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ORIGINAL PAPER

Slow restoration of soil microbial functions in an *Acacia* plantation established on degraded land in Thailand

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Abstract Deforestation diminishes the ecological services that a forest provides (e.g., flood prevention). To restore such services, reforestation is often utilized. The full restoration of the original forest ecosystem, however, can take several decades. The present study was conducted to identify the missing key components for rehabilitation of a degraded plot of land in Thailand on which *Acacia* trees were planted 18 or 19 years ago. Canopy spectral and soil physicochemical profiles of the *Acacia* plantation plot showed more advanced rehabilitation than in the soil microbial functions, as represented by soil dehydrogenase activity and community-level physiological profiles, when compared with those of a natural evergreen forest. The slower restoration of the soil microbial functions was thought to: (1) be attributed to the loss of certain microbes that played important roles in the evergreen forest soil, and (2) restrict the restoration of the entire forest ecosystem which was found to be still progressing towards a full restoration of the land's original conditions. Finally, possible measures for further rehabilitation of the ecosystem were discussed.

Keywords *Acacia auriculiformis* · Components for ecological restoration · Land degradation and rehabilitation · Multivariate analysis

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Introduction

Deforestation has emerged as a challenge to the socio-economic development of Thailand (Anon 2011) and many other countries. It has been estimated that, in the past four decades alone, the forested areas in Thailand have declined by 50 % or more (Fisher and Hirsch 2008). In the tropics, deforestation often leads to land degradation which, in turn, results in degraded soils (Eden and Parry 1996). Deforestation is seen as a major cause of increasingly severe problems associated with drought and flooding (Kraira-panond and Atkinson 1998). Since the late 1980s, the Thai government has taken measures to rehabilitate these degraded lands. Reforestation is one of the measures employed, and trees have been planted on the degraded lands under a government-subsidized program (Sharp and Nakagoshi 2006). Because the native tree species often do not survive under the degraded soil conditions, exotic tree species are often introduced as substitutes (Ashton et al. 2001). Though the strategy is often criticized, these exotic tree species have shown rehabilitative effects in places where native species have failed (Lugo 1997).

Acacia auriculiformis is one of the introduced exotic tree species capable of surviving in the degraded lands found in the Thai savanna (Badejo 1998). Besides its high adaptability in degraded savanna areas (Badejo 1998), *A. auriculiformis* is known for its nitrogen-fixation property (Sprent and Parsons 2000), macrofaunal composition enrichment (Mboukou-Kimbatsa et al. 1998), low allelopathic effects (Bernhard-Reversat 1999), and ability to pump nutrients from the subsoil (Kang 1993). In Sakaerat, Thailand, *A. auriculiformis* was planted in a degraded part, producing evidence of the rehabilitative effects of reforestation through soil physicochemical (Doi and Ranamukhaarachchi 2007) and biotic changes (Doi and Ranamukhaarachchi

2009a) and through changes in plant species community structure (Kamo et al. 2002). As succession progresses following the plantation of exotic tree species, native tree species may return. The restoration in Sakaerat, however, was found to be incomplete even 19 years after the trees were first introduced. In a strict sense, the *Acacia* plantation plots were still considered to be progressing towards full restoration to an evergreen forest. It was hypothesized that the restoration of these plots was being inhibited by the absence of key components required for full restoration (Hobbs and Norton 1996).

Given this, certain ecological aspects were considered potential components for further forest ecosystem rehabilitation and were compared in terms of the progress of the restoration taking place in Sakaerat, Thailand. This study examined whether there were differences in the degree of restoration owing to these components of ecological restoration by planting *A. auriculiformis* trees on degraded land under savanna climatic conditions. Above and belowground components of ecological restoration are often dependent on each other (Kardol and Wardle 2010). Further, in ecological restoration, either the above or the belowground components themselves may also exist in dependent relationships. Therefore, the canopy's spectral profile, the soil physicochemical characteristics, and the functions and the structure of the soil microbial community of the *Acacia* plantation plot were compared with those of the dry evergreen forest, which was the original vegetation type of the study site.

Materials and methods

Site description

This study was conducted at the Sakaerat Environmental Research Station, Thailand. The details were described elsewhere (Doi and Sakurai 2004; Doi and Ranamukhaarachchi 2007). The annual precipitation is 1,260 mm and the average temperature is 26 °C. The climate is classified as savanna (Köppen 1931). The soil is originally an Orthic Acrisol, according to the FAO/UNESCO classification system (FAO/UNESCO 1979). We compared soils of dry evergreen forest (the original vegetation), *Acacia* plantation, and bare ground (the most degraded state of vegetation). These vegetation types represent a land degradation-rehabilitation gradient. The vegetation types were randomly distributed. Thus, the vegetation mosaic was regarded as a completely randomized design (Fig. 1). The numbers of replications were 2, 4 and 2 for dry evergreen forest, *Acacia* plantation and bare ground, respectively. All the sampling points were taken on slight slopes (less than 10°).

The dry evergreen forest is primarily dominated by *Hopea ferrea* and *Shorea* spp. that form the upper storey, 20–40 m above ground level. A typical dry evergreen forest contains more than 1,000 trees (trunk diameter at breast height >5 cm) ha^{-1}, the total basal area at a height of 1.3 m exceeds 30 m^2 ha^{-1}, and the aboveground biomass is over 200 tons ha^{-1} (Kanzaki et al. 1995).

The *A. auriculiformis* plantation plots are scattered throughout the area (Fig. 1). The *Acacia* plantation plots were established in 1986 and 1987 in areas that had previously been subjected to slash-and-burn shifting cultivation (Kaeoniam et al. 1976). In these areas, the original vegetation had been removed and the aboveground biomass had been burned. The cleared land had been cultivated for a few years, and then abandoned when the soil quality deteriorated to the point where it could no longer support crop production. Some of the abandoned portions of Sakaerat had been converted to plantation plots of *Acacia mangium*, *Eucalyptus camaldulensis* and other tree species. *A. auriculiformis* was one of the introduced tree species.

