Original article

Human-induced land degradation gradient shown by antibiotic susceptibility profiles of bacterial communities and physico-chemical soil quality characteristics

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Abstract

In this study, multivariate profiles of soil bacterial communities were used to measure the intensity of land degradation as a result of deforestation of a Thai tropical forest and subsequent human activities. Soils were sampled at a dry evergreen forest (the original vegetative type), a dry deciduous forest (moderately disturbed) and bare ground (the most severely degraded). The soil bacterial communities were profiled by the antibiotic disk diffusion (ADD) method. Based on antibiotic susceptibility profiles of soils, the ADD method may give soil quality measures that are unique in their meanings. Principal component analysis extracted principal components from the antibiotic susceptibility and soil physico-chemical data sets. Both the first principal components showed the degradation gradient in the principal component score plots. Redundancy analysis showed that decreases in soil moisture, total carbon content and electrical conductivity involved in the degradation correlated significantly to changes in the antibiotic susceptibility profile. Multiple regression analyses between values of a soil fertility index (SFI) or a soil evaluation factor (SEF) and scores on the principal components or redundancy analysis ordination axes indicated that the first principal component or redundancy analysis ordination axis significantly explained the SFI and the SEF models ($p<0.002$). The formulae that quantified the intensity of the land degradation are given. This scheme is expected to help the conservation and rehabilitation of lands by predicting results, describing a gradient of interest and suggesting preferable soil conditions for particular goals.

Key words: antibiotic susceptibility, deforestation, land degradation, multivariate analysis, soil bacterial community, Thailand

Introduction

Human activities have been responsible for deforesting the tropics, and deforestation has emerged as a serious challenge to socio-economic development in Thailand, as in many other countries (National Economic and Social Development Board of Thailand, 1996). In the tropics, deforestation often leads to land degradation and results in hard-to-rehabilitate soils under tropical climatic conditions (Eden and Parry, 1996). While it is important to clarify how soils change following deforestation, various changes in the soil quality, e.g., physico-chemical and biotic ones, are involved in land degradation (Mausbach and Seybold, 1998), and soil variables respond to land degradation differently (e.g., Jha et al., 1992). Thus, it is considered advantageous to analyze multiple soil variables and to obtain integrative measures of the soil quality (Sena et al., 2000). Each integrative measure provided by multiple soil variables can be a reliable and specific measure of a gradient of interest (e.g., Francl, 1993).

Because of the above-mentioned multidimensionality of soil quality variations, changes in an area can be determined by various measures. If we put soils sampled from an area into an increasing or a decreasing order based on values of a single variable such as soil moisture, then we can see the gradient of the measure (McCune et al.,

2002). We may not recognize the gradient continuously extending in the area; nontheless, this ordering reveals the gradient. In general, such a gradient is called an environmental gradient (e.g, Hutchinson et al., 1999). On the other hand, the moisture gradient is a specific gradient based on values of soil moisture (e.g., Zak et al., 1994), and there are other specific environmental gradients such as a land degradation gradient (Doi and Sakurai, 2004).

The statistical technique based on putting things into order is called ordination (McCune et al., 2002), and the technique often provides integrative measures, in this case, the measure of soil quality (Doi and Sakurai, 2004). Integrative measures derived from the same data set are independent of one another; thus, ordination can be a tool to extract meaningful measures based on multidimensional differences among soils. Hence, measuring multiple soil variables of samples gathered in an area provides multivariate profiles of the soils and shows particular aspects of the variation of soil quality by providing integrative measures of soil quality (MacMillan, 1991).

In this research, the antibiotic disk diffusion method (ADD method, Doi, 2003) was used to profile soils. The ADD method showed applicability in finding differences between soils supporting a tropical dry evergreen forest (DEF) and bare ground (BG) as a result of deforestation and subsequent human activities (Doi et al., 2004). The ADD method profiles soils based on susceptibility patterns of the soil bacterial communities to antibiotics. The power of the ADD method to discriminate among different soils was comparable (Doi, 2003) to that of the Biolog method (Garland and Mills, 1991), which has demonstrated applicability to multivariate profiling of soils (Insam and Rangger, 1997).

This type of method of profiling soils, however, is subjected to the criticism that the meaning of each variable is not clear. Some methods for multivariate profiling of soils involve measuring quantities of biotic molecules such as 16S rDNA amplification products (Engelen et al., 1998). In such cases, the meaning of each variable is clear. On the other hand, the variables of the Biolog method (Garland and Mills, 1991), the sole carbon source most probable number method (Wren and Venosa, 1996), and the ADD method do not have clear meanings, because the variables are measured by applying

carbon sources or antibiotics that do not necessarily play a role in the ecosystem. However, the multivariate profiles of soils may show specific aspects of change in the soil quality, and may give unique integrative measures that explain gradients of interest (Pankhurst et al., 2001). Despite the difficulty in utilizing a single variable, the Biolog method (e.g., Bossio and Scow, 1995) and the sole carbon source most probable number method (e.g., Doi and Sakurai, 2003) have given multivariate profiles of soils in responding to various effects, and produced integrative measures that were unique in their meanings. The ADD method is relatively new, but a previous comparison with the Biolog method showed that the method could recognize differences among soils uniquely (Doi, 2003). Hence, the ADD method is expected to offer additional observation windows.

