16S rRNA gene analyses of bacterial community structures in the soils of evergreen broad-leaved forests in south-west China

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Abstract

Bacterial community structure was studied in humus and mineral soils of evergreen broad-leaved forests in Ailaoshan and Xishuangbanna, representing subtropical and tropical ecosystems, respectively, in south-west China using sequence analysis and terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes. Clone sequences affiliated to *Acidobacteria* were retrieved as the predominant bacterial phylum in both forest soils, followed by those affiliated to members of the *Proteobacteria*, *Planctomycete* and *Verrucomicrobia*. Despite higher floristic richness at the Xishuangbanna forest than at the Ailaoshan forest, soil at Xishuangbanna harbored a distinctly high relative abundance of *Acidobacteria*-affiliated sequences (80% of the total clones), which led to a lower overall bacterial diversity than at Ailaoshan. Bacterial communities in humus and mineral soils of the two forests appeared to be well differentiated, based on 16S rRNA gene phylogeny, and correlations were found between the bacterial T-RFLP community patterns and the organic carbon and nutrient contents of the soil samples. The data reveal that *Acidobacteria* dominate soil bacterial communities in the evergreen broad-leaved forests studied here and suggest that bacterial diversity may be influenced by soil carbon and nutrient levels, but is not related to floristic richness along the climatic gradient from subtropical to tropical forests in south-west China.

Introduction

The use of rRNA for characterization of bacterial communities has established a new perspective on the biosphere (Pace, 1997). Molecular cloning and sequencing of the 16S rRNA gene has been used to investigate the bacterial composition in soils of various forest ecosystems, including oak, beech and pine forests (Axelrood *et al.*, 2002; Chow *et al.*, 2002; Hackl *et al.*, 2004). These studies provide detailed and valuable information about the composition of microbial communities, but the method is time consuming and expensive. Terminal restriction fragment length polymorphism (T-RFLP) is a DNA-based fingerprinting approach, which allows rapid comparison of bacterial community structure among soil samples (Liu *et al.*, 1997), and the technique has been applied to various forest soils (Dunbar *et al.*, 2000; Hackl *et al.*, 2004). However, culture-independent molecular studies focused on broad-leaved forests are lacking.

Tropical forests are often recognized to contain higher a biodiversity of flora and fauna than forests in subtropical regions, and the floristic richness has long been assumed to have a positive correlation with the bacterial diversity in the soil. This speculation is based on the interaction between plants and microorganisms (Grayston & Germida, 1991). Root exudates, which consist of labile organic carbon, act as a readily available substrate for the microbes in the soil, and shifts in bacterial communities correlated to root exudates have been reported (Marschner *et al.*, 2002; Baudoin *et al.*, 2003). Plant litter provides the main resources of nutrients and carbon input, which may also affect bacterial community structure (Rothstein *et al.*, 2004; Ruan *et al.*, 2004). Differences in the microbial communities in soils under various tree species have been reported (Priha *et al.*, 2001; Grayston & Prescott, 2005) but few studies have examined the correlation between plant diversity and soil bacterial community structure (but see Stephan *et al.*, 2000; Brodie

et al., 2002; Kowalchuk *et al.*, 2002), most investigations being limited to grassland ecosystems.

As a result of decomposition, plant-derived organic material is converted to humus. The humus layer of the forest soil contains higher organic carbon and nutrient content than the mineral soil below. Grayston & Prescott (2005) reported that carbon utilization profiles and microbial community structures were distinct among various layers of the forest soils in British Columbia, Canada. In addition, Priha *et al.* (2001) found that the microbial community in the humus layer correlated to tree species more closely than that in the mineral soil in Finland. Neither of these studies characterized microbial community structure at a fine taxonomic scale, and differences in bacterial communities in humus and mineral soils at the genus or species level remain unknown.

Evergreen broad-leaved forests are formed under a humid and warm climate, are distributed widely in south-west China (Qiu & Xie, 1998) and their soil bacterial communities have been studied using cultivation-based studies in the subtropical region of this area (Jiang & Xu, 1983; Sheng *et al.*, 1983). The total number bacteria in the humus layer was found to be an order of magnitude higher than in mineral soil, and the proportion of the anaerobic bacteria increased from 10% in the humus to 30% in the mineral soil layer (Jiang & Xu, 1983). In addition, Sheng *et al.* (1983) and Jiang & Xu (1983) cultured *Azotobacter* and actinomycetes, respectively, and their distributions with soil depth were investigated, but overall community structure was not studied.

In the present study, the bacterial community structures in the soils of two evergreen broad-leaved forests in the tropical and the subtropical regions of souh-west China were investigated using 16S rRNA gene clone sequencing and T-RFLP analyses, along with floristic richness, soil carbon and nutrient contents, total microbial biomass, live fine root biomass and soil fauna. The aim was to determine the dominant bacterial groups, to examine whether high floristic richness led to higher bacterial diversity in soil of the tropical forest, and to determine whether bacterial community structure correlated with carbon and nutrient content. To our knowledge, this is the first molecular study of bacterial community structure in evergreen broad-leaved forests.

Materials and methods

Study sites

The evergreen broad-leaved forest sites at Ailaoshan and Xishuangbanna in Yunnan Province, south-west China are approximately 400 km apart. The forest at Ailaoshan (24°N, 101°E) is in a subtropical region, and at an elevation of 2500 m; the site at Xishuangbanna (21°N, 101°E) has a tropical climate, and is at a lower attitude of 800 m. Annual mean temperature is 11.3 °C and 21.4 °C and annual precipitation 1931 and 1557 mm for Ailaoshan and Xishuangbanna, respectively (Qiu & Xie, 1998; Zhu, 2000). Both sites are research stations of the Chinese Ecosystem Research Network, which conducts routine monitoring of various terrestrial, aquatic and atmospheric parameters including climate data (http://www.cern.ac.cn:8080/index.jsp). Floristic characteristics at Xishuangbanna have been described by Zhu *et al.* (2006), with 36 tree species in 2500 m^2 (five 25×20-m^2 plots). At the Ailaoshan site, 30 tree species were identified in eight plots of $20 \times 20\,m^2$, according to the *Flora, Reipublicae Popularis Sinicae* (1997).

Soil sampling and handling

Samples were collected in July 2004. Humus layers were collected using a wooden frame (20×20 cm) and mineral soils were taken by core sampler (diameter: 5 cm, depth: 10 cm). Four samples collected at each site, sieved (2 mm mesh) and analysed for chemical composition, total microbial biomass and root biomass. Subsamples were stored immediately at −80 °C prior to nucleic acid analyses. Pooled soil samples of the four sampling points of equal weights were used for cloning and sequencing analyses. Because the humus and mineral soil layers at the Xishuangbanna site could not be clearly distinguished visually, a mixed sample of humus and mineral soil was analysed to avoid excessive redundant DNA sequencing. For T-RFLP analysis, the four independent replicates of humus and mineral soil samples from both study sites were analysed. In addition, floor mass was sampled by use of a wooden frame and used directly for soil fauna analysis.

