Environmental Role of Nitrogen-fixing
Blue-green Algae and Asymbiotic Bacteria
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N_2-FIXING ALGAL BIOMASS IN SENEGAL RICE FIELDS

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Abstract

Qualitative and quantitative variations of the algal flora in paddy fields in Senegal were studied. Between planting and tillering, unicellular eucaryotic algae were dominant. Between tillering and panicle initiation, filamentous green algae and non heterocystous blue-green algae were dominant. After panicle initiation, heterocystous and non heterocystous blue-greens became dominant if the plant cover was sufficiently dense; under a weak plant cover, filamentous green algae and non heterocystous blue-green algae remained dominant.

Total algal biomass was greatest between tillering and panicle initiation; then, after heading, it decreased. The measured values varied from a few hundred kilos to many tons of wet algae per hectare.

The nitrogen-fixing algal biomass, small at the beginning of the cultivation cycle, reached an absolute maximum after heading and a relative maximum at the end of the cultivation cycle. Observed values, generally a few hundred kilos, could exceed a ton per hectare in some exceptional cases. The N_2-fixing algal biomass was positively correlated with soil pH and with density of plant cover.

Intensity of light reaching the soil is an important factor in the evolution of algal flora composition. Growth and nitrogenase activity of N_2-fixing blue-green algae are dependant upon a plant cover sufficiently dense to protect them from the inhibitory effect of very high light intensity occurring in Senegal (70000 lux at 13 00 hrs). Maximum light intensity in the day determined variations of algal N_2-fixing activity. Four types of daily cycles were found.

Values recorded varied from 0 to 60 nmoles $C_2H_4 \cdot cm^{-2} \cdot h^{-1}$. These values, in terms of the N_2-fixing algal biomass, varied from 0.2 to 2.2 nmoles $C_2H_2 \cdot mg\ prot^{-1} \cdot min^{-1}$. Extrapolation of the results indicates algal N_2-fixation in Senegal paddy soils to be in the order of a few kilos of N per hectare per cultivation cycle. Values between 10 and 30 kg were found, but were exceptional.

Introduction

The role of N_2-fixing blue-green algae in the nitrogen economy of rice fields has been studied in Japan (Watanabe, 1951; Okuda & Yamaguchi, 1956), India (Singh, 1961; Venkataraman, 1972), Egypt (El-Nawawy & Hamdi, 1975) and Morocco (Renaut *et al.*, 1975). The present work reports ecological studies made between 1973 and 1975 in Senegal rice fields where qualitative and quantitative variations in the total and N_2-fixing algal flora were studied during the rice cultivation cycle. Acetylene reduction activity was used to measure the specific activity of the nitrogen-fixing algal biomass, and to evaluate how it varies with variation in sunlight.

Materials and methods

Sampling sites

The area studied was characterized by high light intensities, often reaching 70.000 lux at 13.00 hrs. Rice cultivation generally occurred during the rainy season (July – November) and rice fields were dry during

December–June. Thirty soils were sampled. These were acidic, having an average pH value of 5.0 at the beginning of the rice cultivation and 6.2 after two months of submersion.

Biomass estimation of algal flora components

The algal flora was determined by the following three successive steps:

1) *Preparation of a representative sample of the area studied:* Core-samples representing a density of 1600 unit-samples per hectare were collected and carefully mixed. Each core-sample (2 cm diameter) included the top centimeter of soil and the corresponding surface water.

2) *Enumeration of algae.* Algal populations were estimated by agar-plate counts on three selective media (Reynaud & Roger, 1977). The media used to separate the following groups of algae were: eucaryotic algae: BG11 medium (Allen & Stanier, 1968) supplemented with bacitracine 15 ppm; procaryotic algae: BG11 medium supplemented with cycloheximide 20 ppm; nitrogen-fixing blue-green algae: BG11 medium, nitrogen-free medium supplemented with cycloheximide 20 ppm.

