

# INTEGRATIVE EVALUATION OF REHABILITATIVE EFFECTS OF *ACACIA AURICULIFORMIS* ON DEGRADED SOIL

R. Doi\* & S. L. Ranamukhaarachchi

AFE Building, School of Environment, Resources and Development, Asian Institute of Technology, Pathumthani 12120, Thailand

Received August 2006

**DOI, R. & RANAMUKHAARACHCHI, S. L. 2007. Integrative evaluation of rehabilitative effects of *Acacia auriculiformis* on degraded soil.** Multivariate soil profiling was applied to determine the rehabilitative effects of 17- and 18-year-old *Acacia auriculiformis* plantation plots on a degraded land in Thailand. Soils were sampled from the *A. auriculiformis* plantation plots, a dry evergreen forest (the original vegetative type) and bare ground (the most degraded vegetation) in Sakaerat, Thailand. The soils were profiled by (1) physico-chemical measurements; (2) antibiotic disc diffusion method for soil bacterial community; and (3) long bean growth measurements. Soil dehydrogenase activity, as a soil microbial activity indicator, was also measured. No variables showed differences between soils of the dry evergreen forest and the plantation. Most of the physico-chemical and long bean variables showed clear differences between the bare ground soil and soils of the dry evergreen forest and the *A. auriculiformis* plantation. The bacterial variables showed less distinctive differences between the soils profiled. The land degradation was explained in terms of deteriorated soil physico-chemical conditions and crippled biotic functions. The principal components and discriminant analyses of the data sets for the methods and for all the variables indicated that the *A. auriculiformis* plantation soil had been restoring its original soil conditions, including abiotic properties and biotic functions. For rehabilitating degraded lands in the Thai savanna region, it would be practical and effective to plant *A. auriculiformis*.

Keywords: Land degradation and rehabilitation, multivariate analysis, soil profiling, Thailand

**DOI, R. & RANAMUKHAARACHCHI, S. L. 2007. Penilaian bersepadu kesan pemuliharaan *Acacia auriculiformis* terhadap tanah ternyahgred.** Pemprofilan tanah pelbagai pemboleh ubah digunakan untuk menentukan kesan pemuliharaan *Acacia auriculiformis* berumur antara 17 tahun hingga 18 tahun ke atas tanah ternyahgred di negara Thai. Tanah disampel daripada plot *A. auriculiformis*, hutan malar hijau kering (tumbuhan asli) dan tanah gondol di Sakaerat, negara Thai. Tanah diprofil secara (1) ukuran fiziko-kimia; (2) kaedah resapan cakera antibiotik bagi mengesan komuniti bakteria tanah dan (3) ukuran panjang lenggai. Aktiviti dehidrogenase tanah sebagai penunjuk aktiviti mikrob tanah juga disukat. Tiada perbezaan diperhatikan antara tanah hutan malar hijau kering dengan tanah ladang *Acacia*. Kebanyakan pemboleh ubah fiziko-kimia dan panjang lenggai menunjukkan perbezaan ketara antara tanah gondol dengan tanah hutan malar hijau kering dan ladang *A. auriculiformis*. Pemboleh ubah bakteria menunjukkan perbezaan kurang ketara antara tanah berlainan. Tanah dianggap ternyahgred apabila keadaan fiziko-kimianya merosot dan fungsi biotanya lumpuh. Komponen utama dan analisis pembezaan set data untuk kaedah serta semua pemboleh ubah menunjukkan bahawa *A. auriculiformis* dapat memulihkan tanah kepada keadaan asal termasuklah ciri-ciri abiotik dan fungsi biota. Adalah lebih praktik dan berkesan untuk menanam *A. auriculiformis* bagi memulihkan tanah ternyahgred di kawasan savana negara Thai.

## INTRODUCTION

Deforestation is a worldwide environmental issue that also prevails in Thailand (Sayer *et al.* 2004). As in many other countries, it has been a serious challenge to socio-economic development there (Anonymous 1996). In the tropics, deforestation often leads to land degradation under tropical climatic conditions

(Eden & Parry 1996). In such degraded areas, the native plant species are prone to fail in surviving because the degraded soil conditions are different from the original conditions (Kanowski *et al.* 2005). Exotic plant species are then often introduced to rehabilitate the lands (Ashton *et al.* 2001).

\* E-mail: roird2000@yahoo.com, roird@aeiou.pt

*Acacia auriculiformis* is an exotic species that can survive in degraded lands in Thai savanna (Badejo 1998). Besides its high adaptability in degraded savanna areas, *A. auriculiformis* is known for its nitrogen fixation property (Sprent & Parsons 2000), enriching macrofaunal composition (Mboukou-Kimbatsa *et al.* 1998), low allelopathic effects (Bernhard-Reversat 1999), and ability to pump nutrients from the subsoil (Kang 1993). On the other hand, introducing the tree species also introduces constraints: (1) it is an exotic tree species and thus may result in biological deserts and (2) it may escape to adjacent areas, threatening native species (Hartley 2002). Due to these constraints, it is questionable whether introducing the exotic species is truly rehabilitative.

When a degraded land is rehabilitated, the effects can be observed as changes in soil quality (Chazdon 2003) as well as that in above-ground community structure (Kanowski *et al.* 2005). Various changes in soil quality, e.g. physico-chemical and biotic, occur as a result of land degradation and rehabilitation (Mausbach & Seybold 1998). Soil variables respond to land degradation differently (Jha *et al.* 1992).

Integrative measures of soil quality are provided if we measure multiple soil variables to obtain a multivariate data set based on differences between soils in an area (Sena *et al.* 2000). Multivariate data sets on physical, chemical and biological soil characteristics are available. The statistical technique based on putting things into order is called ordination (McCune *et al.* 2002). Ordination provides ordination axes and changes in scores on the axes may describe gradients of interest, such as the intensity of land degradation (Doi & Puriyakorn 2007). Ordination axes 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 between soils. Measuring multiple soil variables of samples gathered from an area provides a data set based on multivariate profiles of the soils. Analysing the data set provides ordination axes that (1) show particular aspects of the variation of soil quality and (2) form integrative soil quality measures (MacMillan 1991). These measures, consisting of the ordination axis/axes, can be reliable and specific measures of gradients of interest (Francé 1993) because of the

above-mentioned independence of the single ordination axis.

The multivariate profiling of soils and obtaining an integrative soil quality measure are expected to contribute to land resource conservation by uncovering soil quality changes (Verburg & Veldkamp 2001), though it has not been applied frequently (Warren *et al.* 2001, Woomer *et al.* 2004). Therefore the objective of this research was to evaluate the effects of planting *A. auriculiformis* on the degraded soil in Sakaerat, Thailand.

## MATERIALS AND METHODS

### General

The soil of *A. auriculiformis* plantation was analysed to obtain multivariate profiles of it. The profile was compared with that of soils of the dry evergreen forest (the original vegetative type of Sakaerat) and the bare ground (the most degraded vegetative type). The following methods for multivariate profiling of soils were used: (1) soil physico-chemical profiling, (2) soil bacterial community profiling by the antibiotic disc diffusion method, and (3) measuring long bean growth variables. A multivariate data set was constructed for each of these methods. The principal components, as the components of integrative soil quality measures, were extracted from each data set to visualize relationships between the soil sample groups in the ordination plane. We also tried to describe soil biotic functions, soil dehydrogenase activity and an integrative plant growth measure. The above principal components derived from each data set were tested for describing the gradients of dehydrogenase activity and plant growth in association with the land degradation and rehabilitation.