Chemical analyses

Soil pH, water content, organic carbon content, total N, hydrolysable N, extractable NH_4^+-N and NO_3^--N, total P, extractable PO_4^--P, and exchangeable Na^+, K^+, Mg^{2+} and Ca^{2+} were analysed according to Forest Soil Analysis Methods (1999). After homogenization of the soil sample to a saturated colloid, pH was measured by a pH-meter (PHS-3C; Shanghai Precision & Scientific Instrument, Shanghai, China) and water content was determined by drying at 105 °C. Organic carbon content was analysed based on the chromic acid wet oxidation method (Walkley & Black, 1934). Total N was detected using an Auto Kjeldahl Unit model K370 (Buchi, Flawil, Switzerland; Jones & Bradshaw, 1989). Hydrolysable N was converted to ammonium by reaction with iron (II) sulfate and sodium hydroxide by a diffusion procedure. NH_4^+-N and NO_3^--N were determined using indophenol and cadmium reduction methods, respectively (Allen, 1989). Total P was determined

by digestion with hydrofluoric acid and perchloric acid, followed by inductively coupled plasma–atomic emission spectrometry (ICP-AES, model IRIS Advantage-ER, TJA, Franklin, MA, USA). Orthophosphate was measured by the molybdenum blue method (Allen, 1989). Exchangeable Na^+, K^+, Mg^{2+} and Ca^{2+} were equilibrium-extracted using ammonium acetate. Extracted Na^+ and K^+ were measured by an atomic absorption spectrophotometer (932AA; GBC Scientific Equipment, Dandenong, Australia) and Mg^{2+} and Ca^{2+} by ICP-AES. Each sampling point was measured in duplicate for all analyses.

Total microbial biomass, root biomass and soil fauna

Soil microbial biomass carbon was measured by a modified fumigation-incubation method based on the difference in CO_2 evolved from fumigated and control soils during incubation for 10 days (Jenkinson & Powlson, 1976; Liu & Zou, 2002). Biomass of live fine roots (diameter <2 mm) in the humus and mineral soil layers at Ailaoshan was measured following Janssens *et al.* (2002). Live fine roots were picked from the bulk soil sample, dried at 70 °C for 24 h and weighed. Soil fauna, mesomicroarthropods, in the floor mass of the forests was separated from the soil by use of a Tullgren funnel and heating to 60 °C (Yang, 2004). The number of individuals was counted and arthropods were classified according to Yin (1992, 1998).

DNA extraction and PCR amplification

Total nucleic acids were extracted using an Ultra High Purity DNA Isolation Kit for Soil (MoBio Laboratories, Solana Beach, CA) following the manufacturer's instructions. Nearly full-length 16S rRNA gene sequences of the bacterial domain were amplified by PCR using the primer set Bact8f–Prok1492r (Amann *et al.*, 1995) (Sangon, Shanghai, China) for cloning and sequencing analyses. For T-RFLP analysis, the bacterial 16S rRNA gene was amplified by Bact8f (5′ end labeled with FAM fluorescent dye) and Bact912r (Liu *et al.*, 1997).

Cloning, sequencing and phylogenetic analyses

PCR products were purified using a DNA agarose gel extraction kit (BioAsia, Shanghai, China), and the purified bacterial amplicons were cloned using the TOPO TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Clone sequences were determined using an ABI 3730 sequencer (Applied Biosystems, Foster City, CA) at BioAsia Biotechnology Ltd, Shanghai, China. Close relatives and phylogenetic affiliation of the sequences were checked using the BLAST search program (Altschul *et al.*, 1997) at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Sequences were manually aligned to the closest relative provided by NCBI using Multicolor Sequence Alignment Editor (Hepperle, 2002) and were submitted to the CHECK_CHIMERA program (Maidak *et al.*, 2001) in the Ribosomal Database Project (RDP) to exclude chimeric artifacts. Phylogenetic trees were constructed based on 100-fold bootstrap analysis using the neighbor-joining, parsimony and maximum-likelihood algorithms with the Phylogenetic Inference Package (PHYLIP) version 3.6 (Felsenstein, 2005). To estimate bacterial diversity, clone sequences were grouped into operational taxonomic units (OTUs). The clone sequences generated were deposited in the GenBank database under accession nos. AY963298–AY963516.

T-RFLP analysis

For T-RFLP analysis, amplicons were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and digested by *Hha*I (Amersham Biosciences, Freiburg, Germany) for 4 h at 37 °C. DNA was precipitated by 75% isopropanol and DNA pellets were washed, dried and suspended in high-performance liquid chromatography water. GeneScan™-1000 ROX™ Size Standard (AB Applied Biosystems, Warrington, UK) was added as an internal lane standard and fluorescence labeled terminal restriction fragments (T-RFs) were size-separated by an automatic sequencer (ABI 3100 Genetic Analyzer; AB Applied Biosystems) equipped with a POP6 polymer-filled capillary under denaturing conditions. T-RFLP electropherograms were analysed by peak area integration of the T-RFs using GeneScan 2.1 software (AB Applied Biosystems). The fragment length of a T-RF was determined by comparison with the internal standard, and the position of the T-RF was indicated as the average of all runs with the corresponding peak. Representative clone samples were analysed by T-RFLP as references. The relative abundance of a single T-RF was calculated as the percentage fluorescence intensity calculated relative to the total fluorescence intensity of all well-resolved peaks with area >1000 fluorescence units or $>2\%$ of the maximum peak of an electropherogram. The relative intensities of T-RFs from individual samples were subjected to principal component analysis (PCA) (Multi Variate Statistic Package, MVSP version 3.1, Kovach Computing Services, Pentraeth, Anglesey, UK) to elucidate major variations in pattern (Blackwood *et al.*, 2003). The scores of the first two components were subsequently used to compare differences between the T-RFLP fingerprint patterns with differences between soil chemical characteristics by calculating Spearman's correlation coefficients ($P \leq 0.05$) (SPSS version 13.0). The average relative intensity of T-RFs of the four independent replicates were subjected to cluster analysis

based on Euclidean distances measured with the unweighted pair-group method arithmetic mean, UPGMA (Multi Variate Statistic Package, MVSP version 3.1, Kovach Computing Services).

Diversity indices

Diversity was indicated using the Shannon–Weaver index (H') (Shannon & Weaver, 1963) and the Simpson index (D) (Simpson, 1949)

$$H' = -\sum_{i=1}^{i=n} p_i \ln p_i$$

$$D = \sum_{i=1}^{i=n} {p_i}^2$$

For estimation of the soil fauna diversity, n is the number of species and p_i the relative abundance of the ith species. For bacterial diversity, n is the number of OTUs or T-RFs, and p_i is the percentage of clones of the ith OTU or the relative abundance of the ith T-RF. For convenience, the Simpson index is usually expressed as $1/D$.

Results

Soil chemical characteristics

In the Ailaoshan evergreen broad-leaved forest site, the humus layer was approximately 7–12 cm deep and could be visually distinguished from the mineral soil layer, the latter being lighter in color. By contrast, the amount of humus at the Xishuangbanna forest site was low (<1 cm deep) and its appearance was less distinct. Both soils were acidic (pH 4.2–4.5, Table 1), and organic carbon content, nutrient content and exchangeable cations were higher in the humus layer than in the mineral soil. The Ailaoshan forest soils had higher organic carbon and nutrients, including total N, hydrolysable N, extractable NH_4^+-N and NO_3^--N, and total *P*, than the soils at Xishuangbanna.

Total microbial biomass, root biomass and soil fauna

Total microbial biomass in the humus layer at the Ailaoshan site ($4.33 \pm 1.64\,g\,C\,kg^{-1}$ dried soil) was approximately double that in the mineral soil ($2.16 \pm 0.88\,g\,C\,kg^{-1}$ dried soil), which was more than threefold that in the mineral soil at Xishuangbanna ($0.63 \pm 0.17\,g\,C\,kg^{-1}$ dried soil). Data on microbial biomass in the humus layer at Xishuangbanna were not obtained. The live fine root biomass in the humus layer of the Ailaoshan forest site ($36.16 \pm 19.53\,g\,m^{-2}$) was more than three times that in the mineral soil ($10.40 \pm 6.04\,g\,m^{-2}$). The Shannon–Weaver index (H') for soil fauna (mesomicroarthropods) in the floor mass at Ailaoshan (0.85 ± 0.21) was less than that at Xishuangbanna (1.06 ± 0.19), suggesting higher arthropod diversity in the latter. However, the abundance of soil fauna at Ailaoshan ($36.24 \pm 29.85\,g^{-1}$ dried weight) was considerably greater than at Xishuangbanna ($8.47 \pm 4.83\,g^{-1}$ dried weight).

