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ORIGINAL ARTICLE

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Microbial diversity of topographical gradient profiles in Fushan forest soils of Taiwan

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Abstract To evaluate the microbial diversity of Fushan forest soils, the variation of soil properties, microbial populations, and soil DNA with soil depth in three sites of different altitude were analyzed. Microbial population, moisture content, total organic carbon (C_{org}), and total nitrogen (N_{tot}) decreased with increasing soil depth. The valley site had the lowest microbial populations among the three tested sites due to the low organic matter content. Bacterial population was the highest among the microbial populations. The ratios of cellulolytic microbes to the total bacteria in organic layers were high, implying their roles in the carbon cycle. The microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) contents ranged from 130.5 to 564.1 μg g^{-1} and from 16.7 to 95.4 μg g^{-1}, respectively. The valley had the lowest C_{mic} and N_{mic}. The organic layer had the highest C_{mic} and N_{mic} and decreased with soil depth. Analysis using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplicons of 16S rDNA showed that the bacterial diversity of the three sites were very similar to each other in the major bands, and the variation was in the minor bands. However, the patterns in PCR-DGGE profile through gradient horizons were different, indicating the prevalence of specific microbes at different horizons. These results suggest that the microbial diversity in the deeper horizons is not simply the diluted analogs of the surface soils and that some microbes dominate only in the deeper horizons. Topography influenced the quantity and diversity of microbial populations.

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Introduction

Natural forests have gained recognition as sites of high biodiversity, where complex relationships among fauna, flora, and microflora are maintained due to the structural richness of the habitat. Soil microbes are essential components of the biotic community in natural forests, and they are largely responsible for ecosystem functioning because they participate in most nutrient transformations (Hackl et al. 2004). Usually, the microbial composition of the surface horizon has been studied because it is supposed to be the most active one, whereas little attention has been paid to the deeper horizons of the soil profile (Agnelli et al. 2004). Few papers take into account the subsurface soil layers (Bundt et al. 2001; Ekelund et al. 2001; Fierer et al. 2003), and fewer, as far as we know, consider the pedogenetic horizons in assessing soil-inhabiting microorganisms (Fritze et al. 2000; Agnelli et al. 2001). Microbes in these deeper horizons play an important role in ecosystem biogeochemistry, soil formation, and maintenance of groundwater quality (Konopka and Turco 1991). However, it is not clear whether the subsurface microbial community is closely tied to the surface-soil microbial community or is an independent ecosystem with a distinct assemblage of microorganisms.

The number of culturable microbes in soil is often only about 1% of the total number of microbe presents (Schoenborn et al. 2004), which hinders microbial diversity analysis. To overcome this limitation, a number of approaches, such as DNA reassociation (Torsvik et al. 1990), DNA–DNA and mRNA–DNA hybridization (Cho and Tiedje 2001), analysis of bacterial 16S rDNA library (Axelrood et al. 2002), and other polymerase chain reaction (PCR)-based methods of denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) (Muyzer 1999), and

ribosomal intergenic spacer analysis (Fisher and Triplett 1999), have been developed to study molecular microbial diversity (Kirk et al. 2004). DGGE has the advantages of being reliable, reproducible, rapid, and inexpensive. Multiple samples can also be analyzed concurrently, making it possible to follow changes in the microbial populations (Muyzer 1999). PCR-DGGE is based on the variation in base composition and secondary structure of the 16S rDNA molecule fragment, and DGGE can separate the DNA with one base-pair difference. Application of DNA fingerprinting methods enables detection of soil microbial community diversity, even including those microbes not yet cultivated.

Fushan Mountain is located in the northern part of Taiwan and is a typical low-altitude, ecosystem-protected area. Fushan forest is identified as one of the four natural forest sites by Taiwan Long Term Ecological Research Network (TERN) to study ecosystem response to environmental disturbances such as typhoons and acidic deposition (Lin et al. 1997, 2000, 2003b; Liu et al. 2004). Although, few studies were conducted in the Fushan forest, such as nitrogen (N) mineralization and nitrification rates (Owen et al. 2003) and fluvial transportation and sedimentation (Jen et al. 2006), the microbial ecology of this forest is not characterized. Hence, in the present study, microbial populations, microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) were determined, and DGGE of PCR-amplified ribosomal RNA (rDNA) genes was performed to evaluate whether the subsurface bacterial community is closely tied to the surface soil bacterial community or is a distinct assemblage of bacteria through different soil profiles from three altitude locations.

Materials and methods

Site description

Fushan forest in northern Taiwan (24°34′N, 121°34′E) has an elevation from 400 m to 1,400 m (King et al. 2003). Fushan is a moist, subtropical, mixed evergreen/hardwood broadleaf forest with a flora of more than 500 species (Mabry et al. 1998). Members of Lauraceae, Fagaceae, and Theaceae are dominant in this area. Mean annual precipitation is about 3,990 mm (Hsia and Hwong 1999). The soil is classified into Hapludults, Dystrochrepts, Udipsamments, and Udorthents according to the Keys of Soil Taxonomy (Lin et al. 1996). The ridge is the richest in vegetation, with the highest species diversity and density. Diversity decreases gradually through the middle slope, and the valley habitats are the least diverse. The ridge also has the greatest elevation, effective soil depth, and mean canopy height; the middle-slope area has the highest slope; and the valley has the greatest canopy gaps and the shallowest soil formation due to erosion. Topography was significantly correlated with soil disturbance, which induced differentiation of microenvironment and species compositions.

