TROPICS Vol. 12 (3) Issued April 30, 2003

Soil environmental factors relating to diversity of culturable soil bacterial communities in the Sakaerat Environmental Research Station, Thailand

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ABSTRACT Soil biodiversity may relate to environmental changes in the soil. To determine how soil environmental factors relate to soil bacterial diversity, we compared culturable bacterial communities in soils under bare ground (BG, highly degraded), dry dipterocarp forest (DDF, moderately disturbed) and dry evergreen forest (DEF, the original vegetation) that form a degradation gradient in the Sakaerat Environmental Research Station (SERS), Thailand. The Shannon diversity and evenness and Simpson indexes were determined using the sole carbon source most probable number method. Variation patterns of these indexes were analyzed for three data sets obtained during the dry (February) to wet (June) seasonal transition. Only the March data set had significant differences in the diversity indexes among the vegetative types. In March, BG soil had a significantly lower Shannon diversity index (1.77) and evenness index (0.64) than the others (2.34 and 0.84 for DDF and 2.41 and 0.87 for DEF), and a higher Simpson index of 0.23 (0.08 for DDF and 0.07 for DEF). Repeated measures analysis of variance at $p=0.05$ indicated that both vegetative type and sampling time were significant sources of variation of the Shannon indexes, while only sampling time was a significant source of variation of the Simpson index. Simple and multiple regression analyses and a redundancy analysis ordination diagram showed that throughout the period of the study, soil water content was the most significant soil environmental factor with regard to the Shannon and Simpson diversity indexes. The soil bacterial communities under all the vegetative types had higher diversity and evenness in the wet months. The transition from dry to wet season had a grater effect on variation of the diversity indexes than did the soil degradation gradient.

Key words: bacterial community, biodiversity, environmental factor, seasonal transition, soil degradation

INTRODUCTION

Soil degradation often results in changes in the soil biotic profile (Pankhurst *et al.*, 1997). Soil degradation involving stress or disturbance may change biodiversity as expressed by a diversity index, e.g., the Shannon diversity index (Wardle and Giller, 1996). Several factors may alter biodiversity in soil. Heavy metal contamination may effect biodiversity in the soil microbial community (e.g., Wenderoth and Reber, 1999). Agricultural (e.g., Cuenca and Meneses, 1996) and forest management practices (e.g., Bird *et al.*, 2000) also have altered biodiversity in the soils. These impacts cause changes in various soil environmental factors. Relationships between soil environmental factors and biodiversity may differ in every case.

A new scheme to determine soil microbial diversity using the sole carbon source utilization (SCSU) method on a Biolog gram negative (GN) plate (Biolog, Hayword, California) has been proposed by Staddon *et al.* (1997). A Biolog GN plate has 95 wells, each of which has a sole carbon source. In the

SCSU method, the plate is inoculated with a microbial community. Then, color changes as determined by the utilization of each carbon source are measured. The color intensity on each carbon source was used as the species frequency in the inoculated microbial community to determine microbial diversity. Gamo and Shoji (2000) developed another Biolog GN plate technique. They analyzed microbial communities using the most probable number (MPN) counting method. In the MPN method, the number of microbial cells that utilize each carbon source in the microbial community is determined. From this information, model microbial communities were successfully distinguished.

Our primary objective was to explore the relationships between diversity within culturable soil bacterial communities and soil environmental factors in relation to soil degradation gradient. For this purpose, we analyzed soils in dry evergreen forest (DEF, original vegetation), dry dipterocarp forest (DDF, moderately disturbed) and bare ground (BG, strongly degraded) scattered in the Sakaerat Environmental Research Station, Thailand. We used a variation of the sole carbon source MPN method proposed by Gamo and Shoji (2000) to determine diversity indexes for a soil bacterial community by applying the concept proposed by Staddon *et al.* (1997). Each MPN on a carbon source was regarded as frequency of the species, then this frequency was used to determine the Shannon diversity and evenness and Simpson indexes and how these indexes possibly reflect soil degradation.

MATERIALS AND METHODS

Site description

The Sakaerat Environmental Research Station (SERS), Wang Nam Kiao district, Nakhon Ratchasima (14° 30'N, 101° 55'E) was established in 1967. At the time of establishment, most of the area had already been disturbed by human activities.

The area is 7,808 hectares and the altitude ranges 250 to 762 m above sea level. The soil is categorized as an Orthic Acrisol according to the FAO/UNESCO scheme (FAO/UNESCO, 1979). The area includes dry evergreen forest (DEF), dry dipterocarp forest (DDF) and plantation plots as the major vegetative types, and others. The climate is classified as Aw according to the Köppen classification (Köppen, 1931). The annual precipitation is 1,260 mm and the average temperature is 26℃. Approximately, the dry season starts in November and ends around late April and the rainy season is from approximately May to October.

The DEF is primarily dominated by *Hopea* spp. and *Shorea* spp. that form the upper story 20 to 40 m above ground. A typical DEF fosters more than 1,000 trees (trunk diameter at breast height, DBH>5 cm) ha^{-1}, and the total basal area at 1.3 m height exceeds 30 m^2 ha^{-1} and the above ground biomass is over 200 tons ha^{-1} (Kanzaki *et al.*, 1995).

The DDF is more open in comparison with the DEF and has uniformly spaced trees. The upper story, 11 to 35 m above ground is formed by canopies of *Shorea obtusa*, *Pentamo suavis*, *Dipterocarpus intricatus*, *Gardenia* spp. and others. In a DDF, 875 trees (DBH>5 cm) ha^{-1} were enumerated, and the total basal area at 1.3 m height was 15 m^2 ha^{-1} and the above ground biomass was 73 tons ha^{-1} (Sahunalu and Dhanmanonda, 1995). An obvious feature of the DDF is that the ground is widely covered by *Arundinaria pusilla* or *Imperata cylindrica*. These grass species spend the dry season as rhizome with dried shoots.

