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Diversity of nitrogen-fixing cyanobacteria under various ecosystems of Thailand: population dynamics as affected by environmental factors

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Summary

Investigation of N_2-fixing cyanobacteria from Thai soil was carried out at 2-month intervals between July 1997 and November 1999 to determine the population number, population dynamics and favourable habitats. Sites were selected in three parts of Thailand; North, Central and Northeast. In each part, various soil ecosystems were used as sampling sites; at highest elevation as on the top of the mountain, in the middle and at the foot of the mountain, as well as in flat areas of agricultural practice and uncultivated areas. Generally, a high population of N_2-fixing cyanobacteria was found in agricultural areas where rice cultivation was practised, rather than in other sites. The population dynamics in the mountain and uncultivated areas were less fluctuating than in agricultural areas. The population densities in agricultural areas increased in the rainy season and decreased during the dry season. Other environmental factors such as temperature, moisture and pH also affected the population densities in different habitats. Cyanobacterial diversity was notably influenced by the type of ecosystem in both dry and rainy seasons. The cultivation area containing rice in rotation with other crops contained the most genetically diverse range of species.

Introduction

The cyanobacteria are by far the largest group of phototrophic prokaryotes, occurring in a wide range of habitats. They are essential components of grassland soils, desert rocks, hot and cold desert soils, tropical and temperate forest soils, agricultural soils, wet soils (silt and mud) and marine marginal areas. Their success appears to be due to a number of features widespread in the group, of which the following are likely to be important in soils and sediments. The temperature optimum for many cyanobacteria is higher by at least several degrees than most eukaryotic algae (Castenholz & Waterbury 1989). Tolerance to desiccation and water stress is widespread, and cyanobacteria are among the most successful organisms even in highly saline environments (Borowitzka 1986).

There are many reports of N_2-fixing cyanobacterial establishment in soils including desert crusts, agricultural soils and rice-field ecosystems (Whitton 1993). The ability of many species to fix N_2 is believed to provide a competitive advantage to these organisms in habitats where levels of combined nitrogen are low. One of the fruitful examples of the role of N_2-fixing cyanobacteria in terrestrial ecosystems is supplementation of nitrogen to rice fields through the *Anabaena–Azolla* symbiosis. In addition, various reports have indicated that cyanobacteria may be important agents in the maintenance of the N-status of soils (De 1939; Singh 1942, 1961; Watanabe 1951, 1959). Hence, the distribution of N_2-fixing cyanobacteria has been investigated almost in every soil ecosystem, and increment or reduction of the cyanobacterial population may continuously affect the nitrogen status of the soil. Therefore, proper management of N_2-fixing cyanobacterial populations in agro-ecosystems has been emphasized for sustainable agriculture. In this study, the environments which promoted the N_2-fixing cyanobacterial persistence and the effect of physical factors on population dynamics have been evaluated in various soil ecosystems in Thailand.

Materials and methods

Site selection

Soil samples were collected at 2-month intervals between July 1997 and November 1999. Sites were selected in three parts of Thailand: North, Northeastern and Central. In each part, seven different ecosystems as elevation areas (top, middle and foot of mountains), agricultural practice areas (crop cultivation, rice fields and rice cultivated in rotation with other crops) and uncultivated areas were used as sampling sites. In each sampling site, soils were collected in five samples at 0–15 cm from the surface with four replications, then kept in an ice-box prior to further analyses.

Meteorological data collection and soil characterization

The heights above sea level of each site were obtained from the metropolitan department or research stations near the sampling site. Soil temperature and moisture were recorded during soil sampling. Soil characterizations for amount of organic matter (OM), pH, amount of available phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were conducted by standard protocols by the Soil Analysis Division, Kasetsart University, Bangkok, Thailand.