Sampling

During September 2005, soil samples were collected from three locations—valley, middle slope, and ridge—at OA (0–15 cm), A (16–35 cm), BW1 (36–55 cm), BW2 (56–75 cm), and BC (76–95 cm) horizons, which differed from each other in altitude, characteristic plant species, and slope (Table 1). Soil profiles were classified into horizons according to the International Soil Classification System (Soil Survey Staff 2003). Three replicate samples were taken at each horizon for plate count, chemical analysis, and DNA extraction. The samples were sieved to 2 mm, and DNA extraction was carried out immediately after reaching the laboratory.

Culture media and conditions

For plate count, 1 g soil was used to prepare the serial dilution, and 1 ml from selected dilutions was pour plated. Bacteria were counted at 25°C after 5 days on nutrient agar (Merck, Darmstadt, Germany), and actinomycetes were cultivated at 25°C for 7 days on

Table 1 Characteristic features of sampling sites of Fushan forest

Properties	Valley	Middle slope	Ridge
Altitude (m)	700	850	1,000
Characteristic plant species	*Aralia bipinnata*, *Callicarpa dichotoma*, *Cyclobalanopsis gilva*, *Villebrunea pedunculata*	*Cinnamomum micranthum*	*Ilex goshiensis*, *I. uraiensis*, *Itea parviflora*, *Myrsine sequinii*, *Rhododendron ellipticum*, *Syzygium buzifolium*, *Ternstroemia gymnanthera*
Soil texture	Lithosols, stony loam	Colluviums, stony loam	Yellow soils, stony loam
Air temperature (°C)[a]	12.4–24.5	13.3–26.6	12.0–25.3
Soil temperature (°C)[a] at 5-cm depth	11.3–21.5	13.1–23.0	12.1–23.3
Light intensity (lux)[a]	142–5,676	414–3,667	742–3,519

[a]Ranges of temperature and light intensity measured during the sampling between October 2002 and March 2005

glycerol–yeast extract medium composed of (g l^{-1}) glycerol 5.0, yeast extract 2.0, K_2HPO_4 1.0, and agar 15.0 at pH 7.0 ± 0.1. Streptomycin and cycloheximide were added to inhibit the growth of bacteria and fungi at a final concentration of 10 μg ml^{-1} (Yang and Yang 2001). Fungi were grown at 25°C for 5 days on Rose Bengal medium containing (g l^{-1}) glucose 10.0, peptone 5.0, K_2HPO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, Rose Bengal 0.033, and agar 15.0 at pH 6.8 ± 0.1. Cellulolytic microbes were assayed at 25°C after 7 days' incubation on Mandels–Reese medium (Mandels and Reese 1957) with modifications, containing (g l^{-1}) carboxymethylcellulose (CMC, Sigma) 10.0, peptone 1.0, urea 0.3, $(NH_4)_2SO_4$ 1.4, KH_2PO_4 2.0, $CaCl_2 \cdot H_2O$ 0.3, $MgSO_4 \cdot 7H_2O$ 0.3, $FeSO_4 \cdot 7H_2O$ 0.005, $ZnSO_4 \cdot 7H_2O$ 0.0014, $MnSO_4 \cdot 4H_2O$ 0.0016, $CoCl_2 \cdot 7H_2O$ 0.002, and agar 20.0 at pH 6.0 ± 0.1. The plates were sprayed with Congo red to show a clear zone around the colonies. Phosphate-solubilizing microbes were measured at 25°C after 5 days on rock phosphate medium by the clear zone around the colonies (Chang et al. 2001). Nitrogen-fixing microbes were counted after incubation for 7 days at 25°C on nitrogen-free mannitol medium (Yang et al. 2003). All experiments were carried out in triplicate.

Microbial biomass carbon and nitrogen

C_{mic} and N_{mic} were determined by the chloroform fumigation extraction method (Brookes et al. 1985; Vance et al. 1987). Fresh soil samples (25 g) at 40% water-holding capacity were placed in a 50-ml beaker and kept in a vacuum desiccator containing a 100-ml beaker with 50 ml chloroform (ethanol removed). The desiccator was evacuated using a vacuum pump until the chloroform boiled rapidly. After 72-h incubation, the soil samples were extracted with 0.5 M K_2SO_4 (100 ml) by shaking at 250 rpm for 30 min, and filtered through Whatman no. 42 filter paper. A nonfumigated incubation (without chloroform) was conducted as the control. For the C_{mic} analysis, 8 ml of soil extract was mixed with 0.066 M $K_2Cr_2O_7$ 2 ml, HgO 70 mg, concentrated H_2SO_4 10 ml, and 85% H_3PO_4 5 ml; digested at 150°C for 30 min; and titrated against 0.033 M ferrous (II) ammonium sulfate using 1,10-phenanthroline–ferrous sulfate mixture as indicator. N_{mic} was determined by a modified Kjeldahl method (Yang et al. 1991). The C_{mic} was calculated using the formula $C_{mic} = E_C/K_{EC}$, where E_C is the difference between extractable C from fumigated and nonfumigated samples, and $K_{EC} = 0.45$ (Wu et al. 1990). The N_{mic} was calculated as $N_{mic} = E_N/K_{EN}$, where E_N is the difference between extractable N from fumigated and nonfumigated samples and $K_{EN} = 0.54$ (Brookes et al. 1985).

DNA extraction

Genomic DNA of the soil samples was extracted following a modified protocol of Krsek and Wellington (1999). Two grams of wet soil was used to extract DNA, and the yields were expressed in dry-weight basis. Soil DNA was extracted with Crombach buffer (33 mM pH 8.0 Tris–HCl, 1 mM pH 8.0 EDTA) containing lysozyme (5 mg ml^{-1}) and sodium dodecyl sulfate (1%). After centrifugation, supernatants were subjected to potassium acetate and polyethylene glycol precipitation, phenol/chloroform/iso-amylalcohol purification, isopropanol precipitation, and spermine-HCl precipitation. The crude DNA was purified by GeneSpin 1-4-3 DNA Extraction Kit (Protech, Protech Technology Enterprise Ltd., Taiwan) according to the manufacturer's recommendations. After extraction, absorbances at 230, 260, and 280 nm were measured to evaluate the levels of protein (A_{260}/A_{280}) and humic acid (A_{260}/A_{230}) contamination. DNA was quantified by measuring the absorbance at 260 nm, and the concentration was determined from a λ phage DNA standard curve (Promega, Madison, WI, USA).