Human–induced fire occurs in the DDF and burns the dry grass shoots in the later months of the

dry season. Sometimes, the fire becomes strong enough to burn relatively large trees also. After the first rain following the dry season, the trees and grasses expand their leaves.

The vegetative types are distributed in a mosaic pattrern in the northeastern part of the site. Bare ground (BG), having no vegetation as a result of past human activities, is also scattered in the mosaic.

Meteorological measurements

During the period of the research in 2001, precipitation and temperature at a meteorological station (marked with ☆ in Fig. 1) were measured, and the data were used to decide sampling dates.

Soil sampling

In this study, DEF, DDF and BG soils were sampled. The vegetative types were randomly distributed, and fire is thought to encourage the continuance of the fire–resistant DDF in the area (Sakurai *et al.*, 1998; Sahunalu and Dhanmanonda, 1995; Stott, 1984). Thus, the vegetative mosaic was regarded as a completely randomized design (CRD, Fig. 1). The replication numbers were 7, 7 and 6 for DEF, DDF and BG, respectively. In each of the 20 grids, one vegetative type was represented (Fig. 1). All the sampling points were on slight slopes (less than 10°).

Soils were sampled three times in 2001, on February 8 and 9, March 15 and 16, and June 10 and 11. Each sampling time took less than 36 hours. At each sampling point, a circle, 10 m in diameter was set, and 6 soil cores were randomly taken in the circle. A hundred ml core samplers, 5 cm in diameter, were inserted from the surface to a depth of 5.1 cm. The 6 soil cores were immediately put into a plastic bag, mixed, passed through a 2 mm screen and kept moist at 5℃ for up to 10 days before the culturable bacterial community was analyzed in the laboratory.

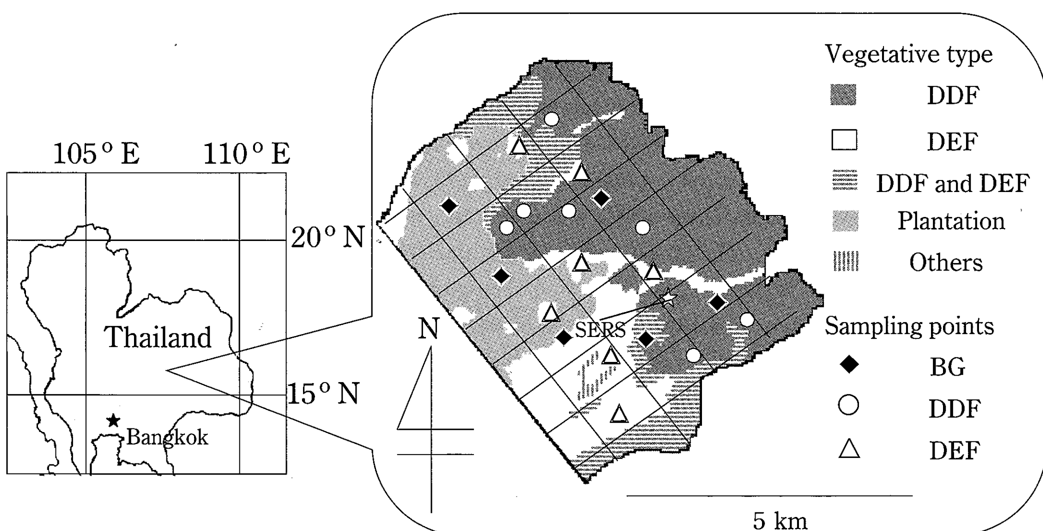

Fig. 1. The vegetative types of the Sakaerat Environmental Research Station (SERS) and the sampling points. BG, DDF and DEF indicate bare ground, dry dipterocarp forest and dry evergreen forest, respectively.

Physico-chemical analyses of soils

Soil water content and bulk density were determined using oven drying at 105℃ for 48 h. The air–dried soils were suspended in water at a soil to solution ratio of 1: 5 and reciprocally shaken at room temperature for 1 h at 120 rpm to determine their pHs and electrical conductivities (ECs). Total carbon and nitrogen in the soils were determined using the NC analyzer (Sumigraph model NC-80, Sumitomo Kagaku Kogyo, Japan). The particle size distribution was determined with a hydrometer method. Exchangeable cations (Ca, K, Mg and Na) were extracted with 1 M ammonium acetate (pH 7.0) and determined with an atomic absorption spectrophotometer. Exchangeable acidity (Al and H) was determined with titration. Cation exchange capacity (CEC) was calculated as the sum of the four exchangeable cations and the exchangeable acidity. Percentage of the four exchangeable cations to CEC was regarded as the base saturation rate. Available phosphorus was determined by the Bray II method.

