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Seasonal variation of microbial populations and biomass in Tatachia grassland soils of Taiwan

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Abstract To investigate the seasonal variations of microbial ecology in grassland of Tatachia forest, soil properties, microbial populations, microbial biomass, and 16S rDNA clone library analysis were determined. The soil had temperatures 6.6–18.4°C, pH 3.6–5.1, total organic carbon 1.11–10.68%, total nitrogen 0.18–0.78%, and C/N ratios 3.46–20.55. Each gram of dry soil contained bacteria, actinomycetes, fungi, cellulolytic, phosphate-solubilizing microbes, and nitrogen-fixing microbes 4.54×10^4 to 3.79×10^7, 3.43×10^2 to 2.17×10^5, 5.74×10^3 to 3.76×10^6, 1.97×10^3 to 1.34×10^6, 8.49×10^2 to 5.59×10^5, and 3.86×10^2 to 3.75×10^5 CFU, respectively. Each gram of soil contained 117–2,482 µg of microbial biomass carbon, 23–216 µg of microbial biomass nitrogen and 9–29 µg of DNA. The microbial populations, microbial biomass, and DNA decreased stepwise with the depth of soil, and they had low values in winter seasons. The microbial populations, microbial biomass carbon, microbial biomass nitrogen, and DNA at the BW2 horizon were 8.42–17.84, 19.26–64.40, 16.84–61.11, and 31.03–46.26% of those at the O horizon, respectively. When analyzing 16S rDNA library, members of Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, candidate division TM1, candidate division TM7, Gammatimonadetes, and Verrucomicrobia were identified. Members of Proteobacteria (44.4%) and Acidobacteria (33.3%) dominated the clone libraries. Within the phylum Proteobacteria, α-, β-, and γ-Proteobacteria were most numerous, followed by δ-Proteobacteria.

Introduction

Forest soil microorganisms and fauna strongly influence the nutrient cycling of ecosystem through regulating the dynamics of organic matter decomposition and plant nutrient availability (Nannipieri et al. 2003). The numbers and species of microbes in soil vary instantly with environmental conditions, nutrient availability, soil texture, and type of vegetation cover (Hedlund 2002; Allen and Schlesinger 2004). Forest soils have very high diversity of prokaryotes compared with other habitats (Torsvik et al. 1996). The complex structure of soil results in the isolation of organisms and partitioning of resources at different substructures of the soil environment, and in relation

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to gradients of soil chemical properties and microenvironments (Zhou et al. 2002; Treves et al. 2003). For microorganisms, local species richness is tightly linked to regional (and even global) species richness, and the environment simply selects which organisms are active (Finlay 2002). The diversity of soil microorganisms is high; it is less clear how that diversity relates to the functioning of grassland ecosystems.

In Taiwan, the microbial distributions of soils had only been determined in acidic soil of Yang-Ming-San, inorganic acidic soil, upland soil, alkaline soil of Tainan and power plant areas (Yang et al. 1998b, 1999; Yang and Yang 2001). There are few data in the microbial populations of forest soils (Yang et al. 1998a, 2003, 2006; Imberger and Chiu 2001; Tsai et al. 2007). Tatachia mountain is located in the central part of Taiwan and is a typical high altitude ecosystem protected area, designated as a National Park. There are three major vegetations: grass, spruce, and hemlock in this temperate area. The present study aimed to use the parameters of microbial populations, biomass, and DNA as index of the environmental impacts in Tatachia grassland soils. To understand the diversity of bacteria in the grassland soil, three clone libraries of 16S rDNA were constructed and the phylogenetic affiliation of these sequences is reported. The effects of season and depth on these parameters are also included in this article.

Materials and methods

Site description

The study was conducted at Tatachia, in the saddle of Jade Mountain, central Taiwan (120°52′ E, 23°28′ N). Tatachia has an elevation from 1,800 to 3,952 m. Some environmental conditions and properties of the tested soils are listed in Fig. 1. The area has been selected as a representative long-term ecological study site of sub-alpine forest ecosystems in Taiwan. The study area consists geologically of metamorphosed sedimentary rock (Miocene epoch), comprising sandstone and shale. Dwarf bamboo (Yushania niitakayamensis) and alpine silver grass (Miscanthus transmorrisonensis) are the dominant vegetation. The annual precipitation of Tatachia grassland was between 2,800 and 3,000 mm.

Soil sampling

Soil samples were collected at Tatachia grassland in organic layer, topsoil (0–20 cm depth) and subsoil (21–40 cm depth). The samples were sieved to 2 mm, homogenized, and stored at 4°C for further studies. Soil DNA was extracted immediately when the soils were received in the laboratory.

Cultural media and conditions

Bacteria were counted at 25°C for 5 days on nutrient agar at pH 6.8 ± 0.1. Actinomycetes were cultivated at 25°C for 7 days on glycerol-yeast extract medium 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 (Yang and Yang 2001). Fungi were grown at 25°C for 5 days on Rose Bengal medium at pH 6.8 ± 0.1 (Yang et al. 1998a). Cellulolytic microbes were assayed at 25°C after 7 days incubation on Mandels–Reese medium with carboxymethylcellulose (CMC, Sigma) as the sole carbon source and sprayed with Congo red to show a clear zone around the colonies (Mandels et al. 1981). Phosphate-solubilizing microbes were counted at 25°C after 5 days on rock phosphate medium at pH 6.6 ± 0.1 by the clear zone around the colonies (Chang et al. 2001). Nitrogen-fixing microbes were characterized at 25°C after 7 days incubation on nitrogen-free mannitol medium (Yang et al. 2003). All the experiments were carried out in triplicates.

Microbial biomass carbon and nitrogen

Microbial biomass carbon and nitrogen were determined by the chloroform fumigation extraction method (Brookes et al. 1985; Vance et al. 1987). Fresh soils at 40% water-holding capacity were fumigated with chloroform (ethanol removed) for 72 h. A non-fumigated incubation (without chloroform) was conducted as the control. Soil extracts 8.0 ml and 0.066 M $K_2Cr_2O_7$ 2 ml, HgO 70 mg, conc. H_2SO_4 10.0 ml and 85% H_3PO_4 5.0 ml were mixed thoroughly. The mixture was 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 an indicator. For microbial biomass