This research was conducted to obtain integrative measures of the intensity of land degradation based on the susceptibility of soil bacterial communities to various antibiotics. Soils were sampled from portions of Thailand's Sakaerat Environmental Research Station variously catergorized as DEF (the original vegetative type), dry deciduous forest (DDF, moderately disturbed) and BG (the most severely degraded); these soils were then profiled with the ADD method. The multivariate profiles were analyzed with principal component analysis to extract principal components. Multiple regression analysis between values of a soil fertility index (Moran et al., 2000) or a soil evaluation factor (Lu et al., 2002) and principal component scores for the soils was performed to obtain integrative measures that quantified the intensity of the land degradation. To obtain another integrative measure, soil physico-chemical characteristics and susceptibility profiles were used for direct gradient analysis (McCune et al., 2002). Specifically, it was determined whether or not any of these ordination axes significantly explained the degradation.

Materials and Methods

Site description

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

1976). The area is 7,808 hectares and the altitude ranges from 250 to 762 m above sea level. The climate is classified as savanna (Köppen, 1931). The area includes dry evergreen forest (DEF), dry deciduous forest (DDF) and plantation plots as the major vegetative types (Fig. 1). The vegetative types are distributed in a mosaic pattern 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.

The DEF is primarily dominated by *Hopea ferrea* 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 >5 cm) ha^{-1}, 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 plot, 875 trees (trunk diameter at breast height >5 cm) ha^{-1} were counted, 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*. Human-induced fire occasionally occurs in the DDF and burns the grass shoots. Sometimes, the fire becomes strong enough to burn relatively large trees, as well.

The soil is originally an Orthic Acrisol, according to the FAO/UNESCO scheme (FAO/UNESCO, 1979). Sakurai et al. (1998) compared the morphological properties of the DEF and the DDF soils. The effective soil depths (A and B horizons) were more than 31 cm and less than 15 cm for typical DEF and DDF pedons, respectively. The colors of the A horizons were dark reddish brown (DEF) and brownish black (DDF). The A horizon of the DEF soil was weak structured, while that of the DDF soil was moderately structured. The depth of the C horizon of the DEF soil ranged from 31 to 80 cm or deeper, while that of the DDF soil from 15 to 51 cm.

Because of past human activities, mainly illegal slash and burn cultivation (Kaeoniam et al., 1976), the BG soil has been intensively deprived of nutrients and have lost other conditions seen in forest soils. At these sampling points, restoration of vegetative cover is difficult and the harsh conditions for plants make the bare ground remain so. Morphological features of bare ground can be seen at some points in the site. For typical bare ground, the A horizon can not be recognized. The uppermost
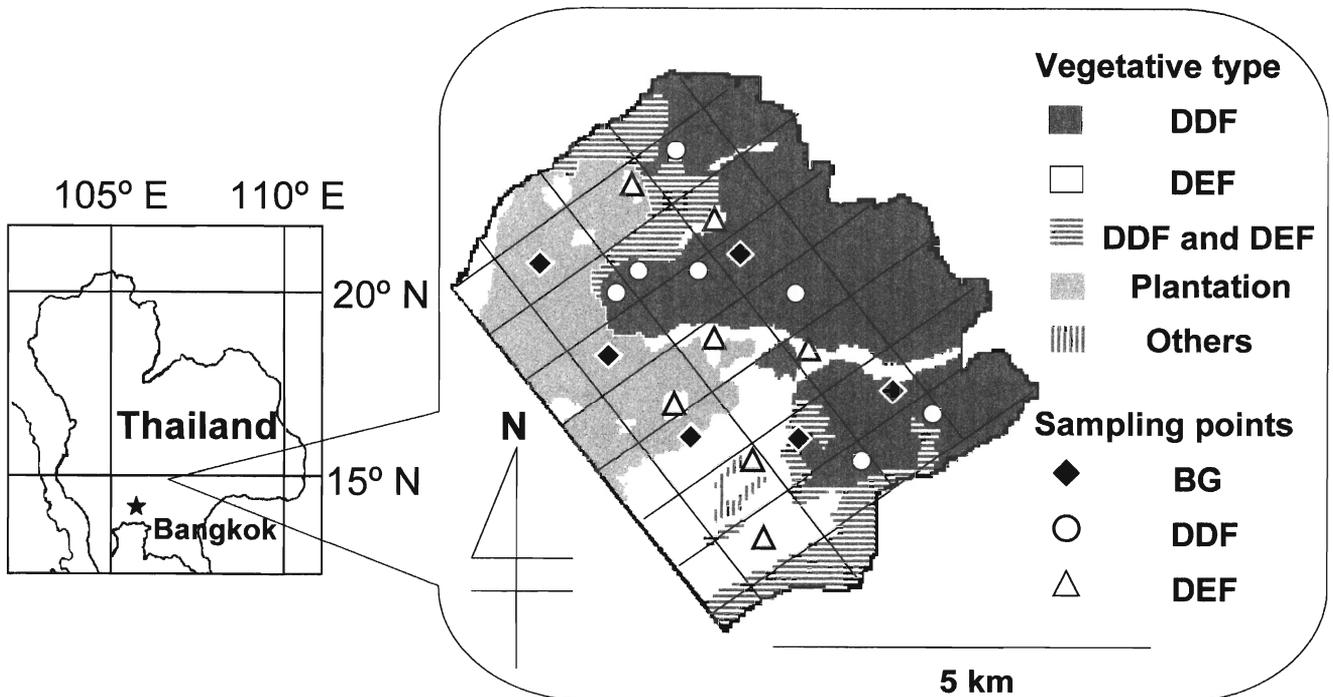