Bacterial community structures assessed by cloning and sequencing

Two clone libraries comprising 72 and 69 nearly full-length 16S rRNA gene sequences were obtained, respectively, from

Table 1. Chemical characteristics of the soils of the evergreen broad-leaved forests at the Ailaoshan and Xishuangbanna study sites

				Nitrogen content				Phosphorus content		exchangeable cations			
	pH	Water content (%)	Organic carbon ($g\,C\,kg^{-1}$)	Total N ($g\,N\,kg^{-1}$)	Hydrolysable N ($mg\,N\,kg^{-1}$)	Extractable NH_4^+-N ($mg\,N\,kg^{-1}$)	Extractable NO_3^--N ($mg\,N\,kg^{-1}$)	Total P ($g\,P\,kg^{-1}$)	Extractable P ($mg\,P\,kg^{-1}$)	Na^+ ($mg\,kg^{-1}$)	K^+ ($mg\,kg^{-1}$)	Mg^{2+} ($mg\,kg^{-1}$)	Ca^{2+} ($mg\,kg^{-1}$)
Ailaoshan subtropical forest site													
Humus	4.5 ± 0.1	71.9 ± 3.8	303.8 ± 57.0	18.38 ± 2.99	820 ± 104	37.03 ± 18.16	5.19 ± 2.08	1.18 ± 0.10	9.00 ± 2.19	0.25 ± 0.04	0.96 ± 0.07	2.45 ± 0.96	5.43 ± 4.72
Mineral soil	4.2 ± 0.1	57.7 ± 3.5	116.1 ± 14.3	6.94 ± 0.98	505 ± 53	10.38 ± 4.27	3.36 ± 0.96	0.82 ± 0.14	< 0.05	0.19 ± 0.02	0.22 ± 0.07	0.70 ± 0.06	0.59 ± 0.20
Xishuangbanna tropical forest site													
Humus	—	—	76.8 ± 24.2	4.49 ± 1.64	334 ± 60	4.00 ± 0.4	1.40 ± 0.59	0.34 ± 0.08	8.14 ± 8.17	0.18 ± 0.04	0.77 ± 0.33	1.45 ± 0.76	2.65 ± 1.16
Mineral soil	4.5 ± 0.1	50.0 ± 3.5	23.9 ± 2.3	1.64 ± 0.28	157 ± 26	1.46 ± 0.97	< 0.05	0.26 ± 0.04	0.23 ± 0.23	0.13 ± 0.01	0.18 ± 0.02	0.25 ± 0.11	0.64 ± 0.41

Values are means and standard deviations of four sampling points. Values for organic carbon, nitrogen content, phosphorus content and exchangeable cations are expressed in dry weight of the soil sample. pH and water content of the humus at Xishuangbanna were not measured.

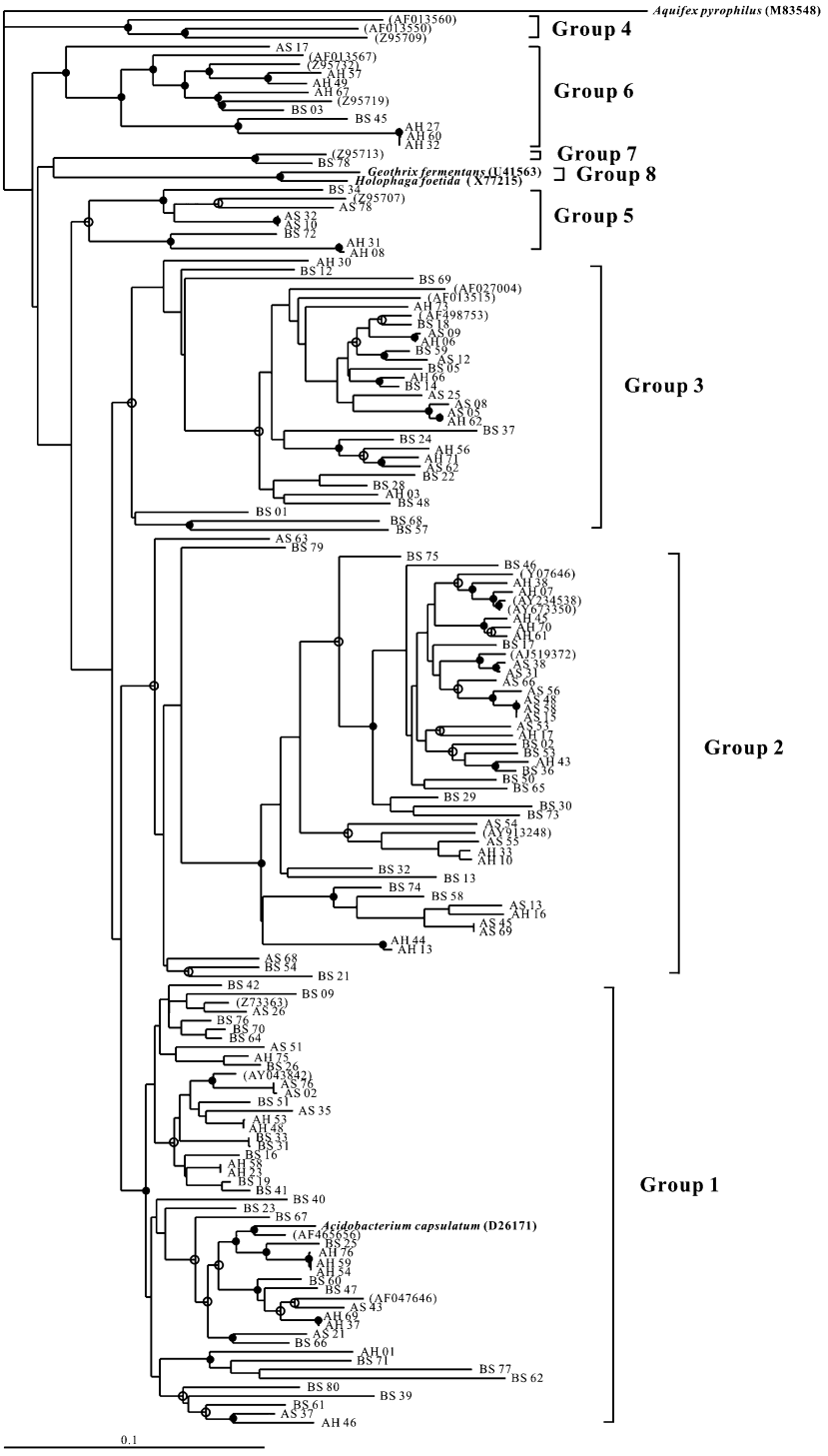

Fig. 1. Phylogenetic relationship of the nearly full-length 16S rRNA gene sequences (~1500 bases) in the phylum *Acidobacteria* retrieved in this study. Classification of the eight subdivisions is according to Hugenholtz *et al*. (2003). The sequences denoted as AH and AS represent the humus and the mineral soil of the evergreen broad-leaved forest at the Ailaoshan study site, respectively, and the sequences denoted as BS represents the mixed soil sample at the Xishuangbanna study site. Phylogenetic trees were constructed based on 100-fold bootstrap analyses using the neighbor-joining, parsimony and maximum-likelihood algorithms with the PHYLIP program. Filled and open circles at the nodes represent bootstrap values >80% and >50%, respectively. The tree is rooted with the rRNA gene sequences of *Aquifex pyrophilus* (M83548). The scale bar indicates 10% sequence divergence.