Sole carbon source MPN method

The most probable number (MPN) method developed by Wren and Venosa (1996) was used with a modification. Their MPN method was used to selectively determine the number of target bacteria that utilize a hydrocarbon. In this research, we used a similar method reported by Gamo and Shoji (2000), who counted MPNs of bacteria that grow on various individual carbon sources. The basal medium reported by Doyle and Stotzky (1993) was slightly modified to give final concentrations of 1 g K_2HPO_4, 0.5 g KNO_3, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, 0.1 g NaCl, 2 mg $FeCl_3$ and 1 g glucose per liter. The pH was adjusted to 6.0. Glucose in the basal medium was substituted with: acetamide; citruline; dextran; fructose; potassium gluconate; glycine; glycolic acid; maleic acid; malic acid; malonic acid; succinic acid; sucrose; potassium sodium tartrate tetrahydrate; xylitol or xylose to introduce different carbon sources. Each carbon source was dissolved in water, adjusted to pH 6.0, filter sterilized, and added to the medium at a final concentration of 1 g L^{-1}. The control was prepared substituting glucose with no carbon source. 2,3,5-triphenyltetrazolium chloride (TTC) was introduced into the media to facilitate detection of physiological activity of bacteria. When bacteria oxidize a carbon source, TTC is reduced, then, a deep red precipitate occurs. TTC was filter sterilized and added to the medium at a final concentration of 0.01% (w/v). A preliminary test showed that employing TTC at higher concentrations than 0.01% (w/v) did not improve the efficiency, thus, we employed this concentration throughout this study. Cycloheximide as a fungicide (Gray, 1990) was filter sterilized and added to the medium at a final concentration of 0.01% (w/v). The media were added to microtiter plates, which was sterilized in 70% (v/v) ethanol for 30 min in advance, at 75 μL/well. Ten g of soil sample were suspended in exactly 9 volumes of sterilized water (90 mL) and reciprocally shaken at room temperature for 1 h at 120 rpm. After 20 seconds, 40 ml of the upper part of the suspension was taken and centrifuged at 1,000 g for 5 min. The pellet was resuspended in 10 ml of sterilized water and successively diluted 10 to 10^7-fold with sterilized water. Each well was inoculated with 25 μL of the soil suspension with 5 replications. The plates were incubated at 30℃ in the dark for 14 days. During the incubation, the plates were wrapped in a plastic film to avoid desiccation. MPNs were determined by observing, with the naked eye, the presence or absence of the color change in the wells. A preliminary test using the glucose plate, under the condition above, resulted in no significant increase in the MPN after an incubation period longer than 14 days. In the same preliminary test, the method scored coefficient of variance (CV) of 0.31 (n=4) after incubation for 14 days, the accuracy was comparable to that reported by Wren and

Venosa (1996). MPNs after 14 days of incubation were used for statistical computation. Negative MPNs by subtraction of the blank MPN were given up to 2 variables for a soil sample. In these cases, the negative results were regarded as 0, because the difference from the negative result was trivial, less than 1% of the total MPN for the sample. The MPN data were used to determine the diversity indexes.

Data analyses

The statistical software, SPSS 10.0.5J (SPSS Japan Inc., Tokyo) was used for the statistical analyses. The LSD t–test was performed to test the significance of the observed differences between means. To test similarity among the sample groups under the vegetative types, we performed discriminant analyses. The Shannon diversity and evenness and Simpson indexes were calculated using the following equations (Staddon *et al.*, 1997).

$$\text{Shannon diversity } (H') = -\sum pi \ln pi \quad (1)$$

Where pi is the proportional MPN for the ith carbon source over total MPN for the soil sample.

$$\text{Shannon evenness } (E) = H'/\ln S \quad (2)$$

Where S is the number of carbon sources with MPN (0<) observed.

$$\text{Simpson } (D) = \sum (ni(ni-1))/N(N-1) \quad (3)$$

Where ni and N are MPN for the ith carbon source and total MPN for the soil sample, respectively.

The Shannon diversity and evenness indexes indicate diversity and evenness of distribution pattern of the community, while the Simpson index indicates dominance by a particular portion of the community (Staddon *et al.*, 1997). Using the same SPSS software, we also performed repeated measures analysis of variance (ANOVA) for analyzation, determination and comparison of sources of variation of the diversity indexes. Simple and multiple regression analyses were performed to determine soil environmental factors significantly correlated to the diversity indexes. In the multiple regression analyses, the stepwise method at the default criteria ($p=0.05$ for inclusion and 0.10 for removal) was chosen. The environmental factors analyzed were the soil physico–chemical characteristics and altitude. The average altitude was 437, 416 or 437 m above sea level for BG, DDF or DEF, respectively. There were no significant differences among the altitudes according to the LSD t-test.

For the overall data set ($n=60$), direct gradient analyses were applied to analyze the relationships among the soil bacterial community profiles, soil environmental factors and the Shannon diversity indexes. Redundancy analysis (RDA), canonical correspondence analysis (CCA, ter Braak, 1986) and exploration of the Shannon diversity indexes in the ordination space were performed using CANOCO for Windows 4.02 and CanoDraw 3.10 (Microcomputer Power, NY), respectively. RDA and CCA are multivariate statistical techniques to relate species distribution patterns and environmental factors in decreased dimensionality. These statistical techniques specify whether environmental factors as gradients in relation to species distribution patterns are statistically more or less significant. RDA detects linear species distribution patterns against the significant environmental gradients, while CCA does bell–shaped unimodal patterns (ter Braak and Šmilauer, 1998). The significant environmental

gradients are shown as vectors from the origin of the ordination diagram. Thus, in this paper, significant environmental gradients and MPNs of soil bacteria that utilize any carbon sources have linear or unimodal correlations. In the same diagram, the soil samples are located according to their scores on the axes. CanoDraw has the additional function of determining the Shannon diversity index for each soil sample located in the RDA or CCA ordination diagram. Furthermore, CanoDraw draws contour lines to show changes in the Shannon diversity in relation to the environmental gradients. The contour lines are interpolated depending on Shannon diversity values for the samples in the ordination diagram. The diagram obtained by this computation explains trends of changes in the Shannon diversity along the significant environmental gradients.

