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NITROGEN FIXING BLUE-GREEN ALGAE IN RICE SOILS OF NORTHERN LUZON (PHILIPPINES)

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A study of the algal population in 13 rice soils of northern Luzon, Philippines showed the presence of N_2-fixing blue-green algae (BGA) in all soils. Colony-forming units of N_2-fixing BGA ranged from 8×10^3 to 9.6×10^5 per cm^2 of soil. In such soils, studies of practical utilization of BGA as a source of N for rice should equally emphasize practices favoring indigenous BGA and algal inoculation. Dominance of strains forming mucilaginous colonies, which are known to be resistant to grazing by invertebrate populations, indicates that grazing may be a major limiting factor for BGA growth in the studied area.

INTRODUCTION

In March-April 1984, the International Rice Research Institute (IRRI), through the International Network on Soil Fertility and Fertilizer Evaluation for Rice (INSFFER) program, the Soil Management Support Services (SMSS) of AID (USA), and the Bureau of Soils of the Philippines Ministry of Agriculture, organized a workshop entitled "Wetland soils: characterization, classification, and utilization" (IRRI, 1985). The site tour during the workshop provided a good opportunity for soil sampling to study the occurrence of N_2-fixing blue-green algae (BGA) in northern Luzon, Philippines. Using the chemical analysis of the soils of the visited sites, performed by the Analytical Service Laboratory of IRRI and SMSS, the relationship between BGA populations and soil properties was studied.

MATERIALS AND METHODS

Sampling Method

Composite samples of surface soil were collected from 15 sites (Table 1). Samples comprised the top 0.5 cm of 10 core subsamples collected with plastic tubes 10 cm long and 3 cm in diameter. Sampling points were at 0.5-m intervals along a transect through the field. In flooded soils, the flood-water was discarded while taking care not to remove the upper soil layer at the soil-water interface. During the trip, soil samples were kept in plastic bottles. Bottles containing wet soil samples were opened daily to avoid anaerobiosis. Samples were processed right after the trip, that is, 5 days after collection for the ones collected during the first day of the trip.

Table 1. Major physicochemical properties of the upper horizon of the soils and algal enumeration.

No.	Site	Major Soil Properties (Upper Horizon)							Number of Colony-Forming Units of Algae per cm^2 of Surface Soil		
		Water status	C %	N %	C/N	P (Olsen) ppm	CEC meq/ 100 g	pH water	Total flora	N_2-fixing BGA	Hetero-cystous BGA
1	Calauan	Submerged	4.3	0.36	11.9	7.8	50	6.8	5.0×10^6	9.6×10^5	3.0×10^4
2	Victoria	Submerged	4.0	0.33	12.1	3.4	53	6.9	2.7×10^6	6.0×10^5	2.6×10^5
3	Famy	Submerged	2.3	0.19	12.1	5.7	36	5.3	1.9×10^6	5.3×10^4	4.9×10^4
4	Teresa	Dry	1.3	0.10	13.0	2.1	43	7.2	4.0×10^5	7.7×10^3	7.7×10^3
5	Gapan	Wet	1.8	0.14	12.9	2.1	47	6.1	2.0×10^6	2.0×10^5	3.0×10^4
6	Maligaya	Submerged	2.6	0.19	13.7	3.0	38	5.5	1.4×10^6	4.0×10^4	4.0×10^4
7	Urdaneta	Dry	1.6	0.13	12.3	12.0	42	7.1	5.3×10^5	1.3×10^4	1.3×10^4
8	Binalonan	Dry	2.0	0.19	10.5	4.0	88	7.8	4.0×10^4	1.8×10^4	1.8×10^4
9	Mangaldan	Dry	1.8	0.09	20.0	12.0	53	6.7	3.3×10^5	4.1×10^4	4.1×10^4
10	Baguio	Wet	5.6	0.40	14.0	2.4	43	4.8	—	—	—
11	Bugallon	Dry	2.9	0.20	14.5	1.0	36	7.3	6.7×10^5	5.0×10^4	5.0×10^4
11 bis	Bugallon	Dry	2.9	0.20	14.5	1.0	36	7.3	9.7×10^4	5.3×10^3	5.3×10^3
12	Mangatarem	Dry	2.2	0.15	13.3	1.6	42	7.5	1.0×10^6	5.0×10^4	5.0×10^4
13	Amucao, Tarlac	Dry	1.1	0.10	11.0	21.0	16	6.1	1.5×10^5	1.2×10^4	1.2×10^4
14	Angeles	Dry	1.0	0.08	12.5	8.5	10	5.3	3.3×10^4	3.3×10^4	3.3×10^4
Average			2.2	0.17	13.1	6.5	43	6.6	1.2×10^6	1.6×10^5	4.9×10^4
Maximum			4.3	0.36	20.0	21.0	88	7.8	5.0×10^6	9.6×10^5	2.6×10^5
Minimum			1.0	0.08	10.5	1.0	10	5.3	3.3×10^4	7.7×10^3	7.7×10^3