Bare ground can still be seen at some points in Sakaerat. The bare ground sampling points had been intensively deprived of soil nutrients and had lost conditions seen in the forest soils (Doi and Sakurai 2004). At these sampling points, recovery of vegetative cover did not occur since the harsh conditions make it too difficult for plants to survive. The uppermost horizon is reddish brown, rich in gravel, and has few roots and other plant organs/debris. The true color remote sensing image was previously reported (Doi and Ranamukhaarachchi 2010).

Spectral profiling of soil sampling points in remote sensing image

In this study, a true color remote sensing image provided by Google Earth was used. The remote sensing image was captured by the satellite Quickbird on 3 April 2006. A virtual altitude of 1,000 m above ground level was chosen when copying the remote sensing image from the Google Earth window using the "copy image" function. The original multispectral image included data on red–green–blue color intensity and panchromatic grayscale values. When the image was copied from the Google Earth window, the data regarding the values of the red–green–blue color intensity were retained. The image was used for the multivariate color profiling of the canopy or bare ground using Adobe Photoshop 7.0 as previously described (Doi 2012; Doi et al. 2010). The intensity values of red, green, blue, cyan, magenta, yellow and L^* and the values of a^* and b^* were read.

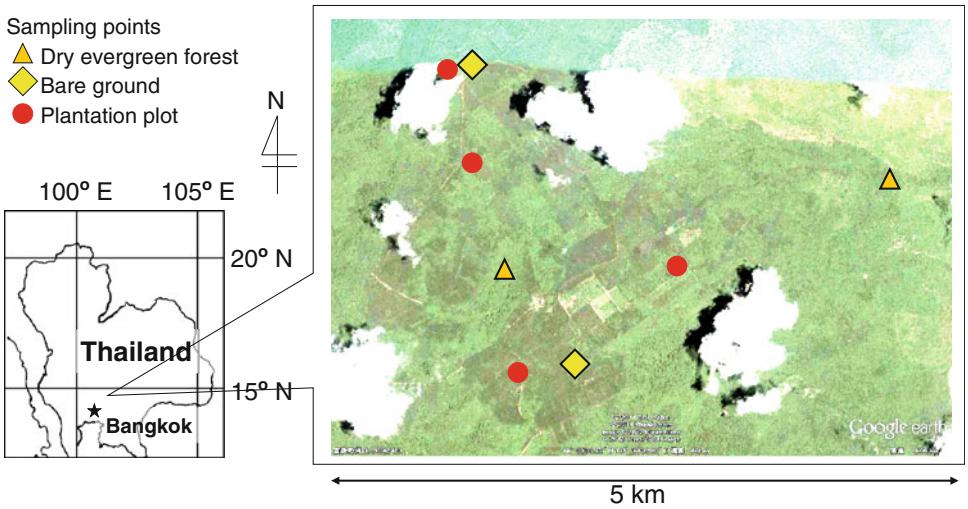

Fig. 1 Map of the research area in Sakaerat, Thailand. Brightness and contrast were increased from the original values for clarification

Soil sampling and physicochemical profiling

Soils were sampled on 24 and 25 December 2005. The sampling was performed within a 26-h period, during which the site experienced 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 from within the circle. In addition, two other cores were randomly taken from within the circle for soil moisture and bulk density measurements. The eight soil cores were immediately placed into a single plastic bag, mixed, and brought to the laboratory. For soil dehydrogenase activity measurement and community-level physiological profiling, described later, the moist soil was immediately passed through a 2-mm sieve and then transported to the laboratory and analyzed within 12 h. For other soil profiling purposes, the soil samples were air-dried, passed through a 2-mm sieve, and then analyzed. Physicochemical profiling of soils was performed as previously described (Doi and Ranamukhaarachchi 2007).

Dehydrogenase activity measurement and community-level physiological profiling

Soil dehydrogenase activity was determined as described by Casida et al. (1964) via colorimetric measurement of the reduction of 2,3,5-triphenyltetrazolium chloride to triphenyltetrazolium formazan. The details of this process are previously described (Doi and Ranamukhaarachchi 2009b).

The bacterial community in each composite sample was profiled with community-level physiological profiling using three Biolog EcoPlates with 31 carbon sources as previously described (Doi and Ranamukhaarachchi 2009a). Five grams of each soil sample were suspended in 45 mL of sterilized 0.85 % (w/v) NaCl and reciprocally shaken at room temperature for 30 min at 120 rpm. The suspension was centrifuged at 1,000*g* for 5 min, decanted, and the pellet was re-suspended in 45 mL of the NaCl solution. Centrifugation and suspension were then repeated twice. The soil suspension was left still for 1 min, and 10 mL of the uppermost section was diluted 40-fold with the NaCl solution. This suspension was used to inoculate a Biolog EcoPlate at a rate of 0.1 mL/well. Oxidation of each carbon source was measured by quantifying purple color development as a result of the reduction of tetrazolium violet. The plates were incubated in the dark at 26 °C, and absorbance at 405 nm was read using a microplate reader (Perlong DNM-9602G, Nanjing, PR China) at 4- to 12-h intervals for 7 days. During the incubation, the plates were wrapped in a plastic film to avoid desiccation. Values for the three above-mentioned pseudo-replicates were averaged, and then used for statistical analyses.