Fig. 1 Properties of grassland soil in Tatachia. (**a**) Environmental conditions, (**b**) pH, (**c**) Moisture content, (**d**) Total organic carbon (TOC), (**e**) Total nitrogen (TN), (**f**) C/N ratio. ■ air temperature, □ soil temperature, ◆ light intensity. ● organic layer, ○ topsoil, ▼ subsoil. Vertical bars represent standard deviations ($n = 9$)
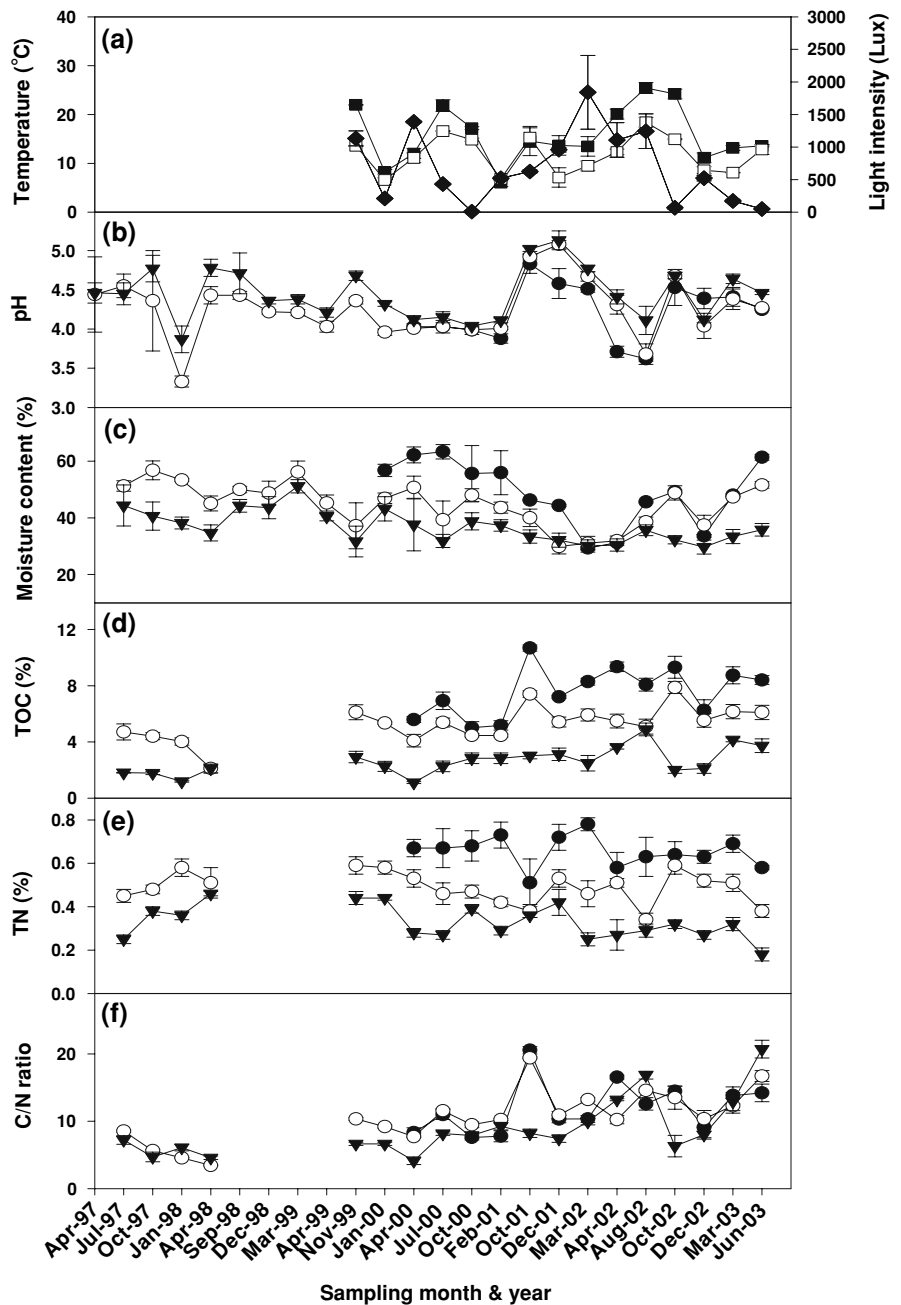
nitrogen, the digested filtrate was distilled by steam distillation with a modified Kjeldahl method (Yang et al. 1991), and titrated against 0.01 N HCl. Microbial biomass carbon (B_C) was calculated according to the equation: $B_C = E_C/K_{EC}$, where E_C is the difference between extractable C from fumigated and non-fumigated samples and $K_{EC} = 0.45$ (Wu et al. 1990). The microbial biomass nitrogen (B_N) was calculated

as $B_N = E_N/K_{EN}$, where E_N is the difference between extractable N from fumigated and non-fumigated samples and $K_{EN} = 0.54$ (Brookes et al. 1985).

DNA extraction

Genomic DNA of the soil was extracted following a modified protocol of Krsek and Wellington (1999). Soil

DNA was extracted with Crombach buffer containing proteinase K at 37°C for 30 min. Sodium dodecyl sulfate (SDS) was added and the samples were incubated at 65°C for 120 min. The supernatant was collected after centrifugation at 4,500 × g for 5 min. Extraction was repeated twice using extraction buffer and 20% SDS, and incubated at 65°C for 10 min. After centrifugation, supernatants were subjected to potassium acetate and polyethyleneglycol precipitation, phenol/chloroform/iso-amylalcohol purification, isopropanol precipitation, and spermine-HCl precipitation. The crude DNA was purified by Gene-Spin™ 1-4-3 DNA Extraction Kit (Protech Technology Enterprise Ltd, Taiwan) and stored at –20°C.

Effect of soil profile on microbial populations, biomass, and DNA

Soil profiles were taken from the grassland and classified into horizons according to the International Soil Classification System (Soil Survey Staff 2003). Soil samples were collected in each horizon from the top of soil to 100 cm depth. The microbial populations, biomass, and DNA at each horizon were measured.

PCR amplification of 16S rDNA

Bacterial 16S rDNAs were amplified by PCR using the primers 10f (5′-AGTTT GATCCTGGCTCAG-3′) and 1507r (5′-TACCTTGTTACGACTTCACCCCA-3′). *Escherichia coli* numbering positions of the primers are 10–27 and 1507–1485, respectively (Heyndrickx et al. 1996). The 50 µl reaction solution containing 25 pmol of each primer, 200 µM of each dNTPs, 1× PCR buffer (Protech, with MgCl$_2$), and 1.5 U Pro *Taq* DNA polymerase was combined with 1 µl DNA. The PCR was performed with a Applied Biosystems 2720 Thermal Cycler (Foster City, CA, USA) with the following reaction conditions: 94°C for 5 min, followed by 35 cycles at 95°C for 1 min, 55°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were examined by electrophoresis on 1× TAE agarose gel (2.0% w/v) with 100 bp DNA ladder (Promega, Madison, WI, USA) as marker to confirm the size and the approximate quantity of generated amplicons.

Construction and analysis of clone libraries

The PCR products of 16S rRNA genes were completely loaded onto a 2% low melting agarose gel. The band with expected size of approximately 1.5 kb was cut and purified with a Gel Extraction Kit (Qiagen, CA, USA), and subsequently ligated into pGEM-T Vector Systems (Promega). The ligation product was transformed into competent *E. coli* JM109 cells and the clones were isolated by blue–white screening with IPTG (isopropyl-β-D-thio-galactopyranoside) and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside). White colonies were plated on LB agar containing 100 µg/ml ampicillin. Approximately 40 clones were randomly selected to represent each of three composite forest soil samples.

Sequencing and phylogenetic analysis

The 120 transformants were sequenced (Mission Biotech., Taiwan) using the primer SP6, and 45 unique bacterial sequences were obtained. The close relatives and phylogenetic affiliation of the sequences obtained were checked using the BLAST search program at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). Sequences were also submitted to the Chimera Check program of RDP for checking the chimeric artifacts.

Chemical analysis

Moisture content was determined by drying the sample at 105°C overnight to a constant weight. The pH was measured in 5 times volume of distilled water equilibrated with soil for 1 h with a pH meter. Air and soil temperatures were determined on site directly or under 5 cm depth of soil with a thermometer. Total nitrogen was measured by a modified Kjeldahl method (Yang et al. 1991). Total organic carbon was determined as Nelson and Sommers (1982) described. Experiments were carried out in triplicates and statistical analysis of the results was performed using analysis of variance and Duncan's multiple range tests ($p = .05$) with the help of the Statistical Analysis System (SAS Institute 2002).

Results

Environmental conditions

Annual average air temperature is around 12°C, and most months have cool weather. During the test period, January and February had low temperature (6.0–8.3°C), while August and October had high temperature (24.2–25.4°C) (Fig. 1). The soil temperature in winter seasons (6.6–8.6°C) was higher than those of air temperature and it was reverse in summer seasons (12.8–18.4°C) due to the high heat capacity of soil. The soil temperature is one of the major factors, that affects the microbial populations and the nutrient cycling in grassland soils.

Soil properties

The grassland soils of Tatachia is sandy loam and acidic. The pH of organic layer was the lowest, topsoil was the next, and subsoil was the highest (Fig. 1). The moisture content, total organic carbon and total nitrogen of organic layer were the highest, while subsoil was the lowest. The C/N ratios of organic layer, topsoil, and subsoil were 7.62–20.55, 3.46–19.42, and 4.14–20.72, respectively.