PCR amplification

Total bacterial 16S rDNA was amplified using the primer set GC−968f (5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TA-3′) and 1,401r (5′-GCG TGT GTA CAA GAC CC-3′), as described by Felske et al. (1997). The DNA template was amplified with 5 U μl^{-1} of Pro *Taq* DNA polymerase (Protech), 10 mM of each primer, 10 mM of each dNTP (Protech), and reaction buffer 1× (Protech, with $MgCl_2$) in a final reaction volume of 50 μl. The PCR was performed with an Applied Biosystems 2720 Thermal Cycler (Foster City, CA, USA) with the following reaction conditions: 94°C for 90 s, followed by 33 cycles at 95°C for 20 s, 56°C for 30 s, 72°C for 45 s, and a final extension step at 72°C for 10 min. The PCR product, 5 μl, was analyzed by electrophoresis on 2% (w/v) agarose gel with 100 bp DNA ladder (Promega) to conform the size and the approximate quantity of generated amplicons.

Denaturing gradient gel electrophoresis

The PCR products, 20 μl, were separated in a vertical denaturing gradient gel using Dcode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) at 60°C. The polyacrylamide (6%) gels with gradients of 45–65% denaturants (where 100% denaturants contains 7 M urea and 40% formamide) (Muyzer et al. 1996) and a running time of 6 h at 150 V were selected, as these conditions optimally separated a maximal number of bands. After electrophoresis, gels were stained with ethidium bromide and photographed under ultraviolet (UV) light.

Chemical analysis

Moisture contents were determined by drying the sample at 105°C overnight to a constant weight; pH was measured in 1:5 soil:water extracts. Air and soil temperatures were measured directly and at 5 cm depth of soil, respectively, with a thermometer. Total nitrogen (N_{tot}) was determined by a modified Kjeldahl method (Yang et al. 1991), and total organic carbon (C_{org}) was estimated using a modified Walkley–Black method, as described by Nelson and Sommers (1982).

Statistical analysis

Analyses were carried out using triplicate samples, and the mean values with standard error (SE) are presented after statistical analyses. All results were reported on a dry-weight basis. Data analysis was performed using analysis of variance (ANOVA) and Tukey's multiple range tests ($P = 0.05$) using the Statistical Analysis System (SAS Institute 2002). For DGGE analysis, samples were run to get at least three identical profiles, and a representative figure was presented. DGGE banding patterns were analyzed by ImageJ software (National Institutes of Health, Bethesda, MD, USA), which used band intensity. Band-matching data and relative band densities were exported via Excel (Microsoft Corp.) for cluster analysis using the BioDiversity Pro ver.2 software (http://www.sams.ac.uk/dml/projects/benthic/bdpro/downloads.htm). The similarity matrices were generated using the Bray–Curtis cluster analysis, and dendrograms were created using group average link.

Results

Soil properties and environmental conditions

Soil properties of the valley, middle slope, and ridge are presented in Fig. 1. Fushan soil is acidic (pH 4.39–4.97), and the pH of deeper horizons was higher than that of the organic layer, but the differences were not statistically significant. The pH of the valley soils was the highest, and the ridge soil was the lowest, although there were no significant differences among them. The C_{org} and N_{tot} contents of the ridge soils were the highest, while the valley samples were the lowest. Further, C_{org}, N_{tot}, and the C/N ratio were high in the organic layer and low in the deeper layer. Air and soil temperatures ranged from 12.0°C to 26.6°C and from 11.3°C to 23.3°C, respectively.

Microbial populations

Microbial populations of three tested sites are presented in Fig. 2. The valley had the lowest microbial population among the tested sites. The organic layer contained the

