NITROGEN FIXATION IN ACID SULFATE PADDY SOILS

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Abstract

Cultivated acid sulfate soils have limited microbial activities and populations related to nitrogen transformations, particularly to N_2 fixation. Nitrogen fixation activity by blue green algae was much weaker than in most of the other soils in Thailand.

The addition of phosphorus increased rice yield by about 1 ton/ha without nitrogen fertilizer. Field acetylene reduction assays in a long-term fertility plot in Thailand demonstrated the enhancement of N_2 fixation by the application of phosphate.

The technique of growing *Azolla* in acid sulfate soil needs to be improved. Blue green algae grow epiphytically on the aquatic parts of deepwater or floating rice, which is widely grown in flooded acid sulfate soils. The nitrogen fixation by those epiphytic blue green algae and its transfer to aerial parts were demonstrated by ^{15}N technique.

Roles of phosphate to stimulate nitrogen fixation are discussed.

In Southeast and East Asia, 60% of about 5 million ha of actual and potential acid sulfate soils are distributed along the sea coast of Indonesia, Thailand and Vietnam. West Africa may have 6.6 million ha of actual and potential acid sulfate soils (Van Breemen and Pons, 1978).

There is adequate information available about genesis, classification, chemistry, toxic properties, and reclamation method of acid sulfate soils, but research on the microbiological aspects, excluding sulfur metabolism, is limited.

Extensive surveys on microbial properties and their activities in acid sulfate soil were reported in Thailand (Araragi and Tongcham, 1974; 1979; Matsuguchi *et al.,* 1970; 1975) and Senegal (Garcia *et al.,* 1973)

Discussion here focuses on rice yield in acid sulfate soil areas. Potential acid sulfate soils, which will undoubtedly become noxious after reclamation as rice fields, are not discussed.

Microbial properties related to nitrogen transformation

Surveys of acid sulfate soils in Thailand were made in the Central Plain. These soils are mainly old acid sulfate soils (Sulfic Tropaquept), characterized by the presence of pyritic substratum in deep layers and less acidic surface horizon (pH 4–5) with medium or high organic matter content (Van Breemen and Pons, 1978). Microbial populations related to nitrogen transformation, except Athioradaceae, and ammonifiers at 1–10 cm were lower in acid sulfate soils than in other soils in Thailand (Araragi and Tangcham, 1974; 1979)

Low populations were more marked in the dry season than in the wet season, because the adverse conditions of acid sulfate soils are alleviated under submergence, except for H_2S formation. Total N and C in mineralizable N contents were not necessarily lower in acid sulfate than in other soils in Thailand. This means that some factors other than organic supply limit the growth of various microorganisms related to nitrogen transformation (Matsuguchi *et al.,* 1975; Van Breemen and Pons, 1978).

In Senegal, nitrogen-fixing activity by blue green algae and rhizosphere bacteria was more sensitive to acidity than were denitrification and nitrification (Garcia *et al.,* 1973).

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Matsuguchi *et al.* (Matsuguchi *et al.*, 1970; 1975) reported extensive surveys of the nitrogen-fixing populations and nitrogen-fixing activities (acetylene reduction activity) of various soils in Thailand. Compared with other soil groups, acid sulfate soils in particular were characterized by a lower population of blue green algae.

Acetylene reduction activities (ARA) of surface 0 – 5 cm soil and floodwater were measured in dark-aerobic, dark-anaerobic, light-aerobic and light-anaerobic conditions in the laboratory. From the frequency distribution of soil activities for the four conditions, it was observed that the acetylene reduction activities of acid sulfate soils were lower than the average values (Fig. 1).