Bacterial populations

The bacterial populations of grassland soils ranged from $(1.29 \pm 0.31) \times 10^6$ to $(3.79 \times 1.01) \times 10^7$ CFU/g organic layer, from $(1.31 \pm 1.05) \times 10^5$ to $(6.02 \pm 2.14) \times 10^6$ CFU/g topsoil and from $(4.54 \pm 0.18) \times 10^4$ to $(5.32 \pm 0.91) \times 10^6$ CFU/g subsoil (Fig. 2a). Most of organic layer and topsoil samples had higher bacterial populations than those of subsoil. The bacterial populations in summer seasons (July–September) were usually higher values than those in winter seasons (December–February). Statistical analysis revealed that 45.4% of the samples exhibited significant differences between topsoil and subsoil.

Actinomycete populations

Actinomycetes are known to have a diverse set of enzymes to degrade complex compounds for energy and biomass production. Actinomycete populations were between $(1.48 \pm 0.54) \times 10^3$ and $(2.17 \pm 0.56) \times 10^5$ CFU/g organic layer, between $(2.38 \pm 0.83) \times 10^2$ and $(1.26 \pm 0.13) \times 10^5$ CFU/g topsoil and between $(3.43 \pm 1.01) \times 10^2$ and $(7.93 \pm 0.41) \times 10^4$ CFU/g subsoil (Fig. 2b). Organic layer and topsoil samples had higher populations than those of subsoil. All tested soils in summer seasons had higher values than those in winter seasons. From the statistical analysis, it was also found that 36.9% of actinomycete populations had significant differences between topsoil and subsoil samples.

Fungal populations

The pH of grassland soil was acidic, which is favorable for fungal propagation. The fungal populations were high in summer seasons [$(1.02 \pm 0.45) \times 10^4$–$(4.49 \pm 1.11) \times 10^5$ CFU/g] and low in winter seasons [$(5.98 \pm 0.17) \times 10^3$–$(6.82 \pm 0.72) \times 10^5$ CFU/g] (Fig. 2c). The populations were also high in organic layer and topsoil. From the statistical analysis, it showed that 45.5% of topsoil samples had significantly higher populations than those in subsoil.

Cellulolytic, phosphate-solubilizing, and nitrogen-fixing microbes

Cellulolytic, phosphate-solubilizing, and nitrogen-fixing microbes are very important in elemental cycle and in plant nutrition of grassland soils. The populations varied with sampling season and soil depth. Each gram contained $(6.94 \pm 0.13) \times 10^4$–$(1.34 \pm 0.41) \times 10^6$, $(1.56 \pm 0.55) \times 10^4$–$(7.32 \pm 0.46) \times 10^5$ and $(1.97 \pm 0.32) \times 10^3$–$(1.17 \pm 0.41) \times 10^5$ CFU cellulolytic microbes in the organic layer, topsoil, and subsoil, respectively (Fig. 2d). The organic layer and topsoil had higher populations than those of the subsoil. Around 53.23% and 45.47% of the samples had significant differences between organic layer and topsoil, and between topsoil and subsoil, respectively. Although the populations of cellulolytic microbes varied at different seasons, the percentage of cellulolytic microbes in total microbial count was very consistent. The percentage was high in topsoil and low in organic layer. The values were 0.46 to 45.81% (average 9.05%), 0.26 to 87.5% (average 19.75%)

Fig. 2 Seasonal variation of microbial populations in grassland soil of Tatachia. (**a**) Bacteria, (**b**) Actinomycetes, (**c**) Fungi, (**d**) Cellulolytic microbes, (**e**) Phosphate-solubilizing microbes, (**f**) Nitrogen-fixing microbes. ● organic layer, ○ topsoil, ▼ subsoil. Vertical bars represent standard deviations ($n = 9$)
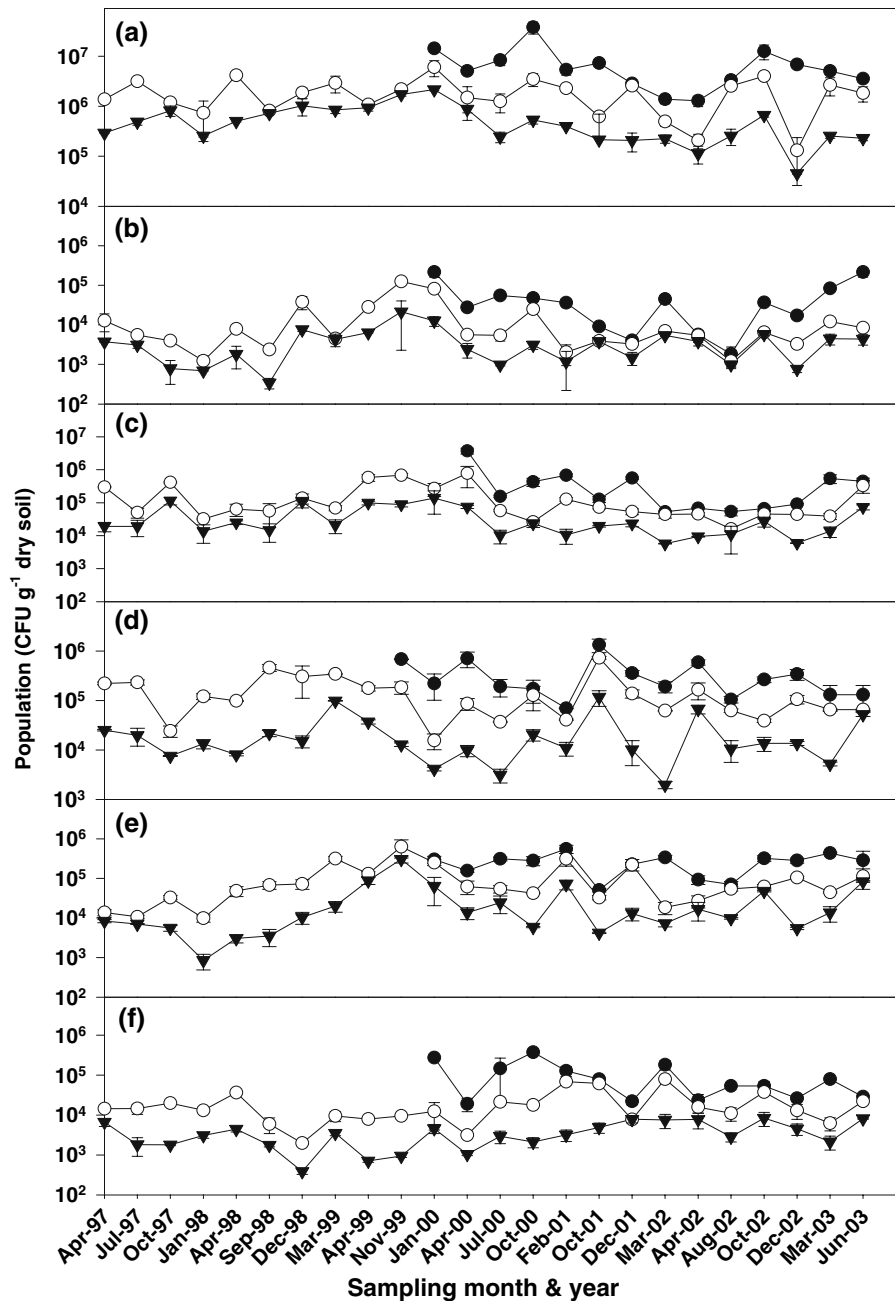
and 0.19 to 58.51% (average 9.46%) in organic layer, topsoil, and subsoil, respectively. Whereas the percentages of cellulolytic microbes were also high in summer seasons (from 2.47 to 7.37%, average 4.09%) and low in winter seasons (from 5.35 to 80.92%, average 20.18%).