### 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 7808 ha and the altitude ranges from 250 to 762 m above mean sea level. The climate is classified as savanna (Köppen 1931). The area includes dry evergreen forest

(DEF), dry deciduous forest and plantation plots as the major vegetative types (Figure 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 soil is originally an Orthic Acrisol, according to the FAO/UNESCO scheme (FAO/UNESCO 1979).

The DEF is primarily dominated by *Hopea ferrea* and *Shorea* spp. that form the upper storey 20 to 40 m above ground. A typical DEF fosters more than 1000 trees (trunk diameter at breast height > 5 cm) ha<sup>-1</sup>, the total basal area at 1.3 m height exceeds 30 m<sup>2</sup> ha<sup>-1</sup> and the above-ground biomass is over 200 tons ha<sup>-1</sup> (Kanzaki *et al.* 1995).

The *A. auriculiformis* plantation plots are scattered in the area. The *A. auriculiformis* plots were established in 1986 and 1987 in the areas that used to be subjected to slash and burn shifting cultivation (Kaeonium *et al.* 1976). For the cultivation, the original vegetation was removed and the biomass was burned. The cleared area was cultivated by the people for a few years, and then abandoned. Some of the abandoned areas were converted to plantation plots of *Acacia mangium*, *Eucalyptus camaldulensis* and other tree species. *Acacia auriculiformis* was one of the introduced tree species.

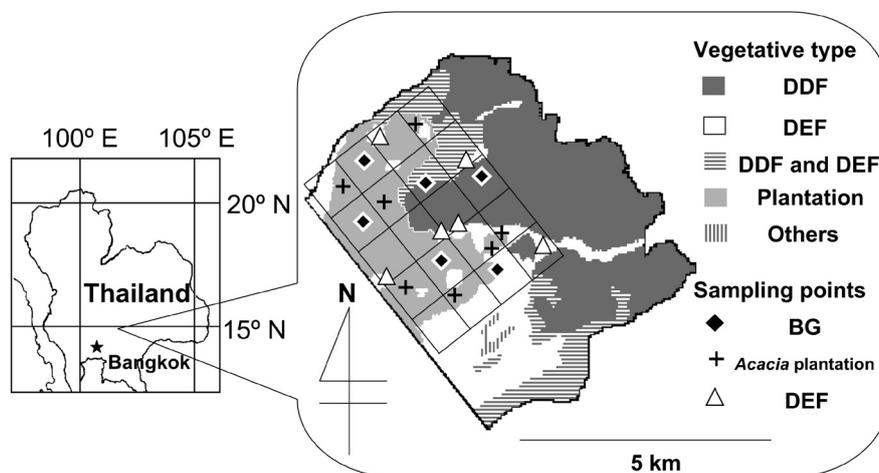
The BG soil has intensively lost the original conditions due to the slash and burn shifting cultivation. At these BG sampling points, restoration of vegetative cover did not occur because of the harsh conditions: poor soil fertility,

direct solar radiation and high temperature. Morphological features of bare ground can still be seen at some points in the site. For typical bare ground, the A horizon can not be recognized. The uppermost 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, soils were sampled at sampling points in the DEF, *A. auriculiformis* plots and BG. The vegetative types were randomly distributed, thus, the vegetative mosaic was regarded as a completely randomized design (Figure 1). The numbers of replications were 6, 7 and 6 for DEF, *A. auriculiformis* plot and BG respectively. All the sampling points were on slight slopes (less than 10°).

Soils were sampled on 22 and 23 May 2005. The sampling took 22 hours. The area had negligible precipitation (< 1 mm). At each sampling point, 100 ml core samplers, 5 cm in diameter, were inserted from the surface to a depth of 5.1 cm. A circle, 10 m in diameter, was established and eight soil cores were randomly taken within the circle. Two additional cores were randomly taken in the circle for soil moisture and bulk density measurements. The eight soil cores were immediately placed into a single plastic bag, mixed and a one-fourth portion was separated for measuring plant growth patterns.



**Figure 1** The vegetative types of Sakaerat and the sampling points. BG, DDF and DEF indicate bare ground, dry deciduous forest and dry evergreen forest respectively.

The one-fourth portion was put into a plastic pot 12 hours after the completion of the sampling. The rest of the soil was passed through a 2 mm sieve, brought to the laboratory, then used for multivariate profiling.

### Physico-chemical analyses of soils

Soil moisture content and bulk density were determined using oven drying at 105 °C for 48 hours. The air-dried soil was suspended in water at a soil to solution ratio of 1:5 and reciprocally shaken at room temperature for one hour at 120 rpm to determine its pH and electrical conductivity. Soil organic matter was determined by the loss of ignition method. Exchangeable cations (Ca, K and Mg) were extracted with 1 M ammonium acetate (pH 7) and determined with an atomic absorption spectrophotometer. Exchangeable acidity (Al and H) was determined with titration. Available phosphorus was determined by the Bray II method (Bray & Kurtz 1945).

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. The following equation was used to calculate SFI values (Lu *et al.* 2002):

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

However, 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. To circumvent these latent drawbacks, another index called SEF was developed (Lu *et al.* 2002). SEF values were calculated using the following equation:

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

### Antibiotic disc diffusion method

The antibiotic disc diffusion method (ADD method, Doi 2003) was used to obtain soil bacterial community profiles. 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 & Mills 1991), which has demonstrated applicability to multivariate profiling of soils (Insam & Ranggner 1997).

Biolog universal growth medium was dissolved in water at 36 g l<sup>-1</sup>, following the producer's instruction. The pH was adjusted to 6. Agar was added to the medium at 1.5% (w/v). The medium was autoclaved and cooled to 55 °C. A volume of 20 ml of this medium was poured into a Petri dish (87 mm in diameter). The soil samples were profiled within 24 hours after the completion of the soil sampling. Twenty grams of the soil were suspended in 20 ml of sterilized water and reciprocally shaken at room temperature for one hour at 120 rpm. Three ml of the suspension were poured and spread onto the agar plate. The plates were then aseptically placed under an airflow at room temperature for one hour to expel excessive moisture.

The following 11 antibiotic solutions were prepared: 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); nalidixic acid (in 50% ethanol at 8.62 mM); neomycin•HCl (in water at 44.0 mM); penicillin G (in water at 300 mM); kanamycin sulfate and neomycin•HCl (in water at 42.9 and 22.0 mM respectively); nalidixic acid and penicillin G (in 25% ethanol at 150 and 4.31 mM respectively) and neomycin•HCl and penicillin G (in water at 150 and 22.0 mM respectively).

Paper discs of 6 mm in diameter were prepared from Whatman No. 2 filter papers and autoclaved. Four µl of the filter sterilized antibiotic solution were loaded onto a disc. The discs were air dried for 30 min and placed onto the plates (three or four discs/plate). The agar plates were incubated at 26 °C in the dark for 24 hours, before the distance between disc and the edge of the inhibitory zone was measured.

Duplicate zones of inhibition around a disc were observed in some cases with erythromycin, kanamycin, penicillin and the three mixtures. In these cases, the inner arc was used for the measurement. Using the following equation, a ratio-transformation was employed and the transformed value was called the relative susceptibility.

$$\begin{aligned} &\text{Ratio-transformed value for the } i\text{-th antibiotic} \\ &= \text{Relative susceptibility to the } i\text{-th antibiotic} \\ &= \frac{\text{disk} - \text{edge distance } i}{\Sigma(\text{disk} - \text{edge distance})} \quad [3] \end{aligned}$$

where the disc-edge distance  $i$  is the raw observed value for the  $i$ -th antibiotic. The transformed values were used for statistical analyses.