Enumeration of N_2-fixing cyanobacteria

Fifty grams of soil sample was shaken in 450 ml distilled water prior diluted with 10-fold dilution technique. Each dilution was then cultivated in BG_{11} medium without an added nitrogen source (Richmond 1986) under a 12 h light/12 h dark cycle with an average light irradiance of 400 $\mu E\ s^{-1}\ m^{-2}$ for 25–30 days at 30 °C. The growth occurring was then enumerated by the standard protocol MPN method. To confirm the N_2-fixing capability, the nitrogenase activity of each isolate was measured by acetylene reduction assay (ARA) and compared with the amount of chlorophyll *a* (Wintermans & Demots 1965).

Morphological characteristics of cyanobacteria

Colony-forming characteristics of each cyanobacterial isolate were observed on BG_{11} medium without sole nitrogen source. Microscopic study of all strains was employed at 400× magnification. The morphological characteristics: heterocyst, vegetative and akinete cells were interpreted with the description technique of Rippka (1988).

Statistical analysis

For the four replications of each sampling site, a completely randomized design was conducted. The mean of population number and analysis of variance were analysed by Duncan's new multiple range test (DMRT) at $P = 0.05$. The correlation of chemical and physical factors with population number of N_2-fixing cyanobacterial isolates was carried out along with multiple regression analysis using the statistical program SPSS version 9.0.

Results

Comparison of N_2-fixing cyanobacterial population that grow in each ecosystem

The population number of N_2-fixing cyanobacteria was enumerated from various ecosystems in three parts of Thailand over 2 years. The population size in each part was different, but followed the same pattern. Generally, the population size in mountain areas (top, middle and foot) and uncultivated areas was not significantly different (Figure 1). The cyanobacterial population densities in these areas were in the range of 1.1–1.3 log (number of cells) g^{-1} dry soil. However, these population numbers were less than in agricultural practice areas. Moreover, differences in the population size were also found among agricultural areas depending on how the agricultural activities were performed. In the North, the highest population number was found where rice was grown in rotation with other crops (average number 2.30 log (number of cells) g^{-1} dry soil) while the crop cultivation area (average number 1.56 log (number of cells) g^{-1} dry soil) contained a lower number than other agricultural practice areas (Figure 1). In the Central region, high population number in agricultural practice area was mostly found in rice fields (average number 1.47 log (number of cells) g^{-1} dry soil) and rice in rotation with other crops cultivation area (average number 1.61 log (number of cells) g^{-1} dry soil) (Figure 2). The Northeast presented a similar population pattern to that in the North and Central regions (Figure 3).

Population dynamics of N_2-fixing cyanobacteria

The fluctuation of population size during the 2 years of investigation showed similar patterns in all three regions. The highest population number always occurred during the rainy season of each year (May–September). This seemed to agree with the trends of soil moisture content (Figures 1A–C). In the North, the population numbers in mountain areas and uncultivated areas were less fluctuating than in agricultural areas. Most agricultural practice areas showed fluctuations of population sizes, which were high in the rainy season and dramatically low in the dry season (November–April). In addition, N_2-fixing cyanobacterial population in the rice in rotation with other crops areas showed seasonal dependent fluctuation pattern (Figure 1C). The Central part showed similar population dynamics as in the North, particularly in the first year of sampling. However, in some agricultural practice areas such as rice, and rice in rotation with other crops, cultivation areas showed the reduction of population number at the end of September and slight increases in November–January (Figure 2C). This might be due to the second crop cultivation system, which provided the appropriate moisture in these areas. In the Northeastern area, the population number in mountain areas, some agricultural practice areas and uncultivated areas showed very