Algae Counts

The total algal flora was evaluated by plating soil suspension-dilutions on solid BG 11 medium (Stanier et al., 1971) containing mineral nitrogen. The same medium depleted of mineral nitrogen was used for enumerating N_2-fixing BGA. The volume of the first soil suspension was adjusted with distilled water to a value in cm^3 equal to ten times the value in cm^2 of the surface corresponding to 10 core samples, thus providing a 10^{-1} dilution on a surface basis. Petri dishes were incubated for 3 weeks at laboratory temperature (22° to 30°C) under continuous light from cool white fluorescent lamps before counting and identifying the algal colonies. Counts were expressed as numbers of colony forming units (CFU) per cm^2 of soil.

The method does not permit one to distinguish between active organisms and spores or propagules dormant in the soil. Also, because of counting and competition problems on dishes having too many colonies, strains present at a density lower than 1% of the total CFU are frequently not recorded. The method is therefore suitable for an inventory of the major strains present in a soil. Strains were classified into broad taxa according to criteria directly observable on the colonies growing on petri dishes (Table 2). The ability to form mucilaginous colonies of defined shape, which is associated with resistance to grazing (Grant et al., in press), was taken as a major character. Taxa having this ability were unicellular, *Nostoc*, and *Gloeotrichia* groups.

Table 2. Definition of the taxa of N_2-fixing BGA[1].

Unicellular: Unicellular strains growing on BG11 medium without nitrogen.

"*Anabaena*" group: heterocystous strains, without branching, not forming mucilaginous colonies of definite shape (*Anabaena*, *Nodularia*, *Cylindrospermum*, *Anabaenopsis* . . .)

"*Nostoc*" group: heterocystous strains, without branching, forming mucilaginous colonies of definite shape.

"*Scytonema*" group: heterocystous strains, with false branching, without polarity, forming velvet like patches on agar medium.

"*Calothrix*" group: heterocystous strains, with false branching, with polarity, forming velvet-like patches on agar medium.

"*Gloeotrichia*" group: heterocystous strains, with polarity, forming mucilaginous colonies of definite shape.

"*Fischerella*" group: heterocystous strains with true branching.

[1]All features refer to strains growing from soil or water samples plated on solid BG 11 medium without nitrogen.

Physicochemical Properties of Soils

Methods of analysis are described elsewhere (Anon., 1984). Classification of the soils is given in Table 3. Major physicochemical properties of the upper horizon, which mostly influence algal growth, are presented in Table 1.

Table 3. Classification of the soils.[1]

No.	Site	Soil Classification
1	Calauan	Fine-loamy, mixed isohyperthermic Andaqueptic Fluvaquent
2	Victoria	Fine, montmorillonitic, isohyperthermic Vertic Haplaquoll
3	Fanny	Fine, mixed isohyperthermic Vertic Tropaqualf
5	Teresa	Fine, montmorillomitic, isohyperthermic, Udorthentic Pellustert
6	Gapan	Fine, montmorillomitic, non-acid, isohyperthermic Aeric Tropaquept
7	Maligaya	Fine, montmorillonitic, isohyperthermic Entic Pellustert
8	Urdaneta	Fine, montmorillonitic, non-acid, isohyperthermic Aeric Tropaquept
9	Binalonan	Coarse loamy, mixed isohyperthermic Fluvaquentic Haplustolls
10	Mangaldan	Fine, mixed, isohyperthermic Entic Chromustert
11	Baguio	Fine, mixed, isohyperthermic Andic Palehumult
12	Bugallon	Fine, loamy, mixed isohyperthermic Typic Haplaquoll
12 bis	Bugallon	Fine loamy, mixed isohyperthermic Typic Haplaquoll
14	Mangatarem	Fine clayey, montmorillonitic, isohyperthermic Cumulic Haplaquoll
15	Amucao, Tarlac	Coarse loamy, mixed, non-acid, isohyperthermic Typic Tropaquept
16	Angeles	Sandy, mixed, isohyperthermic, Aquic Ustifluvent

[1] Workshop "Wetland Soils — Characterization, Classification, and Utilization," 30 Mar 3 Apr 1984, field book, IRRI, Los Banos, Laguna.