RESULTS

Climatic conditions

The climatic conditions were summarized in Fig. 2. The site had no precipitation from the beginning of 2001 to mid March, thus, the soil condition was extremely dry until the first rain of the year. These conditions made the DDF area more susceptible to fire, because the grassy floor supplied dried fuel. However, the fire did not devour all the grassy floor, but burnt some patchy spaces. To avoid complications due to the burning, we sampled soil cores at locations not subjected to fire in the dry season.

In mid March, the rain moistened the environment, but it was dry again for several weeks afterwards in which both the maximum and minimum temperatures were high. From late April, the evening squall brought much rain, increasing surplus soil moisture.

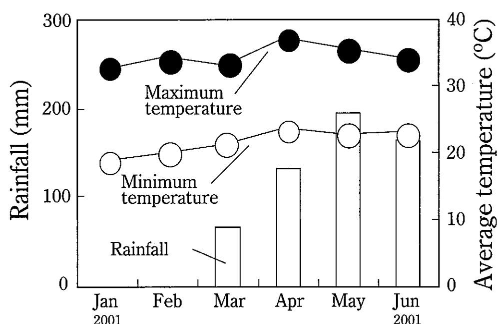

Fig. 2. Changes in precipitation and temperature at the SERS.

Soil physico-chemical characteristics

Table 1 indicates physico–chemical characteristics of the soils. Effects of the degradation were reflected by various soil physico–chemical characteristics. The soil degradation featured relatively high bulk density, sand content and exchangeable acidity, and low soil water content, EC, total C and N, exchangeable phosphorus, K, Ca and CEC. C/N showed a different trend, the intermediately disturbed DDF samples had high C/N values. The ANOVA concluded that sampling time was significant ($p < 0.05$) as a source of variation of soil water content, EC, available P and exchangeable Al.

Sakurai *et al.* (1998) determined physico-chemical properties of soils at the locations in the DEF located west to the SERS and in the DDF area located southeast to the SERS. Our observations of the DEF and the DDF soils in Table 1 are comparable to their report.

Table 1. Physico-chemical characteristics of the soils.

Soil physico-chemical characteristics	Vegetative type	Sampling time			Repeated measures
		February	March	June	
Water content (%)	BG	2.9b+	9.8b	7.4b	**
	DDF	2.9b	15.1a	16.4a	
	DEF	6.4a	13.3a	16.7a	
Bulk density (g/l)	BG	1.42a	1.40a	1.40a	NS
	DDF	1.11b	1.10b	1.02b	
	DEF	1.05b	1.01b	0.98b	
Clay (%)	BG	64.2b	61.5b	64.3b	NS
	DDF	75.8a	72.2a	76.0a	
	DEF	69.2b	70.8ab	70.5b	
Silt (%)	BG	14.4b	14.6b	13.9b	NS
	DDF	13.1b	15.4b	13.3b	
	DEF	18.0a	17.7a	16.3a	
Sand (%)	BG	21.5a	23.9a	21.7a	NS
	DDF	11.0b	12.5b	10.7b	
	DEF	12.7b	11.5b	13.2b	
pH	BG	5.31b	5.26a	5.13b	NS
	DDF	5.82a	5.71a	5.70a	
	DEF	5.94a	5.68a	5.63a	
EC (mS/m)	BG	2.7b	4.6b	3.5b	**
	DDF	4.1b	6.0b	7.2ab	
	DEF	8.7a	12.0a	10.1a	
Total N (mg/g dry soil)	BG	0.78b	0.79c	0.63b	NS
	DDF	0.97b	1.15b	1.25a	
	DEF	1.91a	1.82a	1.68a	
Total C (mg/g dry soil)	BG	10.9c	10.6c	9.7b	NS
	DDF	16.8b	19.7b	21.7a	
	DEF	25.5a	24.2a	22.5a	
C/N	BG	13.8b	13.4b	15.0b	NS
	DDF	17.4a	17.3a	17.3a	
	DEF	13.3b	13.3b	13.4b	
Available P (μg/g dry soil)	BG	3.7b	2.2c	3.6b	*
	DDF	7.8ab	6.4b	4.6b	
	DEF	10.4a	10.7a	7.7a	
Exchangeable K (m eq/100 g dry soil)	BG	0.48b	0.46b	0.37b	NS
	DDF	0.47b	0.56b	0.51ab	
	DEF	0.70a	0.91a	0.73a	
Exchangeable Ca (m eq/100 g dry soil)	BG	0.97b	1.11b	0.85b	NS
	DDF	1.57b	1.98ab	1.92a	
	DEF	3.44a	2.53a	2.21a	
Exchangeable Mg (m eq/100 g dry soil)	BG	1.52a	1.49a	1.16a	NS
	DDF	1.88a	1.99a	2.20a	
	DEF	2.48a	2.69a	2.21a	
Exchangeable Na (m eq/100 g dry soil)	BG	0.15a	0.15a	0.14a	NS
	DDF	0.14a	0.14a	0.13a	
	DEF	0.13a	0.10b	0.14a	
CEC (m eq/100 g dry soil)	BG	8.7b	9.7b	8.4b	NS
	DDF	9.6b	10.7b	11.3ab	
	DEF	12.3a	13.4a	12.3a	
Base saturation (%)	BG	34.3b	33.1a	29.3a	NS
	DDF	41.8ab	43.7a	45.1a	
	DEF	53.6a	51.8a	43.2a	
Exchangeable Al (m eq/100 g dry soil)	BG	0.23a	0.56a	0.46a	*
	DDF	0.15a	0.15b	0.26a	
	DEF	0.16a	0.24b	0.23a	
Exchangeable H (m eq/100 g dry soil)	BG	1.23a	1.08a	1.18a	NS
	DDF	0.18b	0.39a	0.46ab	
	DEF	0.30b	0.40a	0.28b	

+Values within each soil environmental factor followed by the same letter in the same column are not significantly different at $p=0.05$, according to the t–test. *, ** and NS in the repeated measures column indicate significant at $p=0.01$, 0.05 and not significant, respectively.