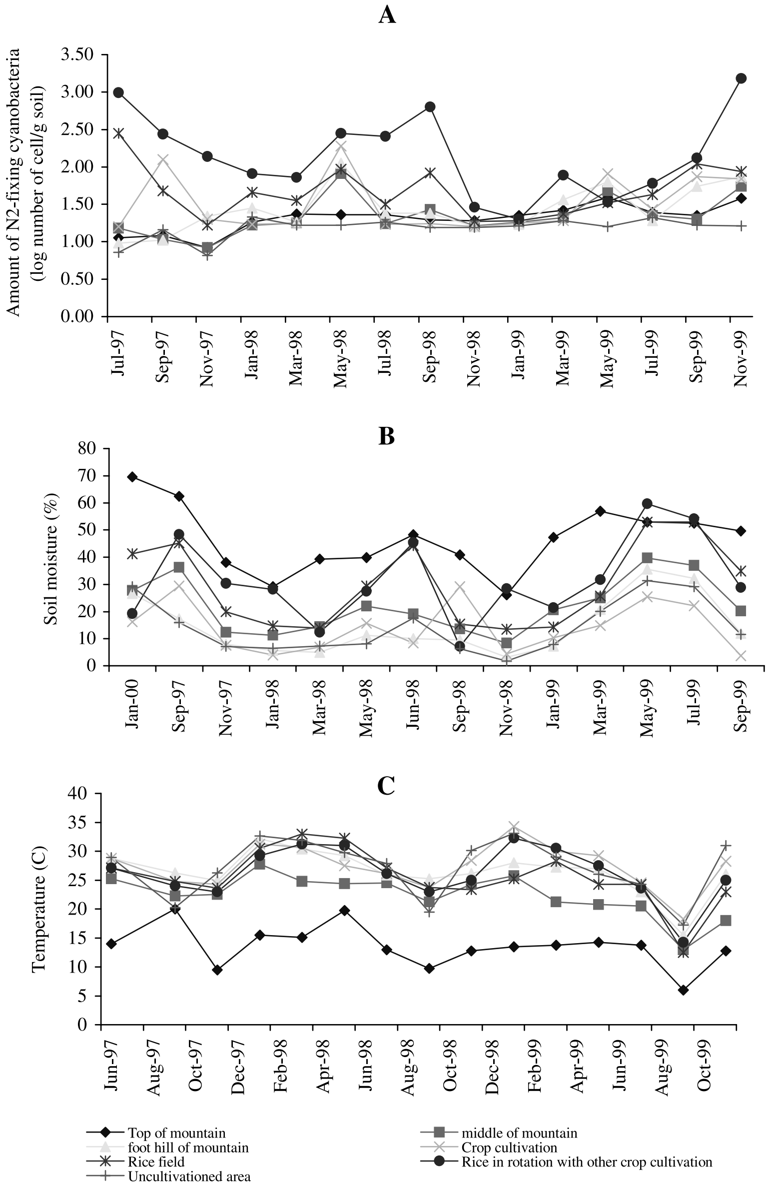

Figure 1. (A) N_2-fixing cyanobacterial population number during 1997–1999 in the Northern region. (B) Soil moisture profile during 1997–1999 in the Northern region. (C) Soil temperature profile during 1997–1999 in the Northern region.

little fluctuation during the period of study. Only two areas of cultivation; rice fields and rice in rotation with other crops, showed increases in population, during the rainy season (Figure 3C).

Correlation between N_2-fixing cyanobacterial population and environmental factors

Besides the seasonal changes, other environmental factors, such as soil characteristics also had direct effects on the population dynamics of N_2-fixing cyanobacteria (Table 1). By using multiple regression analysis, it was found that the population sizes of N_2-fixing cyanobacteria in the North were significantly correlated with soil pH and moisture (Table 2). From Table 1, the average pH value in this area was rather low, thus a negative correlation between pH and population number was found. When effects of environmental factors with N_2-fixing cyanobacterial population in each individual ecosystem were analysed, population number in the Central region was positively correlated only with soil moisture. The population in the Northeast was negatively correlated with soil organic matter and moisture. It was clearly indicated that moisture affected the N_2-fixing cyanobacterial population for all locations (as compared the trends in Figure 1A–C) while organic matter affected only the Northeast because in the Northeast most soil contained slightly less organic matter than other parts (Table 1).