Among the 15 soils, one was an acidic mountain soil (Baguio) and the rest were soils from fields where rice was or had been grown either continuously or as one of the components of the cropping system. Chemical properties of the upper horizon of the rice soils are as follows: pH 5.3 to 7.8 (average 6.6), 1.0% to 4.3% carbon (average 2.2%), 0.08% to 0.36% nitrogen (average 0.17%), and 1 ppm to 21 ppm available Olsen P (average 6.5 ppm). Five sites were sampled when submerged or wet, the eight others were sampled when dry.

At Bugallon site two samplings were made: one in a plot where straw had been harvested (12), one in a contiguous plot where straw had been burned (12 bis).

RESULTS AND DISCUSSION

Occurrence and Abundance of BGA

Results of algal enumerations are presented in Table 1 together with major physicochemical properties of the upper horizon of the soils.

Nitrogen-fixing BGA were recorded in all soils but not in the mountain soils of Baguio. Environmental conditions prevailing in the Baguio site (very low pH, high elevation, dryland soil, relatively low temperatures) are known to be unfavorable to the growth of N_2-fixing BGA (Roger and Kulasooriya, 1980).

Total algal flora ranged from 3.3 x 10^4 to 5 x 10^6 CFU/cm^2 (average 1.2 x 10^6 CFU/cm^2) and nitrogen-fixing BGA population ranged from 7.7 x 10^3 to about 10^6 CFU/cm^2 (average 1.6 x 10^5 CFU/cm^2).

At the Bugallon site, comparison between the plot where straw was harvested (no. 12) and the contiguous one (12 bis) where straw was burned showed a decrease by about ten times of the total algal and the N_2-fixing BGA populations in the plot where straw was burned. In the latter, N_2-fixing BGA flora comprised almost only *Nostoc* spp., indicating that *Nostoc* strains (which produce spores) were more resistant to high temperatures than the strains belonging to the *Calothrix* and *Fischerella* groups.

The presence of N_2-fixing strains in all the rice soils at densities higher than 8.10^3 and averaging 5.10^4 CFU/cm^2 indicates that algal inoculation may not be necessary in most of the soils of the studied area. Considering that high-count, soil-based inocula have an average CFU content of 10^7 CFU/g dry weight (IRRI, 1984), application of 500 kg dry inoculum/ha would be necessary to equal the average indigenous N_2-fixing algal flora.

Dominant Taxa

The relative abundance of major taxa of N_2- fixing BGA is presented in Table 4. Mucilaginous colonies of various *Nostoc* strains were recorded in 12 of the 13 rice soils and dominant in 9 of them. Mucilaginous colonies of unicellular putative N_2-fixing *Aphanothece* were recorded in six soils and dominant in three of them. All wet and submerged soils had unicellular putative N_2-fixing strains. Strains belonging to the *Anabaena* group (*Anabaena* and *Aulosira*) were recorded in six of the rice soils but were dominant only in one. Similarly nonmucilaginous strains with true or false ramifications were recorded in more than half of the soils, but were never dominant.

A general trend among N_2-fixing BGA is that strains forming mucilaginous colonies (such as unicellular N_2-fixing strains *Aphanothece* and *Nostoc* strains) are less efficient fixers and less susceptible to grazing than are the non-colony-forming strains. The fact that non-colony-forming strains were present in 13 soils but were dominant only in one whereas strains forming mucilaginous colonies dominated in 12 of the 13 may indicate that grazing is a major limiting factor for the development of blooms of nonmucilaginous efficient N_2-fixing strains in the wetland soils of northern Luzon. In agreement with this hypothesis is the visual observation at the time of sampling. It showed the presence of snails in all five submerged and wet soils and that of microcrustaceans in three of them. In site 5 (Teresa), the surface of the dry soil was covered with shells of dead ostracods at a density sufficiently high to change the color of the surface

Table 4. Relative abundance of major groups of N_2-fixing BGA in the soils (% of the total N_2-fixing BGA).