3) *Calculation of biomass.* The mean volume of each "unit" (cell, filament or colony according to species) of the most important algae was determined by making 100 measurements on fresh mixed core-samples. This, together with counts of each unit, gave the biomass present. Estimation of algal biomass was made every three weeks in a rice field of 0.25 ha during a cultivation cycle (Roger & Reynaud, 1976). A second series of algal biomass estimations was made on thirty rice-fields of differing geographic location, stages of rice-growth and fertilizer treatments (Roger & Reynaud, 1977). The results of these two studies agreed well and a scheme for the evaluation of the algal flora in paddy soils of the areas studied can be postulated.

Acetylene reduction assays

Six core-samples (soil + surface water) were placed in a 250 ml serum bottle. Acetylene was added through the rubber stopper at zero time without removing the initial gas phase (Stewart *et al.*, 1971) and the bottle was replaced in the sampling site. The composition of the gas phase was determined after 15 and 30 minutes incubation *in situ*.

Results

The algal biomass in Senegal rice-fields

Total algal biomass

After planting and soil submersion, algal biomass increased until tillering with highest values occurring between tillering and panicle initiation and then decreasing after heading (Fig. 1). Total biomass was influenced by fertilizers with maximum yields of only a few hundred kg ha^{-1} occurring in non-fertilized paddy soils, while in fertilized soils several tons ha^{-1} were produced with a maximum of more than 5 tons ha^{-1}.

During the early part of the cultivation cycle (planting to tillering) the algal biomass consisted mainly of diatoms and unicellular green algae (Table 1). After tillering, filamentous green algae and non-N_2-fixing blue-green algae were dominant. After panicle initiation, heterocystous and non-heterocystous blue-green algae became dominant if the plant cover was sufficiently dense, but under a low plant cover filamentous green algae and non-heterocystous blue-green algae predominated.

The biomass of nitrogen-fixing blue-green algae.

The absolute maximum nitrogen-fixing algal biomass (N.F.A.B.) was observed during panicle initiation, but the relative maximum was reached at the end of the cultivation cycle (Fig. 1 and Table 1). During the early stages of the cycle, observed values were

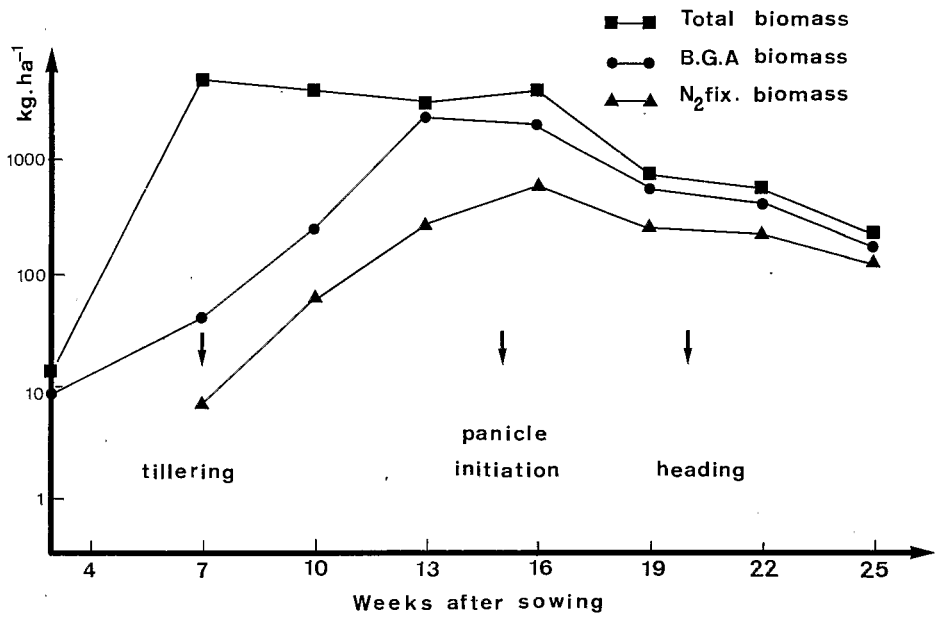

Figure 1. Variations of algal biomass during a cultivation cycle of rice.