Population and diversity of soil bacterial community

The total MPN for each sample was determined by summing up MPNs for the 16 carbon sources. Total MPNs in the soils indicated relatively small numbers of bacteria in the BG soil (Fig. 3). At the drier sampling time, the DEF soil had a significantly higher total MPN than the others. The DDF soil significantly increased the total MPN in wet conditions. While total MPNs in the BG or the DEF soil did not have significant seasonal variation.

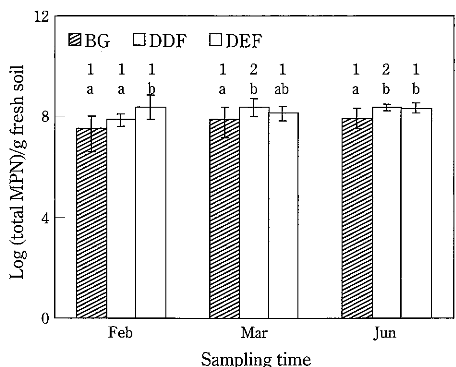

Fig. 3. Total most probable numbers (MPNs) of the bacteria in the soils. The same letter above the columns indicates that the values are not significantly different (p=0.05) among the vegetative types, while the same number among the sampling times.

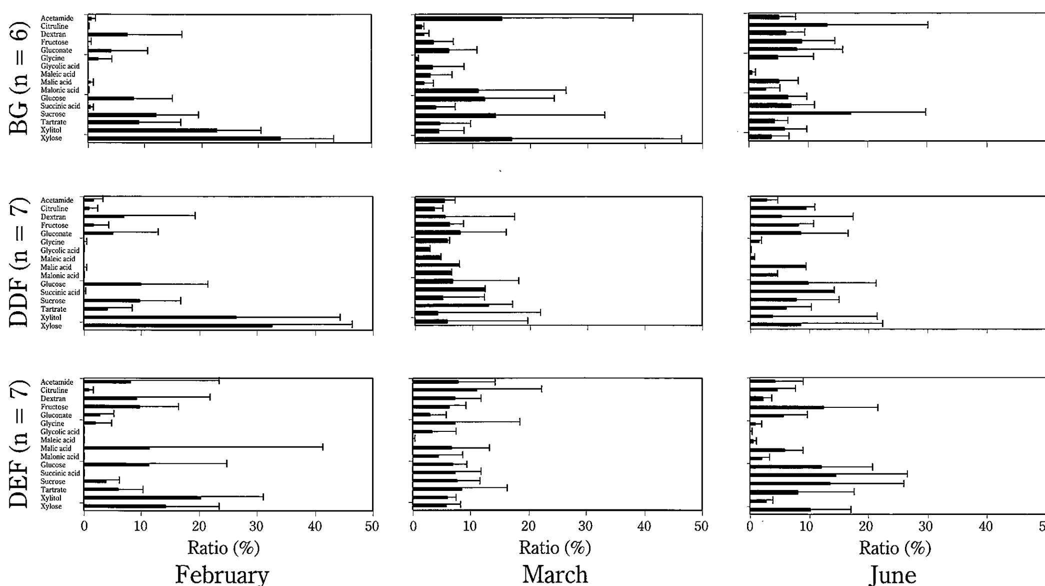

Fig. 4. Profiles of the soils based on the sole carbon source MPN method. The bars indicate ratios and standard deviations for the carbon sources.

The culturable soil bacterial communities were profiled as in Fig. 4. In February, MPNs on xylitol and xylose were relatively high in the BG and the DDF bacterial communities. For all the soil sample groups, the distribution patterns became more even in later months due to increases in some bacterial

portions suppressed in February. Then, dominance by portions on particular carbon sources became less remarkable.

Table 2 indicates that the Shannon diversity index changed within a common trend among the vegetative types: higher biodiversity in the wet months compared to the dry months. In March, the BG soil had significantly lower diversity and higher dominance by a particular portion of the bacterial population. But no significant differences in the diversity indexes were found among the soil sample groups in the other months. The repeated measures ANOVA determined sources of variation of the diversity indexes. The sources of variation of the Shannon indexes, vegetative type, sampling time and the interaction were significant at p=0.05. On the other hand, for the Simpson index, sampling time was a more significant source of variation, while vegetative type was not as significant as it was for the Shannon indexes.

Table 2. The diversity indexes for the soils under the vegetative types at each sampling time.

Diversity index	Vegetative ype	Sampling time			Repeated measures ANOVA		
		February	March	June	Vegetative type (V)	Sampling time (S)	Interaction (V x S)
Shannon	BG	1.628a	1.773b	2.286a	0.045	0.000	0.008
	DDF	1.551a	2.338a	2.300a			
	DEF	1.728a	2.413a	2.208a			
Shannon evenness	BG	0.587a	0.640b	0.824a	0.045	0.000	0.008
	DDF	0.559a	0.843a	0.829a			
	DEF	0.623a	0.870a	0.797a			
Simpson	BG	0.233a	0.230a	0.137a	0.393	0.000	0.082
	DDF	0.278a	0.082b	0.126a			
	DEF	0.261a	0.074b	0.147a			

Values followed by the same letter in the column are not significantly different at p=0.05, according to the t–test. *, ** and NS in the repeated measures column indicate significant at p=0.01, 0.05 and not significant respectively. BG, DDF and DEF indicate bare ground, dry dipterocarp forest and dry evergreen forest, respectively.