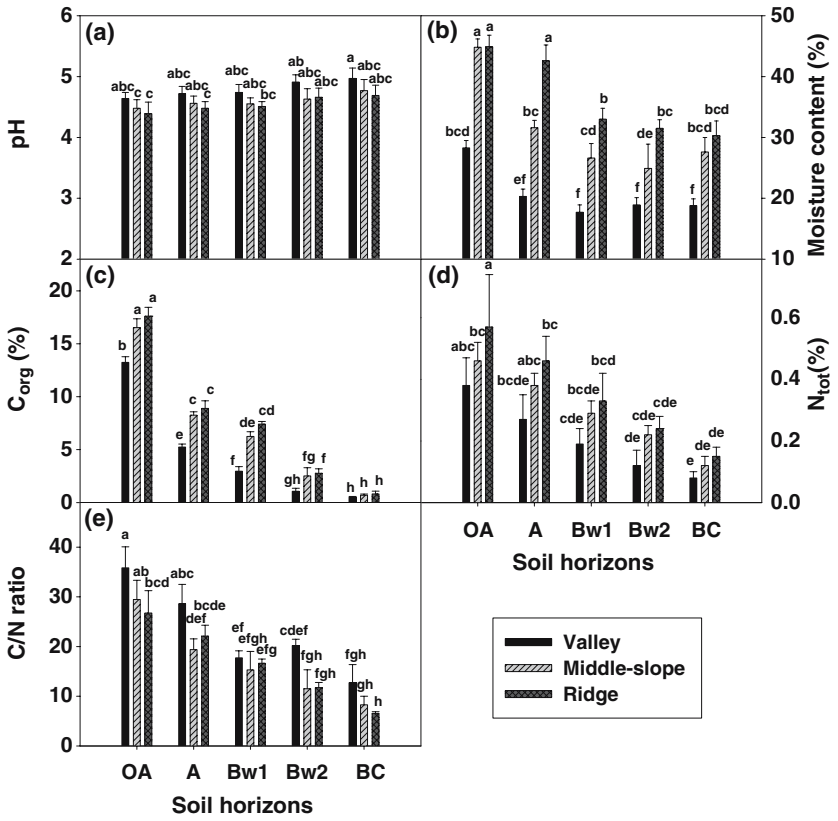

Fig. 1 Physicochemical characteristics of the valley, middle slope, and ridge profile soil samples: **a** pH, **b** moisture content, **c** total organic carbon (C_{org}), **d** total nitrogen (N_{tot}), and **e** C/N ratio

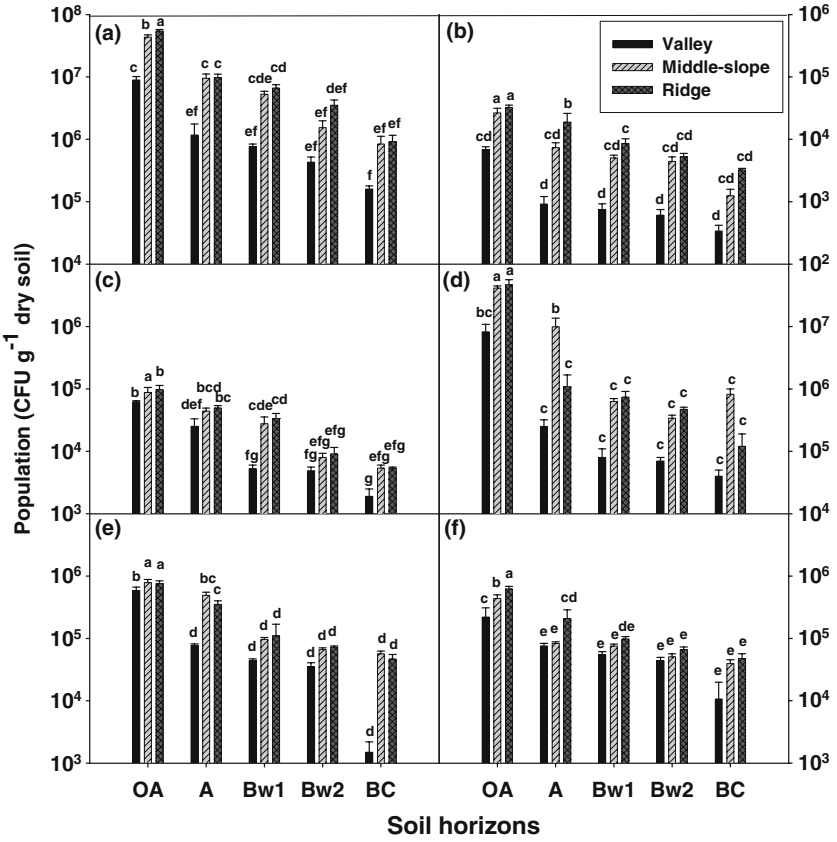

Fig. 2 Microbial population [colony forming units (CFU) g^{-1} dry soil] of the valley, middle slope, and ridge profiles in Fushan forest soils: **a** bacteria, **b** actinomycetes, **c** fungal propagules, **d** cellulolytic microbes, **e** phosphate-solubilizing microbes, and **f** nitrogen-fixing microbes

highest populations, and the populations decreased gradually through deeper layers. The bacterial, actinomycete, fungal, cellulolytic, phosphate-solubilizing, and nitrogen-fixing populations at the BC horizon were 1.7–1.9, 4.7–10.0, 3.1–6.2, 0.3–2.0, 0.3–7.2, and 4.9–9.0% of those at the OA horizon, respectively. The ratios of cellulolytic, phosphate-solubilizing, and nitrogen-fixing microbes to the total bacteria in organic layers were 85.8–95.6, 1.4–6.6, and 1.0–2.5%, respectively.

Microbial biomass carbon and nitrogen

C_{mic} and N_{mic} in the three topographically different sites are presented in Fig. 3. The C_{mic} and N_{mic} contents in the Fushan forest soils ranged from 130.5 to 564.1 μg g^{-1} and from 16.7 to 95.4 μg g^{-1}, respectively (Fig. 3a, b). The valley had low C_{mic} and N_{mic} in all tested sites, and the differences in C_{mic} were significant in the upper layers (OA, A, and BW1), whereas the N_{mic} values were not significantly different among all sites. For all profiles, the organic layer had the highest C_{mic} and N_{mic}, which decreased with soil depth. However, below 55 cm depth in the three tested profiles, C_{mic} and N_{mic} contents remained fairly constant. The proportion of organic carbon present as C_{mic}/C_{org} (Fig. 3c) and C_{mic}/N_{mic} ratios (Fig. 3e) were high in deeper horizons, and the highest values were in the BC horizon, while all values of N_{mic}/N_{tot} were not significantly different (Fig. 3d).