Table 1. Algal biomass composition in relation to rice development (40 paddy soils studied).

Stages of rice development	Dominant flora				N_2-fixing algae		
	Nature	% of total biomass			% of total biomass		
		Mean value	Max. value	Min. value	Mean value	Max. value	Min. value
Tillering	Diatoms. Unicellular green algae	73	99	49	2	4	0,1
Panicle initiation	Filamentous green algae. Non-heterocystous blue-green algae	89	93	86	3	9	0,1
Heading to maturity. Weak plant cover	Filamentous green algae. Non-heterocystous blue-green algae	70	91	62	8	14	0,2
Heading to maturity. Dense plant cover	Blue-green algae	71	99	16	38	99	13

about ten kg ha^{-1}. After panicle initiation, observed values were generally a few hundred kilos per hectare but exceptionally they exceeded a ton per hectare with a maximum of 2260 kg ha^{-1} (Table 2). Overall, the N.F.A.B. represented 38% of total algal biomass at the end of the cycle.

The effect of some physico-chemical factors on N.F.A.B.

Statistical comparison of mean values and calculation of Spearman's coefficient of rank correlation (Spearman, 1904) demonstrated the effect of some physico-chemical factors

Table 2. N_2-fixing algal biomass in 14 soils sampled after panicle initiation.

Class in kg wet weight · ha^{-1}	Number of soils per class	Corresponding N in kg ha^{-1}
50– 100	5	0.4– 0.8
100– 500	10	0.8– 4
500–1000	1	4 – 8
1000–5000	1	8 –40

on N.F.A.B. Fertilization with NPK had a positive effect on absolute N.F.A.B., which was about four times greater in fertilized than in non-fertilized soils. However a negative effect of fertilization on the relative N.F.A.B. was noted. At the end of the rice cycle, heterocystous blue-green algae represented about 30% of total biomass of fertilized soils as opposed to 50% in non-fertilized soils. A positive significant correlation was observed between soil, pH and N.F.A.B. This relationship was only conspicuous in samples from soils which contained rice of a similar stage of development, fertilization and plant density.

An arbitrary Plant Cover Index was given to each sample depending on the density of the higher plant cover. With increase in plant cover there was a corresponding increase in the N.F.A.B. (Fig. 2).

Blue-green algae are more sensitive to high light intensities than diatoms and green algae (Brown & Richardson, 1968). Thus, they grew only when the plant cover was sufficiently dense to protect them from high light intensities (70,000 lux at 1300 hours).

A survey of the nitrogen-fixing blue-green algae present

The enumeration method allowed us to count the major species and associated species if they represented more than 10% of the major one. As Table 3 shows, *Anabaena* and *Nostoc* were ubiquitous and were the major genera present in 60% of the samples. They were observed at lower pH values than other nitrogen-fixing blue-green algae. An average pH of 5.9 was observed in soils where these 2 genera were dominant, while the average pH of other samples was 6.3 (significant at the 95% level). Of the other genera, *Scytonema* was the most common, occurring in approximately 50% of the soils and being dominant in 30%. *Calothrix* was found in 15% of the samples, particularly in sandy soils. *Cylindrospermum* developed large biomasses on wet soils after harvesting and was often located in soil depressions. The other genera: *Scytonematopsis, Microchaete, Westiellopsis, Nodularia* and *Microcoleus* were never dominant.

Nitrogen fixation by blue-green algae

The data above indicate a marked effect of plant cover, as a light screen, on the development of N.F.A.B. Before measuring nitrogenase activity *in situ*, the influence of high light intensities was investigated.