Profiling structure of soil bacterial community

A polymerase chain reaction-denaturing gradient gel electrophoresis (DGGE) method was used to analyze bacterial community structures of the soils. Soil DNA was extracted using a commercial kit (Power Soil™ DNA Isolation Kit, Mo-BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The V3 region of the 16S ribosomal RNA gene of the soil bacterial community was amplified using universal primers GC341F (341F with a GC clamp) and 907R (Ponnusamy et al. 2011) using a Takara Ex Taq™ polymerase kit (Takara Bio Inc., Otsu, Japan). A DNA quantity of between 0.7 and 2.1 ng was used as the template DNA. In amplification of the V3 region, a polymerase chain reaction was initiated by denaturing the sample at 95 °C for 5 min, followed by ×19 polymerase chain reaction cycles [95 °C for 1 min, 62 °C

(−0.8 °C per cycle) for 1 min, and 72 °C for 1 min]. These cycles were followed by 9 cycles of 95 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min, then the final elongation at 72 °C for 10 min. The amplified DNA was separated by DGGE with 20–60 % urea–formamide denaturing gradients (100 % denaturant was defined as 7 M urea and 40 % formamide). The gel contained 6 % polyacrylamide in ×0.5 strength Tris–acetate-ethylenediaminetetraacetic acid buffer (20 mM Tris, 10 mM acetic acid, and 0.5 mM ethylenediaminetetraacetic acid, pH 8.4. The gel was stained with SYBR Gold (Invitrogen, Carlsbad, CA, USA) and photographed with a blue LED transilluminator (Opt-Code, Corp., Tokyo, Japan). Band intensities were determined using the analytical software Gel Analyzer (Istvan Lazer). The most significant bands as indicators of land degradation or rehabilitation/conservation were selected by performing a principal component analysis described later. The most significant indicator bands were recovered from the gel, amplified as above in 20 cycles of polymerase chain reaction (95 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min) followed by a final elongation at 72 °C for 10 min. The amplified DNA fragment was cycle-sequenced using Big-Dye^{TM} Terminator Ver 3.0 (Applied Biosystems, Inc., Foster City, CA, USA), then sequenced by running a PRISM^{TM} 3100-Avant Genetic Analyzer (Applied Biosystems, Inc.). The closest relative of the bacterium represented by the DGGE band was retrieved from the Genbank database, BLAST (National Center of Biotechnology Information).

Data analyses

The following analyses were performed using the statistical software SPSS 10.0.1 (SPSS Inc., Chicago, IL, USA). Analysis of variance for each of the soil and canopy variables was performed. Community-level physiological profiles of the soils were analyzed to construct qualitatively different data sets (Doi and Ranamukhaarachchi 2009a) by applying the kinetic approach proposed by Lindstrom et al. (1998). Continuously measuring the intensity of the purple color development, a value of absorbance known as asymptote (K) was determined statistically for each of the 31 carbon sources. As absorbance value increased with incubation time, the value of asymptote K was determined to be the point at which the absorbance value essentially converged at 405 nm. The 0.95 K time point was regarded as the convergence stage (Lindstrom et al. 1998). This approach thus provided a 0.95 K data set consisting of multivariate profiles of the soils. In addition, another technique for constructing an area data set was applied. In this technique, a soil's multivariate profile was determined by integrating the area [absorbance × incubation time (h)] under the color development curve by reading the absorbance value at 405 nm (Hackett and Griffiths 1997) for each of the 31 carbon sources. Thus the area data set was constructed by gathering all the soils' area profiles. Then, a ratio-transformation was performed, i.e., each observed value was divided by the sum of all the 31 observed values for the soil sample and used for statistical analyses. The same ratio-transformation was also employed to obtain a DGGE profile of the soil by dividing each value of band intensity by the sum of all the values of band intensity for the soil sample.

Principal component analysis was performed to extract principal components from the data set on the remote sensing, soil physicochemical, or soil bacterial profiles. The community-level physiological and the DGGE profiles were used as soil bacterial profiles.

Wilk's lambda statistic was determined to quantify the discriminatory power of the profiling methods to discriminate among the groups which consisted of the evergreen forest, the *Acacia* plantation plot, and the bare ground groups. If the mean values among compared groups for each variable are equal, Wilk's lambda becomes 1. Conversely, as the difference between multivariate profiles increases, Wilk's lambda moves closer to 0. Thus a small Wilk's lambda value indicates the high discriminatory power of the profiling method (Zar 1999).

Results and discussion

Figure 2 shows spectral profiles of the forest canopies and the bare ground. Vegetation type was a significant ($p < 0.05$) source of variation in the color components, except for magenta. The land degradation-rehabilitation gradient is shown as the relationships in which the bare ground was clearly separated from the forest canopies. Between the forest canopies, relatively clearer differences were suggested by yellow and b^*. The similarity in the spectral profile between the *Acacia* plantation plot and the evergreen forest shows that 18 or 19 years after the *Acacia* plantation, the plantation plots had largely restored the original ecosystem.

Figure 3 presents physicochemical profiles of the soils. Vegetation type was a significant source of variation in moisture, pH, organic matter, exchangeable K^+, and soil fertility index ($p < 0.05$). The land degradation-rehabilitation gradient is shown as the relationship in which the bare ground soil was separated from the forest soils according to the soil fertility index, which was the comprehensive indicator of soil fertility. Land degradation was described by dryness, heaviness, poor organic matter content, low value of soil fertility index, high acidity, and the deteriorated soil buffering function indicated by small values of cation exchange capacity. According to Fig. 3,