PCR-DGGE diversity profiles of different soil layers

The molecular profiles of bacterial communities at different topographies and different layers are shown in Fig. 4. The yield of total genomic DNA through the soil profiles decreased from 36.0–43.3 μg g^{-1} in the organic layer to 3.0–10.4 μg g^{-1} in the BC horizon (75- to 95-cm depth), and the valley had the lowest DNA yield among the three tested sites (Fig. 3f). The ratio of A_{260}/A_{230} greater than 2.0 and the ratio of A_{260}/A_{280} larger than 1.7 indicate the yield of high-purity DNA. Our soil DNA extraction method resulted in high yield of DNA and low contamination with humic acid and protein, as revealed by A_{260}/A_{230} and A_{260}/A_{280} ratios (1.79–2.03 and 1.63–1.83, respectively). Total number of bands in the DGGE profile varied from 14 to 17 in the valley, 16 to 18 in the middle slope, and 20–25 in the ridge sites (Fig. 4). Four strong bands were common to all the profiles, and the total number of strong bands was five to six in the valley and the middle slope and five to eight in the ridge site. DGGE profiles showed that bacterial diversity is almost similar when comparing the strong bands, which may represent the numerically dominant bacteria. However, the soil microbial biomass had a

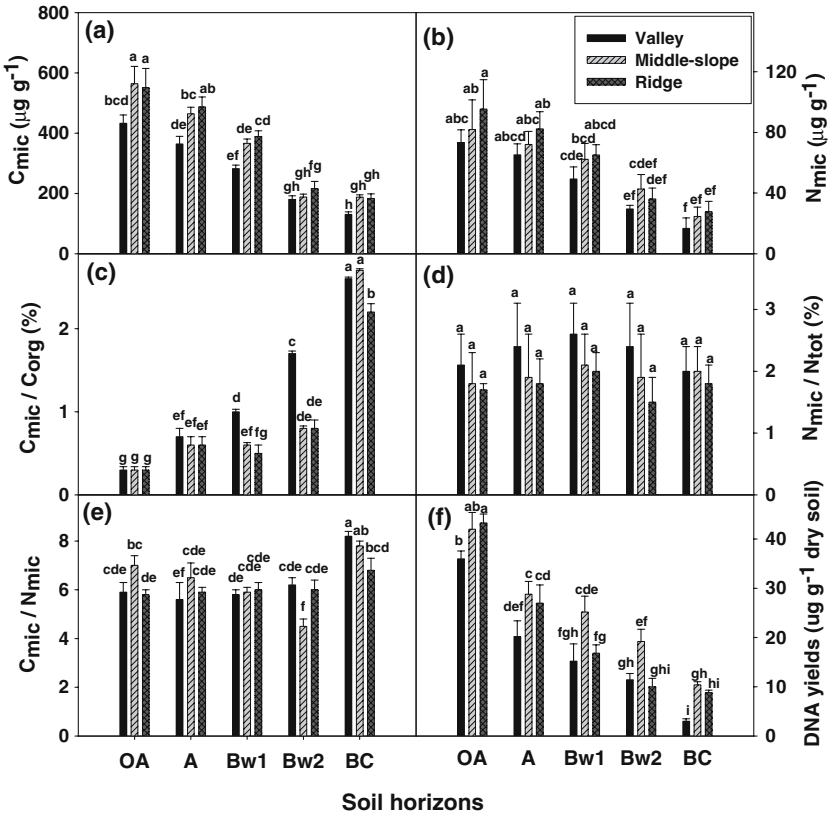

Fig. 3 DNA yield and microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) of the valley, middle slope, and ridge profiles in Fushan forest soils: **a** C_{mic}, **b** N_{mic}, **c** C_{mic}/total organic carbon (C_{org}), **d** N_{mic}/total nitrogen (N_{tot}), **e** C_{mic}/N_{mic}, and **f** DNA yield

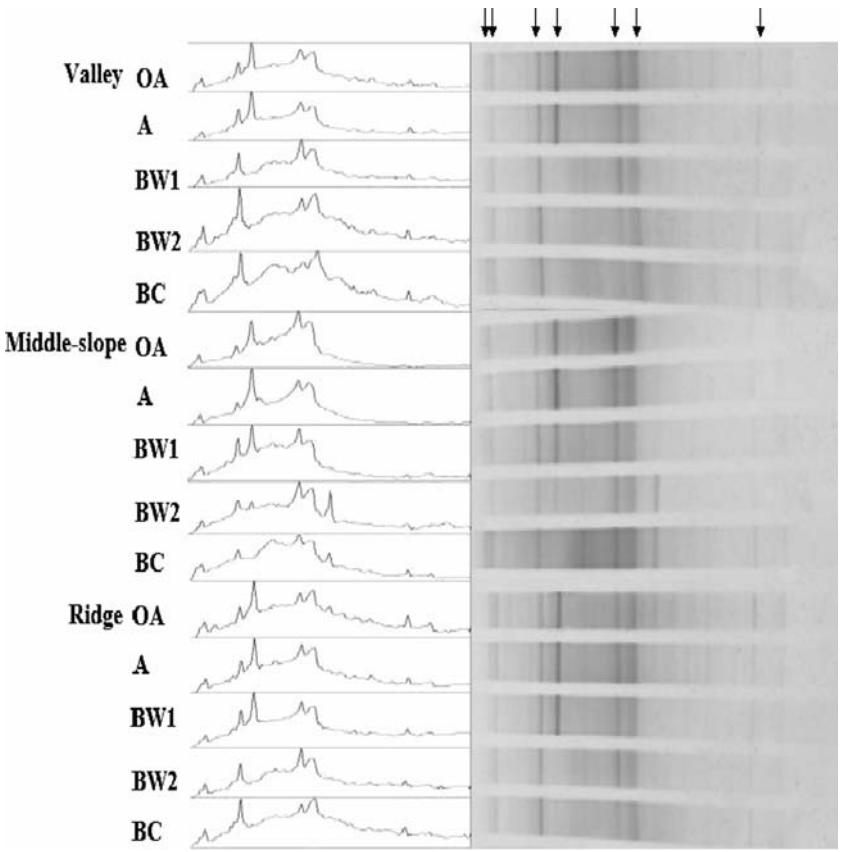

Fig. 4 Bacterial diversity as revealed by polymerase chain reaction—denaturing gradient gel electrophoresis (PCR-DGGE) profiles of soil samples from the valley, middle slope, and ridge sites

gradient through different layers. Together with these strong bands, a greater number of fainter, well-resolved bands appeared in the profiles, which were all considered when the clustering method was applied. A striking diversity was observed in DGGE patterns due to numerous faint bands, indicating that the structure of microbial communities was rather complex. Profiles of replicates were generally highly repetitive (data not shown). The Bray–Curtis cluster analysis was used to cluster data based upon band intensity. The similarity dendrogram (Fig. 5) clustered into two groups: one group comprising the ridge and valley sites and other one from middle-slope, except the BC layer of the middle slope.