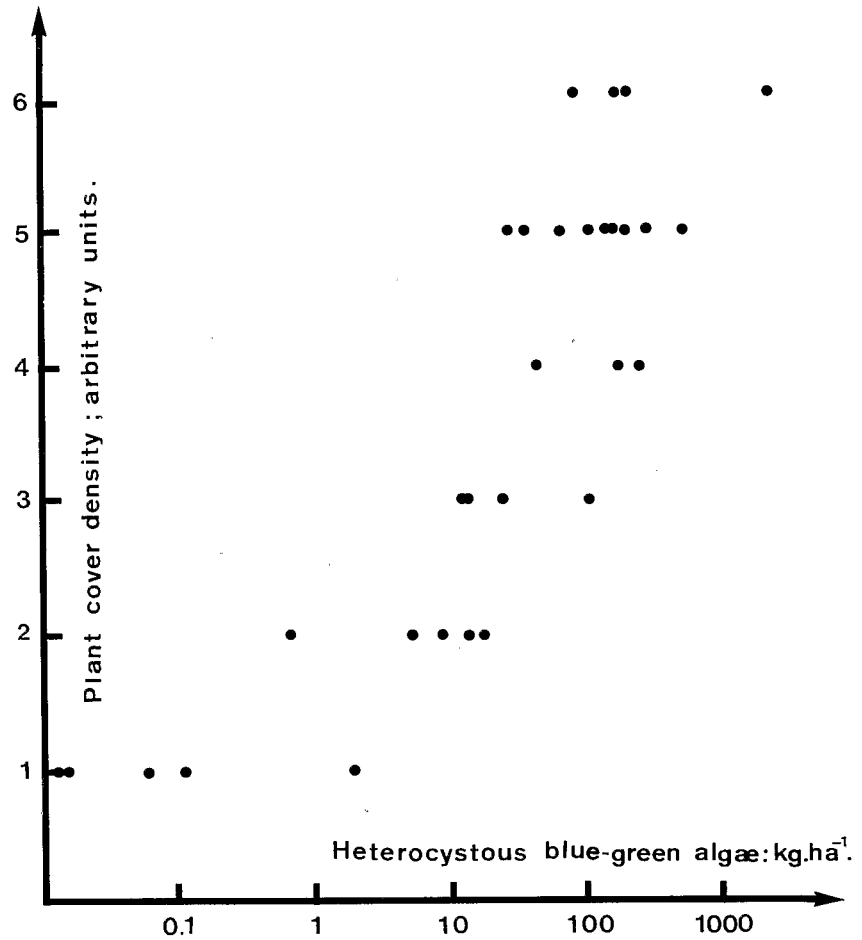

Figure 2. Correlation between plant cover density and the biomass of heterocystous blue-green algae.

Table 3. Nature and frequency of N_2-fixing blue-green algae on 40 paddy soils.

	% of soils in which the genus is present	% of soils in which the genus is the dominant N_2-fixing genus
Anabaena; Nostoc	100	60
Scytonema	47.5	30
Calothrix	15	5
Scytonematopsis	7.5	0
Microchaete	7.5	0
Tolypothrix	7.5	2.5
Cylindrospermum	5	2.5
Westiellopsis	5	0
Nodularia	5	0

The influence of light on N_2 fixation by blue-green algae *in situ*

Cultivation vessels containing non-planted submerged soil were placed under screens permitting transmission of 100%, 60%, 22% or 7% of the incident sunlight. Half of the vessels received PK fertilization and half NPK fertilization. The amounts of fertilizers correspond to 50 kg $KH_2PO_4 \cdot ha^{-1}$ and 50 kg urea $\cdot ha^{-1}$. After one month of submersion, *in situ* activity was measured for each vessel, as described, at 3,000 lux. The results (Fig. 3) showed a negative effect of nitrogen fertilizers and of high light intensities. Maximum nitrogenase activity was obtained for vessels without nitrogen fertilization receiving 7% of the incident light (corresponding to a maximum of about 5,000 lux).