In the North, the effects of chemical factors on the population number were not significantly different. Only soil temperature and moisture were significantly correlated with population sizes. The soil temperature showed a direct effect on population only in the middle of mountains, while moisture affected the population in the areas where soil contained a lower amount of

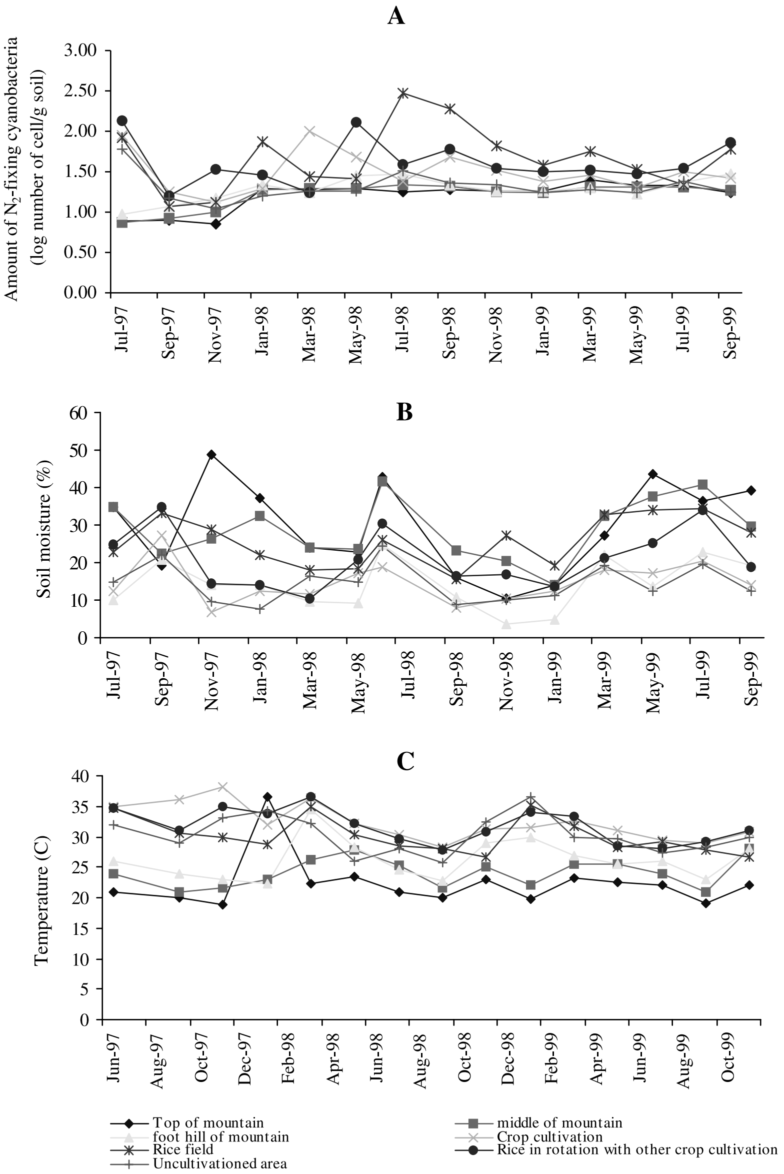

Figure 2. (A) N_2-fixing cyanobacterial population number during 1997–1999 in the Central region. (B) Soil moisture profile during 1997–1999 in the Central region. (C) Soil temperature profile during 1997–1999 in the Central region.

moisture than in middle of mountain (20% moisture) and crop cultivation area (12% moisture).

In the Central region, chemical factors did not have any effects on population size of N_2-fixing cyanobacteria except organic matter. Rice fields where soil contained a rather low amount of organic matter (Table 1) showed a positive effect on population sizes. Soil moisture levels also had a strong positive effect on population size, particularly on top and middle of the mountain (Table 2).

In the Northeast, some chemical and physical factors affected the sizes of N_2-fixing cyanobacterial populations. The population of N_2-fixing cyanobacteria at the top of the mountain and in rice fields were positively affected by soil pH and soil moisture (Table 2).