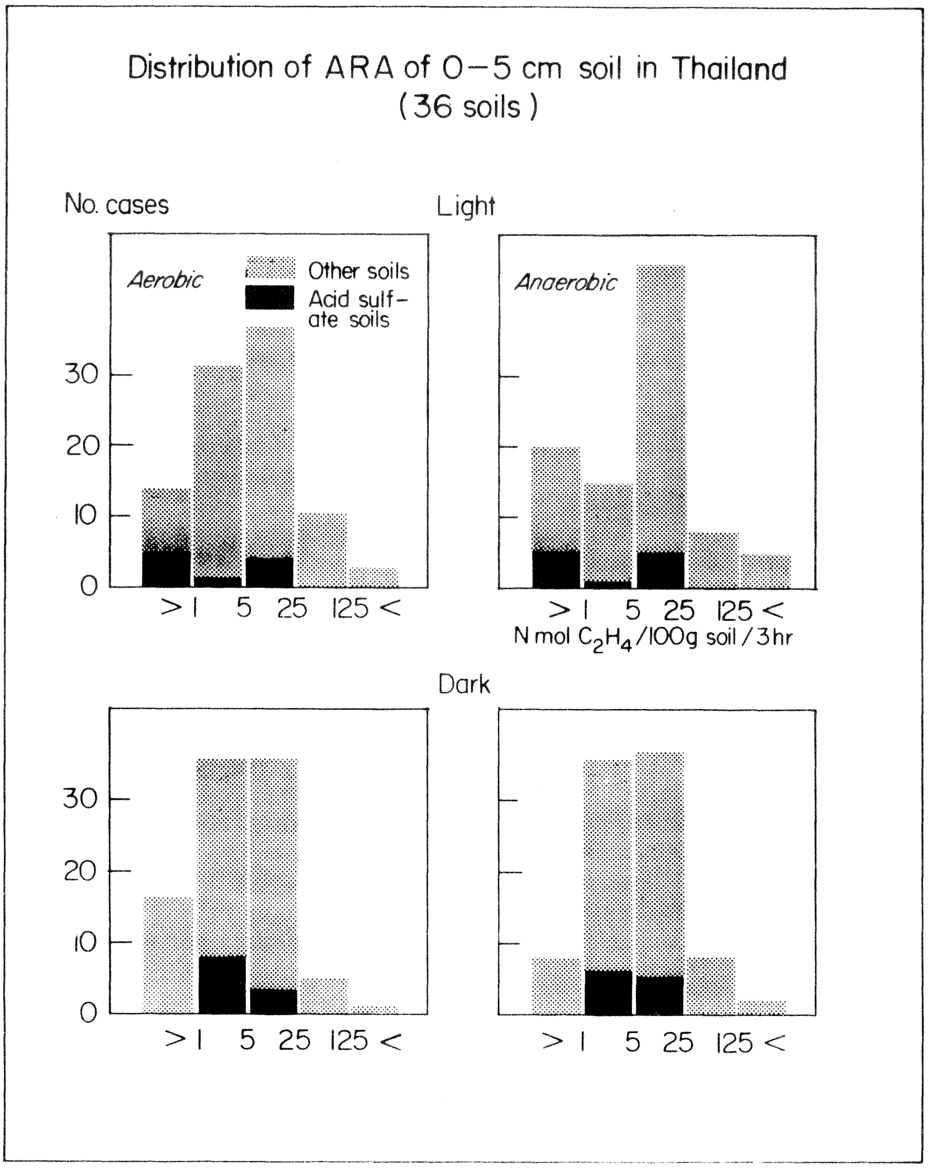

Fig. 1 Distribution of ARA in Thailand soils as compared with acid sulfate soils.

The deviation from the median values toward lower values was more pronounced for photo-dependent acetylene reduction activity, due mostly to the presence of blue green algae and partly to that of photosynthetic bacteria. This finding does not conflict with data of the population surveys.

Factors limiting N_2-fixing activities

Reported adverse conditions in acid sulfate soils include low pH *per se;* toxicity due to Al, Fe^{+2}, H_2S, and CO_2; salt injury; toxicity of organic acids at low pH, and deficiency of phosphorus and other micronutrients (Van Breemen and Pons, 1978). Among these, low pH and phosphorus deficiency were major targets of improvement. Matsuguchi *et al.* (Matsuguchi *et al.,* 1970) reported the effects of phosphate and lime and their combination in Sulfic Tropaquept in Klong-Luang, Thailand on the microbial population and activity related to nitrogen fixation.

Phosphate fertilizer application at the rate of 37.5 and 112 kg P_2O_5/ha, increased soil population of photosynthetic bacteria and, to a lesser extent, that of anaerobic N_2-fixing bacteria. Application of lime at the rate of 1.9 and 5.6 ton/ha enhanced the soil population of photosynthetic bacteria and, to a greater extent, that of blue green algae. Combination of phosphorus and lime further increased the positive effects on the populations of photosynthetic bacteria and blue green algae.

Acetylene reduction activity also was increased by phosphorus application, but the positive effect was most apparent only in a limited period during the cycle of rice growth. Both heterotrophic and phototrophic N_2 fixations were stimulated by phosphorus. Liming similarly increased acetylene reduction activity. The combination of both maintained high levels of ARA during the period of rice growth and phototrophic N_2 fixation was stimulated more than the heterotrophic one.