Table 3. Simple and multiple regression between soil physico–chemical characteristics as independent variables and Shannon or Simpson indexes.

Sampling time	Diversity index	Simple regression			Multiple regression	
		Significant factor	Slope	R^2	Significant factor+	R^2
February	Shannon+	None			None	
	Simpson	None			None	
March	Shannon+	Water	0.075	0.290	C	0.504
		Bulk density	-0.993	0.266		
		C	0.044	0.504		
		N	0.526	0.403		
		P	0.063	0.410		
		Sand	-0.027	0.221		
		CEC	0.096	0.287		
	Simpson	Water	-0.195	0.197	Sand	0.326
		C	-0.011	0.300		
		pH	-0.115	0.209		
		N	-0.124	0.227		
		P	-0.015	0.234		
		Clay	-0.008	0.239		
		Sand	0.010	0.326		
		Exch Al	0.027	0.307		
		Exch H	0.008	0.218		
June	Shannon+	None			None	
	Simpson	None			None	
Overall	Shannon+	Water	0.048	0.410	Water	0.410
	Simpson	Water	-0.011	0.249	Water	0.249

+Both the Shannon diversity and evenness scored the same R^2, therefore, slope and R^2 for only the Shannon diversity index were tabulated.

Results of simple and multiple regression analyses between the indexes and the environmental factors are summarized in Table 3. It is clear that soil water content had a strong correlation to the overall data set. The analyses showed the same significance for both the Shannon diversity and evenness indexes, therefore, results for the Shannon diversity were shown. Several soil environmental factors significantly correlated to the indexes in March. The multiple regression analysis for the March data sets indicated that total carbon and sand were significantly correlated to the Shannon and Simpson indexes, respectively. The March Shannon indexes positively correlated to contents of total carbon, total nitrogen and available phosphorus and CEC, and negatively to bulk density and sand content. The March Simpson index positively correlated to sand content and exchangeable acidity (Al and H), and negatively correlated to pH and water, total carbon, total nitrogen, phosphorus and clay contents. The indexes for the soils in February or June did not correlate to any soil environmental factors.

The first and second axes were extracted by the direct gradient analyses to explore the relationships between the Shannon diversity and soil environmental gradients in decreased dimensionality. The primary two axes extracted by RDA had eigenvalues of 0.352 and 0.050, and that by CCA 0.222 and 0.085. These results indicate that for some carbon sources, linear distribution patterns against the significant environmental gradients were more frequently seen in comparison with the bell-shaped unimodal pattern. Hence, RDA better related environmental gradients and the soil bacterial community profiles. Thus, we used results of the RDA computation in the following analyses. The Shannon diversity in RDA ordination space also showed that soil water content was the most significant factor related to the diversity indexes and soil bacterial community profiles based on the sole carbon source MPN method (Fig. 5).

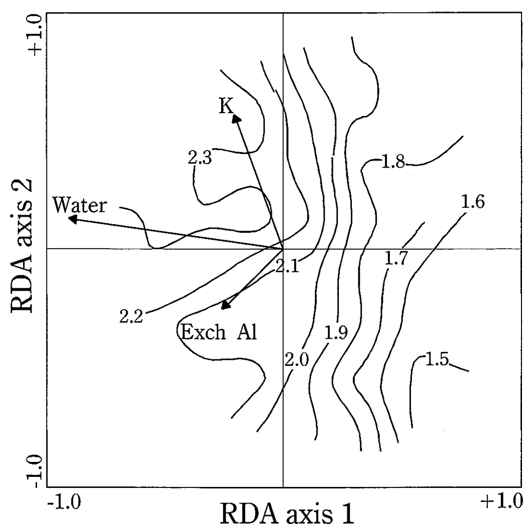

Fig. 5. Shannon diversity in the ordination plane. The figures and contour lines indicate the Shannon diversity for the samples scattered in the RDA ordination diagram. The environmental gradients drawn as vectors were significant at p=0.05.

In the discriminant score plots, each soil sample was scored and located (Fig. 6). The discriminant analyses clearly separated all the sample groups taken at each sampling time without any misclassification, indicating that those sample groups were distinctively different in terms of the bacterial community structures. The difference among the sample groups decreased in the later months along with the increase in soil water content.

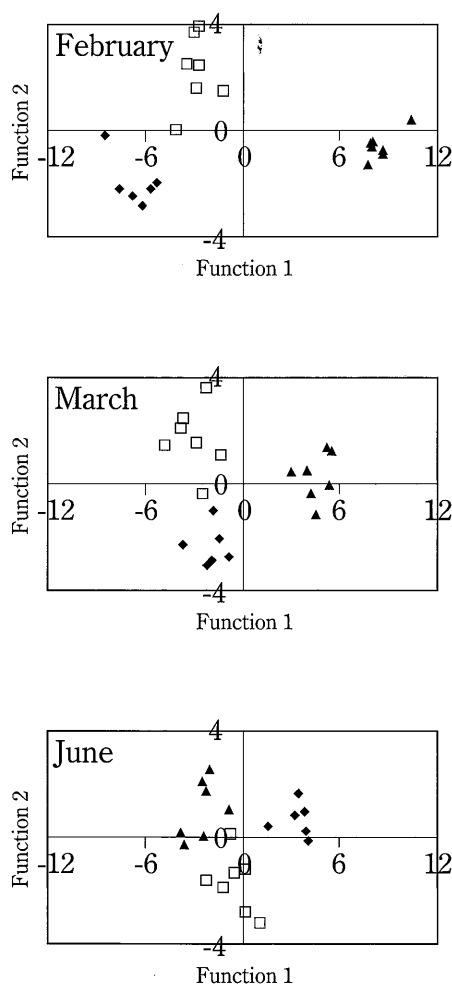

Fig. 6. Discriminant scores for the soil samples at the sampling times. The diamond (◆), the open square (□) and the triangle (▲) indicate BG, DDF and DEF, respectively.