the humus and mineral soil layers at Ailaoshan and 78 clones were obtained from the mixed soil sample at Xishuangbanna. Among these 219 clone sequences, 135 were affiliated to the phylum *Acidobacteria*, and further clustered into five subdivisions, groups 1, 2, 3, 5 and 6 according to the classification of Hugenholtz *et al*. (1998) (Fig. 1). The majority of sequences were affiliated to groups 1–3, and group 6 sequences were found mainly in the humus layer. Twenty-two sequences belonged to two clusters within the *Gammaproteobacteria* (Fig. 2). Most of the sequences in one

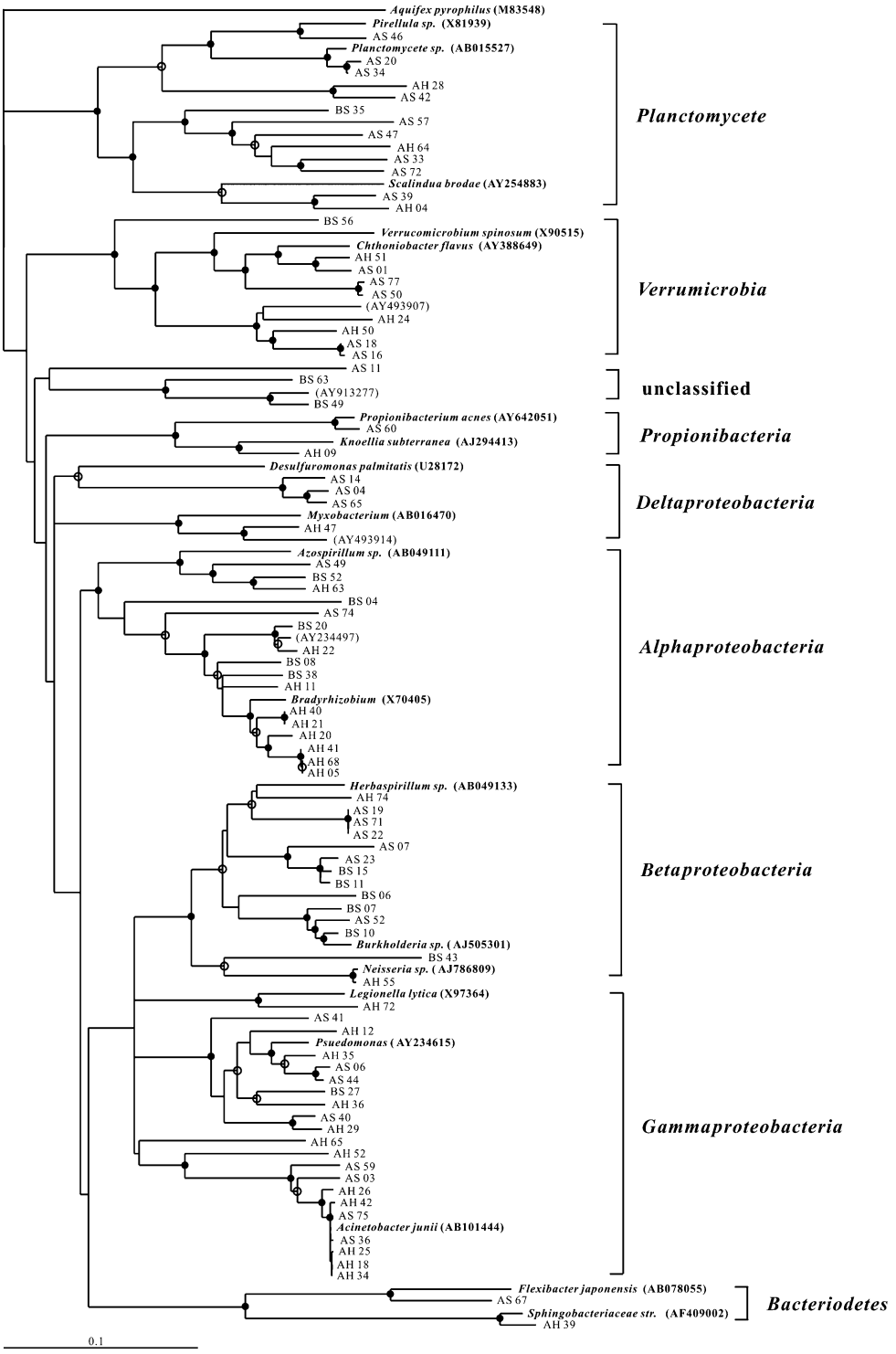

Fig. 2. Phylogenetic relationship of the nearly full-length (~1500 bases) 16S rRNA gene sequences, except for the phylum *Acidobacteria*, retrieved in this study. Specification of the phylogenetic tree is as described for Fig. 1.

of these clusters were related to *Pseudomonas* and seven were closely affiliated to *Acinetobacter* (>99% sequence similarity). Sixteen sequences belonged to the *Alphaproteobacteria*, of which nine sequences from the Ailaoshan humus layer and two from the Xishuangbanna mixed soil were affiliated to the phylum *Bradyrhizobium*. Most other sequences fell within the *Betaproteobacteria*, *Deltaproteobacteria*, *Planctomycete* and *Verrucomicrobia*. The *Acidobacteria* group

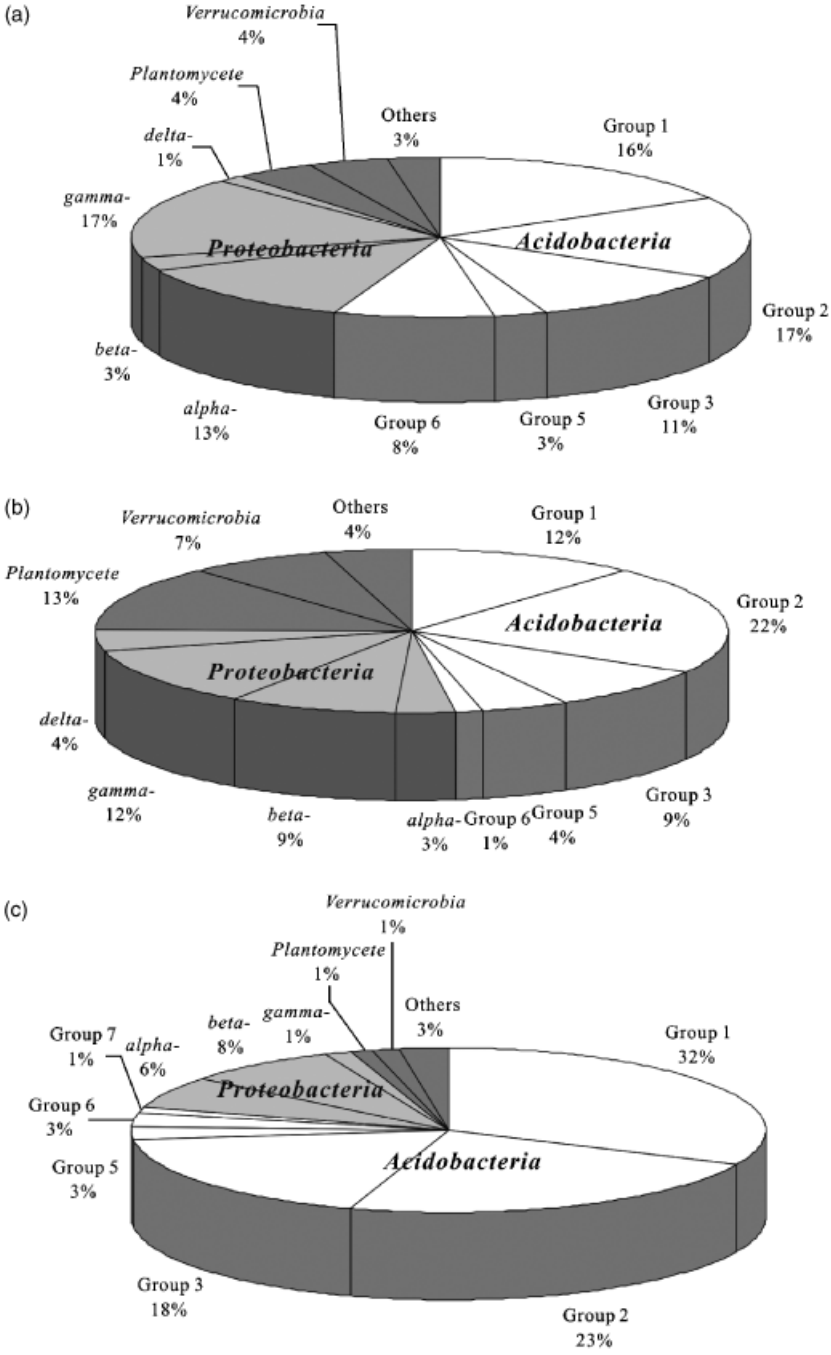

Fig. 3. Distribution of various taxa obtained from the bacterial 16S rRNA gene partial clone sequences retrieved from (a) the humus layer at Ailaoshan, (b) the mineral soil at Ailaoshan and (c) the mixed soil sample at Xishuangbanna.