### Soil dehydrogenase activity and long bean growth as measures of soil functions

The sieved soil samples were kept at 5 °C for three days. We measured the enzyme activity as a good overall indicator of microbial activity and of the capacity of microbes to oxidize soil organic matter (Dick 1997). The activity was determined by colorimetric measurement of the reduction of 2, 3, 5-triphenyltetrazolium chloride to triphenylformazan according to the method of Casida *et al.* (1964). Five grams of fresh soil were suspended in 4 ml of 250 mM tris-HCl buffer containing 0.0625% (w/v) glucose. The enzymatic reaction started when 1 ml of 2.5% (w/v) triphenyltetrazolium chloride solution was added to the soil suspension. The reaction took 16 hours in the dark at 37 °C, mixing the suspension occasionally. The reaction was stopped by adding methanol. The methanol suspension was passed through cotton plug, filled up to 100 ml with methanol, and the formazan was measured colorimetrically at 480 nm.

The long bean experiment was similar to the method reported by Vivas *et al.* (2005). Six seeds of long bean (*Vigna sinensis* cv. Sut Sakhon) were dipped in 70% (v/v) ethanol for 30 s and sterilized with 15 g l<sup>-1</sup> sodium hypochlorite solution including Tween 80® at 0.1% (v/v) for 20 min using a rotary shaker. The seeds were, then, rinsed with sterilized water for 15 min with rotary shaking. This rinsing process was repeated once again. The sterilized seeds were

germinated for two days on a moist sterilized paper towel at 26 °C in the dark, washed with sterilized water and placed in a 250 ml plastic pot containing 200 ml of the soil sample mentioned above at a rate of six seeds pot<sup>-1</sup>. These processes completed within 24 hours after the completion of the sampling. The pot, with small holes at the bottom, was placed on a plastic tray continuously supplemented with a small amount of distilled water. Other sources of water supply were completely avoided. The temperature ranged from 25 to 43 °C. The plants were subjected to the natural day/night cycle, approximately a day length of 12.5 to 13 hours during the period. The plants were exposed to partial shading (80%). On the 10th day, the poorest three seedlings among the six were removed by cutting the basal part. The plants were harvested 30 days after the transplanting. Leaf, stalk, shoot, root and nodule fresh weights were measured and the number of root nodules recorded.

### Data analyses

Analysis of variance for each of the soil physico-chemical characteristics, the SFI and the SEF values, soil dehydrogenase activity, long bean variables and relative susceptibility was performed using the statistical software, SPSS 10.0.1 (SPSS Inc.). Fisher's least significant difference (LSD)  $t$ -test was performed to examine the significant differences between means. To measure the power of each profiling method to discriminate different soils, discriminant analysis was performed with the SPSS software. Discriminant analysis is a statistical technique to classify samples based on multivariate profiles of them. Misclassification occurs if a sample is classified into an untrue category. Misclassification is prone to occur when the compared sample groups are similar. A Wilk's lamda value tells how much the compared sample groups are different.

Principal component analysis to elucidate variation patterns in soil bacterial susceptibility, long bean growth and soil physico-chemical profiles was performed. Principal component analysis is a technique for simplifying a dataset, by reducing multidimensional datasets to lower dimensions for analysis. By providing principal components, this statistical technique shows major and minor variation patterns in a multivariate data set. Thus principal component

analysis can offer principal components to describe various gradients of interest, when a variation pattern in score on each principal component significantly related to a gradient of interest such as the intensity of land degradation (Doi & Sakurai 2004).

To find the principal components (PCs) that significantly explain the soil functions, multiple regression analysis between values of soil dehydrogenase activity or integrative plant growth measure and PC scores was also performed. In the computation, the stepwise method at the default criteria ( $p = 0.05$  for inclusion and 0.10 for removal) was chosen. For each data set only the PC scores on the significant PC's (eigenvector  $> 1$ ) were used.

## RESULTS

### Physico-chemical characteristics of the soils

One-way analysis of variance (Table 1) indicated that most soil variables reflected the degrading and the rehabilitative effects significantly ( $p < 0.05$ ). As measures of the intensity of land degradation, changes in SFI and SEF values also showed degrading and rehabilitative effects significantly ( $p < 0.05$ ). Land degradation was shown by high values of bulk density and exchangeable acidity (Al, H) and low values of moisture content, pH, organic matter content, base nutrients (K, Ca, Mg) and available phosphorus.

The land degradation–rehabilitation gradient observed as the vegetative types was a significant source of variation in SFI value ( $p < 0.001$ ), which decreased as the land degradation became intensive. The average SFI value for the BG soil was significantly lower than that of the others, while

the values for the DEF and the *A. auriculiformis* plantation did not differ significantly. The SEF values also showed significant effects of land degradation and rehabilitation ( $p = 0.028$ ). The SEF value for the BG soil was significantly lower than that of the others, while the values for the other vegetative types did not differ significantly. The BG soil was the most intensively degraded soil, while the soil of the *A. auriculiformis* plantation was restoring the original physico-chemical conditions as in the DEF soil.

### Antibiotic susceptibility profiles of the soil bacterial communities

Susceptibility profiles of the bacterial communities did not show clear effects of land degradation and rehabilitation (Figure 2). For the neomycin–penicillin mixture, vegetative type was a significant source of variation of relative susceptibility at  $p = 0.05$ . The relative susceptibility of the soil bacterial community of the *A. auriculiformis* plantation to chloramphenicol was significantly higher than that of the BG soil bacterial community. The DEF soil bacterial community had a higher relative susceptibility to kanamycin when compared to the BG soil bacterial community. This contrast was reversed for the neomycin–penicillin mixture. No significant differences in relative susceptibility were recognized between the soil bacterial communities for the other antibiotics and mixtures.

### Changes in soil dehydrogenase activity

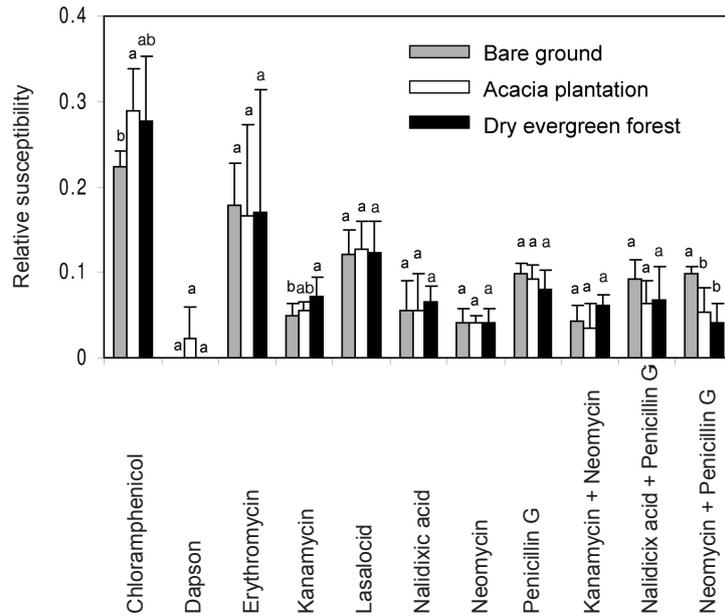
One-way analysis of variance concluded that soil dehydrogenase activity significantly reflected degrading and rehabilitative effects ( $p = 0.002$ , Table 2). The BG soil scored the poorest soil