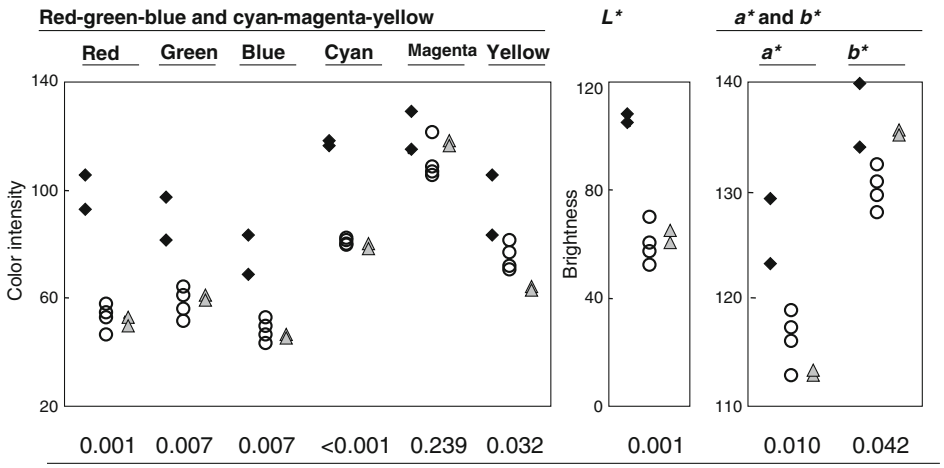

Fig. 2 Multispectral profiles of the bare ground (*filled diamond*), the *Acacia* plantation plots (*open circle*), and the evergreen forest (*filled triangle*) based on the remote sensing image acquired in April 2006

the *Acacia* plantation soil had largely restored the original physicochemical conditions.

These results indicate the completeness of the above components for ecosystem restoration (Figs. 2, 3). However, soil bacterial functions highlighted differences among the three vegetation types as indicated by small p values (< 0.006) provided by the analyses of variance (Fig. 4). The bare ground soil was the poorest, and the evergreen forest soil the most functional. The soil of the *Acacia* plantation plot tended to lie between these two extremes. According to Fig. 4, the *Acacia* plantation soil was still progressing towards full restoration to evergreen forest soil.

To present bacterial community structures of the soils, DGGE profiles of the soils are shown in Fig. 5. The use of gel analyzer eventually detected 40 bands, and the band intensities were ratio-transformed as mentioned above. This transformation provided DGGE profiles of the soils which were used for principal component analysis.

The remote sensing profiles of the forests and the bare ground were most clearly discriminated in the principal component score plot as shown by the smallest p value of 0.045 and a Wilk's lambda value of smaller than 0.001 (Fig. 6). The 0.95 K data set was the second clearest in terms of discrimination of the soils, as indicated by small lambda (<0.001) and p (0.093) values. The area data set, as another community-level physiological data set, and the soil physicochemical data set were comparable in terms of clarity of discrimination of the soil sample groups, while the DGGE data set was the poorest in discriminatory power among the five data sets.

The single remote sensing and the soil physicochemical variables as indicators of the land degradation and

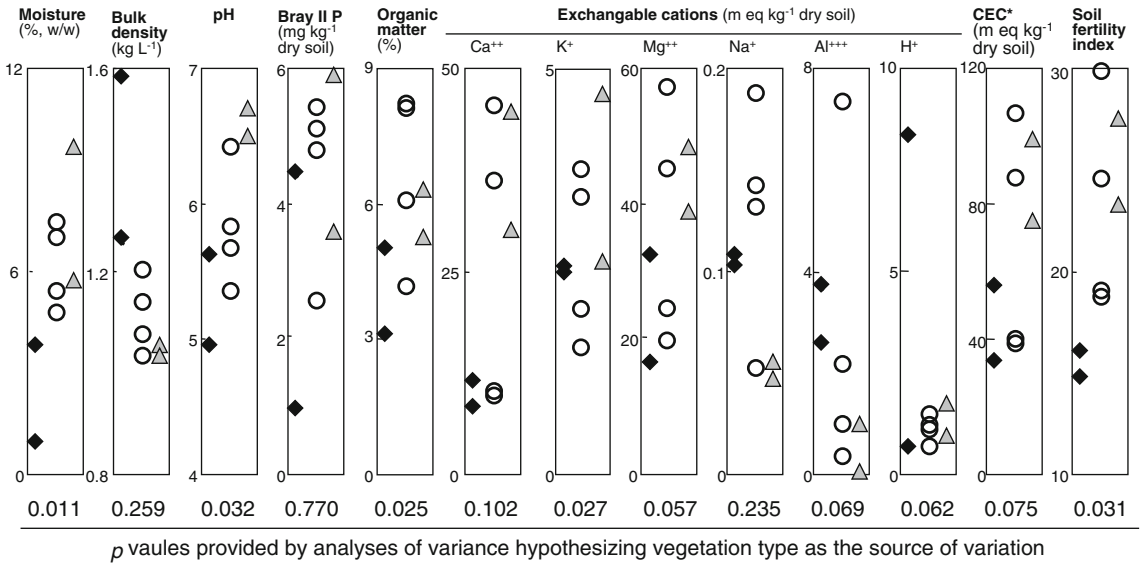

Fig. 3 Physicochemical characteristics of the soils of the bare ground (*filled diamond*), the *Acacia* plantation plots (*open circle*), and the evergreen forest (*filled triangle*). *Cation exchange capacity

rehabilitation are summarized in Table 1. Since the first principal component for these data sets showed the degradation and rehabilitation/conservation (Doi and Sakurai 2004), the single variables with great absolute values of eigenvector are the indicators of the land degradation or rehabilitation/conservation. The eigenvectors for the remote sensing variables indicate that a large value of color intensity or brightness indicates that the bare ground was light-colored. On the other hand, according to the soil physicochemical data, the land rehabilitation/conservation was described by high values of pH, exchangeable cations (Mg^{++}, Ca^{++}, K^{+}), cation exchange capacity, rich organic matter content and greater soil moisture. Great exchangeable acidity (Al^{+++}, H^{+}) and bulk density of soil described the land degradation as shown in Fig. 3.