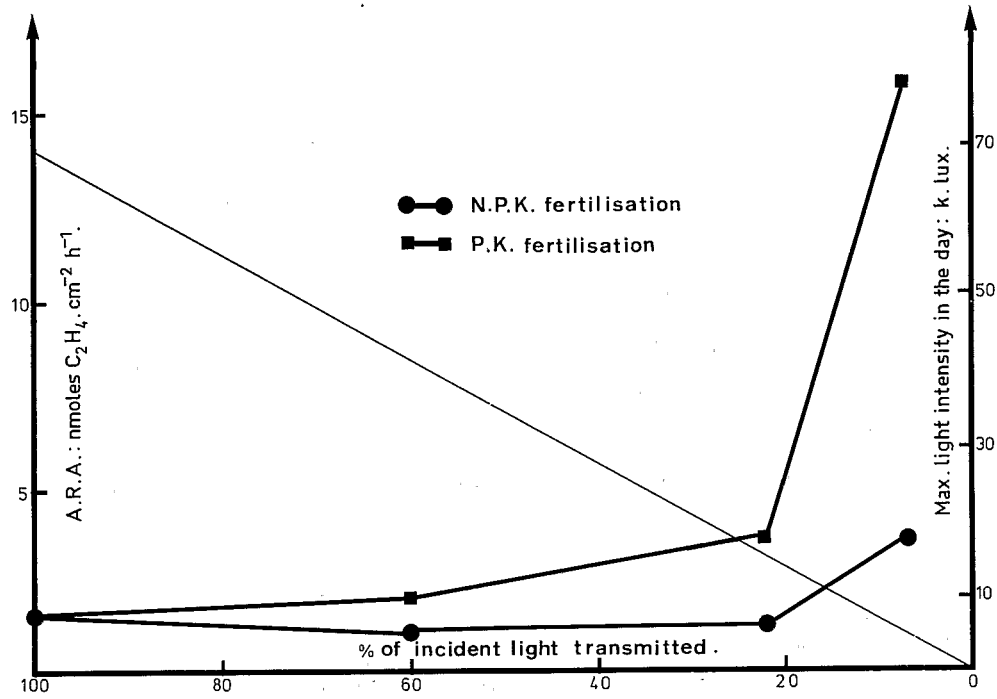

Figure 3. Effect of artificial plant cover on acetylene reduction by soil algae.

Diurnal variations of nitrogenase activity

Diurnal variations of nitrogenase activity were studied in the same rice field. A dense bloom of *Anabaena* sp. and *Westiellopsis prolifica* occurred under a plant cover of small Cyperaceae and in an area without plant cover. The vessels containing the core-samples were exposed outside to sunlight and acetylene reduction activity (A.R.A.) was assayed during a sunny day (Fig. 4A) and a hazy day (Fig. 4B). In both bare soil and shaded soil A.R.A. increased during the morning. When the light intensity reached its usual value of about 70,000 lux at 1300 hours, A.R.A. decreased markedly in bare soil but remained stable during the whole afternoon under cover (Fig. 4A). During a hazy day, when the light intensity did not exceed 30,000 lux, variations of A.R.A. of both