**Table 1** Soil physico-chemical characteristics and values of soil fertility index and soil evaluation factor

Vegetative type	Moisture (%)	Bulk density (kg l <sup>-1</sup> )	pH	Organic matter (g kg <sup>-1</sup> dry soil)	Available phosphorus (mg kg <sup>-1</sup> dry soil)	Exchangeable cations			Exchangeable acidity		Integrative soil quality measures	
						K	Ca	Mg	Al	H	SFI	SEF
Dry evergreen forest	15.6 <sup>a</sup>	0.94 <sup>b</sup>	6.27 <sup>a</sup>	4.75 <sup>a</sup>	6.56 <sup>a</sup>	7.40 <sup>a</sup>	37.8 <sup>a</sup>	29.0 <sup>a</sup>	1.31 <sup>b</sup>	0.45 <sup>a</sup>	23.7 <sup>a</sup>	39.9 <sup>a</sup>
<i>Acacia auriculiformis</i>	13.4 <sup>a</sup>	0.97 <sup>b</sup>	5.89 <sup>ab</sup>	4.52 <sup>a</sup>	5.41 <sup>ab</sup>	6.17 <sup>a</sup>	32.5 <sup>a</sup>	27.7 <sup>a</sup>	0.85 <sup>b</sup>	1.94 <sup>a</sup>	21.6 <sup>a</sup>	36.5 <sup>a</sup>
Bare ground	5.42 <sup>b</sup>	1.32 <sup>a</sup>	5.57 <sup>b</sup>	2.91 <sup>b</sup>	3.18 <sup>b</sup>	3.81 <sup>b</sup>	15.7 <sup>b</sup>	14.7 <sup>a</sup>	3.42 <sup>a</sup>	3.01 <sup>a</sup>	11.7 <sup>b</sup>	13.2 <sup>b</sup>
Analysis of variance	0.000	0.001	0.007	0.027	0.050	0.007	0.002	0.141	0.031	0.276	0.000	0.028

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.

Values in the column followed by the same letter do not differ significantly at  $p = 0.05$  according to the LSD  $t$ -test.



**Figure 2** Antibiotic susceptibility profiles of the soils. The error bar indicates standard deviation. For each antibiotic, bars with the same letter do not differ significantly at  $p = 0.05$  according to the LSD  $t$ -test.

**Table 2** Soil dehydrogenase activity and long bean growth variables in association with the degradation/rehabilitation

Vegetative type	Soil dehydrogenase activity ( $\mu\text{g}$ formazan $\text{day}^{-1}$ $\text{g}^{-1}$ dry soil)	Long bean growth variables						
		Leaf	Stalk	Shoot	Root	Height (cm)	Nodule	
							(g fresh weight)	
Dry evergreen forest	638 <sup>a</sup>	2.23 <sup>a</sup>	2.11 <sup>a</sup>	4.34 <sup>a</sup>	15.1 <sup>a</sup>	39.6 <sup>a</sup>	116 <sup>a</sup>	595 <sup>a</sup>
<i>Acacia auriculiformis</i>	427 <sup>a</sup>	1.87 <sup>ab</sup>	2.02 <sup>a</sup>	3.90 <sup>ab</sup>	14.1 <sup>a</sup>	39.1 <sup>a</sup>	81.3 <sup>a</sup>	362 <sup>a</sup>
Bare ground	126 <sup>b</sup>	1.67 <sup>b</sup>	1.27 <sup>b</sup>	2.93 <sup>b</sup>	13.0 <sup>a</sup>	23.7 <sup>b</sup>	5.83 <sup>b</sup>	96 <sup>b</sup>
Analysis of variance	0.002	0.093	0.019	0.034	0.308	0.043	0.000	0.002

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 LSD  $t$ -test.

dehydrogenase activity, while the other soils did not differ in the enzymatic activity. The BG soil was the poorest in soil microbial activity, while the soil of the *A. auriculiformis* plantation showed restorative effect.

### Long bean growth

The long bean growth variables showed effects of land degradation and rehabilitation (Table 2). One-way analysis of variance indicated that root weight was not significantly affected by land degradation and rehabilitation. A marginally significant variation was recognized for leaf weight ( $0.05 < p < 0.10$ ). The other growth variables reflected degrading and rehabilitative effects significantly ( $p < 0.05$ ). Degradation was explained by poor shoot

growth and nodule formation. Again, no significant differences between the DEF and *A. auriculiformis* plantation soils were detected.

### Principal component analysis

Results of the principal component analyses indicated relative similarity between the DEF and the *A. auriculiformis* plantation soils (Figure 3). On the other hand, the BG soil was shown to clearly differ from the other soils. Analysis of the physico-chemical data set gave three significant PCs with eigenvectors exceeding 1 (Kaiser 1960). The BG sample group was separated from the others by the first principal component axis. The first and second PCs explained a large part of the variation: 53 and 13 respectively. The first axis seemed to explain the degradation–

rehabilitation gradient. Compared with the BG soil samples, soil samples from the DEF and the *A. auriculiformis* plantation scored relatively larger scores on the first PC. Scoring negative first PC scores, the BG sample group was clearly separated from the others by the first PC axis. The other sample groups overlapped scoring positive scores on PC 1, except for two *A. auriculiformis* plantation soil samples.

Analysis of the soil bacterial data set provided four significant PCs (eigenvector > 1). The PC score plot for the bacterial data set did not show clear differences between the soil bacterial communities. The first PC axis explained 27% of the total variation and the second, 22%. The degradation–rehabilitation gradient seemed to be described by the second PC. The BG soil samples tended to score high PC 2 scores, while the DEF soil samples had low PC 2 scores. The *A. auriculiformis* plantation soil samples were widely distributed in the PC score plot.

Analysis of the long bean data also separated the BG sample group in the PC score plot. Only two significant PCs were given by analysing the data set. The first and second PCs explained 67 and 17% of the total variation respectively. The first axis explained the degradation–rehabilitation gradient. The soil sample groups from the DEF and the *A. auriculiformis* plantation were not clearly separated by the two axes. Hereafter, the first PC will be called plant growth factor for the following reasons. The PC largely explained the variation pattern and all the seven growth variables scored high positive eigenvectors (> 0.642, data not shown) on the PC. Thus the plant growth factor is an integrative measure of the soil functions supporting plant growth.

Results of the principal component analysis of all the variables separated the DEF and the *A. auriculiformis* plantation soils, though not clearly. On the other hand, the BG sample group was clearly separated from the other soils by the first PC axis. Seven PCs were significant. The first and second PCs explained about a half of the variation: 37 and 15% respectively.

### Discriminant analysis

Figure 4 indicates results of the discriminant analyses for the data sets. Each discriminant score plot shows a Wilk's lambda value and its significance. The first discriminant functions

for the data sets explained 83% or more of the total variation. In the discriminant score plots, the soil samples were scored and located. The discriminant score plot for the physico-chemical measurements clearly separated the BG sample group from the others by the first discriminant function. A soil sample from an *A. auriculiformis* plot was misclassified as a DEF sample. The Wilk's lambda value was relatively small and the significance was high.