Each gram of soil had $(5.08 \pm 0.49) \times 10^4$ to $(5.59 \pm 1.32) \times 10^5$, $(9.87 \pm 2.05) \times 10^3$ to $(6.38 \pm$

$0.94) \times 10^5$ and $(8.49 \pm 3.63) \times 10^2$ to $(3.08 \pm 0.61) \times 10^5$ CFU phosphate-solubilizing microbes in the organic layer, topsoil, and subsoil, respectively (Fig. 2e). Most of the organic layer samples had the highest populations in all tested samples, and the seasonal variation was statistically significant. The percentage of phosphate-solubilizing microbes in total count ranged from 0.69 to 24.71% (average

6.18%) in organic layer, from 1.17 to 80.92% (average 9.27%) in topsoil and from 0.11 to 70.93% (average 11.24%) in subsoil.

Each gram of soil contained $(1.92 \pm 0.71) \times 10^4$ to $(3.75 \pm 0.62) \times 10^5$, $(1.98 \pm 0.19) \times 10^3$ to $(7.93 \pm 0.39) \times 10^4$, and $(3.86 \pm 0.59) \times 10^2$ to $(8.38 \pm 3.23) \times 10^3$ CFU nitrogen-fixing microbes in the organic layer, topsoil, and subsoil, respectively (Fig. 2f). From the statistical analysis, 38.35 and 50.24% of topsoil had significantly higher populations than those of organic layer and subsoil, respectively. The percentages of nitrogen-fixing microbes in total count ranged from 0.39 to 13.19% (average 2.08%), from 0.11 to 16.09% (average 2.53%), and from 0.04 to 10.00% (average 1.72%) in the organic layer, topsoil, and subsoil, respectively. The percentages in organic layer were significantly lower than that in topsoil or subsoil. The populations and percentages were also higher in summer seasons than in winter seasons.

Microbial biomass carbon and nitrogen

The microbial biomass carbon and nitrogen contents in the organic layer, topsoil, and subsoil are illustrated in Table 1. Organic layer had the highest microbial biomass carbon and nitrogen, followed by topsoil, and subsoil was the least. Summer seasons had high microbial biomass carbon and nitrogen.

DNA yield and purity

DNA yield and purity of grassland soils are shown in Fig. 3. Organic layer had the highest yield of DNA $(21.5 \pm 2.2$ to 29.1 ± 2.5 μg/g) with the highest levels of humate and protein contamination in the three tested layers, followed by topsoil $(14.0 \pm 0.7$ to 17.7 ± 0.6 μg/g), while subsoil had the lowest $(8.6 \pm 0.2$ to 12.2 ± 0.2 μg/g). However, subsoil had the highest purity of DNA with the highest ratios of A_{260}/A_{280} $(1.35 \pm 0.11$ to $1.77 \pm 0.09)$ and A_{260}/A_{230} $(1.35 \pm 0.18$ to $1.49 \pm 0.09)$, while organic layer had the lowest ratios of A_{260}/A_{280} $(1.02 \pm 0.07$ to $1.24 \pm 0.14)$ and A_{260}/A_{230} $(0.94 \pm 0.28$ to $1.18 \pm 0.06)$. DNA yield and humic acid contamination varied significantly with soil depths, and the

molecular size of obtained DNA was higher than 20 kb.

Effect of soil depth on microbial population, microbial biomass, and DNA yield

The soil properties, microbial biomass, and DNA yield at various horizons of grassland soils are shown in Figs. 4 and 5. The soil pH increased slightly with depth, and had the highest pH at the BW2 horizon. The moisture content, total organic carbon, total nitrogen, microbial populations, microbial biomass carbon and nitrogen decreased gradually with increasing depth. The bacteria, actinomycetes, fungi, cellulolytic, phosphate-solubilizing, and nitrogen-fixing microbes at the BW2 horizon were only 12.23, 8.42, 14.13, 16.14, 17.84, and 16.87% of those at the O horizon, respectively. The microbial biomass carbon and nitrogen decreased from 180.71 ± 18.25 to 780.12 ± 20.45 μg C/g and 50.06 ± 5.25 to 283.47 ± 26.96 μg N/g at the O horizon to 58.19 ± 4.62–250.36 ± 11.06 μg C/g and 15.21 ± 1.04–104.92 ± 8.38 μg N/g at the BW2 horizon, respectively. DNA yields and purity at different horizons of grassland are shown in Table 2. The DNA yield decreased with soil depth. However, the purity of DNA indicated by A_{260}/A_{280} and A_{260}/A_{230} ratios was the opposite.

Correlations between soil properties and microbial indicators

Correlations between physical, chemical, and biological properties of grassland soils are summarized in Table 3. Microbial biomass carbon and nitrogen had significant positive correlation with soil moisture content and significant negative correlation with soil pH. Microbial biomass carbon and nitrogen also had positive correlation with microbial populations. Total organic carbon had significant positive correlation with bacteria and actinomycetes, and positive correlation with other microbial populations. DNA yield had significant positive correlation with moisture content, total organic carbon, and total nitrogen. The moisture content and pH seem to be the most important factors influencing the microbial ecology and biomass in Tatachia grassland ecosystem.

Table 1 Microbial biomass carbon and nitrogen contents in grassland soil

Sampling date	Organic layer		Topsoil		Subsoil	
	Biomass C (µg/g dry soil)	Biomass N	Biomass C	Biomass N	Biomass C	Biomass N
Jul. 23, 1997			823 ± 104	99 ± 32	483 ± 89	78 ± 33
Oct. 10, 1997			734 ± 98	115 ± 43	361 ± 78	61 ± 36
Jan. 18, 1998			678 ± 88	128 ± 43	381 ± 83	59 ± 29
Apr. 30, 1998			646 ± 68	134 ± 33	371 ± 83	39 ± 11
Mar. 10, 1999			578 ± 25	94 ± 25	226 ± 26	32 ± 3
Jan. 5, 2000			443 ± 53	41 ± 9	117 ± 35	28 ± 6
Apr. 23, 2000	1,421 ± 469	118 ± 30	922 ± 71	52 ± 11	687 ± 42	30 ± 4
Jul. 15, 2000	2,482 ± 616	216 ± 67	931 ± 92	67 ± 6	716 ± 28	31 ± 6
Oct. 6, 2000	1,906 ± 476	172 ± 48	926 ± 49	74 ± 10	606 ± 60	48 ± 8
Feb. 1, 2001	1,253 ± 270	124 ± 55	827 ± 81	59 ± 8	513 ± 60	28 ± 6
Apr. 19, 2002	1,039 ± 59	129 ± 28	706 ± 5	96 ± 5	545 ± 14	53 ± 1
Aug. 14, 2002	1,146 ± 54	149 ± 23	822 ± 43	111 ± 6	578 ± 15	78 ± 1
Oct. 4, 2002	1,095 ± 11	120 ± 29	695 ± 44	87 ± 2	404 ± 11	70 ± 1
Dec. 17, 2002	818 ± 11	109 ± 23	678 ± 62	82 ± 23	235 ± 12	23 ± 2
Mar. 14, 2003	717 ± 22	122 ± 13	662 ± 49	95 ± 6	357 ± 11	45 ± 2
Jun. 10, 2003	899 ± 72	138 ± 30	876 ± 26	109 ± 5	416 ± 12	49 ± 4

Means ± SD (n = 3)

Sequencing and phylogenetic analysis

Of the 120 clones analyzed by sequencing, 45 unique sequences were identified in the three clone libraries (Table 4). Clones were members of 10 bacterial phyla: Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, candidate division TM1, candidate division TM7, Gammatimonadetes, and Verrucomicrobia. Members of Proteobacteria (44.4%) and Acidobacteria (33.3%) dominated the clone libraries. Within the phylum Proteobacteria, α-, β-, and γ-Proteobacteria were most numerous, followed by δ-Proteobacteria. Each of the remaining bacterial divisions constituted <5% of the clone library.