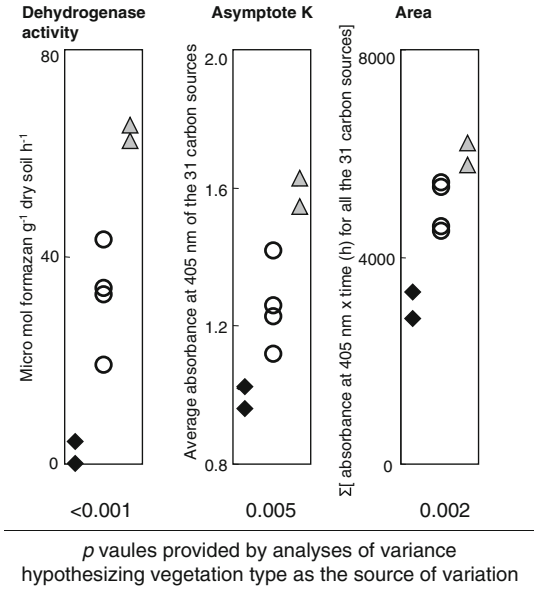

Fig. 4 Bacterial functions of the soils from the bare ground (*filled diamond*), the *Acacia* plantation plots (*open circle*), and the evergreen forest (*filled triangle*)

Table 2 shows that the land rehabilitation/conservation was indicated by the relatively intensive use of glycyl-L-glutamic acid, D-malic acid, α-cyclodextrin, and *i*-erythritol by the soil bacteria, while the land degradation was indicated by the intensive use of putrescine, tween 40, and itaconic acid by the soil bacteria.

The DGGE bands that most significantly contributed to the first principal component axis in Fig. 6 were sequenced. Band 3 could not be sequenced, possibly because of the existence of DNA fragments of multiple bacterial species in the band. Based on the ribosomal DNA sequences, the closest matching relatives were chosen from other soils (Table 3). The closest relative to the bacterium represented by the DGGE band as an indicator of the rehabilitation/conservation was an unidentified bacterium from a Ferralsol in Madagascar, and the second closest was an uncultured bacterium from the soil of a plot of 20-year-old grassland, which is part of a restoration ecosystem from a crop rotation agricultural land in subtropical China (Chen et al. 2012). The land degradation was described by the relatively high occurrence of the bacteria close to those found in disturbed soils under a coniferous monoculture in Taiwan (Lin et al. 2011), a Korean ginseng field (Baek et al. 2011), a pasture land (Jangid et al. 2008), pyrene-exposed soil, or volcanic deposits.

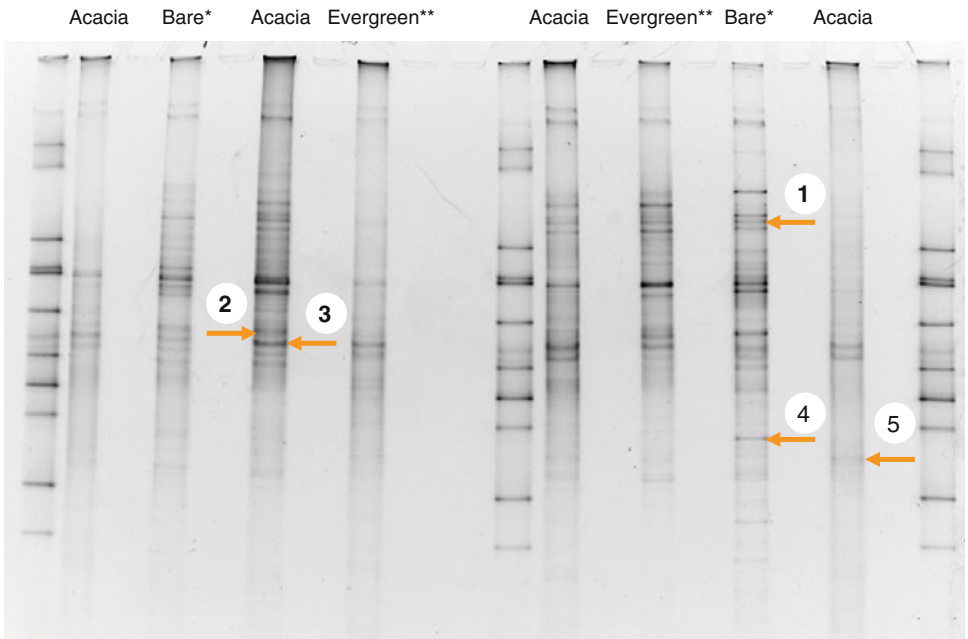

Fig. 5 The denaturing gradient gel electrophoresis profile (*band patterns*) of amplified 16S ribosomal DNA from genomic DNA extracted from the soils of the bare ground, the *Acacia* plantation plots, and the evergreen forest. In the electrophoresis, 20 % (*top*) to 60 % (*bottom*) denaturing gradients were used. The *orange arrows* indicate the bands subsequently analyzed. *Bare, bare ground; **Evergreen, evergreen forest