Fig. 1. The vegetative types of the Sakaerat Environmental Research Station and the sampling points. BG, DDF and DEF indicate bare ground, dry deciduous forest and dry evergreen forest, respectively.

horizon is reddish brown, rich in gravel, and has few roots and other plant organs/debris. The boundary between the uppermost horizon and the deeper horizon is not clear, while the horizon deeper than 50 cm is pale orange.

Soil sampling

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

Soils were sampled on November 4, 2002. At each sampling point, a circle, 10 m in diameter was established, and 6 soil cores were randomly taken within the circle. 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 placed into a single plastic bag, mixed, passed through a 2 mm screen, brought to the laboratory, then analyzed/profiled.

Physico-chemical analyses of soils

Soil moisture content and bulk density were determined using oven drying at 105°C 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 pH and electrical conductivity. Soil organic matter was determined by the loss of ignition method. Total carbon and nitrogen in the soils were determined using an NC analyzer. 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 was calculated as the sum of the four exchangeable cations and the exchangeable acidity. The percentage of the four exchangeable cations to the cation exchange capacity was regarded as the base saturation rate. Available phosphorus was

determined by the Bray II method.

Values of a soil fertility index (SFI, Moran et al., 2000) and a soil evaluation factor (SEF, Lu et al., 2002) were calculated to quantify the intensity of the land degradation. In the humid tropics, Moran et al. (2000) showed the applicability of the SFI model to the evaluation of soil quality and the prediction of the succession rate of secondary tropical forest. The following equation was used to calculate SFI values (Lu et al., 2002):

$$
\begin{aligned}
SFI = {} & pH + \text{organic matter (\%, dry soil basis)} \\
& + \text{available P (mg kg}^{-1}\text{, dry soil)} \\
& + \text{exch K (c eq kg}^{-1}\text{ dry soil)} \\
& + \text{exch Ca (c eq kg}^{-1}\text{ dry soil)} \\
& + \text{exch Mg (c eq kg}^{-1}\text{ dry soil)} \\
& - \text{exch Al (c eq kg}^{-1}\text{ dry soil)} \qquad [1]
\end{aligned}
$$

Latent drawbacks of the SFI model have been pointed out by Lu et al. (2002). SFI may largely depend on pH, but an extremely high pH value is not suitable for plant growth. Moreover, pH is not an independent, but a dependent variable of the relative proportion of Ca, Mg and exchangeable Al in the soil. Thus, the meaning of SFI is not clear. To improve these drawbacks, they developed another index called SEF. SEF values were calculated using the following equation:

$$
\begin{aligned}
SEF = {} & [\text{exch K (c eq kg-1 dry soil)} \\
& + \text{exch Ca (c eq kg-1 dry soil)} \\
& + \text{exch Mg (c eq kg-1 dry soil)} \\
& - \text{Log (1 + exch Al (c eq kg-1 dry soil))}] \\
& \times \text{organic matter (\%, dry soil basis)} + 5 \quad [2]
\end{aligned}
$$

Originally, SFI was developed to measure the quality of soils in cacao fields (Alvim and Rosand, 1974). Moran et al. (2000) found the applicability of this index to the measurement of the quality of forest soils in the humid tropics (Köppen, 1931). Recently, Doi and Sakurai (2004) applied SFI and SEF to the evaluation of soil quality in the SERS, where the climate is classified as savanna.

Antibiotic disk diffusion method

Biolog universal growth medium was dissolved in water at 36 g L^{-1}, following the producer's instruction. The pH was adjusted to 6.0. Agar was added to the medium at 1.5% (w/v); then, the medium was autoclaved, cooled to 55°C, and

supplemented with filter sterilized tetrazolium violet and cycloheximide at final concentrations of 0.20 and 0.36 mM, respectively. Twenty mL of this medium was poured into a petri dish (87 mm in diameter). Ten grams of the soil were suspended in 90 mL of sterilized water and reciprocally shaken at room temperature for 1 h at 120 rpm. The suspension was left for 20 sec, and 60 mL of the upper phase was centrifuged at 1,000 g for 5 min. The supernatant was decanted and the pellet was re-suspended in 3 mL of sterilized water. One mL of this soil suspension was poured and spread onto the agar plate and incubated at 30°C in the dark for 10 h. The plates were then aseptically placed under an airflow at room temperature for 1 h to expel excessive moisture.