DISCUSSION

Change in soil environmental factors

Many soil environmental factors were significantly different among the vegetative types. The degradation in the area (BG) was described as having sandy texture, high bulk density, low pH, high exchangeable acidity, poor mineral and organic nutrients and dryness (Table 1). The moderately disturbed DDF soil had many intermediate soil properties between the BG and the DEF soils, but also had significantly higher C/N ratios throughout the period of this study. *Imperata cylindrica*, a grass species, covers the DDF ground and supplies the organic matter in the dry season. This is probably the cause of the high C/N ratio. Soil water content, EC, available phosphorus and exchangeable Al showed common seasonal trends among the vegetative types, according to the repeated measures ANOVA. The precipitation pattern was thought to have important effects on changes in these soil environmental factors (Fig. 2).

Change in soil bacterial number

It was suggested that vegetative type effects the soil bacterial population. In this study, the BG soil had lower bacterial numbers than the DDF and/or the DEF soils (Fig. 3). Higher bacterial numbers in less degraded soils were observed (Jha *et al.*, 1992). The BG soils had more sand and less clay than the other soils (Table 1). This soil textural condition provides fewer niches where the soil bacteria can be protected from predators (Juma, 1993).

This study also suggested that changes in bacterial number in the DDF soil were due to the seasonal transition. Soil bacterial numbers in more and less degraded soils changed parallel to show an obvious seasonal fluctuation pattern (Jha *et al.*, 1992). In this study, the bacterial number in the DDF soil increased in the late, wet months. Such a seasonal change may occur irregularly, though perceivably (Lundgren and Söderström, 1983). A strong correlation between soil water content and soil bacterial population was suggested (Herman *et al.*, 1994). The increase in bacterial number recognized in the DDF soil under wet conditions was perhaps a result of the higher soil water content.

Change in bacterial community diversity

As Wardle and Giller (1996) commented, it seems to be difficult to generalize how soil biodiversity changes following anthropologic disturbance or stress in soils. Land management practices (Bird *et al.*, 2000; Cuenca *et al.*, 1996; Davis, 1997; Lumley *et al.*, 2001; Marfenina and Mirchink, 1989; Staddon *et al.*, 1997) or met al and other chemicals (Barkey *et al.*, 1985; Hiroki, 1993; Marfenina and Mirchink, 1989; Wenderoth and Reber, 1999; Yang *et al.*, 2000; Val *et al.*, 1999) have been found to alter soil biodiversity. In some instances, those types of human impact decreased soil biodiversity (Barkey *et al.*, 1985; Lumley *et al.*, 2001; Marfenina and Mirchink, 1989; Wenderoth and Reber, 1999; Yang *et al.*, 2000). But other cases showed different trends. Palmer and Young (2000) found higher diversity of a Rhizobium leguminosarum population in an arable soil than the original grassland. In a fungal community in soils subjected to heavy metals, a higher diversity was observed at moderate concentrations than at higher concentrations or the control (Val *et al.*, 1999). No differences in soil microbial diversity were reported based on phospholipid fatty acid profiles in soils under grazed and ungrazed grasslands (Bardgett *et al.*, 1997).

Significant differences in all the diversity indexes between the soils were recognized for the March sample set (Table 2); the most disturbed BG bacterial communities had significantly lower Shannon diversity, evenness and higher dominance by a particular bacterial portion in comparison with the others. Similar trends in soil biodivesity in relation to disturbance had been reported (Bird *et al.*, 2000; Lumley *et al.*, 2001; Marfenina and Mirchink, 1989; Staddon *et al.*, 1997). Considering the current and prior researches, it can be concluded that a decrease in soil biodiversity due to disturbance may be a common event to some extent.

In their phospholipid fatty acid study, Bossio *et al.* (1998) found no significant differences in the Shannon diversity index among agricultural practices, though the phospholipid profiles among the practices were clearly different. As in their case, in all the sampling times of the current study, bacterial community structures clearly differed among the soils (Figs. 4, 6), but the February or June samples did not show significant differences in the indexes among the vegetative types. Forest logging and subsequent pasture establishment in the Amazon Basin little altered the Shannon diversity calculated from distribution patterns of the rDNA library clones, from 2.17 to 2.19 (Borneman and Triplett 1997). But they found a structural difference in microbial community between the original forest and the pastureland. Those instances indicate that changes in soil microbial community structures are not necessarily reflected by the values of biodiversity indexes.

Seasonality was a greater source of variation for the Simpson index than vegetative type (Table 2). While sampling time, vegetative type and the interaction were significant sources of variation for the Shannon indexes. These results imply that sensitivity to a source of variation can differ among diversity indexes. In this work, an increase in soil bacterial diversity and evenness in the dry to wet transition was clear, as shown in Table 2. Such seasonal changes in diversity of soil and litter arthropods were also reported by Bird *et al.* (2000). In the SERS, the primary cause of the seasonal changes in the diversity indexes is the precipitation pattern (Fig. 2). The increase in soil moisture was thought to trigger the increase in soil bacterial diversity (Beare *et al.*, 1995). The high soil bacterial diversity in wet conditions is thought to be related to changes in the soil bacterial community structures, e.g.; decrease in the relative abundance of gram negative bacteria (Kilbertus and Proth, 1979).

