PHIL. AGRI. 69: 599-609
SPECIAL BGA ISSUE
1986

ISSN 0031-7454

NITROGEN-FIXING PHOTOTROPHS IN THE IFUGAO RICE TERRACES (PHILIPPINES)

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Key words: Rice fields; N_2-fixation; Blue-green algae; *Azolla*; ecology; Ifugao rice terraces.

This paper presents quantitative evaluations of populations of N_2-fixing free-living blue-green algae (BGA) and *Azolla* in rice fields in four localities of the Ifugao province, situated at different elevations (300, 800, 1100, and 1500 m). N_2-fixing BGA were found in all fields, with BGA abundance decreasing with an increase in elevation. No *Azolla* was recorded at 300m; *Azolla* abundance increased with elevation. These variations resulted from a vertical gradient of physico-chemical parameters. Low pH and temperatures occurring at higher elevations are more suitable for *Azolla* growth; higher temperatures and pH occurring at lower elevations favor BGA growth. The largest N_2-fixing biomass recorded in a sampling spot was equivalent to about 30 kg N/ha for BGA and 50 kg N/ha for *Azolla*. The average standing photosynthetic N_2-fixing biomasses recorded in localities where no fertilizer was applied ranged from 0 to 16 kg N/ha. In fields where N fertilizer was applied photosynthetic N_2-fixing biomass was negligible.

INTRODUCTION

The Banaue rice terraces in the Philippines are a very ancient agroeco-system where rice has been produced for centuries without chemical fertilizers. A picture of these terraces is frequently used to show that it is possible to grow rice on the same land year after year without chemical N fertilizer and produce modest but constant yields. Under such conditions, biological N_2-fixation (BNF) is the major source for replenishing N exported by the crops. This observation is certainly correct. However, the choice of Ifugao terraces is obviously dictated more by the originality of the magnificent landscape than by knowledge about BNF and N_2-fixing organisms in this ecosystem.

Very little information is available on BNF in the Banaue terraces. The IRRI annual report for 1975 (IRRI, 1976) summarizes observations at two sites in the vicinity of Banaue. In April, about two thirds of the floodwater was covered with *Azolla. In situ* measurements of acetylene reducing activity showed the presence of N_2-fixing BGA in the floodwater. Nitrogen-fixing activity was equivalent to 7 to 125 g N/ha per day. A sampling in May showed no *Azolla* and a low N_2-fixing activity, equivalent to 13-26 g N/ha per day. Laboratory incubation of soil samples showed that Banaue soils are highly responsive to P application. A total N increase of 2.5mg in 4 weeks was observed in 50g soil samples enriched with 50 ppm P.

This paper reports on the quantitative evaluations of populations of N_2-fixing, free-living BGA and *Azolla* in rice fields in four localities of the Ifugao Province, at 300, 800, 1100, and 1500 m elevation.

Because of difficulties in reaching the sampling sites during the rainy season and the distance between the sites and the laboratory where BGA populations were enumerated (IRRI, Los Baños), it was not possible to get samples regularly. Blue-green algae counts were done from November 1982 to April 1983. This period included the end of the fallow period and the first half of the rice crop cycle, when the canopy did not limit algal growth. Direct biomass measurements of algae and *Azolla* were made at various intervals from May 1982 to May 1983.

MATERIAL AND METHODS

The Ifugao Terrace Ecosystem

Ifugao province is located in North Central Luzon, Philippines, on the eastern declivity of the Cordillera Central. It belongs to the Magat-Cagayan drainage system. The area covers about 2500 km^2. Elevation ranges from 200 to 2900 m above sea-level.

The Ifugao rice terraces are part of a complex anthropologic system which connects social life, culture and religion with the environment: forests, swidden, and rice terraces. A comprehensive ethnographic research study on environmental, agronomical, and cultural aspects of the system was presented by Conklin (1980).

When irrigation water is available, almost every suitable slope is terraced (Fig 1). Walls of the terraces are constructed from stones or clay. Terraces have to be continuously flooded to avoid cracking of the walls (Van Breemen et al., 1970).

Climate is characterized by a very short dry season between February and April. Most of the rain falls between July and November with a peak in July due to frequent typhoons. Highest temperatures are recorded in May (monthly average: 25^o C in Kiangan at 750 m elevation and 23.8^o C in Banawe at 1000m elevation), the lowest in January (20.4^o C in Kiangan and 14.2^o C in Banawe). Highest solar radiation is in April (470 cal/cm^2 per day) and the lowest in December (250 cal/cm^2 per day). A thick cloud cover usually decreases temperatures and light availability in July-August. Real-time weather data in Banawe are available from IRRI (1983, 1984).