N_2-fixing cyanobacterial diversification in each ecosystem

The differences in morphological characteristics of each N_2-fixing cyanobacterium were grouped along with the sampling sites. The distribution number of different isolates collected in both dry and rainy seasons was determined. The results revealed relationships between population diversity and ecosystem, indicating that the cyanobacterial diversity was notably influenced by the type of ecosystem in both seasons. The mountain areas (particularly the top of the mountains) showed less biodiversity than agricultural practice areas. Rice cultivated in rotation with other crops gave soil containing the highest diversity of species. Seasonal changes had no effects on species distribution in different localities.

Discussion

The investigation of N_2-fixing cyanobacteria over two consecutive years (1997–1999) in Thailand has shown that the population numbers in the fallow mountain and uncultivated areas were less than in agricultural practice

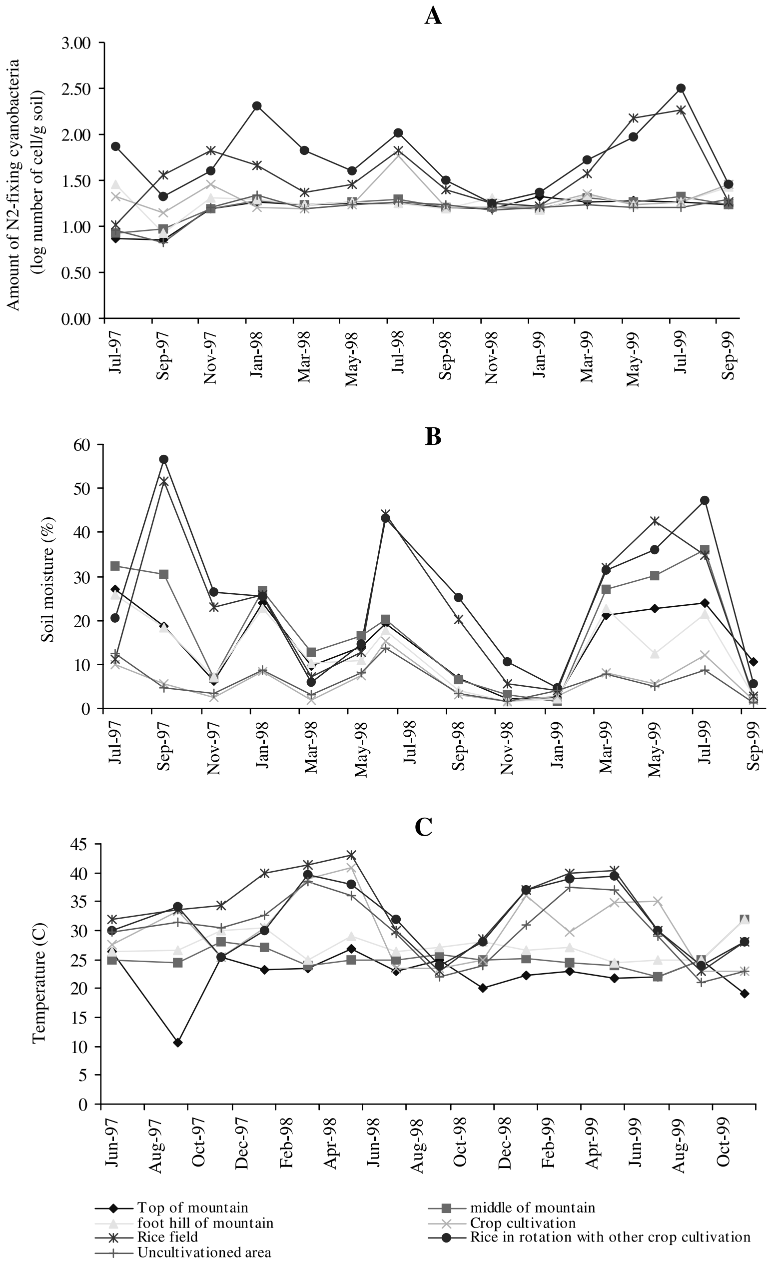

Figure 3. (A) N_2-fixing cyanobacterial population number during 1997–1999 in the Northeastern region. (B) Soil moisture profile during 1997–1999 in the Northeastern region. (C) Soil temperature profile during 1997–1999 in the Northeastern region.