We obtained the Shannon diversity indexes ranging 1.55 to 2.41 (Table 2). The theoretical maximum in this condition is 2.77. After isolating and culturing soil bacteria, Brønstad *et al.* (1996) obtained a soil bacterial diversity of 2.49 from 13 biotypes from a sandy soil and 2.94 from 17 biotypes from an organic soil. Though, the substrate number in our method was comparable to the biotype numbers in their report, the resulted diversity values were lower. In the SERS, the soil bacterial communities may have relatively low diversity. The MPN method used in our work might be a source of the relatively lower values of the diversity index. Different methods may profile the same microbial community differently (Griffiths *et al.*, 1997), and the diversity indexes may also be different depending on methods (Øvreås and Torsvik, 1998). It will be worthwhile to apply various methods to determine diversity indexes in the soils.

Soil environmental gradients and diversity

Relationships between soil bacterial diversity and soil environmental factors were perhaps strongly influenced by the soil water content (Table 1, 3). The low soil water content in the BG soil could be a result of its poor organic matter and sandy texture. Those factors, in addition to stronger solar radiation

due to no vegetative cover, enhance evaporation from the BG soil. Other chemical characteristics are strongly influenced by the changes in soil water content. It is probable that the increase in soil bacterial diversity in the BG soil during the seasonal transition was slower than the increase in the forest soils (Table 1, 2).

The RDA computation also specified soil water content as the most significant factor related to soil bacterial distribution patterns (Fig. 5). Fig. 5 indicates that soil bacterial community divresity becomes higher when soil water content and exchangeable K become greater. Soil exchangeable K did not show a common seasonal fluctuation pattern among the vegetative types (Table 1), indicating that exchangeable K is less dependent on seasonality than water contents is. The regression analyses for the overall data set did not specify exchangeable K content as an environmental factor significantly correlated to the diversity indexes (Table 3). Thus, such an ordination diagram may be better able to find correlations between soil biodiversity and environmental gradients, other than seasonal effects.

Degradation gradient and soil biodiversity

Cause and effect relationships between soil environmental factors and biodiversity may exist. In March, more soil environmental factors correlated to the indexes, especially those related to soil bulk density and soil acidity (Table 3). Soil microbes may affect these environmental factors (Beare *et al.*, 1995), but the opposite is also likely. Previous knowledge on the relationships between such environmental gradients and soil biodiversity is scarce, and therefore, at this point, it is difficult to generalize any trend. N content positively correlated to diversity of *Rhizobium leguminosarum* (Palmer and Young, 2000), but a randomly amplified polymorphic DNA technique showed lower soil microbial diversity and species richness in a N rich soil (Yang *et al.*, 2000). The processes which relate a soil bacterial community to soil environmental factors are complicated (Beare *et al.*, 1995). The extreme environmental conditions in the BG soil may be a direct source of stress for the soil bacterial community. For example, root nodule bacterial activities and functions are inhibited by aluminum toxicity (Flis *et al.*, 1993). As low K content was observed in the degraded BG soil, some environmental factors might be direct or indirect causes of the low soil bacterial diversity. In the experiment conducted by Wenderoth and Reber (1999), the heavy metals were clearly responsible for a decrease in soil microbial diversity. While a change in soil biodiversity may cause functional changes, Salonius (1981) suggested that reducing soil microbial diversity may result in lowering soil physiological activity. Strategies similar to that of the authors are expected to elucidate the cause and effect relationships between soil biodiversity and soil conservation and degradation.

Methodological uncertainty

Observation of phospholipid fatty acid (PLFA) distribution patterns in soils that had been subjected to different farming practices was done by Bossio *et al.* (1998). Despite their successful discrimination of the soils based on the PLFAs, they found no significant differences in the Shannon diversity among the farming practices. They pointed out that each of the PLFAs is produced by plural microbial species, and this was suspected to be the cause. In February and June, we obtained similar results; despite the distinctive differences among the soil bacterial communities, we did not find significant differences in diversity of the bacterial communities (Table 2, Figs. 4, 6). With the currently applied method, the same suspicion is inevitable, since an MPN may include taxonomically different bacteria. Moreover, the

method has another uncertainty: a bacterial portion contributing to an MPN may also contribute to more variables. It is necessary to further investigate these uncertainties possibly favor or inhibit clearer statistical mean separation of the diversity indexes.

CONCLUSION

The current study suggested that the seasonal change in soil water content was the most decisive soil environmental factor controlling the Shannon diversity and evenness and Simpson indexes for soil bacterial communities in the SERS. Degradation gradient as another source of variation was thought to associate with different soil water contents among the soils. The dry to wet seasonal transition increased diversity and evenness of the soil microbial communities. During the seasonal transition, accompanied by increasing evenness, the bacterial communities in the soils had decreasing structural differences among them. Therefore, in order to evaluate the changes in diversity of microbial communities in relation to soil degradation, we should pay more attention to the timing of soil sampling. Based on our results, at the current study site, during the period from extremely dry to moist conditions, degradation gradient based on the diversity of soil bacterial communities can be detected.

ACKNOWLEDGMENTS We thank the Sasakawa Scientific Research Grant from the Japan Science Society for financial support. Prof. Dr. Pongsak Sahunalu, Dr. Chongrak Vatcharinratana and Mr. Sakhan Teejuntuk, Faculty of Forestry, Kasetsart University, Thailand helpfully provided support. Mr. Pramuk Kaeoniam and other staff members of the SERS assisted our activities. We gratefully acknowledge the support from these people.

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Received Sep. 20, 2002
Accepted Feb. 13, 2003