Usually, one crop of rice is grown per year without chemical fertilizer. Nurseries are seeded around December, transplanting is around February, and harvest around July. During the rest of the year, vegetables are grown on compost heaps in the flooded rice fields.

Sampling Sites

Samples were taken from fields in four localities:

1 : *Lawig*, in Lamut municipality, located in the foothills of the Cordillera at an elevation of about 300 m. Two crops of rice are grown per year, using fertilizer at 60 kg N/ha. This locality, which does not belong to

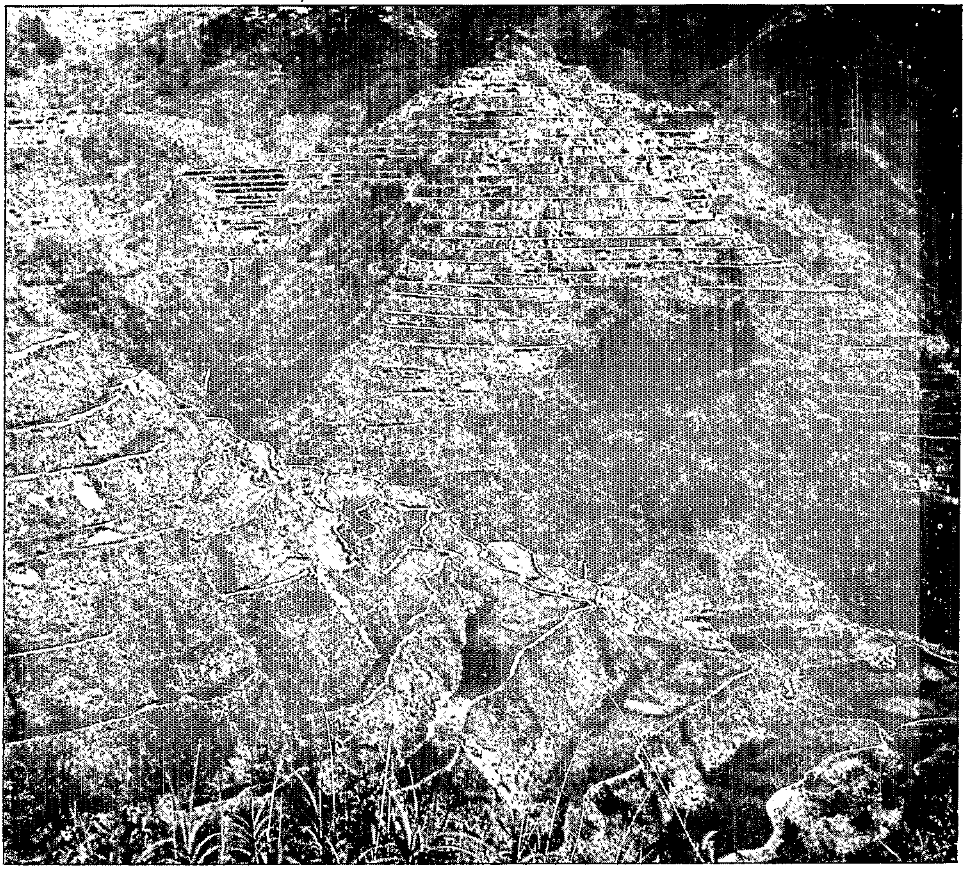

Figure 1. Rice terraces in Ifugao Province.

the rice terraces was selected for comparison with the three other ones where no chemical fertilizer is used and only one rice crop is grown per year.

2 : *Palao*, in the municipality of Kiangan, on the top of a terraced, southeast oriented slope, about 800 m above sea level. Straw and weeds are composted and incorporated into the soil.

3 : *Pitan*, in the municipality of Banawe, southwest oriented, about 1100 m above sea level. No compost applied. The aquatic biomass growing during the fallow period is incorporated before transplanting.

4 : *Apalnga'oh*, in the municipality of Banawe, on the eastern slope of a narrow valley. It is the upper limit of the wetland growing area. One crop is grown per year. Fertilization practices are the same as in Pitan.

Algae Enumeration

Composite samples (10 subsamples) of water and soil were collected from three rice fields at each locality. Subsamples consisted of the top 0.5 cm of soil and the floodwater of cores collected with 3-cm-diameter plastic tubes. Sampling points were located at regular intervals along a transect through the field. Samples were kept in plastic bottles which were opened occasionally to avoid anaerobiosis. They were processed within 2 days of collection.