constituted almost 80% of the clone sequences retrieved from Xishuangbanna and 56% and 48% of sequences from the Ailaoshan humus and mineral soil layers, respectively (Fig. 3). The proportion of sequences from humus at Ailaoshan (13%) was considerably higher than that in the mineral soil, owing to the higher abundance of *Bradyrhizobium*-affiliated sequences in the former. The relative abundances of *Gammaproteobacteria* in Ailaoshan humus and mineral soil layers were higher than in Xishuangbanna. Each subdivision of the *Acidobacteria* and each taxon was identified as an OTU for estimating diversity. The Shannon–Weaver indices (H') for Ailaoshan humus and mineral soil layers were 2.2 and 2.3, respectively, compared with 1.9 for Xishuangbanna. Simpson indices ($1/D$) were 8.2 and 8.5 for Ailaoshan humus and mineral soil layers, respectively, and 5.4 for Xishuangbanna. These values

indicated that the soil in the evergreen broad-leaved forest at the Ailaoshan experimental site exhibited a higher bacterial diversity than at Xishuangbanna.

Bacterial community structures as revealed by T-RFLP

A representative T-RFLP fingerprint electropherogram retrieved from the humus layer sample of the evergreen broad-leaved forest at Ailaoshan study site is illustrated in Fig. 4. Eight peaks were identified as major T-RFs, which exhibited relative intensities of $>2\%$. Unresolved peaks were observed, which were not used in calculating the relative abundance of fragments. T-RFs with fragment lengths of 35, 61, 90, 182 and 283 bases were dominant. T-RFs of 35, 60, 182 and 283 bases showed identical positions to clones AS15, AS43, AH23 and BS31, respectively (data not shown), which were affiliated to *Acidobacteria*. However, clones belonging to other bacterial phyla could generate the same fragment lengths, and thus definite attributes for these T-RFs could not be assigned. The relative abundances of the major T-RFs observed in the T-RFLP bacterial fingerprint patterns derived from the humus and mineral soil layers at Ailaoshan and Xishuangbanna are summarized in Fig. 5. In addition to the dominant T-RFs, 20–30 well-resolved peaks were detected and evaluated, which contributed to $\leq 10\%$ of the total fluorescence intensity. Samples from Xishuangbanna exhibited higher relative abundances of the 35- and 61-base T-RFs and a lower relative abundance of the 292-base T-RF compared with samples from Ailaoshan. Reproducibility of the bacterial 16S rRNA gene community structures among the independent replicate soil samples reflected by the T-RFLP analysis supported characterization of the community retrieved from the cloning and sequencing analysis, which was based on only a single mixed sample. PCA of T-RF data revealed that the four soil samples separated from each other along the first and second principal components (PCs), which together described 84% of the total variance (Fig. 6). Significant correlations were found between the PCs and soil organic carbon (PC 1: $r=-0.52$, $P=0.039$; PC 2: $r=0.60$, $P=0.015$), total N (PC 1: $r=-0.52$, $P=0.039$; PC 2: $r=0.65$, $P=0.0061$), hydrolysable N (PC 1: $r=-0.57$, $P=0.020$; PC 2: $r=0.64$, $P=0.0082$), extractable NH_4^+-N (PC 1: $r=-0.56$, $P=0.025$; PC 2: $r=0.53$, $P=0.035$) and total P (PC 1: $r=-0.61$, $P=0.012$; PC 2: $r=0.56$, $P=0.025$), and between PC 2 and water content ($r=0.54$, $P=0.050$) and microbial biomass ($r=0.59$, $P=0.042$). Cluster analysis of T-RFLP patterns showed that the two soil layers from Ailaoshan formed one cluster and the two layers from

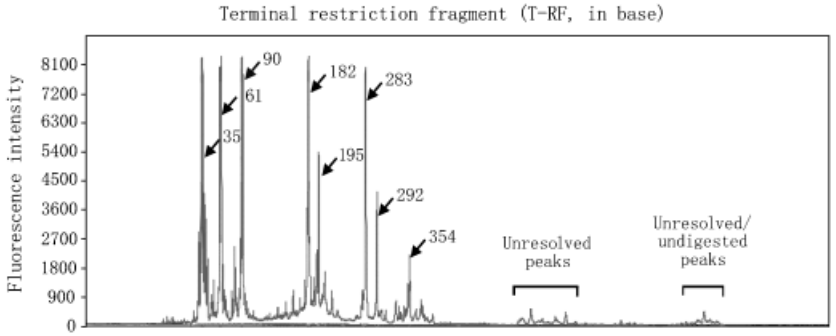

Fig. 4. A representative bacterial 16S rRNA gene terminal restriction fragment length polymorphism (T-RFLP) fingerprint electropherogram of four independent replicates derived from the humus layer sample of the evergreen broad-leaved forest at the Ailaoshan study site.

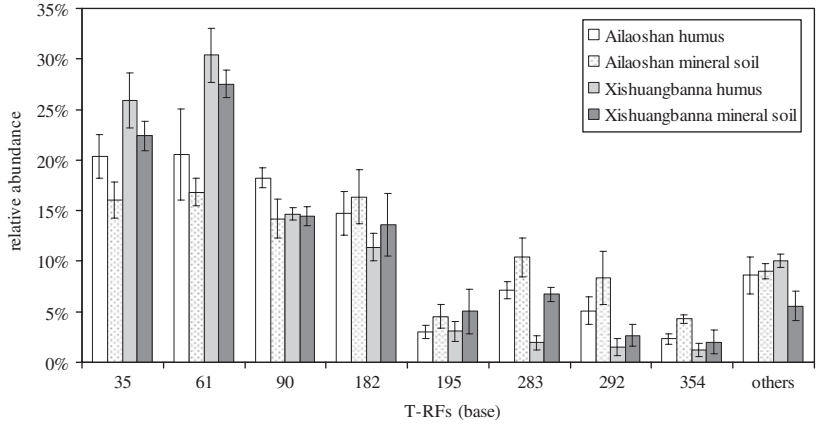

Fig. 5. Relative abundances of the dominant terminal restriction fragments (T-RFs) of the bacterial 16S rRNA gene terminal restriction fragment length polymorphism (T-RFLP) fingerprint patterns derived from the humus and the mineral soil layers of the evergreen broad-leaved forests at the Ailaoshan and Xishuangbanna study sites. Error bars represent standard deviations of four independent replicates.