areas. This was possibly due to rather low amount of phosphorus, potassium, calcium and magnesium in those fallow areas, particularly in the Central and Northeast regions. However, this is somewhat different from axenic cultural studies of *Nostoc linckia*, which demonstrated that varying concentration of macronutrient salts such as K_2HPO_4, $MgSO_4 \cdot 7H_2O$, NaCl, $CaSO_4 \cdot 2H_2O$ and $CaCO_3$ did not significantly affect the algal yields (Rodulfo & Polo 1986). In addition, the average high temperature might increase the rate of decomposition of organic matter. Therefore, a continued high availability of nutrient supply exists in soils such as paddy soils in the tropics. This hypothesis was similar to the determination of phytoplankton community-structure and bloom dynamics in the Neuse river estuary, North Carolina which suggested that high dissolved inorganic-nitrogen during the summer months promoted major blooms of *Cryptomonas*, chlorophytes and cyanobacteria (Pinckney *et al.* 1998). However, the correlation of nutrient and N_2-fixing cyanobacterial population may be much more complex, involving interaction of other factors, such as light intensity, community structure and interaction between species (Berard *et al.* 1998). The low ratio of P and N may also be a factor which directly promotes the growth of some *Anabaena* strains (Rapala & Sivonen 1999). This observation also supported the results of relationships between N_2-fixing cyanobacteria and environmental factors in Spanish rice fields (Quesada & Fernandez-Valiente 1996). The soluble reactive phosphate (SRP)

Table 1. Summary of soil characterization.

Type of Ecosystem	H (m)	OM (%)	pH	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
Northern part							
1. Top of mountain	2565	10.92	4.60	47	253	1060	140
2. Middle of mountain	1018	5.47	5.38	26	178	730	100
3. Foot hill of mountain	705	2.04	5.73	24	105	738	88
4. Crop cultivation area	205	2.46	5.87	39	143	1000	235
5. Rice field	235	2.46	5.63	14	105	1540	448
6. Rice in rotation with other crops area	235	2.14	5.36	43	93	160	78
7. Uncultivated area	205	4.68	6.21	196	160	1590	403
Central part							
1. Top of mountain	1700	6.45	4.45	1	75	430	35
2. Middle of mountain	654	5.11	4.68	8	83	1980	66
3. Foot hill of mountain	325	2.22	5.94	20	165	1170	110
4. Crop cultivation area	16	1.52	6.44	56	80	1700	205
5. Rice field	13	1.36	5.55	23	100	1080	213
6. Rice in rotation with other crops area	16	1.62	5.95	54	100	1440	171
7. Uncultivated area	20	4.10	5.80	40	120	1820	268
Northeastern part							
1. Top of mountain	1310	3.67	4.50	10	33	143	13
2. Middle of mountain	1000	3.72	4.88	4	48	61	15
3. Foot hill of mountain	705	2.39	5.02	9	80	235	82
4. Crop cultivation area	200	1.08	6.51	10	68	1200	188
5. Rice field	230	1.93	6.12	11	118	1890	308
6. Rice in rotation with other crops area	238	1.63	5.43	17	110	1310	330
7. Uncultivated area	205	1.50	6.84	29	123	1270	148

H: average heights from sea level, OM: organic matter, P: available phosphorus, K: extractable potassium, Ca: extractable calcium and Mg: available magnesium. Data are means of four replicates.

Table 2. Multiple regression between cyanobacterial population and soil characteristics in the North, Central and Northeast.