N_2-fixing BGA populations were evaluated using the plating method (Roger et al., 1986,) with agarized BG 11 medium (Stanier et al., 1971) depleted of mineral nitrogen. Counts were expressed as number of colony forming units (CFU) per cm^2 of soil. The method is suitable for making a quantitative record of the major strains present in a soil.

Strains were classified into broad taxa, according to morphological criteria observed on the material growing in petri dishes. In addition to characters used by Rippka et al. (1979), the ability to form mucilaginous colonies of defined shape (which is associated with resistance to grazing) was considered a major character. Definition of the taxa is given in Roger et al., (1986, this issue).

Biomass Measurements of Algae and Azolla

Algae and *Azolla* biomasses were estimated from composite samples obtained by harvesting 0.25 m^2 areas randomly selected in 10 field at each locality. Dry weights were measured on air dried material (about 5% water left as compared with oven dried material).

Chemical Analysis

Chemical properties known to influence the photodependent N_2-fixing activity of the soils (pH, total carbon, total nitrogen, C/N, Olsen available P, and CEC) were studied. Soil (Ap 1 horizon) and water analysis were made in duplicate on composite samples (10 subsamples) by IRRI's analytical services, according to their standard procedures (Benckiser et al., 1982).

RESULTS AND DISCUSSION

Soil and Water Analysis

Soils were acidic to almost neutral, with pH ranging from 4.9 to 6.8 (Table 1). There was a negative correlation between pH and elevation. Carbon content ranged from 1.26 to 2.19%. Nitrogen content ranged from 0.14 to 0.23%. There was no correlation between C or N content and elevation (Table 2). Available P ranged from 4.8 to 18.5 ppm. The low available P content in Pitan soils (6-9 ppm) explains why BNF of these soils was highly responsive to P application (IRRI, 1976). CEC ranged from 28 to 56 meq./100g. Phosphorus content and CEC were negatively correlated with elevation.

These results indicate that a gradient of chemical properties was associated with elevation, most probably because of leaching of cations from the soil, thus the lower pH and available P at higher elevation.

No water analysis was performed in the fields of 1500m elevation. At the three other elevations, irrigation water had a pH between 7 and 8, and a P content of about 2 ppm. In the fields, floodwater pH was higher, increasing up to 10 when an algal bloom was present. Phosphate content ranged from 2 to 4 ppm.

Table 1. Chemical properties of the soils

Location	Elevation (m)	pH	C (%)	N (%)	C/N	P ppm	CEC meq/ 100g
Apainga'oh 1	1500	5.0	1.68	0.14	12.0	4.8	30
Apainga'oh 2	1500	4.9	1.26	0.15	8.4	5.7	27
Apainga'oh 3	1500	5.1	1.70	0.16	10.6	5.4	28
average	1500	5.0	1.55	0.15	10.3	5.3	28
Pitan 1	1100	5.3	2.19	0.23	9.6	8.4	34
Pitan 2	1100	5.3	1.78	0.17	10.1	5.7	36
Pitan 3	1100	5.0	2.02	0.20	9.9	8.8	34
average	1100	5.2	2.00	0.20	9.9	7.6	35
Palao 1	800	6.5	1.88	0.19	9.7	12.5	51
Palao 2	800	6.6	1.86	0.21	9.0	10.5	53
Palao 3	800	6.2	1.82	0.19	9.8	16.5	50
average	800	6.4	1.85	0.20	9.5	13.2	51
Lawig 1	300	6.4	1.52	0.16	9.7	18.5	53
Lawig 2	300	6.8	1.44	0.15	9.5	13.5	54
Lawig 3	300	6.4	1.55	0.17	9.1	15.0	56
average	300	6.5	1.51	0.16	9.4	15.7	55

Table 2. Cross correlations between soil properties and abundance of N_2-fixing BGA (R 1% = 0.68).

	elevation	pH	C	N	C/N	Avail.P	CEC
pH	−0.89	—	—	—	—	—	—
C	+0.16	−0.14	—	—	—	—	—
N	−0.07	−0.10	+0.85	—	—	—	—
C/N	+0.39	−0.37	+0.23	−0.30	—	—	—
Avail.P	−0.89	+0.82	−0.11	+0.12	−0.35	—	—
CEC	−0.93	+0.98	−0.09	+0.14	−0.37	0.88	—
BGA	−0.93	+0.83	−0.35	−0.14	−0.31	+0.91	+0.87