Discussion

Tatachia mountain is located in the central part of Taiwan and is divided into three parts according to the vegetation, grassland, spruce, and hemlock. The pH of grassland soils was weakly acidic due to the leaf litter on the surface, the decomposition of leaf litter, and the accumulation of organic acids (Chen and Yang 2000). The subsoil exhibited higher pH values than those of the organic layer or topsoil because of the lower organic matter content, microbial activity and organic acid accumulation. The pH values were slightly lower in summer seasons than in winter seasons due to the high temperature in summer season for active microbial activity and organic acid accumulation. Berg et al. (1998) indicated that the Wekerom forest soil in the Netherlands had a soil pH of 3.8 for the high organic matter content. Yang and Yang (2001) also reported that the nuclear and thermal power plants in northern Taiwan had soil pH ranging from 4.34 to 4.47 because of the organic matter covering the surface and the inceptisol.

In the spruce and hemlock soils of Tatachia, the pH value of organic layer was also the lowest, followed by topsoil, and subsoil was the highest. These ecosystems were characterized by the heavy leaf residue on surface and the accumulation of organic acids during leaf residue decomposition (Yang et al. 2003, 2006). The soil pH values of grassland were slightly higher than spruce or hemlock soils due to the less leaf deposit on the surface of grassland soils.
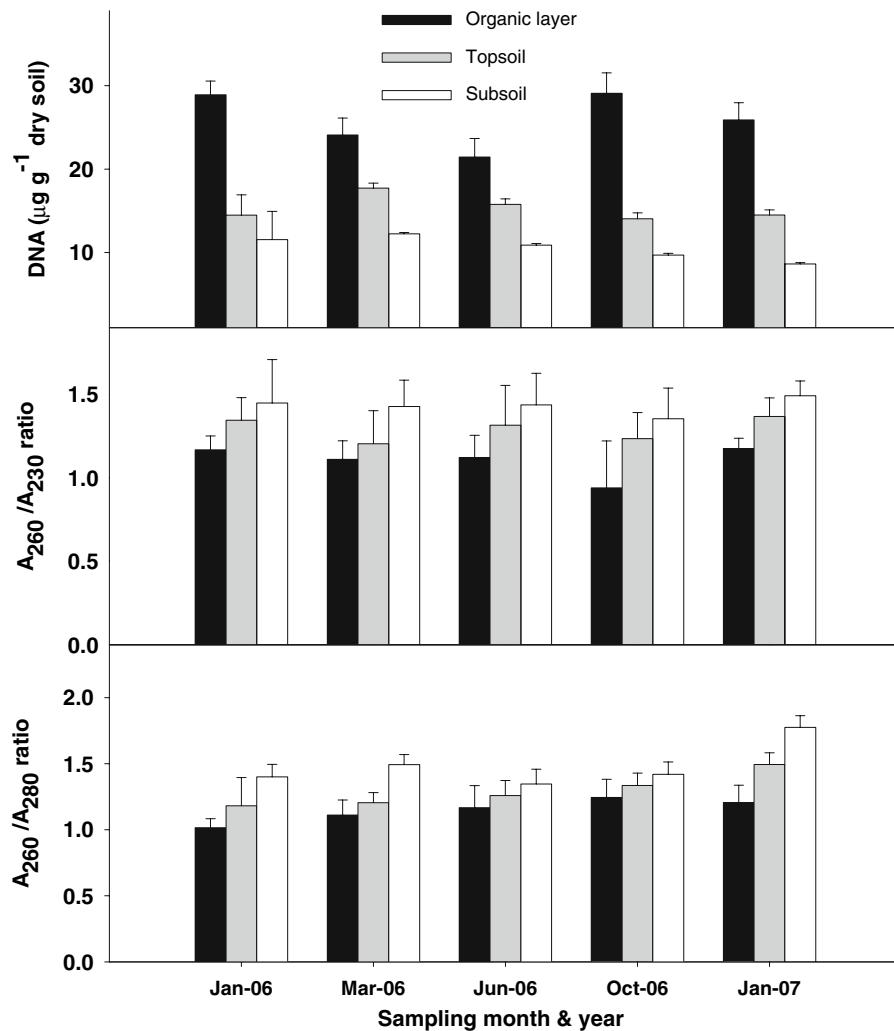
Fig. 3 DNA yield and purity of grassland soil. (**a**) DNA yield, (**b**) A_{260}/A_{230} ratio, (**c**) A_{260}/A_{280} ratio ($n = 6$)

The soil moisture content depends on the environmental conditions. Seasonal variation of the moisture content in organic layer was higher than in the topsoil or subsoil due to the greater environmental change and the heavier organic matter covering. The soil moisture in summer seasons was also higher than in winter seasons, mainly attributable to the raining season during July–September. Same phenomena were also found in power plant areas, spruce, and hemlock soils of Tatachia. The water-holding capacities of organic layer in grassland soils were higher than those of topsoil or subsoil because of the high organic matter content in the organic layer. The result is same as the spruce and hemlock forest soils of Tatachia (Yang et al. 2003, 2006).

Although the variation of culturable microbes was large during the tested periods, the microbial populations were low in subsoil due to the low total organic carbon, total nitrogen, moisture content, and comparatively less oxygen concentration. The microbial populations and microbial biomass were higher in summer seasons because of high soil temperature (12.8–18.4°C in summer seasons, and 6.6–8.6°C in winter seasons), which might favor the microbial activity. Srivastava and Singh (1988) showed that tropical soils had low carbon (0.6%) and nitrogen (0.01%) contents due to the dry condition and less vegetation. Fisk et al. (2003) reported that microbial activity of peat land was greater in surface than
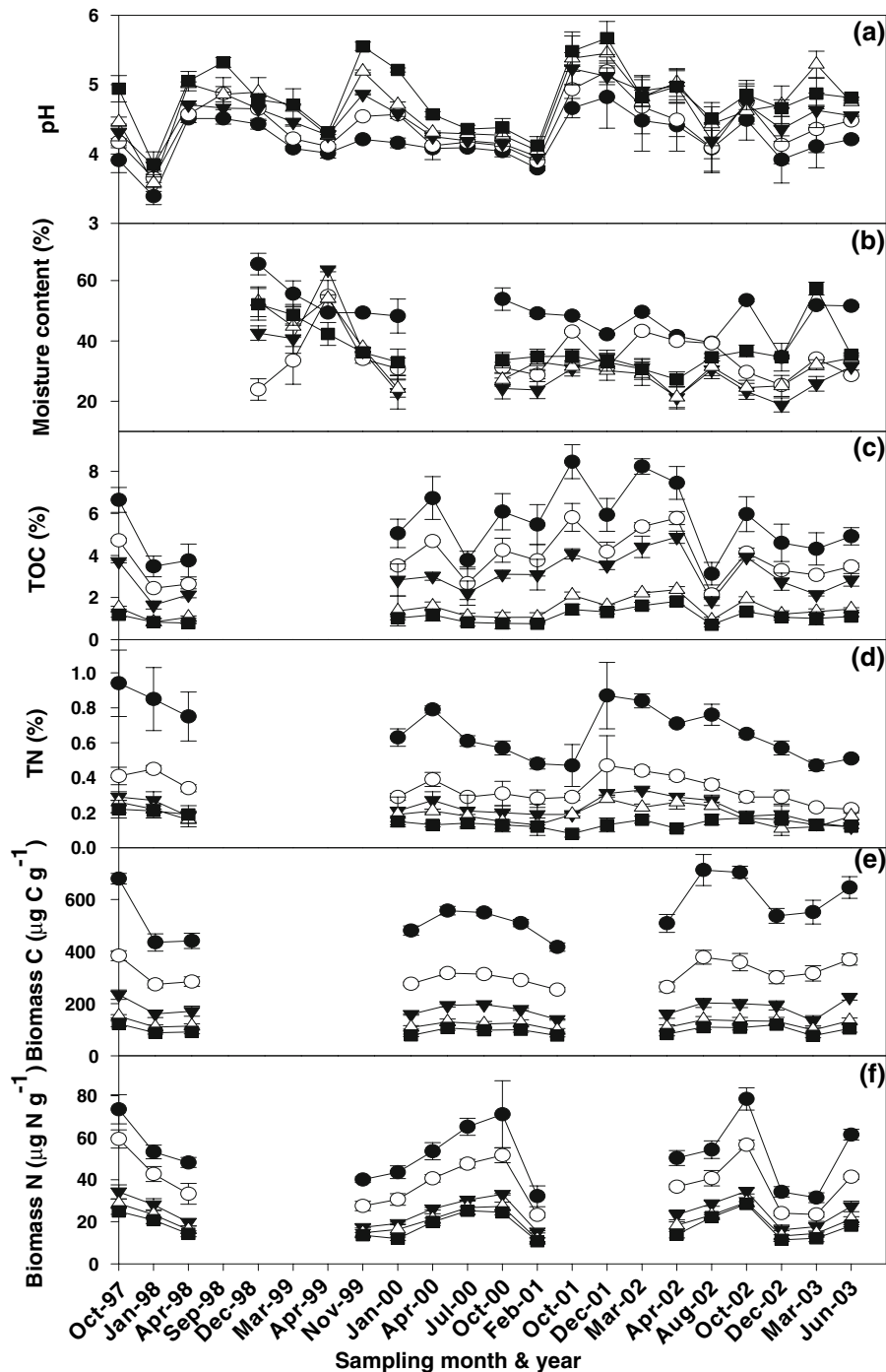
Fig. 4 Seasonal variation of soil properties at different horizons of grassland in Tatachia. (**a**) pH, (**b**) Moisture content, (**c**) Total organic carbon (TOC), (**d**) Total nitrogen (TN), (**e**) Microbial biomass carbon, (**f**) Microbial biomass nitrogen. ● O layer, ◒ Oa layer, ▼ A layer, ▽ BW1 layer, ■ BW2 layer. Vertical bars represent standard deviations ($n = 6$)