The following antibiotic solutions were prepared: Ampicillin (in 25% 1 N NH3 solution, pH adjusted to 6.0); chloramphenicol (in 50% ethanol at 155 mM); dapson (in 50% ethanol at 66.7 mM); erythromycin (in 50% ethanol at 68.1 mM); kanamycin sulfate (in water at 85.8 mM); lasalocid (in 50% ethanol at 8.46 mM); nafcillin (in 50% ethanol at 242 mM); nalidixic acid (in 50% ethanol at 8.62 mM); neomycin•HCl (in water at 44.0 mM); novobiocin (in water at 63.0 mM); penicillin G (in water at 300 mM); spectinomycin•2HCl (in water at 247 mM); streptomycin sulfate (in water at 137 mM); tetracycline (in water at 180 mM); trimethoprim (in 50% ethanol at 68.9 mM); spectinomycin•2HCl and streptomycin sulfate (mixture 1, in 25% ethanol at 68.6 and 19.8 mM, respectively) and tetracycline and trimethoprim (mixture 2, in 25% ethanol at 90.0 and 34.5 mM, respectively).

Paper disks 6 mm in diameter were prepared from Whatman No. 2 filter paper and autoclaved. Four µL of the filter sterilized antibiotic solution was loaded onto a disk. The disks were air dried for 30 min, and placed onto the plates (6 disks/plate). The agar plates were incubated at 30°C in the dark for 24 h, before the distance between disk and the edge of the inhibitory zone was measured. Duplicate zones of inhibition around a disk were observed in some cases with streptomycin, tetracycline and mixture 1. In these cases, the outer arc was used for the measurement. Using the following equation, a ratio-transformation was employed, i.e., each observation was divided by the sum of all the observations for the sample and used for statistical analyses. In this paper, the transformed value is called the relative susceptibility.

Ratio-transformed value for the i-th antibiotic
= Relative susceptibility to the i-th antibiotic
= disk-edge distance $i/\sum$ disk-edge distance [3]

where the disk-edge distance i is the raw observed value for the i-th antibiotic. The transformed values were used for statistical analyses. This transformation excludes effects of the number of viable bacterial cells (Doi, 2003).

Data analyses
Analysis of variance of each of the soil physico-chemical characteristics, the SFI and the SEF values, and the values of relative susceptibility was performed using the statistical software, SPSS 10.0.5J (SPSS Japan Inc., Tokyo). Using the same software, the Dunnett T3 t-test was performed to test the significance of the observed differences between means. Principal component analysis to elucidate patterns of variation in soil bacterial and soil physico-chemical profiles was performed using the SPSS software.

The statistical techniques for direct gradient analyses, canonical correspondence analysis (ter Braak, 1986) and redundancy analysis (McCune et al., 2002), were performed using the statistical software CANOCO for Windows 4.02 (Microcomputer Power, NY). The canonical correspondence analysis is a statistical technique to detect bell-shaped unimodal species distribution patterns against a significant environmental gradient. The redundancy analysis detects linear species distribution patterns against a significant environmental gradient. A diagram to summarize the result was drawn using the software CanoDraw 3.10 (Microcomputer Power, NY). In running CANOCO, a Monte Carlo permutation test was performed with 199 permutations to test the significance of each environmental factor (ter Braak and Šmilauer, 1998). Multicolinearity was checked to find environmental factors having significant interactions with other environmental factors. In this paper, only environmental gradients significant at p=0.05 and 0.10 were shown in the diagrams. In canonical correspondence analysis and redundancy analysis, the ratio-transformed values in the ADD method were used.

Table 1. Soil physico-chemical characteristics

Vegetative type	Moisture	Bulk density	Clay	Silt	Sand	pH	Electrical conductivity	Organic matter	Total nitrogen	Total carbon	C/N	Available phosphorus	Exchangeable cations				Cation exchange capacity	Exchangeable acidity		Base saturation
													K	Ca	Mg	Na		Al	H	
	(%)	(kg L⁻¹)		(%)			(mS m⁻¹)	(g kg⁻¹ dry soil)				(mg L⁻¹)	(c eq kg⁻¹ dry soil)							(%)
Dry evergreen forest	18.2ᵃ‡	0.97ᵃ	71.3ᵃ	15.9ᵃ	12.9ᵃᵇ	5.73ᵃ	11.3ᵃ	47.3ᵃ	1.83ᵃ	24.4ᵃ	13.4ᵇ	8.28ᵃ	0.82ᵃ	2.83ᵃ	2.94ᵃ	0.14ᵃ	7.35ᵃ	0.23ᵇ	0.37ᵇ	91ᵃ
Dry deciduous forest	16.8ᵃ	1.04ᵃ	75.3ᵃ	13.1ᵃ	11.6ᵇ	5.86ᵃ	7.3ᵇ	39.9ᵃ	1.22ᵃ	21.6ᵃ	17.4ᵃ	4.10ᵇ	0.55ᵃᵇ	2.11ᵃ	1.84ᵃᵇ	0.13ᵃ	5.14ᵃᵇ	0.36ᵃ	0.57ᵃ	80ᵃ
Bare ground	6.8ᵇ	1.40ᵇ	65.2ᵃ	13.8ᵃ	21.0ᵃ	5.00ᵇ	3.1ᶜ	19.2ᵇ	0.60ᵇ	8.2ᵇ	13.5ᵇ	2.83ᵇ	0.34ᵇ	0.66ᵇ	1.05ᵇ	0.14ᵃ	4.18ᵃ	0.55ᵃ	1.44ᵃ	52ᵇ
Analysis of variance†	0.000	0.000	0.108	0.402	0.024	0.000	0.000	0.000	0.000	0.002	0.001	0.000	0.001	0.000	0.004	0.421	0.004	0.001	0.000	0.000

† The one-way analysis of variance was performed hypothesizing vegetative type to be the significant source of variation. The p value for each soil characteristic is indicated.