BGA Enumerations

N_2-fixing strains were present in all samples (Table 3). Densities ranged from 3 x 10^3 CFU/cm^2 (Apainga'oh in November) to 7 x 10^5 CFU/cm^2 (Lawig in April). In the fields located at 800 m and at higher elevation, there was almost no variation in the density of N_2-fixing BGA during the period studied. In the fields at Lawig (300 m), significant changes in density were observed with a peak in April, mainly due to the growth of unicellular *Gloeocapsa*. Recorded values were about the same as those we reported in

Table 3: Colony forming units (10^3 /cm^2) of N_2-fixing BGA in the soils at different sampling times

Location	Elevation (m)	Nov 5 1982	Dec 3 1982	Feb 19 1983	April 22 1983	Average
Apainga'oh 1	1500	10	13	11	13	12
Apainga'oh 2	1500	3	7	31	11	13
Apainga'oh 3	1500	10	18	7	3	9
average	1500	8	13	16	9	11
Pitan 1	1100	22	45	39	13	30
Pitan 2	1100	56	25	64	87	58
Pitan 3	1100	12	25	8	7	13
average	1100	30	32	37	36	34
Palao 1	800	180	150	227	120	169
Palao 2	800	125	110	166	115	129
Palao 3	800	167	193	157	178	174
average	800	157	151	183	138	157
Lawig 1	300	184	105	134	1177	400
Lawig 2	300	375	140	103	344	240
Lawig 3	300	306	127	240	623	324
average	300	288	124	159	715	321

Northern Luzon (7.7 x 10^3 to 2.6 x 10^5 CFU/cm^2) (Roger et al., 1986, this issue). However, two thirds of the samples from Northern Luzon came from dry soils, while Ifugao samples were from wet soils. This indicates a lower abundance of N_2-fixing BGA in Banawe soils compared with Northern Luzon soils.

Highly significant positive correlations were observed between density of N_2-fixing BGA and pH, available P, and CEC of the soil. A highly significant negative correlation was observed between density and elevation (Table 2).

The positive correlations observed between abundance of N_2-fixing BGA and pH, as well as available P (Table 2), agree with earlier reports (Garcia et al., 1973; Matsuguchi et al., 1975; Roger and Reynaud, 1977; Roger et al., this issue) and the general observation that N_2-fixing BGA are usually more abundant in neutral to alkaline soils rich in P (Roger and Kulasooriya, 1980).

The positive correlation between abundance of N_2-fixing BGA and CEC is a complex one because CEC itself is positively correlated with available P and pH. Similarly, the negative correlation between the abundance of N_2-fixing BGA and elevation is a result of several factors. Available P, pH, and CEC (being highly negatively correlated with elevation) are responsible for the negative correlation between abundance of N_2-fixing BGA and elevation. In addition, altitude is generally negatively correlated with temperature which is known to be positively correlated with abundance of BGA.

A study of the relative composition of the N_2-fixing algal flora (Table 4) showed the dominance of *Nostoc* and *Calothrix* in fields at higher elevations. In fields at lowest elevation (300 m), unicellular forms were the

Table 4. Relative composition of the N_2-fixing BGA flora (%)