Correlation analysis

Soil depth had a significant positive correlation with soil pH ($R > 0.94$) and a negative correlation with DNA yield ($R > -0.95$), C_{mic} ($R > -0.97$), N_{mic} ($R > -0.99$), C_{org} ($R > -0.91$), N_{tot} ($R > -0.99$), and C/N ratio ($R > -0.94$) in the three tested profiles (Table 2). There was significant positive correlation between DNA yield and microbial biomass (C_{mic} and N_{mic}) ($R > 0.93$) and DNA yield and microbial population ($R > 0.86$). The correlation between moisture content and DNA yield was significant in the ridge ($R = 0.95$) and valley ($R = 0.88$) sites but not in the middle slope. A high correlation was found between microbial biomass and C_{org} ($R > 0.86$) and between microbial biomass and N_{tot} contents ($R > 0.96$) (Table 2). Further, C_{mic} had high correlation with N_{mic} in all tested sites ($R > 0.95$).

Discussion

C_{org} and N_{tot} were high in the organic layer and decreased through the deeper horizons, which indicated that the major nutrient pool in Fushan forest was the organic layer. The ridge and the middle-slope soils had higher tree density and above-ground tree biomass than those of the valley (Lin et al. 2003a). The woody debris biomass of the ridge and the middle slope (36.1 Mg ha^{-1}) and the valley (8.5 Mg ha^{-1}) in Fushan forest (Lin et al. 2003a) were lower than those in the temperate forests (58–381 Mg ha^{-1}) (Carmona et al. 2002), and was similar to those in tropical forests (1.6–51.5 Mg ha^{-1}) (Delaney et al. 1998). There were large areas of canopy gaps in the valley patches, and the effective soil was shallow due to erosion, which led to reduce leaf litter and higher pH in the valley soils when compared with the middle slope and ridge soils. Diaz-Ravina et al. (1995) reported that lack of water seemed to limit the microbial biomass more than temperature did, since lower microbial biomass contents were observed in the dry period than in the wet period. Several studies reported a close relationship between soil moisture and microbial biomass (Chen et al. 2005; Devi and Yadava 2006). The moisture content had a positive correlation with microbial populations especially in the valley due to the topographic effect (Table 2). Low leaf littering in the valley site makes the soil vulnerable to erosion, and the moisture content also is less when compared with the other two sites, which also results in lower C_{org} and N_{tot} content in the valley soils. Moreover, the soil type is stony lithosols in the valley site, and stones of <2 mm size can also account for the comparatively lower microbial population and DNA recovery from this site. Since the correlation among different microbial populations and abiotic factors (C_{org} and N_{tot}) are consistent in the valley site, we recommend that this site would be appropriate to monitor environmental effects such as acid rain in the Fushan forest ecosystem. Further, if the forest ecosystem needs to be monitored by microbial populations as an indicator, the topographic variations should be appropriately considered.

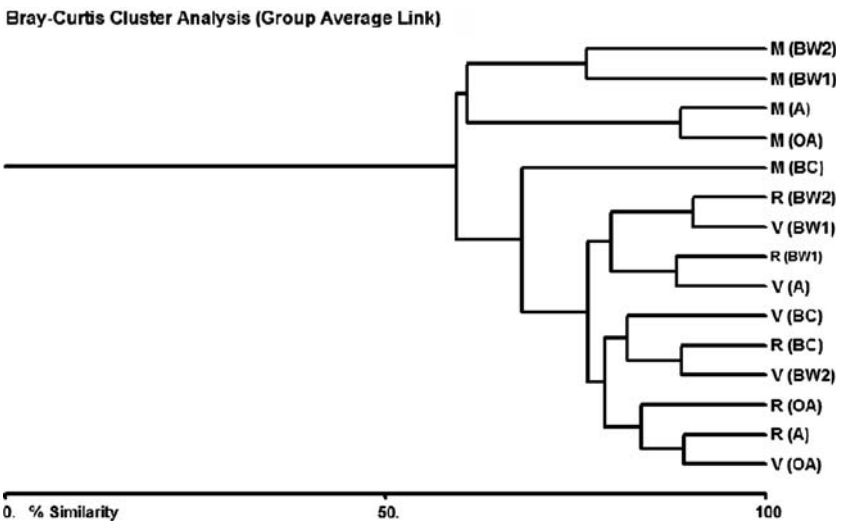

Fig. 5 Dendrogram indicating the relationships among soil samples according to polymerase chain reaction—denaturing gradient gel electrophoresis (PCR-DGGE) bacterial diversity profiles of soil samples from the valley, middle slope, and ridge sites. Soil sites were compared using Biodiversity Pro software. The tree was constructed using Bray–Curtis distance equation and group average linkage method. *V* valley, *M* middle-slope, *R* ridge

Table 2 Correlations between physical, chemical, and biological properties of the valley, middle slope and ridge soil profiles