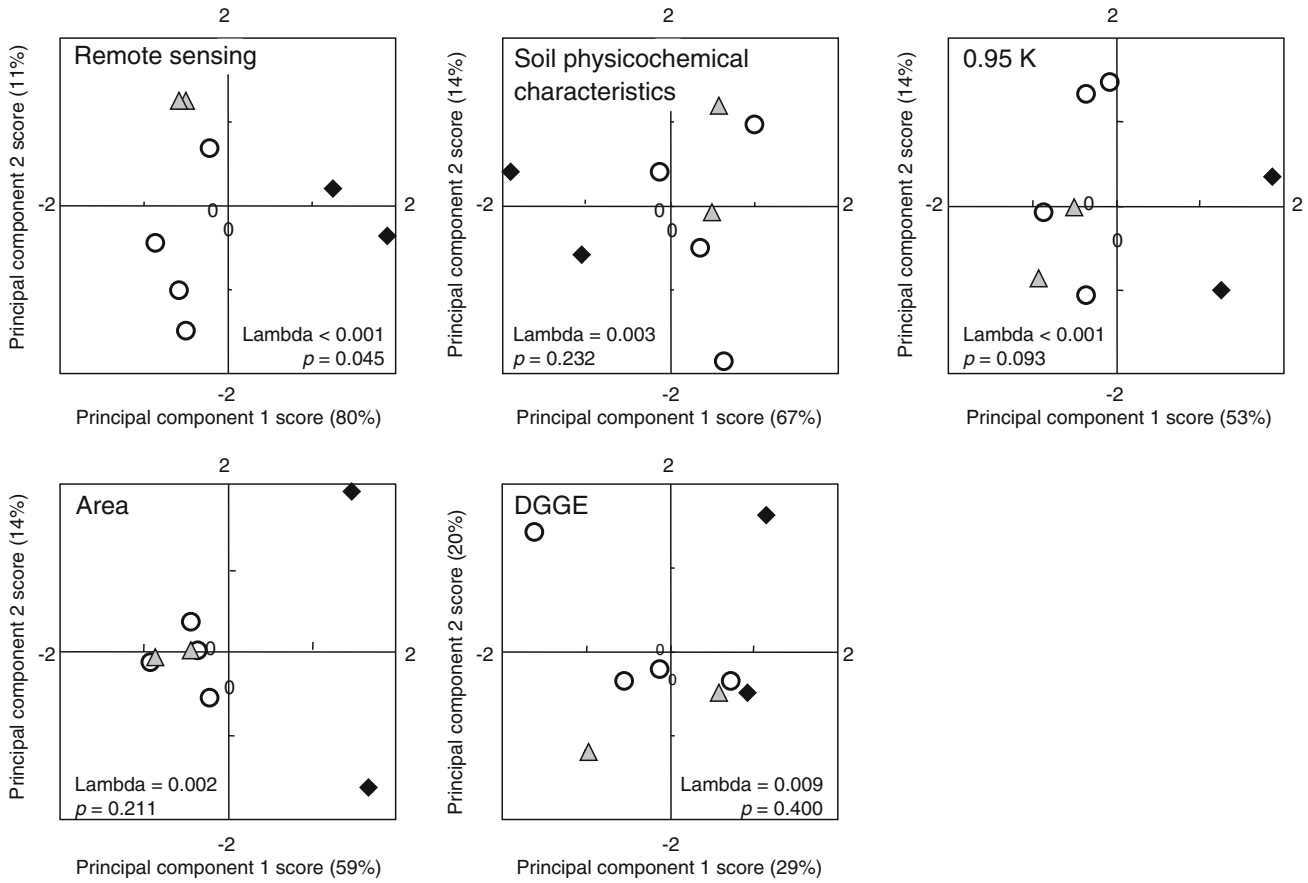

Fig. 6 Principal component score *plots* to show principal component scores for the bare ground (*filled diamond*), the *Acacia* plantation plots (*open circle*), and the evergreen forest (*filled triangle*) for each of the aspects of the land degradation and rehabilitation due to the *Acacia* reforestation

Apparently, the remote sensing and soil physicochemical data sets show that the *Acacia* plantation had largely restored the conditions seen in the evergreen forest canopy and soil (Figs. 2, 3). The same rehabilitative trend was also shown by the principal component score plots from the data sets in which the *Acacia* plantation and the evergreen forest had comparable scores on the first principal components (Fig. 6). These comparisons highlight the proximity of the *Acacia* and the evergreen forest ecosystems (Mitchell et al. 2000). However, there was evidence of incomplete restoration of the original ecosystem as suggested by other variables. Here, the mechanisms behind this incomplete restoration and the measures for the enhancement of the rehabilitation will be discussed.

Slow restoration of soil microbial functions

The scores on the first principal component for the *Acacia* plantation and the evergreen forest are comparable (Fig. 1), but the values of soil dehydrogenase activity, asymptote K, and area (Fig. 4) show a delay in the restoration of the soil microbial functions in the *Acacia* plantation soil compared to the aboveground and soil physicochemical restoration. Soil dehydrogenase activity is quantified measuring the reddish coloration generated when the tetrazolium compound accepts electrons released from the substrate as a result of the oxidation. Hence, the value of dehydrogenase activity represents activities of various dehydrogenases, which play fundamental roles in microbial activity, such as driving the citrate cycle (Vonmersi and Schinner 1991). Therefore soil dehydrogenase activity is an integrative measure of total soil microbial activities and thus could still show the difference between the *Acacia* plantation and the evergreen forest soils in Fig. 4, possibly by integrating subtle differences in the activities of various single dehydrogenases. The high significance (<0.001) of vegetation type as a source of variation of soil dehydrogenase activity may be contributed to by soil fungi that do not oxidize the tetrazolium compound in the Biolog EcoPlate (Preston-Mafham et al. 2002) which provided the values of asymptote K and area. Therefore, the values of asymptote K and area represented the integrative bacterial functions of the *Acacia* plantation soil moving towards full restoration to evergreen forest in terms of soil bacterial function

Table 1 Eigenvectors for the single variables contributed to the first principal components from the remote sensing and the soil physicochemical data sets

Indication	Remote sensing data set		Soil physicochemical data set	
	Variable	Eigenvector	Variable	Eigenvector
Rehabilitation/ conservation	None	–	pH	0.98
			Mg^{++}	0.95
			CEC[a]	0.94
			Ca^{++}	0.91
			K^{+}	0.91
			Moisture	0.85
			Organic matter	0.84
Degradation	Red	1.00	Al^{+++}	−0.91
	Luminosity	1.00	H^{+}	−0.89
	Green	0.98	Bulk density	−0.89
	Blue	0.98		
	*L**	0.98		
	Cyan	0.97		
	Yellow	0.82		

Variables with |Eigenvector| > 0.80 were selected

[a] Cation exchange capacity

Table 2 Eigenvectors for the single variables contributed to the first principal components from the community-level physiological data sets