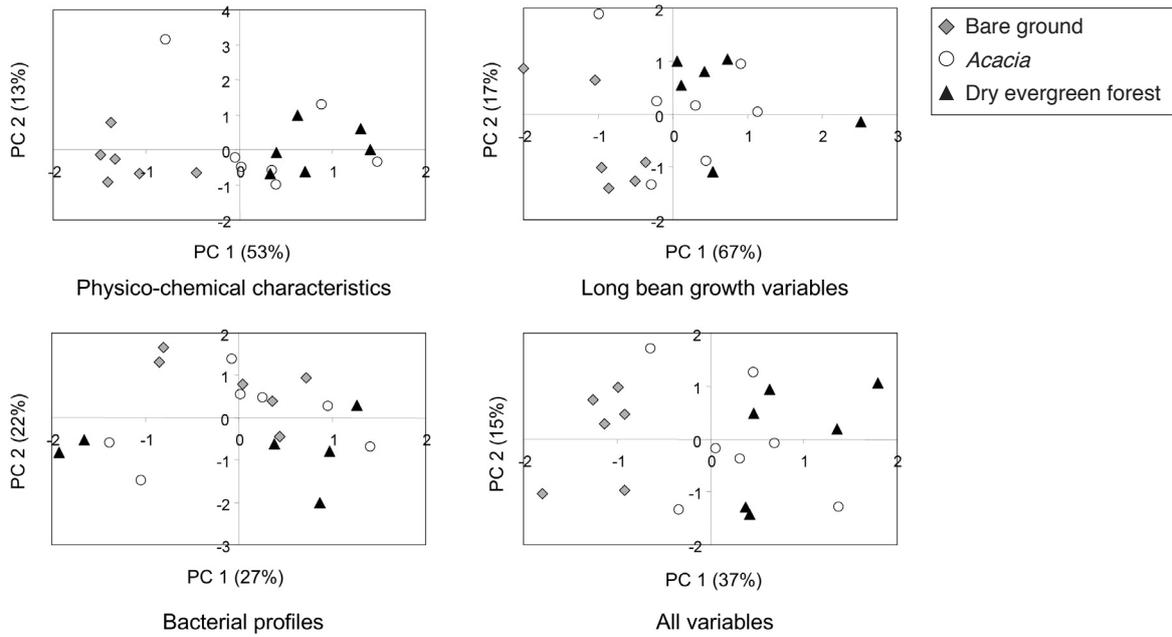
Differences between the sample groups in the bacterial profile were obscure. The BG samples distributed near the other sample groups in the discriminant score plot. One DEF sample was misclassified as an *A. auriculiformis* plantation sample, while an *A. auriculiformis* plantation sample was misclassified as a DEF sample. The significance of the lambda value was low.

The long bean variables showed a similar discriminatory pattern to the above two cases. In the discriminant score plot, differences between the sample groups were less clear than those for the physico-chemical data set but more clear than those for the bacterial. One of the *A. auriculiformis* plantation samples was misclassified as a BG sample. Also, a DEF sample was misclassified as an *A. auriculiformis* plantation soil sample and an *A. auriculiformis* plantation soil sample was misclassified as a DEF sample.

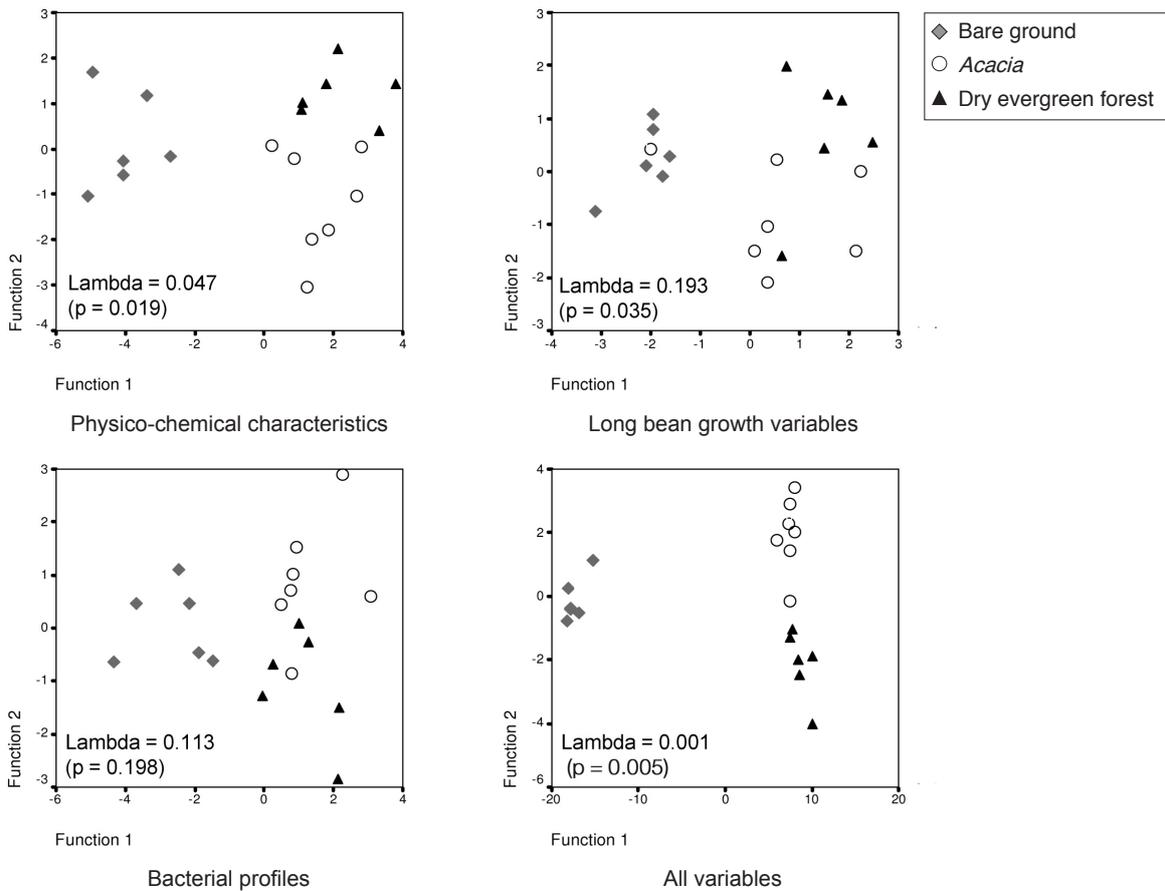
The most successful discrimination was achieved by analysing all the variables together. In the analysis, no misclassification occurred. The lambda value was small with a relatively high significance value of 0.005. The discriminatory pattern was similar to the above three cases. The resolution was the best among the four data sets. All the above discriminatory patterns showed the BG and the others are distinctively different because the separation was done by the first discriminant function.

