidity of 75 %. At humidities as low as 40 % no reduction takes place. No activity occurred at the relative humidity where potassium sodium tartarate was used. It was suspected that this salt was toxic to the algae.

The blue-green algae present in the crusts have a wide pH tolerance with an optimum near pH 8 but with good activity at pH 5 and pH 10 (Fig. 4).

The crust samples showed little activity at 5°C, but activity increased with increasing temperature up to 40°C. Activity stopped above this temperature (Fig. 5).

No clear-cut trend in nitrogenase activity was observed with variations in light intensity; the crusts did not reach saturation even at 34,000 lux, the point at which the experiment was stopped.

Table 1. Acetylene reduction by algal soil crust samples from various habitats in Nigeria.

Location (Zone according to Keay, 1959)	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
Mean of Sudan, Guinea and derived savanna zones	8.2	25.8

Table 2. The effect of various relative humidities on acetylene reduction by algal crusts from Nigeria.

Relative humidity (%)	% Relative C_2H_2 reduction in comparison with reduction at almost 100 % RH
97.5	118
75	149
55	58
40	0

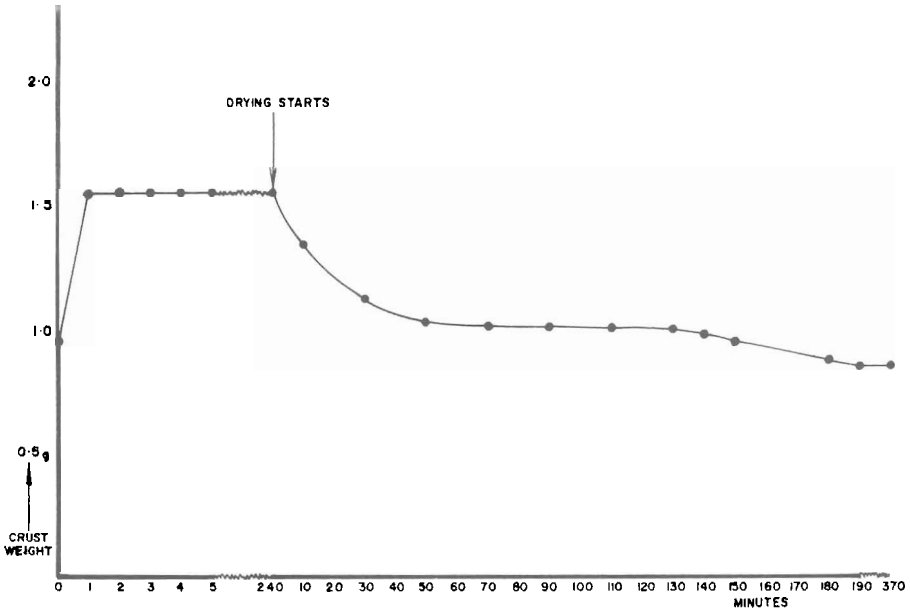

Figure 2. Water uptake and loss by algal crust sample at 20°C and 40 % relative humidity.

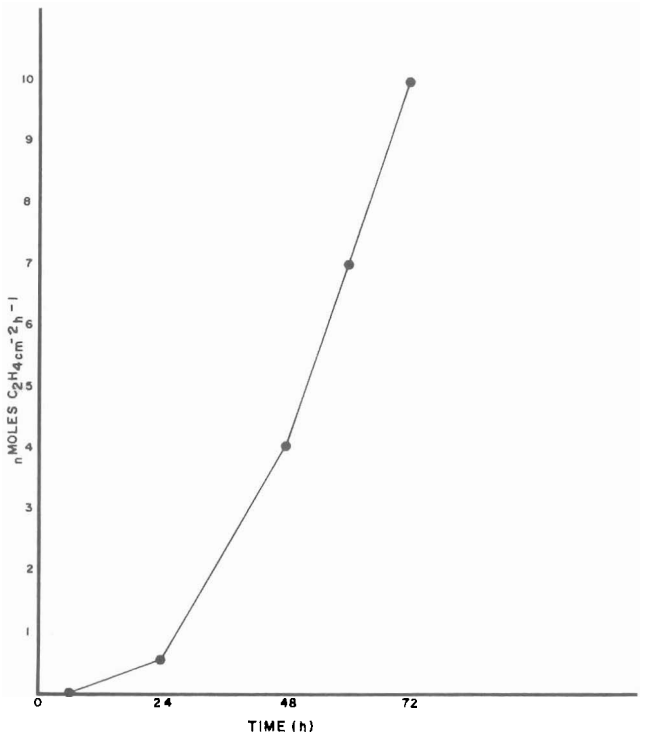

Figure 3. Time course of acetylene reduction by crust sample after rewetting.

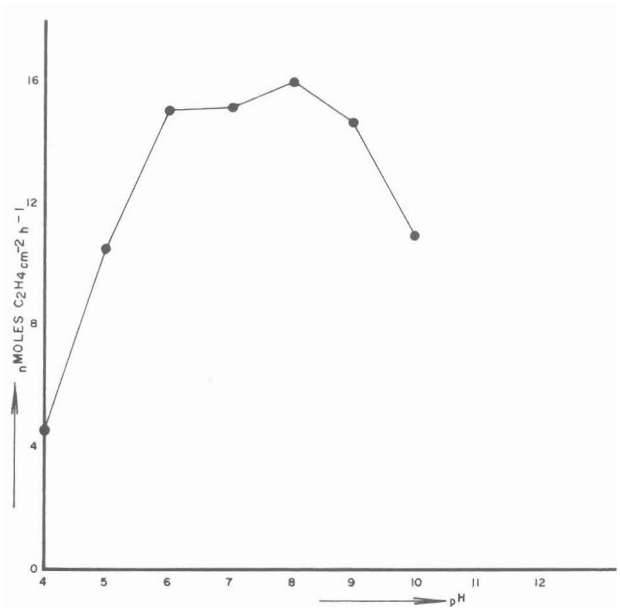

Figure 4. The effect of pH on acetylene reduction by blue-green algal crust.