Location / Dates	1st Field Nov. 82	1st Field Dec. 82	1st Field Feb. 83	1st Field Apr. 83	2nd Field Nov. 82	2nd Field Dec. 82	2nd Field Feb. 83	2nd Field Apr. 83	3rd Field Nov. 82	3rd Field Dec. 82	3rd Field Feb. 83	3rd Field Apr. 83	Mean
Apalnga'ho (1500 M)													
Unicellular	—	35	—	—	4	—	—	—	—	39	—	9	7
Anabaena	—	8	—	8	—	10	1	6	3	2	—	9	4
Nostoc	43	48	24	37	81	19	67	79	40	33	20	21	43
Scytonema	—	—	—	—	—	—	—	—	—	—	—	—	0
Calothrix	9	8	76	55	15	70	32	15	53	26	80	61	42
Gloeotrichia	48	—	—	—	—	—	—	—	3	—	—	—	4
Fischerella	—	—	—	—	—	—	—	—	—	—	—	—	0
Pitan (1100 M)													
Unicellular	—	35	—	—	—	36	—	—	—	24	—	—	8
Anabaena	—	5	—	—	—	9	2	—	14	—	4	—	3
Nostoc	84	47	87	57	88	9	64	57	48	52	43	47	57
Scytonema	—	—	—	—	—	—	—	—	—	—	—	—	0
Calothrix	16	12	13	43	12	45	34	43	27	24	53	53	31
Gloeotrichia	—	—	—	—	—	—	—	—	—	—	—	—	0
Fischerella	—	—	—	—	—	—	—	—	11	—	—	—	1
Palao (800 M)													
Unicellular	—	38	—	—	—	45	—	—	—	12	—	1	8
Anabaena	2	5	4	3	4	3	2	2	2	2	—	1	3
Nostoc	92	48	72	84	59	45	60	66	84	60	49	65	65
Scytonema	—	2	—	—	—	—	—	—	—	—	—	—	0
Calothrix	4	7	21	10	10	7	36	18	2	14	38	21	16
Gloeotrichia	—	—	—	—	—	—	—	—	—	—	—	—	0
Fischerella	2	—	3	3	27	—	2	15	12	12	13	12	8
Lamut (300 M)													
Unicellular	—	25	—	88	77	41	—	81	61	29	—	78	40
Anabaena	—	—	5	5	—	5	3	3	1	5	—	3	3
Nostoc	69	50	80	3	23	48	59	8	23	34	72	12	40
Scytonema	—	—	—	—	—	2	—	—	1	13	—	—	1
Calothrix	16	21	15	3	—	2	38	6	14	16	28	5	14
Gloeotrichia	—	—	—	—	—	—	—	—	—	—	—	—	0
Fischerella	15	4	—	1	—	2	—	2	—	3	—	2	2

second most abundant group after *Nostoc*. Table 5 shows *Nostoc* to be dominant in 67% of the cases, followed by *Calothrix* (19%), and unicellular forms (15%). The dominance of *Nostoc* and unicellular BGA was also reported in rice fields at low elevation in Northern Luzon (Roger et al., 1986, this issue) and India (Roger et al., 1986).

N_2-fixing BGA strains that form mucilaginous colonies (unicellular, *Nostoc*, and *Gloeotrichia* groups) are generally observed to be less susceptible to grazing than strains that do not form such colonies (Grant et al., 1985). Mucilaginous strains (*Nostoc* and unicellular forms) were dominant in

Table 5. Occurrence and dominance of major taxa of N_2-fixing BGA in the samples.

Taxa	Dominant %	Next dominant %	Recorded[a] %	Total %
Unicellular	15	17	8	40
Anabaena	0	2	66	68
Nostoc	67	29	4	100
Scytonema	0	0	8	8
Calothrix	19	48	31	98
Gloeotrichia	2	0	2	4
Fischerella	0	4	33	37

a — but not dominant

more than 80% of the soils; strains that do not form mucilaginous colonies (*Anabaena*, *Calothrix*, *Fischerella*) were present in most soils but were rarely dominant. This may indicate that grazing is a major limiting factor in the development of blooms of nonmucilaginous strains active in N_2-fixation in rice fields.

Algal Biomass

Visual observations showed that algal growth started after transplanting, when the floodwater was becoming clear and there were little submerged macrophytes. Epiphytic BGA were observed on the submerged parts of the rice plants and on the weeds later in the cycle. Epiphytic BGA were also abundant on decaying submerged stems remaining in the field after the cropping season. Green algae became dominant late in the crop cycle.

Algal biomass was measured only when a bloom was visible. Algal growth was very variable and blooms did not occur at the same time in the fields at the same elevation. Usually, blooms established and disappeared in a few days. Individual measurements of standing crops of algae ranged from 1 g to 2 kg fresh weight/m^2. The highest biomass recorded in a subsample was 130g/m^2 dry weight (at Palao). The maximum average value in a locality (average of 10 fields) was about 20 g/m^2 (Table 6). Average blue-green algal biomass in a locality was less than 1g dw/m^2 in 15 out of 20 samples.

Assuming an average content of 52% ash and 4.8% N in the field growing BGA (Roger et al., 1986), the largest biomass recorded in a subsample was equivalent to about 30 kg N/ha and the maximum average value recorded in a locality was equivalent to 5 kg N/ha. These data indicate a moderate fertilizer potential of BGA blooms in terms of kg N/ha.

Highest counts were recorded at 300 m elevation (Lamut); the largest biomass was recorded at 800 m elevation. This is most probably due to application of N fertilizer and the dominance of unicellular BGA at the lowest elevation. Unicellular BGA yield higher CFU counts and have a lower dry matter content than filamentous forms (Roger et al., 1986). Nitrogen fertilizer application was also reported to inhibit the formation of efficient N_2-fixing blooms (Roger and Kulasooriya, 1980).