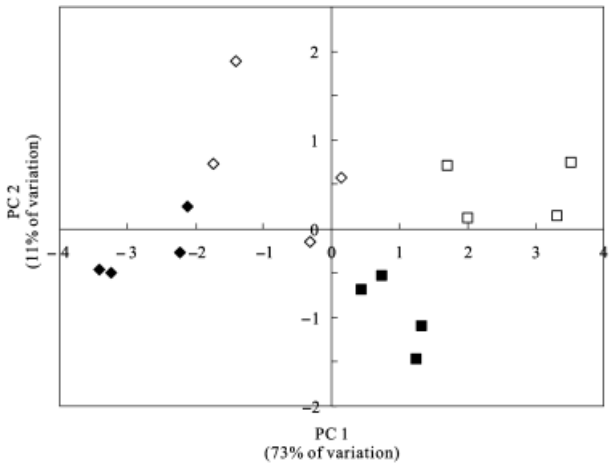

Fig. 6. Principal component analysis of terminal restriction fragments (T-RFs) data retrieved from individual samples. Open and closed diamonds denote humus and mineral soil of the evergreen broad-leaved forest at the Ailaoshan study site, respectively; and open and closed squares humus and mineral soil at the Xishuangbanna study site, respectively.

Xishuangbanna formed another (data not shown). This revealed that similarity of the bacterial community structures between the humus and the mineral soil layers was higher than between the two forest sites. H' values for Ailaoshan humus and mineral soil layers calculated from the T-RFLP patterns were 2.03 ± 0.02 and 2.18 ± 0.04, respectively, and for Xishuangbanna soils were 1.82 ± 0.10 and 1.96 ± 0.03, respectively. Simpson indices were 6.45 ± 0.27 and 7.80 ± 0.38, respectively, for Ailaoshan humus and mineral soil layers, and 4.95 ± 0.52 and 5.67 ± 0.18, respectively, for Xishuangbanna humus and mineral soil layers. As for cloning results, this reflected higher bacterial diversity in the forest soil at Ailaoshan than at Xishuangbanna.

Discussion

Bacterial community structure

A distinctly high relative abundance of *Acidobacteria*-affiliated clone sequences, comprising up to 80% of the total clones from the Xishuangbanna study site, was retrieved from the soils of the evergreen broad-leaved forests compared with other forest soils reported. For various pine forest soils in British Columbia reported by Axelrood *et al.* (2002) and Chow *et al.* (2002), *Proteobacteria* contributed to approximately 50% of the total clone library, and *Acidobacteria*-affiliated clone sequences comprised only 10–20% of the total bacterial community. Hackl *et al.* (2004) reported that the *Acidobacteria* group comprised 12% of the total clones derived from pine forest soils and 28–35% from oak–hornbean and spruce–beech forests. Among the 50 rRNA gene clones retrieved from soil of the rain forest in the Amazon region by Borneman & Triplett (1997), only eight clone sequences were related to *Acidobacteria*. Although PCR artifacts may cause biases (von Wintzingerode *et al.*, 1997) and the relative proportions of different bacterial groups represented in clone libraries may not reflect the relative proportions present in template DNA samples (Becker *et al.*, 2000), the 16S rRNA gene clone libraries derived from Ailaoshan and Xishuangbanna reflected a particularly high proportion of *Acidobacteria*-affiliated clone sequences in the soils of the evergreen broad-leaved forests under study.

Acidobacteria is a recently recognized bacterial division covering a broad phylogenetic spectrum with eight monophyletic subdivisions (Hugenholtz *et al.*, 1998). *Acidobacteria*-affiliated sequences have been retrieved from a wide variety of environments (Barns *et al.*, 1999), including extreme environments such as hot springs and deep-sea sediments (Lopez-Garcia *et al.*, 2003). To date, there are only three well-characterized representatives: *Acidobacterium capsulatum* (group 1), and *Geothrix fermentans* and *Holophaga foetida* (group 8). *Acidobacterium capsulatum* is a moderately acidophilic aerobic heterotroph (Hiraishi *et al.*, 1995) and the mild acidity of the forest soils studied could thus account for the high relative abundance of *Acidobacteria*. Subdivision 8 did not cluster with the clone sequences retrieved from forest soil samples (Hugenholtz *et al.*, 1998; Barns *et al.*, 1999), including those derived from forest soils at Ailaoshan and Xishuangbanna in the present study. Although group 4 is represented by numerous environmental sequences (Hugenholtz *et al.*, 1998), no clone sequences retrieved from Ailaoshan and Xishuangbanna affiliated to this phylum. Subdivision 6 was found in the humus layer but not the mineral soil in this study, which suggests that it might exhibit different ecological significance, such as substrate and nutrient versatility or rhizosphere association. *Acidobacteria* groups 1–3 may favor lower carbon and nutrient conditions, explaining their higher relative abundance in the soil at Xishuangbanna than at Ailaoshan. Recently, a number of isolates of subdivisions 1–4 have been cultivated (Joseph *et al.*, 2003) but their physiological characteristics and potential ecological role in the environment are still unknown.

The relative abundance of 16S rRNA gene sequences that were closely related to *Bradyrhizobium* was higher in the humus layer at Ailaoshan than in the mineral soil, which could correlate with the live fine root biomass. *Bradyrhizobium* is a well-known root-associated bacterial species and its presence indicates the ecological significance of symbiotic nitrogen-fixation in the rhizosphere (Dupuy *et al.*, 1994). Although *Burkholderia* and *Pseudomonas* were also common residents of the rhizosphere, there were no differences in their relative abundances in the two soil layers, indicating

that these bacterial groups may not be restricted to the rhizosphere and exist in significant proportions in the bulk soil of the forest studied. *Verrucomicrobia–Planctomycete* is also a recently described bacterial taxon found in diverse habitats (Ward *et al.*, 1995; Hugenholtz *et al.*, 1998; Buckley & Schmidt, 2001). The most well-studied species in the *Verrucomicrobia* lineage, *Verrucomicrobia spinosum*, is a heterotrophic, prosthecate bacteria originally isolated from freshwater (Schlesner, 1987). More recently, Buckley & Schmidt (2001) investigated the environmental factors influencing the *Verrucomicrobia* 16S rRNA gene distribution in soils, and found that abundance correlated with soil moisture. Planctomycetes are known to oxidize ammonium under anaerobic or anoxic conditions in wastewater treatment plants (Jetten *et al.*, 2005) and in marine and freshwater habitats (Jetten *et al.*, 2003; Risgaard-Petersen *et al.*, 2004), but no related study has been carried out in soils. Members of the *Verrucomicrobia–Planctomycete* may be anaerobic bacteria, explaining their higher relative abundance in the mineral soil than the humus layer above. The water content in the soil at Ailaoshan was high, especially during the rainy season between May and October, which facilitated the existence of anaerobic microenvironments within the soil matrix. Approximately 10% and 30% of the total bacteria in the humus and mineral soil layers, respectively, were reported by Sheng *et al.* (1983) to grow in anaerobic consortia.

It is surprising that a significant number of clone sequences (6%) closely related to *Acinetobacter* were retrieved from Ailaoshan forest soils. This bacterial genus was not reported in forest soils (Axelrood *et al.*, 2002; Chow *et al.*, 2002; Hackl *et al.*, 2004), but is recognized from oil-contaminated environments and wastewater treatment plants, exhibiting an ability to degrade refractory and toxic compounds, including hydrocarbons (Vanbroekhoven *et al.*, 2004), phenolics (Vasudevan & Mahadevan, 1992) and lignin (Lopretti *et al.*, 1993), and it is believed to have phosphorous removal capability in activated sludge systems (Seviour *et al.*, 2003). Sheng *et al.* (1983) and Jiang & Xu (1983) cultured *Azotobacteria* and *Actinomycetes* from the forest soil at Ailaoshan, but sequences affiliated to these bacterial genera were not retrieved in this study, suggesting that they may not be dominant in the overall community.