subsurface for the high organic matter content. Grassland soils had slightly lower total organic carbon and total nitrogen contents than those of spruce or

hemlock soils. Therefore, the microbial populations of grassland soils were also lower than the other two forest ecosystems. Miethling et al. (2000) also

Fig. 5 Seasonal variation of microbes at different horizons of grassland in Tatachia. (**a**) Bacteria, (**b**) Actinomycetes, (**c**) Fungi, (**d**) Cellulolytic microbes, (**e**) Phosphate-solubilizing microbes, (**f**) Nitrogen-fixing microbes. ● O layer, ○ Oa layer, ▼ A layer, ▽ BW1 layer, ■ BW2 layer. Vertical bars represent standard deviations (*n* = 6)
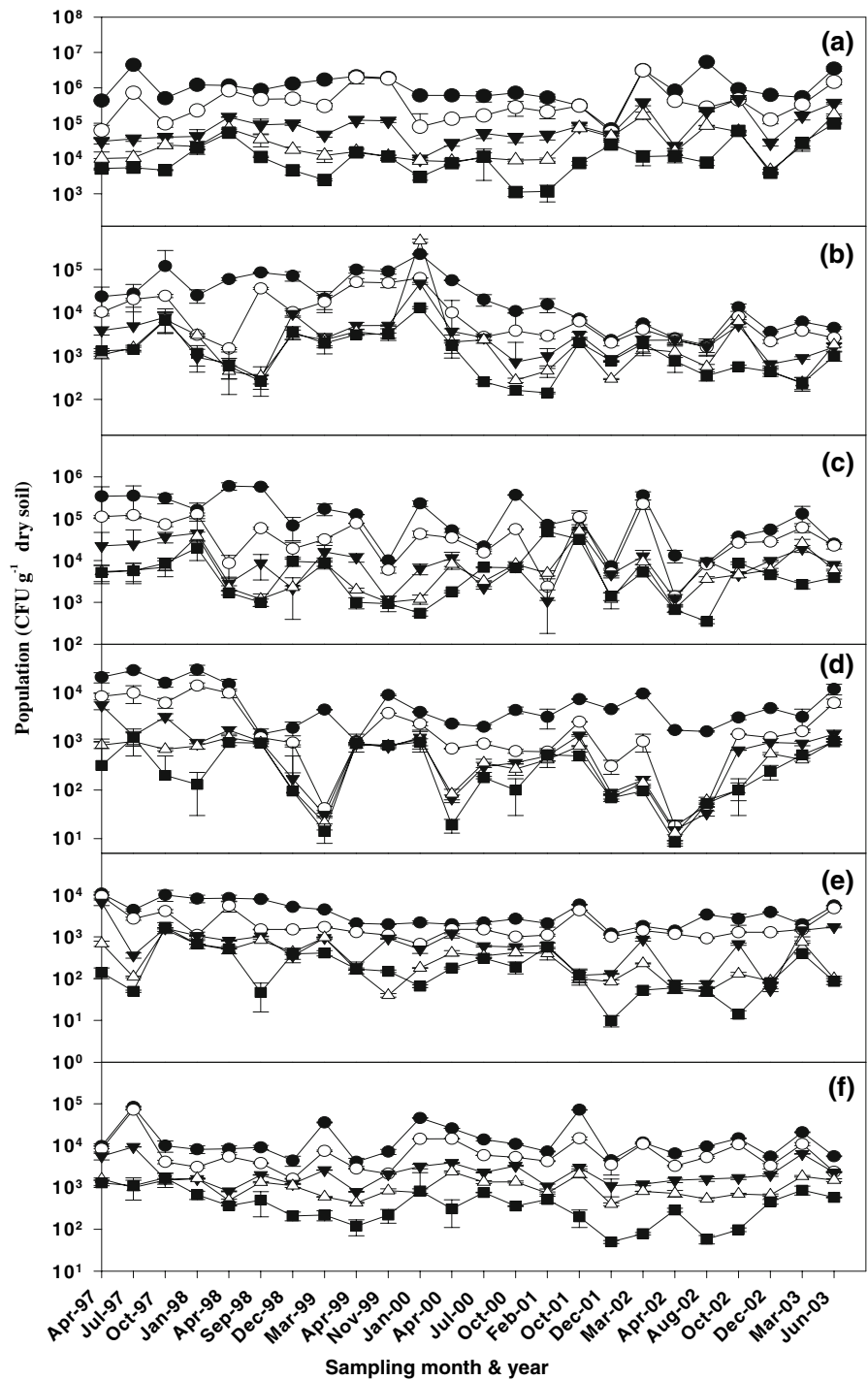
showed that same soil type with different plant species affected the microbial populations. The microbial populations in subsoil were 11.05–96.51, 4.99–70.71, 1.74–82.50, 1.54–74.88, 2.11–89.26, and 1.23–40.06% of bacteria, actinomycetes, fungi, cellulolytic,

phosphate-solubilizing, and nitrogen-fixing microbes, respectively, as in organic layer. The percentages of microbial populations in subsoil of Tatachia spruce soils were 24.86–91.26, 11.63–97.98, 12.63–93.18, 14.65–90.64, 27.22–81.64, and 24.86–74.55% as in