These studies by Matsuguchi *et al.* (1970; 1975) clearly demonstrated the beneficial effects of phosphate and lime on heterotrophic and, to a greater extent, phototrophic N_2 fixation in the acid sulfate soils. Rice yield in this experiment was increased to 2 ton/ha by applying higher levels of phosphorus and lime without nitrogen. N_2 fixation enhanced by phosphate and lime, may partly explain the increased yield of rice without nitrogen fertilizer. But, long-term fertility trials could help determine the role of phosphorus in the maintenance of soil N level.

Field acetylene reduction activity assays in long-term fertility plots in Klong-Luang

To confirm the beneficial effect of phosphorus fertilizer on N_2 fixation in acid sulfate soils, field *(in situ)* acetylene reduction activity assays were conducted in two long-term fertility treatments (no fertilizer and no nitrogen treatments). Similar assays were made in two other low humic gley soils in Chainat, and Suphanburi in Thailand for comparison (Cholitkul *et al.,* 1980).

A plastic bag was used to cover a rice hill with and without elimination of surface algae and acetylene reduction activity assays were run for 24 hours. The plastic bag method measures mainly acetylene reduction activity by blue green algae in floodwater and soil surface, and heterotrophic microorganisms on surface soil and associated with rice. To eliminate algal activity, the surface water and soil (1 cm) were removed and replaced by water. This procedure aimed to measure acetylene reduction activity mainly associated with wetland rice roots and the basal portion of shoots.

From rice yields (Table 1) it is seen that the Klong-Luang soil is most deficient in phosphorus and that the Chainat soil is not deficient in phosphorus for rice growth. Potassium is, from soil and analysis, not considered deficient in the three soils.

From the average values of 9 assays during 4 cycles of rice growth (Table 2), N_2 fixation by blue green algae and bacteria associated with rice root and basal portion of shoot was demonstrated to be lowest in non fertilized plots of an acid sulfate soil among the three soils. The continuous application of phosphorus for about 10 years in this soil improved soil characteristics resulting in an almost similar N_2 fixation rate to that observed in other fertile paddy soils.

Table 1 Yield of paddy (ton/ha) in Thailand soils

	Chainat		Suphanburi		Klong-Luang	
	– NPK	+ PK	– NPK	+ PK	– NPK	+ PK
1976 (wet season)	3.64	4.05	1.91	3.24	0.88	1.78
1977 (dry season)	1.84	2.56	1.32	1.75	0.90	2.35
1977 (wet season)	3.79	3.75	2.16	2.77	0.82	1.35
1978 (dry season)	1.73	2.02	1.82	2.08	0.67	1.33

Cholitkul *et al.*, 1980.

Table 2 Means of nine measurements of daily acetylene reduction activity

Sites	Fertilizer treatment	Daily acetylene reduction activity (mmol C_2H_4 m^{-2})	
		Rice and algae	Associated with rice
Chaint	– NPK	0.83 b	0.21 ab
	+ PK	1.8 a	0.26 ab
Suphanburı	– NPK	1.5 ab	0.12 b
	+ PK	2.1 a	0.40 a
Klong-Luang	– NPK	0.43 c	0.068 c
	+ PK	0.92 ab	0.23 ab

Values with common letters are not statistically different from each other at the 90% significance level.
Cholitkul *et al.*, 1980.[3)]

Use of *Azolla* in improving nitrogen nutrition of rice

The aquatic fern *Azolla* has high nitrogen content and is capable of growing rapidly in a nitrogen-deficient environment due to symbiotic relation with *Anabaena.* The use of this plant as green manure for rice and fish and animal feed has been attracting the attention of many scientists and farmers (Watanabe, 1978). Since 1979, the International Network of Soil Fertility and Fertilizer Evaluation for Rice (INSFFER) has had a collaborative project to test the effect of *Azolla* on rice yield. In Thailand, trials of *Azolla* growth at Rancit Agricultural Experiment Station in acid sulfate soils showed poor *Azolla* growth because of acidity (pH 4.1) (P. Swatdee pers. communication).

Cultural practices to grow *Azolla* successfully in acid sulfate soils must be developed.

Blue green algae associated with deepwater rice

The quality of water in deepwater areas has little to do with the adverse characters of acid sulfate soils if there is no tidal effect. Little is known about the sources of nitrogen for deepwater or floating rice. In Thailand, in the Central Plain, where acid sulfate soils are widely distributed, deepwater or floating rice (water depth $>$ 100 cm) or medium-deepwater rice (water depth $<$ 100 cm) is grown on a large scale. For many years deepwater rice in that area has been grown without fertilizer nitrogen and yet rice biomass production was high.