‡ The values in the column followed by the same letter do not differ significantly at p=0.05 according to the Dunnett T3 t-test.

Table 2. Soil fertility index (SFI) or soil evaluation factor (SEF) reflecting the land degradation

Vegetative type	SFI		SEF	
Bare ground	6.3ᶜ†	±3.6	7.5ᵇ	±2.3
Dry deciduous forest	14.8ᵇ	±2.9	20.7ᵃ	±9.7
Dry evergreen forest	23.0ᵃ	±3.6	34.4ᵃ	±12.5
ANOVA*	0.000		0.000	

† The values in the column followed by the same letter do not differ significantly according to the Dunnett T3 t-test (p=0.05).
* Significance of vegetative type as the source of variation

To compare the discriminatory power of susceptibility and soil physico-chemical profiling to discriminate among/between the soils, Wilk's lambda statistic and its significance were determined with the SPSS software. Wilk's lambda is the most widely used statistic in determining the difference between multivariate data sets (Zar, 1999). If the means among compared groups for each variable are equal, Wilk's lambda becomes 1. The more different the data sets, the closer Wilk's lambda comes to 0.

Results

Physico-chemical characteristics of the soils

Physico-chemical characteristics of the soils are summarized in Table 1. The values are comparable to those described in the previous reports on the current research site, the Sakaerat Environmental Research Station (Sakurai et al., 1998; Doi and Sakurai, 2003, 2004; Doi et al., 2004). One-way analysis of variance indicated that most soil variables reflected the human-induced degradation significantly. The degradation was explained by high values of bulk density, sand content and exchangeable acidity, and low values of moisture content, pH, organic matter content, base (K, Ca, Mg) contents, electrical conductivity, cation exchange capacity, base saturation rate, total nitrogen and total carbon contents and available phosphorus. The DDF soil had the highest C/N value. These results also suggested that the human

activities resulted in several soil environmental gradients.

SFI and SEF values for the soils are summarized in Table 2. The degradation gradient represented by the vegetative types was a significant source of the variation in SFI value (p=0.000) that decreased as the degradation became intensive. The averages for the three vegetative types were significantly different at p=0.05 according to the t-test. The SEF value for the DEF soil was also significantly decreased by the human-induced degradation at p=0.000. The SEF value for the BG soil was significantly lower than that for the others, while the values for the DDF and the DEF soils did not differ significantly at p=0.05. It was clear that the BG soil was the most intensively degraded soil.

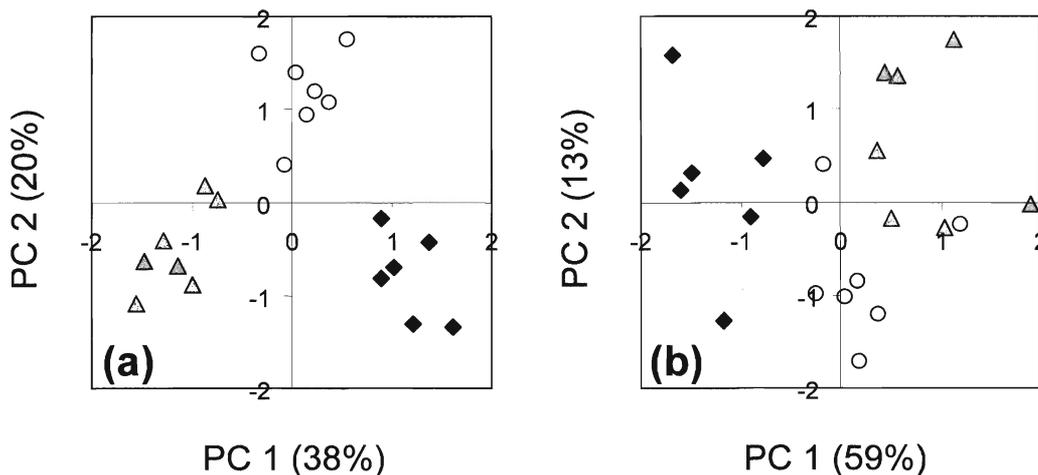### Antibiotic susceptibility profiles of the soil bacterial communities

Susceptibility profiles of the bacterial communities reflected the effects of deforestation and degradation (Fig. 2). For all the antibiotics, except for streptomycin, the degradation was a significant source of variation of relative susceptibility at p=0.05. The soil bacterial communities had different susceptibility patterns. For example, the relative susceptibility of the DEF soil bacterial community to chloramphenicol was significantly lower than that of the other soil bacterial communities. On the other hand, the DEF soil bacterial community had a higher relative susceptibility to erythromycin when compared to the others. Because the degradation resulted in changes in the susceptibility profiles, the applicability of the profiles to extracting integrative measures of the intensity of the land degradation was suggested.