Table 2 DNA yield and purity of grassland profile

Sampling date	O	Oa	A	BW1	BW2
(1) DNA yield (µg/g dry soil)					
Jan. 14, 2006	29.0 ± 2.3*	21.7 ± 0.7	17.2 ± 1.0	12.4 ± 0.7	9.0 ± 0.3
Mar. 26, 2006	31.6 ± 1.5	25.4 ± 0.6	17.9 ± 2.1	13.9 ± 1.1	11.9 ± 1.6
Jun. 27, 2006	28.1 ± 1.6	22.6 ± 3.7	22.6 ± 1.2	20.1 ± 2.2	13.0 ± 3.7
Oct. 1, 2006	25.0 ± 2.8	20.1 ± 0.7	17.9 ± 3.3	16.8 ± 2.4	10.5 ± 1.9
Jan. 12, 2007	22.3 ± 1.5	17.9 ± 3.6	17.7 ± 1.1	13.9 ± 1.3	8.3 ± 1.6
(2) A_{260}/A_{230} ratio					
Jan. 14, 2006	1.05 ± 0.27	1.24 ± 0.22	1.26 ± 0.09	1.46 ± 0.17	1.49 ± 0.07
Mar. 26, 2006	1.10 ± 0.08	1.24 ± 0.22	1.47 ± 0.14	1.53 ± 0.22	1.77 ± 0.15
Jun. 27, 2006	1.02 ± 0.09	1.14 ± 0.27	1.36 ± 0.16	1.48 ± 0.26	1.60 ± 0.17
Oct. 1, 2006	0.94 ± 0.02	1.05 ± 0.10	1.25 ± 0.29	1.64 ± 0.24	1.75 ± 0.19
Jan. 12, 2007	1.17 ± 0.04	1.31 ± 0.12	1.56 ± 0.08	1.65 ± 0.12	1.79 ± 0.08
(3) A_{260}/A_{280} ratio					
Jan. 14, 2006	1.01 ± 0.09	1.03 ± 0.07	1.04 ± 0.12	1.35 ± 0.24	1.32 ± 0.33
Mar. 26, 2006	1.07 ± 0.10	1.17 ± 0.08	1.31 ± 0.09	1.66 ± 0.15	1.83 ± 0.12
Jun. 27, 2006	1.22 ± 0.14	1.33 ± 0.12	1.50 ± 0.13	1.89 ± 0.22	1.97 ± 0.17
Oct. 1, 2006	1.24 ± 0.14	1.35 ± 0.18	1.51 ± 0.11	1.72 ± 0.10	1.79 ± 0.12
Jan. 12, 2007	1.13 ± 0.11	1.30 ± 0.09	1.34 ± 0.10	1.69 ± 0.18	1.70 ± 0.14

* Mean ± SD (n = 3)

Table 3 Correlations between physical, chemical, and biological properties of grassland soil

Soil properties	A	B	C	D	E	F	G	H	I	J	K	L	M
pH (A)	1.000												
MC (B)	−.332	1.000											
C_{org} (C)	.023	.578	1.000										
N_{tot} (D)	−.065	.181	.323	1.000									
C/N (E)	.146	.283	.455	.097	1.000								
Bac (F)	−.177	.279	.502	.082	−.076	1.000							
Act (G)	−.075	.487	.729	.044	.171	.157	1.000						
Fungi (H)	−.016	.441	.392	.275	.258	.160	.340	1.000					
CMC (I)	−.152	.445	.262	.194	.203	.696	.435	.395	1.000				
PSB (J)	−.117	.296	.178	.163	.084	.092	.214	.522	.424	1.000			
NFB (K)	−.161	.467	.348	.282	.276	.046	.238	.683	.343	.793	1.000		
C_{mic} (L)	−.690	.680	.120	.118	−.001	.273	.246	.390	.313	.389	.517	1.000	
N_{mic} (M)	−.607	.641	.302	.150	.183	.220	.373	.445	.339	.333	.493	.862	1.000
DNA (N)	.033	.690	.659	.705	.369	.409	.065	.246	.019	.039	.190	.427	.401

Linear regressions were used. $n > 90$. MC: moisture content, C_{org}: total organic C content, N_{tot}: total N content, Bac: bacteria, Act: actinomycetes, 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

topsoil, respectively (Yang et al. 2003). Similarly, the percentages of hemlock soils in subsoil of Tatachia were 11.58–111.96, 2.61–96.96, 4.66–92.10, 8.91–55.56, 16.82–66.44, and 7.83–60.74% as in topsoil, respectively (Yang et al. 2006). The ranges of microbial populations in grassland soils were less than those of spruce and hemlock soils due to the stick soil structures for nutrition and water penetration from

Table 4 Identification of bacterial strains in 16S rDNA clone library isolated from Tatachia grassland soil

Name	Closest related organism in the database	Accession number of reference strain	Identity (%)	Taxon
TG1	Acidobacteria bacterium Ellin7184	AY673350	97	Acidobacteria
TG2	Acidobacteriaceae bacterium KBS89	AY587227	97	Acidobacteria
TG3	Bacterium Ellin6071	AY234723	99	Acidobacteria
TG4	Uncultured Acidobacteria bacterium FAC3	DQ451442	98	Acidobacteria
TG5	Uncultured Acidobacteria bacterium FAC8	DQ451447	96	Acidobacteria
TG6	Uncultured Acidobacteria bacterium FAC9	DQ451448	96	Acidobacteria
TG7	Uncultured Acidobacteria bacterium JG36-GS–69	AJ582046	97	Acidobacteria
TG8	Uncultured Acidobacteriales bacterium EB1134	AY395453	98	Acidobacteria
TG9	Uncultured Acidobacterium UA3	AF200699	97	Acidobacteria
TG10	Uncultured bacterium AH61	AY963354	97	Acidobacteria
TG11	Uncultured *Holophaga* sp. JG30-KF-C5	AJ536863	96	Acidobacteria
TG12	Uncultured *Holophaga* sp. JG37-AG-74	AJ519380	97	Acidobacteria
TG13	Bacterium Ellin504	AY960767	98	Acidobacteria
TG14	Uncultured Acidobacteria bacterium G08_WMSP1	DQ450718	89	Acidobacteria
TG15	Uncultured Acidobacteria bacterium JG36-GS-126	AJ582044	97	Acidobacteria
TG16	Alpha proteobacterium A0902	AF236003	97	Alphaproteobacteria
TG17	Uncultured alpha proteobacterium EB1127	AY395446	97	Alphaproteobacteria
TG18	Uncultured alpha proteobacterium JG37-AG-11	AJ518764	98	Alphaproteobacteria
TG19	Uncultured bacterium GKS2-215	AJ290032	94	Alphaproteobacteria
TG20	Uncultured *Bradyrhizobium* sp. TM16_2	DQ303345	99	Alphaproteobacteria
TG21	Bacterium Ellin321	AF498703	97	Betaproteobacteria
TG22	*Burkholderia* sp. LMG 22929 RG3-1	AY949190	98	Betaproteobacteria
TG23	*Ralstonia pickettii* strain ATCC 27511	AY741342	99	Betaproteobacteria
TG24	Uncultured bacterium HSM-SS-006	AB238769	98	Betaproteobacteria
TG25	Uncultured beta proteobacterium B-BH93	AY622261	99	Betaproteobacteria,
TG26	Uncultured beta proteobacterium NE45	AJ575695	98	Betaproteobacteria
TG27	Uncultured beta proteobacterium JG36-GS-10	AJ582037	95	Betaproteobacteria
TG28	*Frateuria aurantia* strain:IFO13331	AB091200	98	Gammaproteobacteria
TG29	*Frateuria* sp. WJ64	AY495957	99	Gammaproteobacteria
TG30	Gamma proteobacterium RI-34-2	AY539825	98	Gammaproteobacteria
TG31	*Lysobacter ginsengisoli* strain:Gsoil 357	AB245363	95	Gammaproteobacteria
TG32	Uncultured gamma proteobacterium FAC23	DQ451462	95	Gammaproteobacteria
TG33	Uncultured gamma proteobacterium YNPRH65B	AF465652	97	Gammaproteobacteria