Sites			Relative Abundance (%)						
No.	Name	Water Status	Unicellular	Anabaena group	Nostoc	Scytonema group	Calothrix group	Gloeotrichia group	Fischerella group
1	Calauan	Submerged	96	+	4	—	—	—	+
2	Victoria	Submerged	60	5	30	—	—	—	5
3	Famy	Submerged	11	18	60	—	11	—	—
5	Teresa	Dry	—	—	59	+	27	—	14
6	Gapan	Wet	85	15	—	—	—	—	—
7	Maligaya	Submerged	+	68	11	+	6	—	15
8	Urdaneta	Dry	+	—	92	—	8	—	+
9	Binalonan	Dry	—	—	88	—	—	—	12
10	Mangaldan	Dry	—	—	99	+	1	—	—
11	Baguio[1]	Wet	—	—	—	—	—	—	—
12	Bugallon	Dry	—	—	77	—	20	—	3
12 bis	Bugallon	Dry	—	—	100	—	—	—	—
14	Mangatarem	Dry	—	—	87	—	4	—	9
15	Amucao, Tarlac	Dry	—	2	98	—	—	—	—
16	Angeles	Dry	—	—	94	—	3	—	3
Number of rice soils where the taxon was dominant			3	1	9	0	0	0	0
Number of rice soils where the taxon was recorded			6	6	13	3	8	0	9

[1] Mountain soil — all others are rice soils.

soil. Mucilaginous colonies of *Aphanothece* and *Nostoc* were visible with the naked eye in all submerged or wet soils except in site 5 (Gapan) where herbicide had recently been applied.

Relations Between Soil Properties and N_2-Fixing BGA

The low number of soil samples and the fact that submerged, wet, and dry soils were sampled severely limited the study of correlations between soil properties and abundance of N_2-fixing BGA. In dry soils, only dormant propagules or spores of BGA were recorded whereas in wet soils, both active and dormant organisms were recorded.

On the average, CFU of total (unicellular + heterocystous) N_2-fixing BGA were about ten times higher in the submerged and wet soils (average 3.7×10^5 CFU/cm^2) than in dry soils (average 2.8×10^4 CFU/cm^2), whereas CFU of heterocystous N_2-fixing BGA were only three times higher in the submerged and wet soils (average 8.2×10^4 CFU/cm^2) than in the dry soils (average 2.8×10^4 CFU/cm^2). This indicates that heterocystous BGA can better withstand desiccation than unicellular ones can.

Cross correlations between physicochemical properties and algal flora in the 13 soils sampled, and the 8 of them collected in dry state are shown in Table 5. Previous studies by Garcia et al. (1973), Matsuguchi et al. (1974)

Table 5. Cross correlation between physiochemical properties and algal flora in the rice soils (upper, right) and in 8 of them collected in dry state (lower, left). Only correlations having a level of significance better than 10% are indicated in the table.

13 rice soils (8 dry + 4 submerged + 1 wet)

	C	N	P	CEC	pH	Total algae	N_2-fixing BGA
C		0.97 1%				0.66 1%	0.77 1%
N	0.85 1%					0.57 5%	0.74 1%
P	-0.59	-0.56					
CEC		0.55			0.64 2%		
pH	0.68 5%	0.74 5%	-0.58	0.80 1%			
Total algae	0.49						0.57 5%
N_2-fixing BGA	0.60 10%						

8 dry rice soils

and Roger and Reynaud (1977) have shown positive correlation between pH and number of N_2-fixing BGA in soils. The absence of correlation between these two variables when considering the 13 soils together may be due to the fact that soils with different water status were considered for the calculation. A similar observation was made by Roger and Reynaud (1977). For dry soils, the correlation remained insignificant perhaps because the number of samples was too low and the range of pH too narrow. The positive corrrelations observed between 1) total algae or N_2-fixing algae, and 2) total carbon or total nitrogen in the 13 soils are, at least, partly due to an artifact. Soils submerged or wet at the time of sampling were udic soils with higher organic matter content (average N = 0.24%, average C = 3.0%) than the dry soils (average N = 0.13%, average C = 1.72%) belonging mainly to the ustic group. Because of the season of sampling, therefore, high organic matter content was associated with submersion and with the resulting higher numbers of algae. This becomes obvious in a graphic representation of the correlation (Figure 1). However, the correlation observed between total carbon and N_2-fixing BGA was