Relationship between floristic and bacterial diversity

Surprisingly, bacterial diversity revealed by clone sequence and T-RFLP analyses of 16S rRNA genes in the tropical evergreen broad-leaved forest soil at Xishuangbanna was not higher than that of the subtropical forest at Ailaoshan, despite a greater floristic richness and higher rate of leaf litter decomposition rate (Liu *et al.*, 2000) at the former. The amount of humus in the forest at Xishuangbanna was low, which could due to the higher temperature and thus higher turnover rate. However, the higher floristic richness and decomposition rate did not correlate with higher bacterial diversity and higher microbial biomass at Xishuangbanna. Although estimation of the diversity based on the phyla and the limited number of T-RFs may not reflect actual bacterial diversity (Dunbar *et al.*, 2000), the data indicate that Xishuangbanna forest soil harbored a particularly high relative abundance of *Acidobacteria*-affiliated 16S rRNA gene sequences, resulting in a lower overall bacterial diversity than that in Ailaoshan soil. A broader investigation would be essential to understand the relationship between floristic and bacterial diversity, but our data revealed that higher floristic richness of the tropical forest did not necessarily correlate with higher soil bacterial diversity. In a survey of grassland ecosystems, Stephan *et al.* (2000) demonstrated that plant species richness had a positive influence on bacterial diversity, but Brodie *et al.* (2002) reported an inverse correlation across a floristic gradient and suggested that soil chemical characteristics are as influential in determining bacterial diversity as floristic richness. Soil pH, water content and cation exchange capacity of the two forests were similar, but organic carbon and nutrient (notably N) contents and total microbial diversity in Xishuangbanna soils were lower than in Ailaoshan soils. Substrate and nutrient availability appeared to be a factor accounting for differences in bacterial community structures between the two sites. Soil fauna at Ailaoshan was more abundant than that at Xishuangbanna, and their excretions may have increased organic carbon and nutrient input to the soil.

Significant correlations were found between organic carbon and nutrient content and forest soil bacterial community structure through PCA of T-RFLP patterns. Grayston & Prescott (2005) reported that the microbial community structures were distinct among various layers of the forest soils in British Columbia, and were related to tree species and nitrogen availability. Deposition of anthropogenic nitrogen was reported to alter microbial communities in forest soils by Compton *et al.* (2004) and Sinsabaugh *et al.* (2004). In the soils of the natural forests in Austria, Hackl *et al.* (2004) reported that the bacterial communities were well differentiated according to the vegetation and soil chemical properties, including C/N ratio. These studies demonstrate correlations between bacterial community structures and organic carbon and nutrient contents in forest soils. However, soil bacterial community structure is determined by a wide range of environmental factors. The physical characteristics of the soils studied here, including water content, texture and porosity, have been recognized to affect bacterial community structure (Juma, 1993; Sessitsch *et al.*, 2001; Chiu *et al.*, 2006). Biotic factors,

such as grazing by soil fauna (Mamilov *et al*., 2001) and protozoa (Jjemba, 2001; Ronn *et al*., 2002), as well as viral infection (Weinbauer, 2004), have also been reported to influence soil bacterial community. In the present study, carbon and nutrient content was found to correlate with bacterial structure in the soils of evergreen broad-leaved forests, but an effect of other determinants could not be excluded.

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References

Allen SE (1989) *Chemical Analysis of Ecological Material*, 2nd edn. Blackwell Scientific Publications, Oxford, UK.

Altschul SF, Madden TL, Shaffer AA, Zhang JH, Zhang Z, Miller W & Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Amann RI, Ludwig W & Schleifer K-H (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* **59**: 143–169.

Axelrood PE, Chow ML, Radomski CC, McDermott JM & Davies J (2002) Molecular characterization of bacterial diversity from British Columbia forest soils subjected to disturbance. *Can J Microbiol* **48**: 655–674.

Barns SM, Takala SL & Kuske CR (1999) Wide distribution and diversity of members of the bacterial kingdom *Acidobacterium* in the environment. *Appl Environ Microbiol* **65**: 1731–1737.

Baudoin E, Benizri E & Guckert A (2003) Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biol Biochem* **35**: 1183–1192.

Becker S, Boger P, Oehlmann R & Ernst A (2000) PCR bias in ecological analysis: a case study for quantitative Taq nuclease assays in analyses of microbial communities. *Appl Environ Microbiol* **66**: 4945–4953.

Blackwood CB, Marsh T, Kim S-H & Paul EA (2003) Terminal restriction fragment length polymorphism data analysis for quantitative comparison of microbial communities. *Appl Environ Microbiol* **69**: 926–932.

Borneman J & Triplett EW (1997) Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl Environ Microbiol* **63**: 2647–2653.

Brodie E, Edwards S & Clipson N (2002) Bacterial community dynamics across a floristic gradient in a temperate upland grassland ecosystem. *Microb Ecol* **44**: 260–270.

Buckley DH & Schmidt TM (2001) Environmental factors influencing the distribution of rRNA from *Verrucomicrobia* in soil. *FEMS Microbiol Ecol* **35**: 105–112.

Chiu CY, Chen TH, Imberger K & Tian GL (2006) Particle size fractionation of fungal and bacterial biomass in subalpine grassland and forest soils. *Geoderma* **130**: 265–271.

Chow ML, Radomski CC, McDermott JM, Davies J & Axelrood PE (2002) Molecular characterization of bacterial diversity in Lodgepole pine (*Pinus contorta*) rhizosphere soils from British Columbia forest soils differing in disturbance and geographic source. *FEMS Microbiol Ecol* **42**: 347–357.

Compton JE, Watrud LS, Porteous LA & DeGrood S (2004) Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. *Forest Ecol Manage* **196**: 143–158.

Dunbar J, Ticknor LO & Kuske CR (2000) Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. *Appl Environ Microbiol* **66**: 2943–2950.

Dupuy N, Willems A, Pot B, Dewettinck D, Vandenbruaene I, Maestrojuan G, Dreyfus B, Kersters K, Collins MD & Gillis M (1994) Phenotypic and genotypic characterization of *Bradyrhizobia* nodulating the leguminous tree *Acacia albida*. *Int J Syst Bacteriol* **44**: 461–473.

Felsenstein J (2005) *PHYLIP (Phylogeny Inference Package) version 3.6*. Distributed by the author. Department of Genome Sciences and Department of Biology, University of Washington. Seattle, WA.

Flora, Reipublicae Popularis Sinicae (1997) *(Delectis Florae Reipublicae Popularis Sinicae*, Agendae Academiae Sinicae Edita, ed). Science Press, Beijing.

Forest Soil Analysis Methods (1999) LY/T 1210∼1275-1999. (State Forestry Administration, P.R. China, ed). Standard Press of China, Beijing.

Grayston SJ & Germida JJ (1991) Sulfur-oxidizing bacteria as plant-growth promoting Rhizobacteria for Canola. *Can J Microbiol* **37**: 521–529.

Grayston SJ & Prescott CE (2005) Microbial communities in forest floors under four tree species in coastal British Columbia. *Soil Biol Biochem* **37**: 1157–1167.

Hackl E, Zechmeister-Boltenstern S, Bodrossy L & Sessitsch A (2004) Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. *Appl Environ Microbiol* **70**: 5057–5065.

Hepperle D (2002) *Multicolor sequence alignment editor*. Distributed by the author available from: http://science.do-mix.de/.

Hiraishi A, Kishimoto N, Kosako Y, Wakao N & Tano T (1995) Phylogenetic position of the menaquinone-containing acidophilic chemo-organotroph *Acidobacterium capsulatum*. *FEMS Microbiol Lett* **132**: 91–94.

Hugenholtz P, Goebel BM & Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* **180**: 4765–4774.

Janssens IA, Sampson DA, Curiel-Yuste J, Carrara A & Ceulemans R (2002) The carbon cost of fine root turnover in a Scots pine forest. *Forest Ecol Manage* **168**: 231–240.

Jenkinson DS & Powlson DS (1976) The effects of biocidal treatment on metabolism in soil-V. A method for measuring soil biomass. *Soil Biol Biochem* **8**: 209–213.