The submerged deepwater rice stems produce clusters of nodal roots that grow freely and may absorb nutrients from the floodwater. To examine the contribution of nitrogen from floodwater, deepwater rice was grown in pots with ^{15}N ammonium sulfate. Pots were placed in shallow (5 cm) water, semi-deepwater (50 cm), and deep water (110 cm). The experiments were made in

Thailand and at IRRI. In all cases, rice plants in shallow water had high ^{15}N content, suggesting that nitrogen from deep water contributes to nitrogen of deepwater rice.

In Thailand, however, the pots in shallow water were not placed at the same sites as the experiments in deepwater rice and, the results must be considered with reservation (Table 3) (Watanabe *et al.*, 1981).

Table 3 Acetylene reducing activity in the component parts of a deepwater rice plant at maturity

Component parts of plant	Biomass (g (fw) / plant)	Light: Specific ARA (nmol C_2H_4 g(fw) / hr)	Light: ARA1) (nmol C_2H_4/hr)	Dark: Specific ARA (nmol C_2H_4/g (fw) / hr)	Dark: ARA1) (nmol C_2H_4/hr)
Floating					
Exposed roots	2.5	24	61	0.06	0.15
Leaf sheath	62	54	3,348	1.5	93
Inner roots	0.25	69	17	0.03	0.01
Culm	98	2.5	245	0.5	49
Submerged					
Exposed roots	64.3	0.54	35	0.08	5
Leaf sheath	15	12	180	0.1	1.5
Inner roots	1.3	3	4	0.05	0.06
Culm	100	0.3	30	0.02	2

1) Specific ARA x component biomass. Kulasooriya *et al.*, 1981.[7)]

Kulasooriya *et al.* (1981) reported the presence of epiphytic blue green algae on the deepwater rice's leaf sheath, leaf blade, nodal root exposed from the node and nodal root covered by leaf sheath under water, and, to a lesser extent, on the culm. *Nostoc, Anabaena* and *Calothrix* were found on the nodal root and leaf sheath. Blue green algae were seen inside the air cavities of leaf sheath and blades, but not inside host tissues. *Gloeotrichia* was mainly grown on the decaying tissues. Acetylene reduction activity of various plant parts under water was always higher in light than in darkness. This finding demonstrated that phototrophic N_2 fixation is associated with aquatic parts of deepwater rice (Table 4).

To demonstrate N_2 fixation associated with deepwater rice, ^{15}N labeled N_2 gas was fed to the shoots under water for 9 days at the heading stage and ^{15}N analysis of plant parts was made at maturity. Leaf sheath and nodal root and, to a lesser extent, the culm under water were enriched with ^{15}N (Table 5). The ^{15}N enrichment was roughly parallel to the specific phototrophic acetylene reduction activity of various parts as shown in Table 4. This finding demonstrates that N_2 fixation takes place through blue green algae associated with deepwater rice. ^{15}N was also found in the aerial portions that were not directly exposed to $^{15}N_2$, indicating that the nitrogen fixed under water is transported to the aerial parts and grains. A total of 8 mg nitrogen plant was fixed for 9 days. This rate was much higher than the reported rate of $^{15}N_2$ fixation by heterotrophic N_2-fixing bacteria associated with root and basal portion of shoot.

In $^{15}N_2$ feeding experiments made in artificial deepwater plots at IRRI algal growth on rice was less profuse than that observed in Thailand's Central Plain area. This indicates that the contribution of blue green algae's nitrogen fixation to nitrogen nutrition would be greater in actual deepwater area than that in experiments at IRRI (Watanabe *et al.*, 1981).

Table 4 Total N and ^{15}N contents and dilution of ^{15}N in total plant portions

Site	Season	Water depth (cm)	Total N mg/pot A	Total ^{15}N mg/pot B	Dilution A/B	Percentage contribution of N from water [c]
Philippines						
IRRI	1979 wet [a]	shallow (5)	879 ± 47	235 ± 11	3.7	
		deep (110)	983 ± 67	212 ± 17	4.6	18
	1980 wet [a]	shallow (5)	939 ± 13	380 ± 1	2.5	
		deep (110)	777 ± 31	283 ± 10	2.7	10
Thailand						
Bangkhen-1	1979 wet [a]	shallow (5)	829 ± 65	112 ± 8	7.4	
Huntra-1		semi-deep (< 50)	1,031 ± 115	74 ± 5	13.9	46
Ongkarak-1		deep (120)	988 ± 79	98 ± 11	10.1	27

a) Atom % excess was 6.948, 17.210, 12.54 in 1979 wet, 1980 at IRRI and in Thailand respectively.