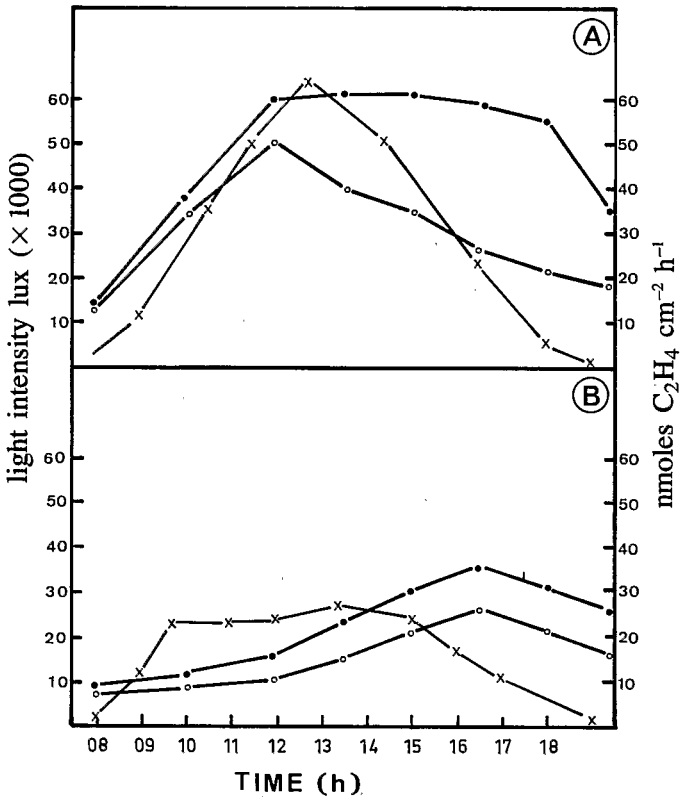

Figure 4. Diurnal variations of acetylene reduction by soil algae. Bare soil: o—o; soil with plant cover: •—•; A: during a sunny day; B: during a hazy day. Incident light intensity: x—x.

samples were similar during the day (Fig. 4B).

Data from these *in situ* tests were consistent with measurements of the A.R.A. of an *Anabaena* sp. bloom placed under screens permitting passage of 100% (Fig. 5A), 60% (Fig. 5B), 22% (Fig. 5C), 7% (Fig. 5D) of the incident sunlight. Identical temperature and moisture were maintained during all experiments. From the observed variations, 4 general forms of curves can be characterized. First, an asymmetrical curve with a maximum in the morning (Figs. 4A and 5A) could be distinguished. This kind of curve was observed under a very low plant cover with a high incident light intensity (maximum >30,000 lux). Second, a curve with two maxima at approximately 1100 and 1600 hours. The data in Fig. 5B were obtained in samples with high incident light intensity (maximum >50,000 lux) and a weak or fairly dense plan cover. Third, an asymmetrical curve with a maximum around 1600 hours (Fig. 4B and Fig. 5C). This was obtained when the incident light intensity did not reach high values (20,000 to 30,000 lux), remained stable most of the day, and when plant cover was fairly dense. Fourth, a symmetrical curve which varied according to variations of incident light intensity and with a slight delay in response (Figs. 4A and 5D). This type of curve was observed under a dense plant cover. The data suggest an inhibitory effect of high light intensities on nitrogenase activity *in situ*.