Jetten MSM, Sliekers O, Kuypers M, *et al.* (2003) Anaerobic ammonium oxidation by marine and freshwater planctomycete-like bacteria. *Appl Microbiol Biot* **63**: 107–114.

Jetten MSM, Cirpus I, Kartal B, *et al.* (2005) 1994–2004: 10 years of research on the anaerobic oxidation of ammonium. *Biochem Soc T* **33** (Part 1): 119–123.

Jiang CL & Xu LH (1983) A Study on the population of soil Actinomycetes in Ailao Mts. *Research of the Forest Ecosystem on Ailao Mountains, Yunnan* (Wu ZY, Qu ZX & Jiang HQ, eds), pp. 376–384. Yunnan Science and Technology Press, Yunnan.

Jjemba PK (2001) The interaction of protozoa with their potential prey bacteria in the rhizosphere. *J Eukaryot Microbiol* **48**: 320–324.

Jones M & Bradshaw D (1989) Copper: an alternative to mercury; more effective than zirconium in Kjeldahl digestion of ecological materials. *Commun Soil Sci Plant Anal* **20**: 1513–1524.

Joseph SJ, Hugenholtz P, Sangwan P, Osborne CA & Janssen PH (2003) Laboratory cultivation of widespread and previously uncultured soil bacteria. *Appl Environ Microbiol* **69**: 7210–7215.

Juma NG (1993) Interrelationships between soil structure texture, soil biota, soil organic matter and crop production. *Geoderma* **57**: 3–30.

Kowalchuk GA, Buma DS, de Boer W, Klinkhamer PGL & van Veen JA (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Anton Leeuw Int J G* **81**: 509–520.

Liu ZG & Zou XM (2002) Exotic earthworms accelerate plant litter decomposition in a Puerto Rican pasture and wet forest. *Ecol Appl* **12**: 1406–1417.

Liu WT, Marsh TL, Cheng H & Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol* **63**: 4516–4522.

Liu WY, Fox JED & Xu ZF (2000) Leaf litter decomposition of canopy trees, bamboo and moss in a montane moist evergreen broad-leaved forest on Ailao Mountain, Yunnan, south-west China. *Ecol Res* **15**: 435–447.

Lopez-Garcia P, Duperron S, Philippot P, Foriel J, Susini J & Moreira D (2003) Bacterial diversity in hydrothermal sediment and epsilon proteobacterial dominance in experimental microcolonizers at the Mid-Atlantic Ridge. *Environ Microbiol* **5**: 961–976.

Lopretti MI, Mathias AL & Rodrigues AE (1993) *Acinetobacter anitratus* and the degradation of *Pinus pinaste*r lignin. *Process Biochem* **28**: 543–547.

Maidak BL, Cole JR, Lilburn TG, Parker CT, Saxman PR, Farris RJ, Garrity GM, Olsen GJ, Schmidt TM & Tiedje JM (2001) The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **1**: 173–174.

Mamilov AS, Byzov BA, Zvyagintsev DG & Dilly OM (2001) Predation on fungal and bacterial biomass in a soddy-podzolic soil amended with starch, wheat straw and alfalfa meal. *Appl Soil Ecol* **16**: 131–139.

Marschner P, Neumann G, Kania A, Weiskopf L & Lieberei R (2002) Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (*Lupinus albus* L.). *Plant Soil* **246**: 167–174.

Pace NR (1997) A molecular view of microbial diversity and the biosphere. *Science* **276**: 734–740.

Priha O, Grayston SJ, Hiukka R, Pennanen T & Smolander A (2001) Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. *Biol Fert Soils* **33**: 17–24.

Qiu XZ & Xie SC (1998) Physical geography. *Studies on the Forest Ecosystem in Ailao Mountains Yunnan, China*, pp. 1–5. Yunnan Science and Technology Press, Yunnan.

Risgaard-Petersen N, Meyer RL, Schmid M, Jetten MSM, Enrich-Prast A, Rysgaard S & Revsbech NP (2004) Anaerobic ammonium oxidation in an estuarine sediment. *Aquat Microb Ecol* **36**: 293–304.

Ronn R, McCaig AE, Griffiths BS & Prosser JI (2002) Impact of protozoan grazing on bacterial community structure in soil microcosms. *Appl Environ Microbiol* **68**: 6094–6105.

Rothstein DE, Vitousek PM & Simmons BL (2004) An exotic tree alters decomposition and nutrient cycling in a Hawaiian montane forest. *Ecosystems* **7**: 805–814.

Ruan HH, Zou XM, Scatena E & Zimmerman JK (2004) Asynchronous fluctuation of soil microbial biomass and plant litterfall in a tropical wet forest. *Plant soil* **260**: 147–154.

Schlesner H (1987) *Verrucomicrobium spinosum* new genus new species a fimbriated prosthecate bacterium. *Syst Appl Microbiol* **10**: 54–56.

Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H & Kandeler E (2001) Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Appl Environ Microbiol* **67**: 4215–4224.

Seviour RJ, Mino T & Onuki M (2003) The microbiology of biological phosphorus removal in activated sludge systems. *FEMS Microbiol Rev* **27**: 99–127.

Shannon CE & Weaver W (1963) *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.

Sheng LL, Guo GY, Pu WM, Zhang CS, Cheng YT, Su R & Yang S (1983) Vertical distribution of bacteria in the soil of the subtropical humid evergreen broad-leaved forests on the northern part of Ailao Mts. *Research of the Forest Ecosystem on*

Ailao Mountains, Yunnan (Wu ZY, Qu ZX & Jiang HQ, eds), pp. 369–375. Yunnan Science and Technology Press, Yunnan.

Simpson EH (1949) Measurement of diversity. *Nature* **163**: 688.

Sinsabaugh RL, Zak DR, Gallo M, Lauber C & Amonette R (2004) Nitrogen deposition and dissolved organic carbon production in northern temperate forests. *Soil Biol Biochem* **36**: 1509–1515.

Stephan A, Meyer AH & Schmid B (2000) Plant diversity affects culturable soil bacteria in experimental grassland communities. *J Ecol* **88**: 988–998.

Vanbroekhoven K, Ryngaert A, Wattiau P, De Mot R & Springael D (2004) Acinetobacter diversity in environmental samples assessed by 16S rRNA gene PCR-DGGE fingerprinting. *FEMS Microbiol Ecol* **50**: 37–50.

Vasudevan N & Mahadevan A (1992) Utilization of complex phenolic compounds by Acinetobacter sp. *Appl Microbiol Biot* **37**: 404–407.

von Wintzingerode F, Goebel UB & Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* **21**: 213–229.

Walkley A & Black IA (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci* **37**: 29–38.

Ward N, Rainey FA, Stackebrandt E & Schlesner H (1995) Unraveling the extent of diversity within the order Planctomycetales. *Appl Environ Microbiol* **61**: 2270–2275.

Weinbauer MG (2004) Ecology of prokaryotic viruses. *FEMS Microbiol Rev* **28**: 127–181.

Yang XD (2004) Dynamics and community structure of soil meso-microarthropods during leaf litter decomposition in tropical seasonal rain forests of Xishuangbanna, Yunnan. *Biodiversity Sci* **12**: 252–261.

Yin WY (1992) *Subtropical Soil Animals of China*. Science Press, Beijing.

Yin WY (1998) *Pictorial Keys to Soil Animals of China*. Science Press, Beijing.

Zhu H (2000) General geography. *Ecology and Biogeography of the Tropical Dipterocarp Rain Forest in Xishuangbanna*, pp. 1–4. Yunnan Science and Technology Press, Yunnan.

Zhu H, Cao M & Hu HB (2006) Flora and vegetation of Xishuangbanna, southern Yunnan. *Biotropica* **38**: 310–317.