Principal component analysis

Susceptibility profiling discriminated among the
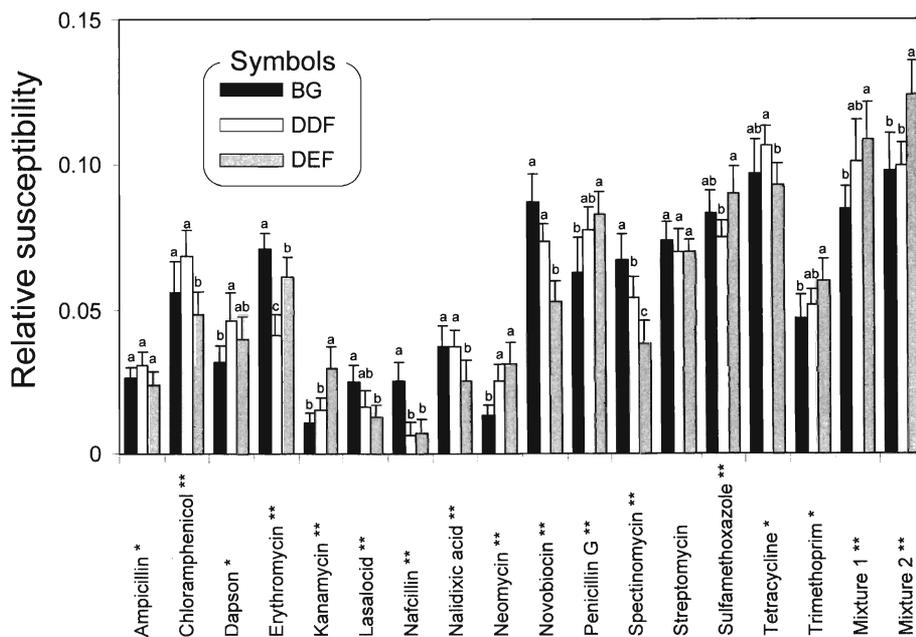
Fig. 2. Antibiotic susceptibility profiles of the soils. BG, DDF and DEF indicate bare ground, dry deciduous forest and dry evergreen forest, respectively. The antibiotic name followed by * and ** indicate that the degradation was the significant source of variation of the relative susceptibility of soil bacterial community to the antibiotic at p=0.05 and 0.01, respectively. (See formula [3] for the calculation.) The error bar indicates the standard deviation (n=6, bare ground; n=7, dry deciduous forest or dry evergreen forest). For each antibiotic, the bars indexed with the same letter do not differ significantly at p=0.05 according to the Dunett T3 t-test.

Fig. 3. Principal component score plots based on (a) the antibiotic susceptibility profiles and (b) the physico-chemical characteristics. The diamond, the open square and the triangle indicate bare ground, dry deciduous forest and dry evergreen forest, respectively. The value in the parenthesis indicates the percentage of the variability explained by the principal component.

sample groups by the first and second principal component axes (Fig. 3a). The first and second principal components explained more than a half of the variation; 38% and 20%, respectively. The first axis seems to explain the degradation gradient for the following reasons: 1) the results shown in Fig. 2 indicated that the land degradation was a significant source of the variation of antibiotic susceptibility profile, and the t-test showed significant differences among the averages for most antibiotics, and 2) the scores on the first principal component axis were positive for the BG soil, around 0 for the DDF soil

and negative for the DEF soils. The second principal component axis differentiates the DDF sample group from the others. This secondary significant part of the variation is thought to owe its significance to the highest or the lowest values of relative susceptibility of the DDF bacterial community to some antibiotics. For example, the DDF soil bacterial community had the lowest relative susceptibility to erythromycin, and the loadings on the second axes was a low value of -0.905, nearly at the lowest theoretically possible value.

Table 3. Wilk's lambda and p values for the comparisons

Data set	Comparisons							
	BG-DDF-DEF		BG-DEF		DDF-DEF		BG-DEF	
	Lambda	p	Lambda	p	Lambda	p	Lambda	p
Antibiotic susceptibility	0.000	0.219	0.006	0.203	0.000	0.050	0.003	0.137
Physico-chemical characteristics	0.000	0.015	0.000	0.012	0.000	0.001	0.000	0.028

Soil physico-chemical profiling also discriminated among the sample groups in the principal component score plot (Fig. 3b). The first and second principal components explained 59% and 13% of the variation, respectively. The first axis explained the degradation gradient, but with poorer resolution than the principal component score plot based on susceptibility profiles (Fig. 3a). The DDF sample group was not clearly separated from the others by the second axis, largely contributed by the variation patterns of clay and silt contents (the loadings were –0.754 and 0.652, respectively) and C/N ratio (the loadings was –0.472).

Wilk's lambda values

In all the comparisons, the ADD method, scoring larger p values, showed poorer discriminatory power than physico-chemical profiling (Table 3). The Wilk's lambda computation takes all the variations into account, while each of the principal component score plots in Fig. 3 shows only the most significant parts of the total variation. The variation of antibiotic susceptibility profiles was relatively well explained by the most significant principal components (Fig. 3), while the ADD method as a whole scored large p values (Table 3). This contrast indicated that the minor parts of the total variation of the susceptibility profile did not strongly correlate to the differences among the soils. On the other hand, minor parts of the total variation of the soil physico-chemical profile correlated to the differences among the soils, thus resulting in the relatively smaller p values, and hence higher overall significance.