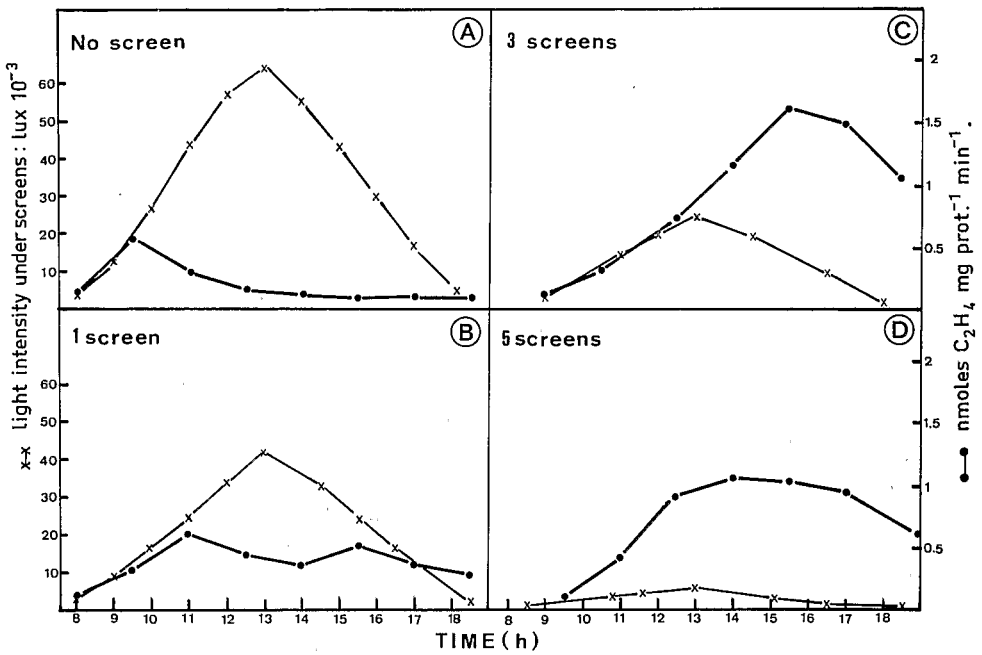

Figure 5. Diurnal variations of acetylene reduction by an *Anabaena* bloom placed under artificial plant cover.

The relationship between nitrogen-fixing algal biomass and nitrogenase activity *in situ*

Nitrogen fixing algal biomass and nitrogenase activity were measured in 25 samples. Assuming a protein content of 5% in blue-green algae (fresh weight), it was possible to evaluate specific algal nitrogenase activity *in situ*. The results obtained varied from 0.1 to 2.2 nmoles $C_2H_4 \cdot$ mg prot$^{-1} \cdot$ min^{-1} and were rather similar to values obtained with axenic cultures. Extrapolation of these results to a rice field in which N.F.A.B. reached a maximum value of 700 kg wet weight $\cdot$ ha^{-1} (corresponding to 5.6 kg of N), gave a contribution from algal nitrogen fixation during a cultivation cycle of 8 kg N $\cdot$ ha^{-1}.

Discussion

Qualitative and quantitative studies on the variation of the algal flora during the rice cultivation cycle in paddy soils of Senegal show that N.F.A.B. reaches its absolute maximum around panicle initiation and its relative maximum at the end of the cycle. Thus, here the N_2-fixing algal flora develops later than in other areas (Watanabe & Lee, 1977). At the beginning of the cultivation cycle, when the paddy soils just become submerged they are characterised by: 1) a low pH, advantageous to development of Chlorophyceae but unfavourable to growth of Cyanophyceae (Shapiro, 1973); 2) a high light intensity at the air-water interface which is also favourable to the development of Chlorophyceae and Xanthophyceae (Whitford, 1960) and unfavourable to Cyanophyceae (Brown & Richardson, 1968). Growth and nitrogenase activity of N_2-fixing blue-green algae are dependent upon a plant cover sufficiently dense to protect them from the inhibitory effects of the very high light intensity occurring in Senegal (up to 70,000 lux); 3) the

presence of mineral nitrogen due to organic nitrogen mineralization after soil remoistening and, for fertilized soils, to manure (Shapiro, 1973); 4) a high level of available CO_2 due to soil remoistening, which favours the growth of Chlorophyceae (King, 1970). During the cultivation cycle, an evolution toward the opposite situation is observed (higher pH, lower mineral nitrogen level, lower light intensities etc.) and this favours the growth of N_2 fixing blue-green algae. A similar evolution of the composition of the algal flora has been observed in freshwater by Wager & Schumacher (1970). Other factors such as nutrition and competition are important in affecting algal successions (Vance, 1965) but cannot be shown using the above results.

In Table 2 values of nitrogen-fixing algal biomass present on 17 soils collected between panicle initiation and maturity of rice are given; 60% of the soils had a N_2 fixing algal biomass between 100 and 500 kg wet weight · ha^{-1}, corresponding to approximately 0.8 to 4 kg of N ha^{-1}. A maximum value of 2260 kg ha^{-1} was found, corresponding approximately to 18.1 kg of N ha^{-1}. *In situ* algal nitrogenase activity varied from 0 to 60 nmoles C_2H_4 $cm^{-2}h^{-1}$. These values, on the basis of the N_2-fixing algal biomass, varied from 0 to 2.2 nmoles C_2H_4 mg $prot^{-1}$ min^{-1}. Extrapolation of the results indicate that algal nitrogen fixation in Senegal paddy soils is in the order of a few kilos of N per hectare per cultivation cycle. Values between 10 and 30 kg were found, but these were exceptional.

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