### Principal components describing the soil functions

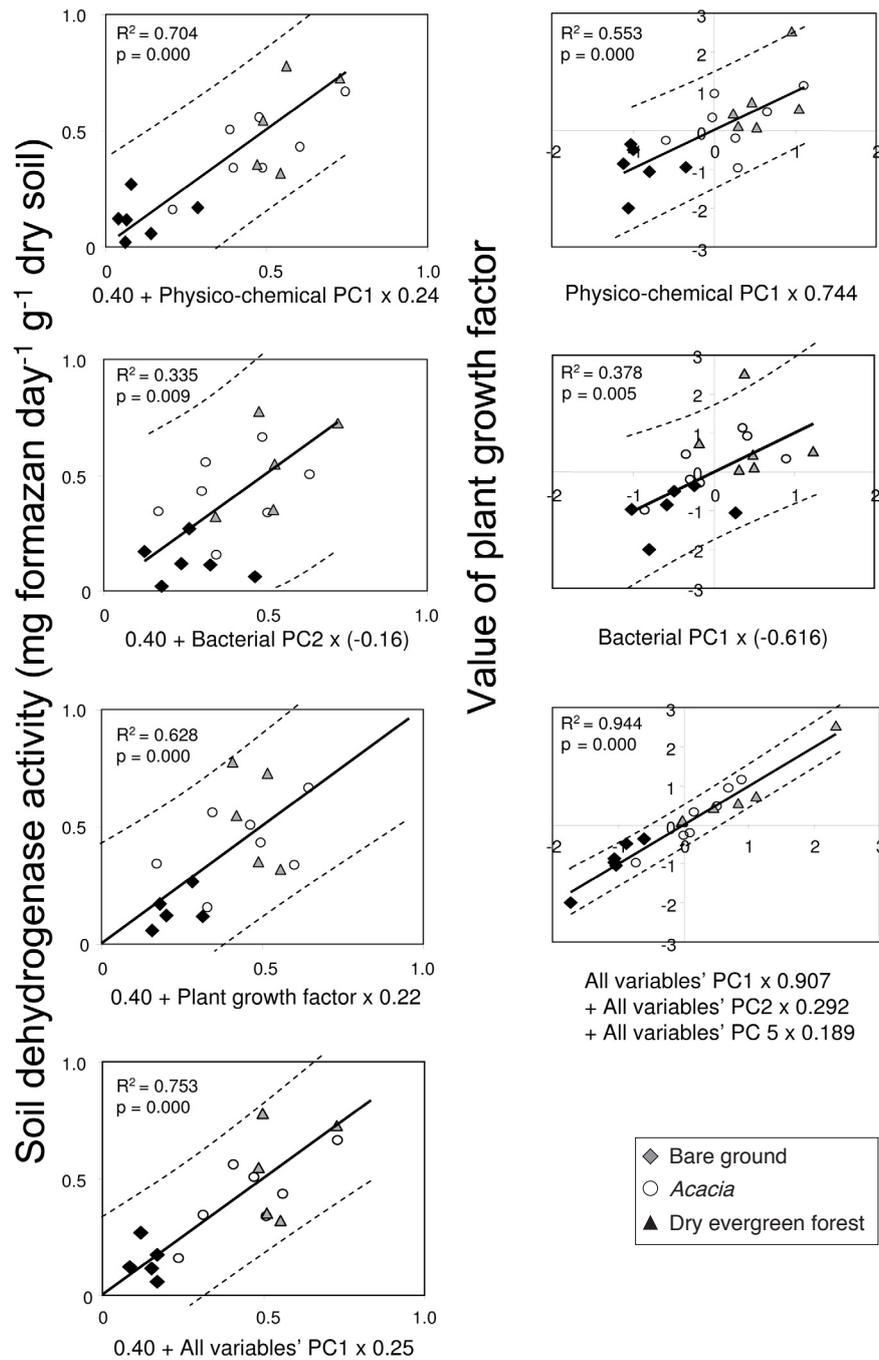
Multiple regression analyses between values of dehydrogenase activity or the plant growth factor and the values of PC scores provided the formulae that describe and predict the soil functions (Figure 5). The first PC derived from all the variables as well as the first PC from the physico-chemical data set described soil dehydrogenase activity relatively well. However, PCs derived from



**Figure 3** Principal component score plots based on physico-chemical characteristics, bacterial profiles, long bean growth variables and all variables. Values in parentheses indicate the percentage of variability explained by the principal component.



**Figure 4** Discriminant score plots for the data sets. Values in parentheses following the Wilk's lambda values indicate the significance of the differences between soils.



**Figure 5** Regression models describing the variations of plant growth factor and soil dehydrogenase activity as soil functions. The formulae along the horizontal axes were provided by the multiple regression analysis. PC<sub>i</sub> indicates the *i*-th principal component for the data set. Values following the R<sup>2</sup> values indicate the significance of the regression models. For each plot, the broken lines indicate 95% confidence interval for the linear regression.

the bacterial data set explained the variation of dehydrogenase activity less precisely. Plant growth was precisely described by the regression model based on the PCs derived from all the variables. However, the single data sets gave less reliable predictors of plant growth.

## DISCUSSION

The *A. auriculiformis* plantation had rehabilitated the degraded soil in Sakaerat. The soil had restored its characteristics similar to the DEF soil. Most physico-chemical and long bean

variables and some bacterial ones showed rehabilitative effects of the plantation; no significant differences were noted between the DEF and the *A. auriculiformis* plantation soils, while the BG soil was separated from the others.

The variables had linear response patterns against the intensity of land degradation. Thus the multivariate data sets on the physico-chemical and the long bean variables had simple data structures; the first PCs explained more than half of the variation. The physico-chemical data set was expected to have a simpler structure than the long bean and the bacterial data sets (Oline & Grant 2002). However, the long bean data set also had a simple data structure.

Some of the long bean variables, especially nodule variables, were expected to show bell-shaped unimodal relationships (McCune *et al.* 2002) against the land degradation–rehabilitation gradient because many *Acacia* rhizobia nodulate crop legumes, including *Vigna sinensis* (Zahran 2001). However, the DEF soil resulted in a nodule number and a weight comparable to that for the *A. auriculiformis* plantation soil. A possible explanation for the DEF soil is the high soil bacterial diversity (Doi & Sakurai 2003), revealed by the sole carbon source most-probable-number method (Wren & Venosa 1996). The DEF soil bacterial community could have the rhizobia that nodulate long bean because of the high diversity.

The following information suggests that the *A. auriculiformis* plantation soil has already been restored beyond the soil of the dry deciduous forest of Sakaerat. The deciduous forest is disturbed by human activities, mainly frequent running fire (Doi & Sakurai 2004). The soil of the deciduous forest had significantly poorer available phosphorus than the DEF soil (Doi 2005). As the cause, the significantly stronger exchangeable acidity than the DEF soil could be responsible because at low pH values, Al, Fe, and Mn are soluble and can react with available P decreasing the availability. This mechanism could contribute to a significantly lower value of SFI for the deciduous forest soil than the DEF soil (Doi 2005). In Sakaerat, being free from human-induced fire, the succession is enhanced (Sahunalu & Dhanmanonda 1995), and the soil variables restore (Sakurai *et al.* 1998). This confirms that planting trees that will be free

from fire should be important for restoring the above-ground vegetation and soil quality in the *A. auriculiformis* plots (Stott 1984).

Self-thinning seems to be an advantage of the *A. auriculiformis* plantation from a viewpoint of restoring ecosystem diversity by enhancing succession (Ashton *et al.* 2001). In 1998, among the six tree species introduced into Sakaerat, the *A. auriculiformis* plantation recorded the second highest species richness of plants (51), following the *Dalbergia cochinchinensis* plantation, which scored a species richness of 59, when the DEF had 114 species (Kamo *et al.* 2002). There were many tree species in the *A. auriculiformis* plots on the sampling days in 2005. This perceivable progress in succession is thought to contribute to the relative proximity of the *A. auriculiformis* plantation soil to the DEF soil. Increasing plant community diversity can help enrich soil fertility (El-Keblawy & Ksiksi 2005) and establish the soil ecological structure (Beare *et al.* 1995) formed by plant roots and root exudates (Garland 1996) on which soil animals and microbes rely. These results of the succession are thought to have been restoring the soil functions observed in this research.