Table 6. Azolla and algal biomass measurements.

Location	Date	*Azolla* (g dw/m^2)	BGA (g dw/m^2)	N_2-fixing Phototophs (kgN/ha)	Green Algae (g dw/m^2)
Pitan	06/17/82	6.8	3.0	3.1	3.2
	07/28/82	2.7	0.0	0.9	24.4
	10/04/82	17.7	0.1	6.2	0.0
	10/17/82	3.9	0.0	1.4	0.0
	03/15/83	46.7	0.1	16.4	0.0
	05/19/83	3.2	epiphytic	1.1	0.0
Palao	05/10/82	29.1	0.3	10.2	0.0
	06/10/82	0.0	0.0	0.0	0.0
	08/06/82	0.0	0.0	0.0	0.0
	09/22/82	0.2	0.0	0.1	0.0
	03/28/83	2.9	6.3	2.8	0.0
	05/13/83	2.4	19.5	5.7	0.0
Lamut	05/12/82	0.0	0.0	0.0	0.0
	06/23/82	0.0	0.0	0.0	0.0
	08/10/82	0.0	0.0	0.0	0.0
	10/30/82	0.0	4.2	1.0	0.0
	12/08/82	0.0	0.0	0.0	0.0
	03/23/83	0.0	0.4	0.1	0.6
	04/30/83	0.0	0.0	0.0	0.0

Azolla Biomass

No *Azolla* was recorded at the lowest elevation site (Lamut). Visual observations in Banawe and Kiangan (no quantitative measurements performed) showed a peak of *Azolla* growth in December/January when temperature and solar radiation were low (16-22^{o}C, 240 cal cm^{-2}.day^{-1}) and rainfall was moderate (313 mm in December, 156 mm in January). After *Azolla* population collapsed in February, a second peak of growth was observed in March at Banawe and in May at Kiangan.

The highest biomass recorded in a subsample was 2.3 kg fresh weight per m^2 (23 t/ha) or 140 g dw per m^2 (1.4 t/ha). The maximum average biomass recorded in a locality was about 0.5 ton dry weight/ha (Table 6). In Banawe, *Azolla* frequently exhibited symptoms of nutrient deficiencies (small purple fronds and long roots) consistent with a relatively high dry matter content (6%). Assuming an average N content of 3.5% in natural *Azolla* samples, these values correspond to 50 kgN/ha (highest biomass in a subsample) and 17.5 kgN/ha (maximum average biomass at a locality). Larger *Azolla* biomasses were recorded at higher elevations.

CONCLUSION

N_2-fixing phototrophs were found at all sites. Blue-green algae were ubiquitous, whereas no *Azolla* was recorded in the fields located at the lowest elevation (300 m).

BGA abundance decreased with elevation, while *Azolla* abundance increased. This crossed gradient resulted from a verticial variation of physico-chemical parameters. Lower pH (5-6.4) and temperatures (22-25°C) occurring at higher elevations favor *Azolla* growth. Higher temperatures (28-31°C) and pH (6.4-6.8) at lower elevations favor BGA growth.

The highest N_2-fixing biomass recorded in a subsample can be considered an estimate of the potential of a given organism. This was equivalent to about 30 kg N/ha for BGA and 50 kg N/ha for *Azolla.*

The average standing photosynthetic N_2-fixing biomasses recorded at localities where no fertilizer was applied ranged from 0 to 16 kg N/ha and averaged 4 kg N/ha. On the average, *Azolla* contributed more N than BGA despite the nutrient deficiency symptoms it exhibited. Soil analysis and visual observations indicated that available P is a major limiting factor for the activity of N_2-fixing phototrophs. Fields close to the villages receive water that contains soap and detergents. In such fields, P concentration was higher, N_2-fixing phototrophs were more abundant, and rice plants were taller and greener.

Biomass sampling was not frequent enough to allow an estimation of N contribution by N_2-fixing phototrophs in the ecosystem. However, from the value of the maximum biomass recorded, a significant contribution can be expected in the fields where no fertilizer was applied. In the fields where N fertilizer was applied, photosynthetic N_2-fixing biomass was negligible.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. H.C. Conklin (Yale University, USA) for stimulating discussions on this work, Dr. R. Oldeman for providing weather data, and Drs. J.K. Ladha (IRRI) and M. Martinez (UPLB) for their very useful comments on the manuscript. This research was conducted under a scientific agreement between IRRI and ORSTOM (France) and partly supported by the United Nations Development Programme.

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