Direct gradient analyses

Canonical correspondence analysis resulted in

quite a low eigenvalue of 0.023 for the first ordination axis, and 0.009 for the second one. These pieces of information indicated that the unimodal model fitted poorly. The eigenvalues for the first and second redundancy analysis ordination axes were 0.449 and 0.199, respectively. Thus, in this paper, the redundancy analysis ordination diagram is shown in Fig. 4, but the canonical correspondence analysis ordination diagram is not.

The redundancy analysis ordination diagram indicates relationships between the susceptibility profiles and the soil environmental gradients. Only the significant environmental gradients at $p=0.05$ and $p=0.1$ are shown as solid and broken arrows, respectively. No multicolinearity among the environmental factors was detected. The DEF soil was explained by its relatively high values of moisture, total carbon content and electrical conductivity. High values of C/N and clay conent explained the DDF soil.

Relationships between the environmental gradients and susceptibility patterns are also shown in the redundancy analysis ordination diagram. For example, the BG soil bacterial community had relatively high susceptibility to novobiocin, and the high susceptibility was related to the lower moisture and total carbon content and electrical conductivity in the BG soil.

Principal component and redundancy analysis ordination axes as the integrative measures

Multiple regression analyses between the SFI or the SEF values and the principal component or the redundancy analysis ordination scores provided the following formulae that quantify the intensity of the degradation based on the susceptibility profiles.

SFI = –6.360 × Score on the first principal component
+ 15.135 (R = 0.836, p = 0.000)   [4]

SEF = –9.796 × Score on the first principal component
+ 21.511 (R = 0.686, p = 0.001)   [5]

SFI = –12.076 × Score on the first redundancy
analysis ordination axis + 15.135
(R = 0.818, p = 0.000)   [6]

SEF = –18.992 × Score on the first redundancy
analysis ordination axis + 21.511
(R = 0.685, p = 0.001)   [7]

These formulae indicate that the first principal component or redundancy analysis ordination axis alone explains the land degradation. The large correlation coefficients and high significance show that the first principal component and redundancy analysis ordination axes are reliable measures of the intensity of the degradation.

Discussion

The average SFI values for the vegetative types were separated more significantly than those for the SEF (Table 2). A possible explanation is that the SFI model takes pH and available phosphorus into account (Formula 1). The SEF model was expected to find changes in the soil quality more sensitively than the SFI model (Doi and Sakurai, 2004). However, the SEF values summarized in Table 2 have large standard deviations that seem to make the mean separation difficult. The differences between the variations in the SFI and SEF values imply the multidimensionality of variation in soil quality (van Straalen, 2002): soil environmental variables show various patterns in response to an impact. At present, it is difficult to conclude which index is more reliable; hence, more comparisons must be made.

A soil has its own ecological structure consisting of various components (Beare et al., 1995). The DEF soil has a unique ecological structure represented by the physico-chemical (Table 1) and the bacterial community structures (Doi and Sakurai, 2003) that differ from the ecological structures of other soils. Deforestation and subsequent human activities result in the destruction of the original soil ecological structure, as seen in the decreases in soil

organic matter and available nutrients, and the increases in bulk density and acidity, in addition to the alteration of bacterial community structure (Doi and Sakurai, 2003). The DEF soil should have antibiotic-producing microbes as a component of the ecological structure (Gottlieb, 1976; Stevenson, 1954); hence, actions of the naturally produced antibiotics should be likely to be reflected on the susceptibility profile of the DEF soil bacterial community. The destruction of the original soil ecological structure could affect the relationships among the population of the antibiotic producers, the production of antibiotics by the microbes (Singleton, 1999), and the effects of naturally produced antibiotics on the bacteria.

Changes in soil bacterial community structure are thought to contribute to the differentiation of susceptibility profiles (Doi, 2004). Such linkages between changes in the soil microbial community structure and those in non-structural aspects such as community level physiological profiles (Zak et al., 1994) have been discovered (Mondini and Insam, 2003). Some soil environmental changes involved in the land degradation are thought to induce stress in living things (Sakurai et al., 1998; Giuffre et al.,
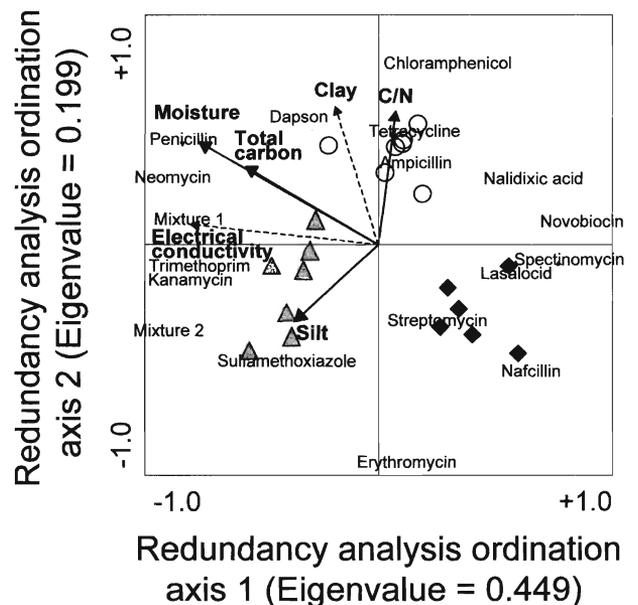
Fig. 4. Redundancy analysis ordination diagram based on antibiotic susceptibility profiles and physico-chemical characteristics. The diamond, the open square and the triangle indicate bare ground, dry deciduous forest and dry evergreen forest, respectively. The solid and broken arrows indicate significant environmental gradients at p=0.05 and 0.10, respectively. Each antibiotic name indicates scores on the redundancy analysis ordination axes for the antibiotic/mixture.