Sites	Part					
	North		Central		Northeast	
	Affecting factor	R^2	Affecting factor	R^2	Affecting factor	R^2
M_1	Ts**	0.557	W*	0.642	pH*	0.282
M_2	W**	0.497	W*	0.499	NS	0.238
M_3	W*	0.126	NS	0.296	NS	0.114
C	NS	0.637	NS	0.195	NS	0.222
R	NS	0.131	OM**	0.548	W*	0.287
Cr	NS	0.198	NS	0.180	NS	0.098
U	NS	0.216	NS	0.225	NS	0.336
All sites combined	pH* + W*	0.440	W*	0.508	OM** + W**	0.740

*, ** Significant different at $P < 0.05$ and $P < 0.01$, respectively. Ts = soil temperature, W = water/moisture, U = uncultivated area, OM = organic matter, M_1 = top of mountain, M_2 = middle of mountain, M_3 = foot hill of mountain, C = crop cultivation, R = rice field and Cr = rice with other crop rotation area, NS = not significant.

was positively correlated with the heterocystous cyanobacteria population. Furthermore, dissolved inorganic nitrogen (DIN) and the ratio of DIN/SRP were negatively correlated with cyanobacterial abundance. Nevertheless, the main environmental factor which clearly affected population dynamics of N_2-fixing cyanobacteria in this study was seasonal changes. The results obtained from this study corresponded to the report from Currin & Paerl (1998) who studied the epiphytic N_2-fixing cyanobacteria in dead *Spatina alterniflora* stems in a North Carolina salt marsh. The heterocystous cyanobacteria (*Calothrix* spp. and *Nostoc* spp.) were dominant in spring. We have observed that *Anabaena* spp. and *Nostoc* spp. were the dominant native strains in Thai soil. Similar results with abundance and seasonal fluctuation of N_2-fixing cyanobacteria in Spain have been reported (Quesada *et al.* 1998).

Soil pH also had some negative effects on the population size in the top of the mountain in the Northern and Northeastern areas. The pH of these soils was very acidic (4.5–4.6), which is much lower than optimum pH (7–10) (Rippka *et al.* 1979). Temperature showed positive effects on population number only in the top of the mountain in the Northern area. At this location, the highest in Thailand (2565 m above sea level), the lowest temperature is approximately 0–9 °C while the maximum is at 20 °C and the average for all the year round is 14 °C. Therefore, this range of temperature is not optimum for general growth of N_2-fixing cyanobacteria, which is actually in the range between 25 and 35 °C (Welch 1952; Allen & Stanies 1968).

The diversity of N_2-fixing cyanobacteria in Thai soils varied in different soil ecosystems. A relatively lower diversity was observed in ecosystems such as mountain areas, where light intensity is low due to the high plant canopy. Some aquatic and terrestrial cyanobacterial diversity investigations revealed that variability in light supply has a strong effect on species diversity. The low average irradiance might decrease the diversity both in aquatic ecosystems (Litchman 1998) and in rice fields (Quesada *et al.* 1998). This may be due to the photosynthetic dependence of these microbes. However, this study used only one condition for isolation and cultivation. Therefore, using other methods or conditions might result in additional cyanobacterial genera for which ASM medium is not appropriate, for example *Anacystis nidulans*, while other media such as BG_{11} or Z8 are more favourable (Rippka 1988).

The study of N_2-fixing cyanobacterial population dynamics as affected by environmental factors could lead to understanding of population changes due to human activities. Imbalances of chemical fertilizer application somehow inhibit N_2-fixation in cyanobacteria and other bacteria such as *Azotobacter* spp. (DeLuca *et al.* 1996). On the other hand, the responses of some cyanobacteria to the application of herbicides and other agricultural activities also depend on the sensitivity of each species as well as environmental factors such as nutrients, light and temperature. As reported by Berard (1998) growth of cyanobacteria was always inhibited by atrazine in low temperature conditions. Furthermore, community structure, interaction between species and other environmental parameters are important factors controlling the response of cyanobacteria to herbicides. Therefore, this exploration might lead to the proper environmental management for a sustainable agroecosystem.

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