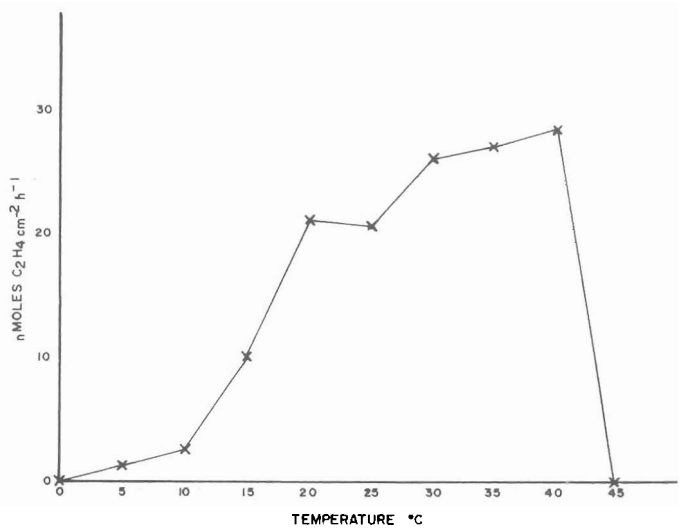

Figure 5. The effect of temperature changes on acetylene reduction in blue-green algal crust samples.

Discussion

The algal crusts are able to survive drought and are fairly resistant to fire because of the extensive mucilaginous sheaths of the filaments, but the sheaths do not impede water absorption. In fact, the slow rate at which water is lost once absorbed make the sheaths a necessary tool for survival and for active growth in the savanna where water is limiting for a major part of the year.

Atmospheric humidity varies tremendously with distance above soil surface, and the usually expressed values of relative humidity may not coincide with the situation at ground level. One can certainly assume that during the rainy season, when water is available for growth, humidity will be high enough for nitrogenase activity most of the time. Some activity may also occur during part of the dry season.

The response of the algae to temperature is not unexpected, because soil surface temperatures in the savanna are often high (see Jones & Wild, 1975). It is interesting, however, that there was nitrogenase activity at low temperatures unknown in Nigeria. This is an example of the physiological versatility for which blue-green algae are noted.

The crusts showed activity under a wide pH range. The algae must possess an efficient pH buffering mechanism, because *in vitro* the nitrogenase enzyme of Cyanophytes is susceptible to pH change out of the 7.0 to 7.5 range (Stewart *et al.*, 1977).

The algae were not light-saturated under the prevailing experimental conditions, probably because their dark pigmented sheaths served as a light screen; if this should be so in nature, it would be a disadvantage, because light intensity is lowest in the rainy season when there is enough moisture for growth. Stewart (pers. com.) believes that the algae adapt to the prevailing light intensity in their growth area by varying their pigmentation. Jones (1977) also found that *Nostoc* mats, depending on the amount of radiation received during the day, may fix nitrogen at night. This claim cannot be evaluated without more thorough work on the nitrogenase activity of blue-green algae and their use of stored photosynthates in the fixation reaction.

The relevance of studying the effects of environmental factors is to see how these factors affect the contribution made by soil crust algae to the nitrogen economy of the savanna ecosystem. Mean values of nitrogenase activity for the various savanna zones are given in Table 1. A maximum mean value of 25.8 moles C_2H_4 cm^{-2} crust h^{-1} was produced by the crust samples from the derived, Guinea and Sudan savanna zones; the corresponding mean value was 8.0 kg N ha^{-1} yr^{-1}. Nitrogen fixation at these levels of activity would represent between 3.3 and 9.2 kg ha^{-1} yr^{-1} of nitrogen. (The ratio of 3:1 of ethylene produced to nitrogen is assumed. In making these estimates, it was also assumed that fixation occurred for 70 % of the time of a rainy season 180 days in length with 10 daily hours of sunlight and 30 % cover of the soil surface by the crusts.)

The actual amount of nitrogen fixed may, however, be smaller or larger. Moisture may not necessarily be available all the time during the rainy season and may very occasionally be available during the dry season. Light, the role of which is not yet clearly understood, may also be limiting. On the other hand, 30 % cover may be an underestimate of algal cover in many locations (Isichei, unpublished data). Also, cover varies considerably over the year and one can reasonably assume that a thicker crust may fix more nitrogen than a thin one. Generally, it can be expected that field conditions vary more widely than is assumed in the laboratory.

With all this in mind, one can say with certainty that the blue-green algae may replace

some of the nitrogen lost in the annual fires on the savanna (Isichei & Sanford, 1979). This goes a long way in explaining how production can be maintained year after year in West African grasslands. In this connection, it is perhaps worth mentioning that Stewart *et al.* (1977) found that growing crusts release ammonia and/or amino acids when dried and later rewetted. This clearly indicates that fixed nitrogen in blue-green algae is not necessarily leached into the soil only at the death of the blue-green algae. E.A. Obot (unpublished) also found the dry matter production of maize seedlings grown in nitrogen-free medium to be significantly greater when algal crusts grew on the pot surface.

Acknowledgements

I thank the University of Ife and the Inter-University Council for Higher Education Overseas of Britain for financing this project. I am grateful to Professor W.D.P. Stewart and his laboratory staff for guidance.

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