Soil properties	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Valley														
Depth (A)														
pH (B)	**													
MC (C)	NS	NS												
C_{org} (D)	*	NS	*											
N_{tot} (E)	**	*	NS	**										
C/N (F)	*	NS	NS	*	*									
Bac (G)	NS	NS	**	**	NS	NS								
Act (H)	NS	NS	**	*	NS	NS	**							
Fungi (I)	NS	NS	**	**	*	*	**	*						
CMC (J)	NS	NS	**	*	NS	NS	**	**	*					
PSB (K)	NS	NS	**	**	NS	NS	**	**	**	**				
NFB (L)	NS	NS	**	**	*	*	**	**	**	**	**			
C_{mic} (M)	**	**	NS	*	**	*	NS	NS	NS	NS	NS	NS		
N_{mic} (N)	**	**	NS	NS	**	*	NS	NS	NS	NS	NS	NS	**	
DNA (O)	**	*	*	**	**	*	*	**	*	NS	*	**	*	*
Middle slope														
Depth (A)														
pH (B)	*													
MC (C)	NS	NS												
C_{org} (D)	**	NS	*											
N_{tot} (E)	**	*	NS	*										
C/N (F)	**	NS	*	**	**									
Bac (G)	NS	NS	**	*	NS	*								
Act (H)	NS	NS	**	*	NS	*	**							
Fungi (I)	*	NS	*	**	*	**	*	*						
CMC (J)	NS	NS	**	*	NS	*	**	**	*					
PSB (K)	*	NS	*	*	*	*	*	*	**	*				
NFB (L)	NS	NS	**	*	NS	*	**	**	*	**	NS			
C_{mic} (M)	**	NS	NS	*	**	*	NS	NS	*	NS	*	NS		
N_{mic} (N)	**	**	NS	*	**	*	NS	NS	*	NS	NS	NS	*	
DNA (O)	**	*	NS	**	**	**	*	*	**	NS	*	NS	*	*
Ridge														
Depth (A)														
pH (B)	**													
MC (C)	*	*												
C_{org} (D)	**	**	*											
N_{tot} (E)	**	**	**	**										
C/N (F)	**	**	*	*	**									
Bac (G)	NS	NS	NS	*	NS	NS								
Act (H)	*	*	*	**	**	*	*							
Fungi (I)	*	*	*	**	**	*	*	**						
CMC (J)	NS	NS	NS	NS	NS	NS	**	NS	*					
PSB (K)	*	NS	*	*	*	*	*	**	**	*				
NFB (L)	NS	NS	NS	*	NS	NS	**	**	**	**	**			
C_{mic} (M)	**	**	*	*	**	**	NS	*	*	NS	NS	NS		
N_{mic} (N)	**	**	*	*	**	**	NS	*	*	NS	NS	NS	**	
DNA (O)	*	*	*	**	**	*	*	**	**	NS	**	**	*	*

Linear regressions were used. $n = 15$, negative correlations are underlined
MC moisture content, C_{org} total organic C content, N_{tot} total nitrogen content, *Bac* bacteria, *Act* actinomyces, *CMC* cellulolytic microbes, *PSB* phosphate-solubilizing microbes, *NFB* nitrogen-fixing microbes, C_{mic} soil microbial biomass C, N_{mic} soil microbial biomass N, *DNA* DNA yield, *NS* not significant
*$P < 0.05$
**$P < 0.01$

Microbial populations were significantly high in the organic layers and decreased gradually through the deeper layers. Similar patterns in microbial abundance had been reported in the profiles of agricultural fields (Taylor et al. 2002) and the spruce profile of Tatachia Mountain (Yang et al. 2003). There is much leaf deposit on the surface of Fushan forest soil. The soil pH increased slightly with increasing depth from the OA horizon (0–15 cm) to the BC horizon (75–95 cm) due to abundant leaf litter on the surface, incomplete decomposition of the litter, and accumulation of organic acids (Yang et al. 2003). Berg et al. (1998) also reported that Wekerom forest soil in the Netherlands had a pH of 3.8 due to organic matter content. The differences in chemical composition of litter and root exudates would be expected to influence the availability of different carbon sources, which subsequently influenced the prevalence of microbes. C_{org} and N_{tot} also decreased

with increasing depth—from 13.25–17.62% and 0.38–0.57% in the OA horizon to 0.51–0.82% and 0.08–0.15% in the BC horizon, respectively. The differences among C_{org} contents of different layers are statistically significant, where as such, a clear distinction was not observed in N_{tot}.

The microbial populations of the BC horizon of the valley, middle slope and ridge sites were less than 10% of those in the OA horizon. Many studies using different methods—such as conventional microbial counts (Priha et al. 2001), respiration and ergosterol content (Imberger and Chiu 2001), microbial activity, respiration, biomass, and adenosine triphosphate (ATP) content (Lavahun et al. 1996)—have reported that the microbial populations in the organic layer were higher than those in the surface or subsurface layers. The low microbial populations in the BC horizon may be attributed to the limitation of organic matter, N_{tot}, and oxygen contents. The valley had the lowest microbial populations because of the lowest amount of organic matter and N_{tot} contents among the three tested locations. However, the differences in microbial populations of the valley site in comparison with other sites are not significant, except at the organic layer. Among these abiotic factors, the C_{org} correlates significantly with the microbial population, indicating its importance on that population.