At present, however, it is hard to conclude the full restoration of the *A. auriculiformis* plantation soil, though no soil variables showed statistically significant differences between the DEF and the *A. auriculiformis* plantation soils. Slight soil environmental changes ( $p > 0.10$ ) may significantly associate with soil biotic variables, such as soil enzyme activity (Jha *et al.* 1992) and/or soil bacterial community structure (Doi & Sakurai 2003). Rather, the *A. auriculiformis* plantation soil seemed to be restoring as indicated by the discriminant analysis (Figure 4), which still discriminated between the DEF and the *A. auriculiformis* plantation soils. Therefore in the current research site, the period occupied by the *A. auriculiformis* plantation in the area appeared inadequate to fully restore the degraded soil. Yemefack *et al.* (2005) investigated soil fertility restoration that resulted in a comparable rehabilitation period of 15 years after shifting cultivation in Cameroon; yet incomplete soil fertility restoration was suggested. As mentioned by Young (1997), it should take a longer period to restore the original soil quality so as to recognize no differences from the DEF soil by discriminant analysis. In the southern Yucatan

of Mexico, under similar climatic conditions, a period of 40–60 years was estimated to recover total above-ground biomass following shifting cultivation, based on the most optimistic estimate (Chazdon 2003). Thus the *A. auriculiformis* plantation soil and the ecosystem were very likely to be in succession towards the climax.

This research showed the advantage of employing multiple soil variables in describing a soil quality variation, as Thanasoulis *et al.* (2002) had demonstrated. However, it is difficult to predict which data set would be the best for describing a gradient. Thus employing different multivariate profiling methods would be advantageous. One multivariate profiling method may find differences between soils but another may not (Widmer *et al.* 2001). In this research, the bacterial data set was the poorest in detecting differences. This failure could be due to the relatively high soil moisture contents for the current soil samples (Doi & Sakurai 2004). Previous precipitation could decrease differences between the soil bacterial communities by (1) increasing the bacterial cell numbers in the soils (Ozawa & Doi 1996), and then (2) increasing the diversity of the soil bacterial communities as reported by Doi and Sakurai (2003) for moist soils, though the values of SFI and SEF are little affected by soil moisture changes (Doi & Sakurai 2004). The antibiotic disc diffusion method may work better when the soils are drier.

This study showed a relevance of (1) the multivariate profiling of soils for monitoring rehabilitative effects and (2) the *A. auriculiformis* plantation as a strategy for rehabilitating degraded lands in the region. The current methods for multivariate profiling of soils are feasible and cost-effective. In these days, various multivariate methods for biotic profiling of soils are available (Kirk *et al.* 2004), besides the relatively well-established physico-chemical measurements. Sometimes, a soil-biotic profile sensitively responds to particular impacts when soil's physico-chemical variables do not respond clearly (Kourtev *et al.* 2003). It would be worth testing the biotic profiling methods that have been proving their discriminatory power in monitoring soils in efforts to monitor rehabilitative effects on forest soils. In some cases, it is more favourable to find a multivariate data set that has unimodal distribution patterns

against the most dominant environmental gradient because plant and animal species may have optima (McCune *et al.* 2002, Oline & Grant 2002) on the distinctive environmental gradient (Doi & Sakurai 2004). The vegetative survey may be more sensitive in finding such unimodal patterns, though it is more labour-intensive. It was shown to be rehabilitative to plant *A. auriculiformis* trees on degraded lands in the region if the plantation plot is kept free from running fire. This is an important prerequisite to enhance the rehabilitation because running fire causes poorer conditions in the soil of the fire-adapted dry deciduous forest (Sakurai *et al.* 1998) than in the *A. auriculiformis* plantation soil. After a period of 18 or 19 years as plantation plots, the soil conditions were converging to that in the DEF. At present, the plantation seems to be effective as a strategy to rehabilitate the degraded lands in the Thai savanna region.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the staff of the Sakaerat Environmental Research Station who assisted in this project.

## REFERENCES

- ANONYMOUS. 1996. *The Eighth National Economic and Social Development Plan (1997–2001)*. National Economic and Social Development Board of Thailand, Bangkok.
- ASHTON, M. S., GUNATILLEKE, C. V. S., SINGHAKUMARA, B. M. P. & GUNATILLEKE, I. A. U. N. 2001. Restoration pathways for rain forest in southwest Sri Lanka: a review of concepts and models. *Forest Ecology and Management* 154: 409–430.
- BADEJO, M. A. 1998. Agroecological restoration of savanna ecosystems. *Ecological Engineering* 10: 209–219.
- BEARE, M. H., COLEMAN, D. C., CROSSLEY JR, D. A., HENDRIX, P. F. & ODUM, E. P. 1995. A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. *Plant and Soil* 170: 5–22.
- BERNHARD-REVERSAT, F. 1999. The leaching of *Eucalyptus* hybrids and *Acacia auriculiformis* leaf litter: laboratory experiments on early decomposition and ecological implications in Congolese tree plantations. *Applied Soil Ecology* 12: 251–261.
- BRAY, H. R. & KURTZ, L. T. 1945. Determination of total organic and available forms of phosphorus in soil. *Soil Science* 59: 39–45.
- CASIDA JR, L. E., KLEIN, D. A. & SANTORO, T. 1964. Soil dehydrogenase activity. *Soil Science* 98: 371–376.
- CHAZDON, R. L. 2003. Tropical forest recovery: legacies of human impact and natural disturbances. *Perspectives in Plant Ecology, Evolution and Systematics* 6: 51–71.