2001). For example, the drier condition in the BG soil was suggested to be the most significant factor in reducing the number of culturable bacterial cells (Doi and Sakurai, 2003; Doi et al., 2004). The drier condition in the BG soil may make it hard for some soil bacteria to survive, while others may not be affected (Kilbertus and Proth, 1979). The number of bacterial cells determined by a plate count method was 4.8×10^8 g^{-1} fresh soil for the DEF soil, while the number was 3.3×10^8 g^{-1} fresh soil or less for the BG soil (Doi et al., 2004). Such a selective force was thought to result in changes in soil bacterial community structures, and hence susceptibility profiles.

While selective forces involved in the land degradation were thought to be important, another possible important factor is the change inside bacterial cells in response to environmental changes. Some environmental changes such as those seen in the Sakaerat Environmental Research Station (Table 1, Fig. 4) may alter bacterial antibiotic resistance phenotypically (McInroy et al., 1996) and genotypically (Pote et al., 2003). The resistance of bacteria to particular antibiotics has been shown to be linked to the possible environmental stresses in the BG soil such as acidity (Ramos et al., 1987) or high temperature (Pillai and Pepper, 1991), while the effects of the significant soil environmental changes shown in Fig. 4 are unknown. These possibly related mechanisms are partially known and remain a problem to be investigated. It is likely both changes in the community structure and changes inside the bacterial cells themselves resulted in the variation of susceptibility profiles. As in the case of the Biolog method, the above factors may contribute to profiling soils with the ADD method. At present, it is difficult to conclude whether the above factors are helpful or inhibitory in obtaining integrative measures. Thus, the effects of the above factors need to be clarified.

The structures of the physico-chemical and the susceptibility data sets were simpler than those given by the sole carbon source most probable number and the antibiotic resistance most probable number methods (Doi and Sakurai, 2002). The most significant principal component or redundancy analysis ordination axis alone explained the land degradation at a high significance level of p<0.002. The first principal component extracted from the susceptibility data explained 38% of the total

variation. This ratio was lower than that for the first principal component axis derived from the physico-chemical data, supporting the hypothesis that a data set on communities in an area has a more complex structure than the soil physico-chemical one (Oline and Grant, 2002).

In the principal component score plot, the first and second principal components derived from the susceptibility data separated the sample groups (Fig. 3a) better than those derived from the physico-chemical data (Fig. 3b). On the other hand, each of the Wilk's lambda values based on the susceptibility data set was less significant than that based on the physico-chemical data (Table 3). These results indicate that the ADD method could well detect the degradation gradient by the most significant sources of the variation, while the rest of the variation did not tell much about the degradation gradient represented by the vegetative types. Detecting a gradient of interest as the most significant source of variation of the multivariate profile is thought to be a prerequisite for finding an integrative measure of the gradient by direct gradient analysis (Jhonson, 1996; Kourtev et al., 1998). This assumption was supported in this research, i.e., the first redundancy analysis ordination axis was found to explain the degradation gradient in association with the significant environmental gradients (Fig. 4, Formulae 6, 7).

The larger eigenvalue for the first redundancy analysis ordination axis when compared to that for the first canonical correspondence analysis ordination axis indicated that few susceptibility variables had unimodal relationships with the significant environmental gradients. Against the distinctive environmental gradients summarized in Table 1, many plant (e.g., Wali, 1999) and soil animal (e.g., Hemerik and Brassaard, 2002) species have optima; they show unimodal distribution patterns. Perhaps the contrast is related to the relatively wide adaptability of soil bacteria to particular environmental changes (Fenchel et al., 1998). Hence, when analyzed using linear statistical techniques, the susceptibility data set could provide the desired information.

Monitoring soils helps conservation and rehabilitation of the land. The ADD method is cost-effective, easy to implement and gives a data set that is easy to analyze and use. Once we obtain susceptibility and soil physico-chemical data sets on

soils on a land degradation gradient, we can choose either susceptibility or physico-chemical profiling to analyze other soils on the gradient, depending on availability. This empirical approach may tell us the best soil environmental condition for a particular goal (e.g., suppression of plant disease, Francl, 1993), and may predict a result upon which there is no or little experimental data (e.g, increase in above ground biomass, MacMillan, 1991). Thus, this approach is worth pursuing as a part of the strategy for conservation and rehabilitation of lands subjected to destructive human activities.

Acknowledgements

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