The C_{mic}/N_{mic} ratio is often used to describe the structure and state of the microbial community. A high C_{mic}/N_{mic} ratio indicates that the microbial biomass contains a high proportion of fungi, whereas a low value suggests that bacteria predominate in the microbial population (Joergensen et al. 1995). Paul and Clark (1996) also indicated that bacteria had a C/N ratio as low as 3.5 and fungi had the values from 10 to 15. In this study, the C_{mic}/N_{mic} ratio of the valley, middle slope, and ridge were 5.6–8.2, 4.5–7.8, and 5.8–6.8, respectively. The differences in C_{mic}/N_{mic} ratios among different sites are not significant ($P < 0.05$), with a few exceptions that are not consistent. The bacterial population was significantly higher than the fungal population, which resulted in low C_{mic}/N_{mic} ratio, as reported by Joergensen et al. (1995). Moreover, the bacteria-to-fungi ratios of the valley, middle slope, and ridge were 46–143, 155–495, and 170–555, respectively. It showed that the valley had the lowest bacteria-to-fungi ratio, which resulted in high C_{mic}/N_{mic} ratio. Further, Joergensen et al. (1995) suggested that forest soil had comparatively low C and N availability and C_{mic}/N_{mic} ratio, which was lower than the optimum value (5–8). Similar results were shown in the valley, middle slope, and ridge sites of Fushan forest soils.

C_{mic} and N_{mic} also decreased with increasing soil depth and showed significant positive correlation with C_{org} and N_{tot}, respectively. Agnelli et al. (2004) reported that the C_{mic}/C_{org} ratio increased with soil depth and had the highest value in the BCb2 horizon. The same phenomenon was found in this study, which might be due to the low organic matter in the deep soil layer. Moore et al. (2000) reported that the N_{mic} showed more pronounced fluctuations than those of C_{mic} because the microorganisms differed much more in their nitrogen contents than in their carbon contents. Therefore, small shifts in the structure of the microbial community can result the large changes in N_{mic}. The C_{mic}/N_{mic} ratio was affected by soil properties such as moisture content, texture, and pH (Moore et al. 2000). In this study, the C_{mic}/N_{mic} ratio increased with soil depth (Fig. 3) in the valley and ridge sites, whereas a fluctuation was observed in the middle slope. The gradual increase of the C_{mic}/N_{mic} ratio may be due to the presence of microbes, which has low nitrogen content. The C_{mic} and N_{mic} of Fushan forest soils (130.5–564.1 and 16.7–95.4 μg g^{-1}, respectively) were similar to the tropical forest soil in India (341–872 and 38–78 μg g^{-1}, respectively) (Raghubanshi 1991) but lower than the spruce (438–2,018 and 43–210 μg g^{-1}, respectively) and hemlock (100–1,582 and 11–147 μg g^{-1}, respectively) soils in Tatachia Mountain of Taiwan (Yang et al. 2003, 2006) and the forest soil in Germany (317–2,116 and 30–347 μg g^{-1}, respectively) (Joergensen et al. 1995). The low value could be due to the relatively warm climate, which enhanced decomposition of soil organic matter and retained less microbial biomass.

C_{mic}/C_{org} ratios of the valley (0.3–2.6%), middle slope (0.3–2.7%), and ridge (0.3–2.2%) sites were lower than that of the tropical forest (1.5–5.3%) (Luizao et al. 1992) and the tropical wet evergreen forest (4–6%) (Barbhuiya et al. 2005) and similar to the subtropical humid forests (0.7–1.7%) (Maithani et al. 1996). However, N_{mic}/N_{tot} ratios of the valley (2.0–2.6%), middle slope (1.8–2.1%), and ridge (1.5–2.0%) sites were also lower than that of the forest soils (3.4–5.9%) (Martikainen and Palojarvi 1990) and the forest regrowths (7.3–8.3%) (Maithani et al. 1996) and similar to the tropical wet evergreen forest (1.3–1.7%) (Barbhuiya et al. 2005). This indicates low C and N immobilization as microbial biomass in Fushan forest soils. The dynamic nature of C and N circulation of forest floor and microbial populations is very important in nutrient conservation and regeneration in Fushan forest. Soil moisture had a strong influence on soil properties, and the dynamics of C_{mic} and N_{mic}. Similar relationship, i.e., a positive correlation ($R > 0.74$ and significant at $P < 0.001$) between moisture content and C_{mic} and N_{mic} was reported from the subtropical humid forest of northeast India (Arunachalam and Arunachalam 2000).

The 16S rDNA-DGGE analysis can be used to study changes in the structure of the microbial community (Fritze et al. 2000). The 16S rDNA-DGGE analysis showed the structure of the microbial community changed with increasing soil depth (Fig. 4). Comparatively more bands were observed in ridge soil DGGE profiles, implying higher diversity of the bacterial population. DGGE analysis had very complex banding patterns in the upper horizons, indicating a high diversity and density of microorganisms in contrast to less diversity in the deeper horizons. Numerically dominant

bacterial types in the upper layers (bands with high intensity) gradually decrease through the deeper horizons, and a few bacterial types prefer the deeper regions and exist in large numbers (high-intensity bands appeared in the BW2 and BC horizons of the middle slope), which may not be numerically dominant in the upper layers (Fig. 4). Cluster analysis of bacterial DGGE banding patterns in the three tested sites resulted in two groups: (1) middle slope and (2) ridge and valley samples (Fig. 5). Within each group, different layers, especially OA-A, formed different groups, suggesting that the microbial community in the deeper horizons (BW1-BW2-BC horizons) was not simply the diluted analogs of the surface soils and that some microbes dominate only in the deeper horizons. These results indicate that topography influenced both microbial populations and bacterial diversity.

In conclusion, the valley site had the lowest microbial populations (microbial biomass) among the three tested sites due to the low organic matter content, and these populations decreased with soil depth. The PCR-DGGE community profiles of three tested sites were very similar to each other in the major bands, and the variation was in the minor bands only. However, the pattern through gradient profiles was different, indicating the prevalence of specific microbes at different horizons. Topography influenced the quantity and diversity of microbial populations.

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