- DICK, R. P. 1997. Soil enzyme activities as integrative indicators of soil health. Pp. 121–156 in Pankhurst, C. E., Doube, B. M. & Gupta, V. V. S. R. (Eds.) *Biological Indicators of Soil Health*. CAB International, New York.
- DOI R. 2003. Application of the antibiotic disk diffusion method to multivariate profiling of soil bacterial community: comparing the power to discriminate different soils and dimension of the discrimination with that of the Biolog method. *Brazilian Journal of Microbiology* 34: 313–320.
- DOI R. 2005. Human-induced land degradation gradient shown by antibiotic susceptibility profiles of bacterial communities and physico-chemical soil characteristics. *Nature and Human Activities* 9: 33–45.
- DOI, R. & PURIYAKORN, B. 2007. Physico-chemical and bacterial profiling of soils for describing a land degradation gradient. *Current Science* 92: 1050–1054.
- DOI, R. & SAKURAI, K. 2003. Soil environmental factors relating to diversity of culturable soil bacterial communities in the Sakaerat Environmental Research Station, Thailand. *Tropics* 12: 185–200.
- DOI, R. & SAKURAI, K. 2004. Principal components derived from soil physico-chemical data explained a land degradation gradient, and suggested the applicability of new indexes for estimation of soil productivity in the Sakaerat Environmental Research Station, Thailand. *International Journal of Sustainable Development and World Ecology* 11: 298–311.
- EDEN, M. J. & PARRY, J. T. 1996. *Land Degradation in the Tropics: Environmental and Policy Issues*. *Global Development & the Environment Series*. Pinter, London.
- EL-KEBLAWY, A. & KSIKSI, T. 2005. Artificial forests as conservation sites for the native flora of the UAE. *Forest Ecology and Management* 213: 288–296.
- FAO/UNESCO 1979. *Soil Map of the World. IX, Southeast Asia*. UNESCO, Paris.
- FRANCL, L. J. 1993. Multivariate analysis of selected edaphic factors and their relationship to *Heterodera glycines* population density. *Journal of Nematology* 25: 270–276.
- GARLAND, J. L. 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biology and Biochemistry* 28: 213–221.
- GARLAND, J. L. & MILLS, A. L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology* 57: 2351–2359.
- HARTLEY, M. J. 2002. Rationale and methods for conserving biodiversity in plantation forests. *Forest Ecology and Management* 155: 81–95.
- INSAM, H. & RANGGER, A. 1997. *Microbial Communities: Functional Versus Structural Approaches*. Springer Verlag, Berlin.
- JHA, D. K., SHARMA, G. D. & MISHRA, R. R. 1992. Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. *Soil Biology and Biochemistry* 24: 761–767.
- KAONIAM, P., KHOORAT, P., SUNTHORNAN, W., ISSAREEYA, M., CHERDCHUN, C. & BUACHUM, W. 1976. *A Study of Illegal Deforestation in the Reserved Forest Area at the Sakaerat Environmental Research Station*. Applied Scientific Research Corporation of Thailand, Bangkok.
- KAISER, H. F. 1960. The application of electronic computers to factor analysis. *Educational and Psychological Measurement* 20: 141–151.
- KAMO, K., VACHARANGKURA, T., TIYANON, S., VIRIYABUNCHA, C., NIMPILA, S. & DOANGRISEN, B. 2002. Plant species diversity in tropical planted forests and implication for restoration of forest ecosystems in Sakaerat, Northeastern Thailand. *JARQ* 36: 111–118.
- KANG, B. T. 1993. *Sustainable Agroforestry Systems for the Tropics: Concepts and Examples*. IITA Research Guide 26. IITA, Ibadan.
- KANOWSKI, J., CATTERALL, C. P. & WARDELL-JOHNSON, G. W. 2005. Consequences of broadscale timber plantations for biodiversity in cleared rainforest landscapes of tropical and subtropical Australia. *Forest Ecology and Management* 208: 359–372.
- KANZAKI, M., YODA, K. & DHANMANONDA, K. 1995. Mosaic structure and tree growth pattern in a monodominant tropical seasonal evergreen forest in Thailand. Pp. 495–513 in Box, E. O. (Eds.) *Vegetation Science in Forestry*. Kluwer Publishers, Netherlands.
- KIRK, J. L., BEAUDETTE, L. A., HART, M., MOUTOGLIS, P., KLIRONOMOS, J. N., LEE, H. & TREVORS, J. T. 2004. Methods of studying soil microbial diversity. *Journal of Microbiological Methods* 58: 169–188.
- KOURTEV, P. S., EHRENFELD, J. G. & HÄGGBLUM, M. 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biology and Biochemistry* 35: 895–905.
- KÖPPEN, W. 1931. *Grundriss der Klimakunde*. Walter de Gruyter, Berlin.
- LU, D., MORAN, E. & MAUSEL, P. 2002. Linking Amazonian secondary succession forest growth to soil properties. *Land Degradation and Development* 13: 331–343.
- MAUSBACH, M. J. & SEYBOLD, C. A. 1998. Assessment of soil quality. In Lal, R. (Ed.) *Soil Quality and Agricultural Sustainability*. Ann Arbor Press, Chelsea.
- MACMILLAN, D. C. 1991. Predicting the general yield class of Sitka spruce on better quality land in Scotland. *Forestry* 64: 359–372.
- MBOUKOU-KIMBATSIA, I. M. C., BERNHARD-REVERSAT, F. & LOUMETO, J. J. 1998. Change in soil macrofauna and vegetation when fast-growing trees are planted on savanna soils. *Forest Ecology and Management* 110: 1–12.
- MCCUNE, B., GRACE, J. B. & URBAN, J. B. 2002. *Analysis of Ecological Communities*. M and M Software Design, Glenden Beach.
- MORAN, E. F., BRONDIZION, E. S., TUCKER, J. M., DA SILVA-FORSBERG, M. C., MCCracken, S. & FALES, I. 2000. Effects of soil fertility and land-use on forest succession in Amazônia. *Forest Ecology and Management* 139: 93–108.
- OLINE, D. K. & GRANT, M. C. 2002. Scaling patterns of biomass and soil properties: an empirical analysis. *Landscape Ecology* 17: 13–26.
- OZAWA, T. & DOI, R. 1996. Increase in the competitive nodulation ability of *Bradyrhizobium japonicum* strains grown in purified water. *Microbes and Environments* 11: 87–90.

- SAHUNALU, P. & DHANMANONDA, P. 1995. Structure and dynamics of dry dipterocarp forest, Sakaerat, northeastern Thailand. Pp. 465–494 in Box, E. O. *et al.* (Eds.) *Vegetation Science in Forestry*. Kluwer Publishers, Netherlands.
- SAKURAI, K., TANAKA, S., ISHIZUKA, S. & KANZAKI, M. 1998. Differences in soil properties of dry evergreen and dry deciduous forests in the Sakaerat Environmental Research Station. *Tropics* 8: 61–80.
- SAYER, J., CHOKKALINGAM, U. & POULSEN, J. 2004. The restoration of forest biodiversity and ecological values. *Forest Ecology and Management* 201: 3–11.
- SENA, M. M., POPPI, R. J., FRIGHETTO, R. T. S. & VALARINI, P. J. 2000. Avaliação do uso de métodos quimiométricos em análise de solos. *Química Nova* 23: 547–556.
- SPRENT, J. I. & PARSONS, R. 2000. Nitrogen fixation in legume and non-legume trees. *Field Crops Research* 65: 183–196.
- STOTT, P. 1984. The savanna forests of mainland Southeast Asia: an ecological survey. *Progress in Physical Geography* 8: 315–335.
- THANASOULIAS, N. C., PILLIOURIS, E. T., KOTTI, M-S. E. & EVMIRIDIS, N. P. 2002. Application of multivariate chemometrics in forensic soil discrimination based on the UV-Vis spectrum of the acid fraction of humus. *Forensic Science International* 130: 73–82.
- VERBURG, P. H. & Veldkamp, A. 2001. The role of spatially explicit models in land-use change research: a case study for cropping patterns in China. *Agriculture, Ecosystems and Environment* 85: 177–190.
- VIVAS, A., BAREA, J. M. & AZCON, R. 2005. Interactive effect of *Brevibacillus brevis* and *Glomus mosseae*, both isolated from Cd contaminated soil, on plant growth, physiological mycorrhizal fungal characteristics and soil enzymatic activities in Cd polluted soil. *Environmental Pollution* 134: 257–266.
- WARREN, A., BATTERBURY, S. & OSBAHR, H. 2001. Soil erosion in the West African Sahel: a review and an application of a “local political ecology” approach in South West Niger. *Global Environmental Change* 11: 79–95.
- WIDMER, F., FLIEßBACH, A., LACZKÓ, E., SHULZE-AURICH, J. & ZEYER, J. 2001. Assessing soil biological characteristics: a comparison of bulk soil community DNA-, PLFA, and Biolog™-analyses. *Soil Biology and Biochemistry* 33: 1029–1036.
- WOOMER, P. L., TOURÉ, A. & SALL, M. 2004. Carbon stocks in Senegal’s Sahel Transition Zone. *Journal of Arid Environment* 59: 499–510.
- WREN, A. B. & VENOSA, A. D. 1996. Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure. *Canadian Journal of Microbiology* 42: 252–258.
- YEMEFACK, M., ROSSITER, D. G. & NJJONGANG, R. 2005. Multi-scale characterization of soil variability within an agricultural landscape mosaic system in southern Cameroon. *Geoderma* 125: 117–143.
- YOUNG, A. 1997. *Agroforestry for Soil Management*. Second edition. CAB International and ICRAF, New York.
- ZAHARAN, H. H. 2001. Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *Journal of Biotechnology* 91: 143–153.