b) Mean ± standard error.

c) $\dfrac{^{14}N\ (A-B)\ \text{in deep water} - {}^{14}N\ \text{in shallow water}}{\text{Total N (A) in deep water}} \times 100$

Watanabe *et al.*, 1981.[14)]

Discussion

As Table 1 shows, without nitrogen fertilizer the addition of phosphorus and potassium (unlikely to be effective) increased grain yield by 0.9 ton/ha. Enhancement of soil nitrogen availability may be one factor. If the effect of phosphorus consisted only of the acceleration of nitrogen availability from soil, soil nitrogen supply should have been exhausted. But soil nitrogen contents were similar regardless of phosphate addition (Cholitkul *et al.*, 1980). Increased uptake of nitrogen must be compensated by biological N_2 fixation or other sources.

Assuming 50 kg of grain is produced by 1 kg of absorbed nitrogen and 30% absorption efficiency for rice plant, a 0.9 ton/ha grain yield corresponds to 60 kg nitrogen supplied to soil. In the long-term experiment, 37.5 kg P_2O_5/ha were applied. If nitrogen came exclusively from biological N_2 fixation, ratio of nitrogen gain to P_2O_5 applied would be 1.6.

No doubt, this presumed ratio is maximal, because sources other than biological N_2 fixation may contribute to nitrogen supply. Because the current price of 1 kg N as urea is almost the same as the price of 1 kg P_2O_5 in superphosphate, the presumed ratio of nitrogen gain to P_2O_5 applied is economically attractive. *Azolla* can produce 2 kg nitrogen or more by applying 1 kg P_2O_5 (Watanabe *et al.*, 1980).

In laboratory experiments on the effect of phosphorus on the growth of free-living blue green algae, nitrogen gain/P_2O_5 applied was found to be 0.5 – 1.2 at IRRI (1976), 0.3 – 0.5 by De and Mandal (1958) and 0.5 by Nishigaki and Shioiri (Nishigaki and Shioiri, 1959).

As far as the ratio of nitrogen gain/P_2O_5 applied is concerned, *Azolla* is most efficient.

If rock phosphate were used in acid sulfate soils and were effective, the role of phosphorus in promoting nitrogen gain to improve nitrogen nutrient would be more economically attractive.

Table 5 N_2 fixation by deepwater rice exposed to $^{15}N_2$ for 9 days, 1980 wet season, IRRI

Plant parts	Atom % excess	Fixed N μg N/pot [a]
Aerial parts		
Grain	0.039 ± 0.02	149
Leaf blade	1.77 ± 0.88	2,780
Leaf sheath	0.30 ± 0.21	208
Culm	0.11 ± 0.07	57
Floating parts		
Leaf sheath	3.12 ± 1.14	2,300
Culm	0.48 ± 0.15	264
Root	2.01 ± 0.77	267
Submerged part		
Leaf sheath	1.37 ± 0.46	831
Culm	0.27 ± 0.07	153
Root	0.66 ± 0.28	625
Root in soil	0.23 ± 0.11	330
Whole plant		7,970
Submerged weed	0.97 ± 0.48	823

a Assuming the average of 48.1 atom % excess of $^{15}N_2$ during 9 days of exposure.

Watanabe *et al.*, 1981.[14)]

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Discussion

Li, C.K. (China): Is the ferrous toxicity in the acid sulfate soils induced by the low redox potential or is there any evidence of ferrous toxicity *per se*?

Answer: In the acid sulfate soils ferrous toxicity is always associated with a low pH because of the formation of ferric sulfate. The existence of a low pH *per se* or the presence of aluminum may decrease the resistance of the plant to ferrous ion. Iron toxicity in acid sulfate soils must be considered as a different phenomenon from the iron toxicity in other soils.

Kyuma, K. (Japan): I would like to point out that acid sulfate soils are not widely distributed in the Irrawady delta of Burma.

Answer: Thank you for your comment.

Araragi, M. (Japan): As you mentioned it, phosphate deficiency and low pH in the acid sulfate soils interfere with nitrogen fixation processes. What is your experience of the effect of pH stress on nitrogen fixation?

Answer: As shown by Matsuguchi *et al.* the addition of lime without phosphorus stimulates population growth and activities of micro-organisms related to nitrogen fixation. However it does not appear that pH values *per se* limit microbial properties. Probably, aluminum is also toxic to the micro-organisms.