Indication	0.95 K data set		Area data set	
	Variable	Eigenvector	Variable	Eigenvector
Rehabilitation/ conservation	Glycyl-L-glutamic acid[a]	−0.90	Alpha-cyclodextrin	−0.93
	D-Malic acid	−0.89	Glycyl-L-glutamic acid	−0.93
	Alpha-cyclodextrin	−0.88	Gamma-hydroxybutyric acid	−0.89
	Glycogen	−0.82	D-Malic acid	−0.87
	i-Erythritol	−0.81	2-Hydroxy benzoic acid	−0.85
	D, L-Alpha-glycerol phosphate	−0.78	*i*-Erythritol	−0.83
Degradation	Putrescine	0.86	A-D-lactose	0.93
	D-Cellobiose	0.90	Itaconic acid	0.93
	Tween 40	0.90	D-Galacturonic acid	0.95
	Itaconic acid	0.91	Pyruvic acid methyl ester	0.96
	D-Mannitol	0.92	Putrescine	0.97
	A-D-lactose	0.97	Tween 40	0.98

Variables with |Eigenvector| > 0.75 were selected

[a] Underlined carbon sources are common between the data sets

(Fig. 4). These results reveal the incomplete restoration of the original soil microbial functions in the *Acacia* plantation plot.

A community-level physiological profile given as an asymptote K or an area profile may be strongly related to the bacterial community structure (Gamo and Shoji 1999). Therefore, the DGGE profiles were expected to discriminate among the soils, though unsuccessful discrimination was seen in Fig. 6. In Table 3, ribosomal DNA sequences from the same Ferralsol in Madagascar were the closest relatives to the ribosomal DNA sequences from the soils of the bare ground and the evergreen forest. As shown in Fig. 5, all the soils shared some of the same major bands. The existence of the soil bacteria commonly seen in the soils should have made the discrimination ambiguous (Fig. 6). In this study, DGGE profiles did not clearly discriminate between the soils, but at present, no study has been conducted to compare the discriminatory power of community-level physiological profiling and DGGE based on statistics such as Wilk's lambda. This seems to indicate that a variety of multivariate soil profiling methods are worth being examined in the evaluation of land ecosystem restoration (Ramsey et al. 2006). Another possible explanation was that the bare ground soil contained extracellular DNA fragments from the evergreen forest bacteria that could firmly attach to the bare ground soil particles because of the low pH and poor cation content (Trevors 1996). The remaining DNA fragments from the evergreen soil bacterial community might, therefore, be a cause of the ambiguous discrimination among the soils.

While the values of soil microbial activity shown in Fig. 4 represent the integrative redox activities of the microbial cells, they may reflect biochemical diversity in the soil (Table 2). A rhizosphere soil bacterial community showed its unique carbon source utilization profile revealed by community-level physiological profiling (Garland 1996). Changes in soil amino acid profile were recognized along a successional sequence of boreal forest (Werdin-Pfisterer et al. 2009). Furthermore, Fujii et al. (2010) found differences in the profile of the occurrence of low molecular compounds which consisted of monosaccharides and organic acids among soils of Japanese forests with different vegetation types. In the latter study, the low molecular organic compounds were rapidly and continuously produced and consumed. Therefore, it is possible that the evergreen forest soil has richer diversity in, and possibly faster metabolic turnover of, available low molecular organic compounds for microbes. Werdin-Pfisterer et al. (2009) concluded that the composition of soil amino acids did not significantly change across a successional sequence in Alaska, while Strobel et al. (1999) found significant differences among concentrations of aliphatic carboxylic acids in soils under different vegetation types and arable

Table 3 Identity of 16S ribosomal DNA sequences that indicate land rehabilitation/conservation and degradation

Indication	Band in Fig. 5	Eigenvector[a]	Closest relative from soils	BLAST accession number	Soil sample's origin	Similarity (%)[b]	Total score[c]	Reference
Rehabilitation/ conservation	5	−0.510	Unidentified bacterium	FQ732438	Ferralsol	99.2	641	Unpublished
			Uncultured bacterium	EU881212	Natural restoration site	95.7	813	Chen et al. (2012)
Degradation	1	0.764	Unidentified bacterium	FQ703663	Ferralsol	97.9	850	Unpublished
			Uncultured *Firmicutes*	GU016126	Monoculture forest	97.9	846	Lin et al. (2011)
	2	0.766	*Tumebacillus ginsengisoli*	AB245375	Ginseng field	99.1	946	Baek et al. (2011)
			Uncultured bacterium	FR687484	Pyrene-degrading soil	98.9	941	Unpublished
			Uncultured Bacilli	EF075265	Pasture land	98.5	929	Jangid et al. (2008)
	4	0.513	Uncultured bacterium	JN093291	*Vetiveria* rhizosphere	100	726	Unpublished
			Micrococcaceae bacterium	DQ490463	Volcanic deposits	99.0	702	Unpublished

[a] The most significant single bands (|Eigenvector| > 0.5) were selected. Band 3 in Fig. 5 could not be sequenced

[b] The first and second best matching relatives, or those with similarity >98 %, were selected

[c] The length coverage (number of bases) of the sequenced DNA fragment

lands. If the *Acacia* plantation soil has a simpler profile of microbiologically available organic compounds than does the evergreen forest remains to be investigated in the future.