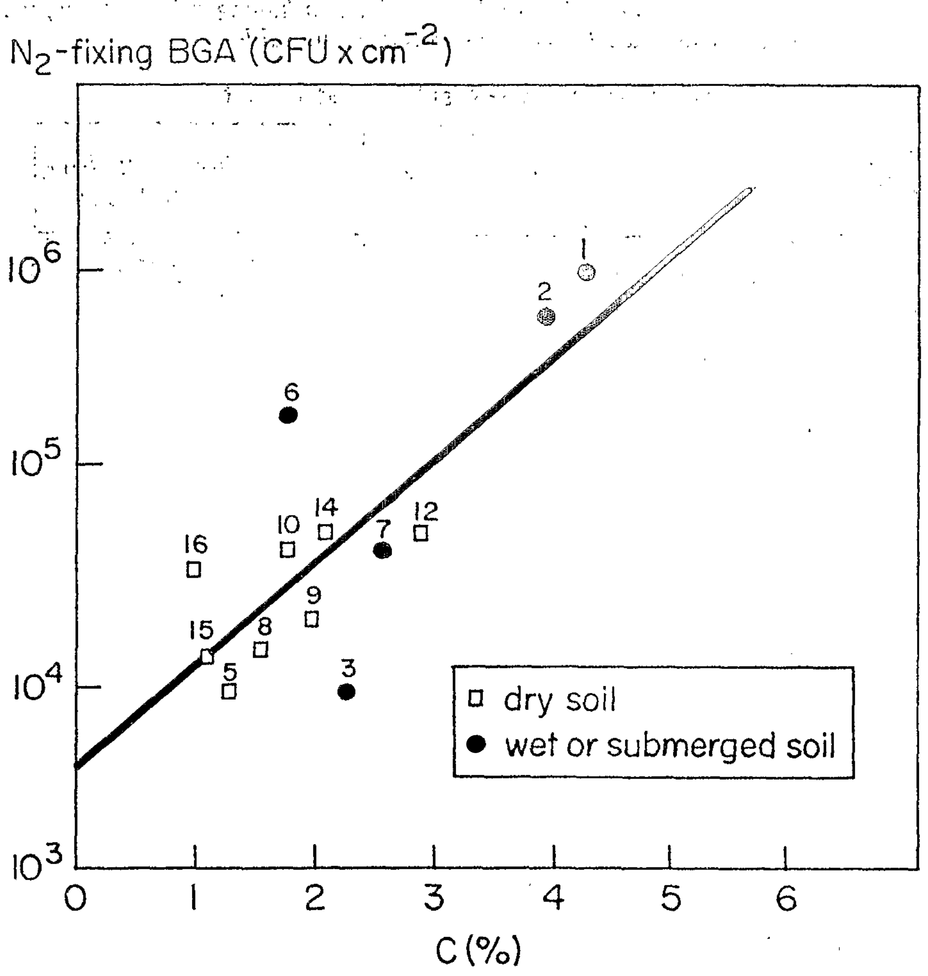

Figure 1. Correlation between carbon content and CFU of N_2-fixing BGA in 13 rice soils. The number of the site is over each symbol.

still weak when considering only dry soils. This may indicate that in the soils of the ustic group, N_2-fixing BGA contribute significantly to the orgaic matter input in the Ap horizon.

CONCLUSION

Research on methods for using N_2-fixing BGA as a source of nitrogen in wetland soils has mostly emphasized algal inoculation alone or together with agricultural practices favoring the growth of inoculated strains. This arose from the earlier belief that N_2-fixing strains were not normally present in many rice fields. It appears now that results concerning the occurrence of N_2-fixing BGA in rice fields are controversial. In qualitative surveys, Watanabe and Yamamoto (1971) found that only 5% or 911 soil samples from Asia and Africa harbored N_2-fixing species. Venkataraman(1975) reported that 33% of 2,213 soil samples from rice fields in India contained N_2-fixing strains.

Okuda and Yamaguchi (1952) reported the presence of N_2-fixing strains in 71% of the samples they collected in Japan. Reynaud and Roger (1978) found N_2-fixing strains in 95% of the samples they collected in Senegal. In a survey of 40 rice fields in Thailand, Matsuguchi et al. (1974) found BGA in all soils.

The study of the algal flora in the surface soil of 13 sites in northern Luzon showed the presence of N_2-fixing BGA in all the rice soils sampled. This suggests that N_2-fixing BGA are more common in rice fields than was previously thought. Unsuitable survey methodology, especially sampling method and methods of growth, probably caused the low estimates of occurrence previously reported in some areas (Roger and Reynaud, 1982).

N_2-fixing BGA populations ranged from 7.7×10^3 to $9.6 \times 10^5/cm^2$ of soil and averaged 1.6×10^5. These values are within the range of values reported by other authors (Okuda and Yamaguchi, 1952; Matsuguchi et al., 1974; Roger and Reynaud, 1977).

The average level of indigenous N_2-fixing BGA was equivalent to half a ton of a good soil-based inoculum (IRRI, 1984). This indicates that research on practical utilization of BGA as source of N in rice fields of the surveyed area should equally emphasize inoculation and indigenous strain enhancement. In 12 of the 13 sites studied, strains forming mucilaginous colonies were dominant. As those strains are known to be resistant to grazing but are frequently poor N_2 fixers, grazer control may be one of the methods for enhancing the growth of the more efficient N_2-fixing strains which are present in these soils.

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