Diversity of vegetation

If the relative microbial inactiveness of the *Acacia* plantation soil compared with the evergreen forest soil is due to a simpler composition of the tree community resulting in the availability of a simpler composition of microbiologically available soil organic compounds, then the spectral profile of the canopy may also be simple and thus would have a smaller value of coefficient of variation of the canopy spectral variable expressed as:

$$\text{Coefficient of variation } (\%) = \text{standard deviation} \times 100/\text{mean}$$

To investigate the above hypothesis, Fig. 7 indicates coefficients of variation for the canopy spectral variables. For most variables, vegetation type was not significant as a source of the variations ($p > 0.05$), and the coefficients of variation were comparable between the canopies of the *Acacia* plantation plot and the evergreen forest. Vegetation type significantly influenced the coefficients of variation of magenta and b^*, but the coefficients of variation for the *Acacia* canopy tended to be greater. These results did not support the hypothesis that the evergreen forest canopy indicates greater diversity of the tree community than does the *Acacia* canopy. Succession was likely to be ongoing in the *Acacia* plantation plots as reported by Kamo et al. (2002) who found 51 plant species in the *Acacia* plantation plot, in 1998, and 114 species in the evergreen forest. This transitional status of the *Acacia* plantation plot must have resulted in the apparent diversity of the canopy spectral profile by allowing other plant species to take root and to be remote sensing-captured through the gaps caused by self-thinning (Ashton et al. 2001). As the number of plant species is greater for the evergreen forest, the relatively more uniform spectral profile of the canopy should be attributed to the canopy leaves' spectral proximity among the upper storey species of *Shorea* species and *Hopea ferrea* (Kanzaki et al. 1995). These differences in the coefficient of variation also indicate that the plant community of the *Acacia* plantation plot was still changing towards the plant community structure of the evergreen forest. The difference in plant species composition (Kamo et al. 2002) could have significant effects on soil physicochemical and biological profiles. At the same time, soil physicochemical and biological conditions can affect the vegetative succession. However, this mutual relationship does not necessarily mean that

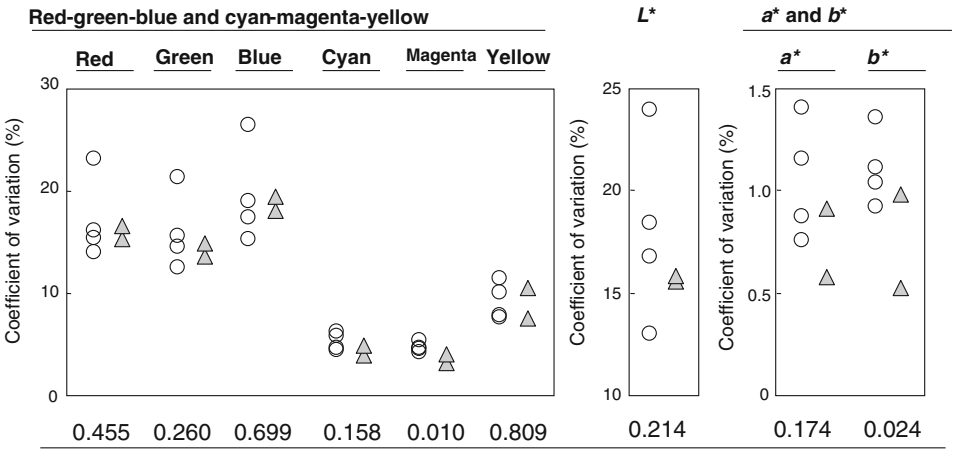

Fig. 7 Coefficients of variation of the color components for canopies of the *Acacia* (*open circle*) and the evergreen forest (*filled triangle*) based on the remote sensing image acquired in April 2006

restoration of a single ecosystem component is always followed by restoration of the others (Kardol and Wardle 2010). In this study, the restoration of soil microbial functions was found to be left behind that of vegetation and soil physicochemical conditions.

Consequences and perspective of the *Acacia* plantation in the region

Thus, the *A. auriculiformis* plantation soil and the ecosystem were very likely to be moving in succession towards full restoration. It will take at least several more years for the *Acacia* plantation plots to fully restore the original ecosystem to the point where there are no discernible differences from the evergreen forest. In humid tropics under similar climatic conditions, Yemefack et al. (2005) recognized incomplete soil fertility restoration in a comparable rehabilitation period of 15 years after shifting cultivation in Cameroon. In the southern Yucatan peninsula of Mexico, under similar climatic conditions, 40–60 years was estimated for restoration of total aboveground biomass after shifting cultivation, based on the most optimistic estimate (Chazdon 2003). In the current study site, the *Acacia* plantation resulted in relatively faster soil physicochemical and aboveground restoration, while the restoration of the soil microbial functions was slower. A similar delay in soil microbial restoration after establishment of vegetation has been previously reported (Tscherko et al. 2004). The *Acacia* plantation in Sakaerat was thought to have restored the soil physicochemical conditions that could further enhance the restoration of the plant community. On the other hand, the aforementioned results also suggest the lack of certain microbes that used to play significant roles in the evergreen forest soil. Hence, inoculation of effective microbes may be a way to further enhance the ecological restoration, especially by targeting specific microbial functions (Chanway 1997). The addition of particular inorganic nutrients (Table 1) may also be effective for enhancing the ecological restoration (Mataji et al. 2010; Kuramae et al. 2011). These actions are expected to further rehabilitate the belowground components for ecosystem restoration in the *Acacia* plantation plots so as to overcome the barrier (Ren et al. 2007) that the in-succession ecosystem seems to be encountering.

Conclusion

This study revealed a delay in the restoration of soil microbial functions related to the redox activities of the microbes compared with the restoration of the vegetation and soil physicochemical characteristics, which suggests a lack of soil microbes that used to play important roles in the original evergreen forest ecosystem. Therefore, once a tropical forest soil is severely degraded, some key microbes can be lost from the soil (Okot-Uma and Endeley 2004). Because microbes cannot move freely, it is difficult for them to be reintroduced to the reforestation site. The lack of such lost microbes may result in crippled ecological services (Bradshaw et al. 2007), which should be evaluated so that effective measures to restore the lost functions and ecological services can be taken, thereby enhancing the land rehabilitation processes following reforestation.

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