Table 4 continued

Name	Closest related organism in the database	Accession number of reference strain	Identity (%)	Taxon
TG34	Uncultured delta proteobacterium JG37-AG-113	AJ518798	95	Deltaproteobacteria
TG35	*Byssophaga cruenta* DSM 14553T	AJ833647	96	Deltaproteobacteria
TG36	Uncultured Actinomycetales bacterium TM146	X92699	98	Actinobacteria
TG37	Uncultured Actinomycetales bacterium TM146	X92699	94	Actinobacteria
TG38	Cytophagales str. MBIC4147	AB022889	95	Bacteroidetes
TG39	Uncultured Termite group 1 bacterium S-J147	AY622271	94	candidate division TM1
TG40	Uncultured bacterium NoosaAW16	AF269015	93	candidate division TM7
TG41	Uncultured bacterium NoosaAW07	AF269011	96	candidate division TM7
TG42	Uncultured Chloroflexi bacterium E11_WMSP1	DQ450736	98	Chloroflexi
TG43	Bacillaceae bacterium KVD-1790-02	DQ490383	99	Firmicutes
TG44	Gemmatimonadetes bacterium Ellin7146	AY673312	99	Gemmatimonadetes
TG45	Bacterium Ellin5102	AY234519	98	Verrucomicrobia

topsoil to subsoil. Similarly, the microbial populations in subsoil of power plant areas had only 19.20–71.62% of bacteria, 21.58–87.34% of actinomycetes, and 15.41–72.92% of fungi as those in topsoil due to the stickiness of tested soils (Yang and Yang 2001). Microbial biomass carbon and nitrogen, and DNA in subsoil of grassland were 26.41–77.20, 28.05–80.46, and 59.31–79.31% as those in topsoil, respectively. The microbial populations, microbial biomass and DNA of the BW2 horizon in grassland soils were 8.42–17.84, 16.84–64.40, and 31.03–46.26% of those at the O horizon, respectively. Similar phenomenon was also reported in other studies (Yang et al. 2003, 2006). The microbial activity, biomass, ATP content, respiration, and relative abundance of denitrifying and dinitrogen-fixing bacteria decreased with increasing depth in forest soils of southern Appalachian Mountains (Garten 2004). The bacterial populations and the mycelial content decreased with increasing depth in pine forest soils (Berg et al. 1998), and the nitrifying bacterial counts also decreased in acidic forest soils of Douglas (de Boer et al. 1992).

Although the populations of cellulolytic, phosphate-solubilizing, and nitrogen-fixing microbes varied with season and location, the ratios of these microbes to total viable counts could be used to assay the change of microbial ecology. The ratios of cellulolytic microbes to total viable counts were between 11.53 and 25.15% (average 20.53%) in grassland soils. The value was lower than 41.84–86.37% (average 63.74%) in spruce, 8.91–94.42% (average 52.90%) in hemlock, and 17.21–60.20% in nuclear and thermal power plant areas (Yang and Yang 2001; Yang et al. 2003, 2006) due to the less coverage of organic matter lying on the surface of grassland soils. Bradley and Fyles (1995) suggested that basal respiration was 2–3 times high in the active rhizosphere due to the high amount of root-labile C in this area. Tietema and Wessel (1994) also reported that microbes associated with oak leaf decomposition were very active in forest soils and essential for the elemental cycle and the nutritional supplement. The differences in the chemical composition of litters and root exudates would be expected to result in different availabilities of various carbon sources among stands of different tree species (Grayston et al. 1996). The ratios of phosphate-solubilizing microbes to total viable counts were 1.88–5.67% (average 3.78%). The values were lower than nuclear and thermal power plants areas (2.11–40.75%, average 22.26%), spruce soils (7.11–21.30%, average 12.00%) and hemlock soils

(6.82–66.48%, average 25.75%) in Tatachia. In addition, the percentages of nitrogen-fixing microbes in total viable counts were between 2.83 and 7.26% (average 5.05%). The values were also lower than hemlock soils (2.20–51.77%, average 19.32%), spruce soils (22.53–68.72%, average 38.02%) in Tatachia, and nuclear and thermal power plants areas (1.20–60.73%, average 23.86%) (Yang and Yang 2001; Yang et al. 2003, 2006). Both of these were due to less organic matter content on the surface of grassland soils.

Microbial biomass carbon of grassland soils (Table 1) was higher than that in tropical soils of India (149–667 µg C/g dry soil) (Srivastava and Singh 1988), but lower than spruce soils (893–2,801 µg C/g organic layer, 644–2,018 µg C/g topsoil and 438–832 µg C/g subsoil) and hemlock soils (678–2,801 µg C/g organic layer, 355–1,013 µg C/g in topsoil and 99–832 µg C/g subsoil) (Yang et al. 2003, 2006) in Tatachia because of the low organic matter content in grassland soils.

Microbial biomass nitrogen of grassland soils (Table 1) was higher than those in tropical forest soils of India (38–78 µg N/g soil) (Raghubanshi 1991), slightly less than those of spruce soils (75–190 µg N/g organic layer, 55–229 µg N/g topsoil, and 21–139 µg N/g subsoil) and hemlock soils (53–166 µg N/g organic layer, 35–147 µg N/g topsoil, and 11–137 µg N/g subsoil) in Tatachia (Yang et al. 2003, 2006) and lower than forest soils in Germany (30–347 µg N/g dry soil) (Joergensen et al. 1990). The microbial biomass carbon of pine and spruce forest soils in Finland was also higher in 0–3 cm depth of mineral soil than that in 0–6 cm depth of mineral soil (Priha et al. 2001). The microbial biomass nitrogen content was consistent with soil total nitrogen content. Therefore, soil microbial biomass along with soil organic carbon had been recommended as indicators of soil carbon (Carter et al. 1999). The similarity between these forests and Tatachia grassland suggested that the type of vegetation cover, total organic carbon content, and total nitrogen contents played the important roles on microbial biomass.

The microbial biomass carbon/total organic carbon ratios of organic layer, topsoil, and subsoil in grassland were 0.49–1.47, 0.25–1.27, and 0.64–2.43%, respectively. The ratios were lower than tropical forest (1.5–5.3%) (Luizao et al. 1992) and

tropical wet-evergreen forest (4–6%) (Barbhuiya et al. 2005), similar to subtropical humid forests (0.7–1.7%) (Maithani et al. 1996), but higher than spruce and hemlock of Tatachia (0.13–1.25%) (Yang et al. 2003, 2006). However, the ratios of microbial biomass nitrogen/total nitrogen of the organic layer, topsoil and subsoil in grassland were 1.77–2.32, 1.36–1.85, and 0.87–3.60%, respectively. The ratios were also lower than forest soils (3.4–5.9%) (Martikainen and Palojarvi 1990) and forest regrowth (7.3–8.3%) (Maithani et al. 1996), similar to tropical wet-evergreen forest (1.3–1.7%) (Barbhuiya et al. 2005), but higher than spruce and hemlock of Tatachia (0.13–1.25%) (Yang et al. 2003, 2006). The alpine ecosystem of Tatachia belonged to acidic type, and the temperature was rather low, so the decomposition rate of litter was low in forest and grassland. The ratios of microbial biomass carbon/total organic carbon and microbial biomass nitrogen/total nitrogen were higher in grassland than in other forest ecosystems, indicating that the organic matters in grassland could decompose and be used by microorganisms easier than in the forest. Therefore, the degree of humification was higher in O layer of grassland than that of forest.

Acidobacteria and Proteobacteria are generally the most numerically dominant phyla in soil, while members of Bacteroidetes and Firmicutes are less common (Dunbar et al. 1999; Chow et al. 2002; Fierer et al. 2005). In this study, we also found Proteobacteria and Acidobacteria to be dominant. Members of α-Proteobacteria were the most abundant bacteria in 16S rDNA clone libraries derived from Long-Term Soil Productivity (LTSP) forest soil from British Columbia, Canada (Chow et al. 2002), Australian forest soils (Stackebrandt et al. 1993), Scotland grassland rhizosphere soil (McCaig et al. 1999), and fertilizer-applied soil (Toyota and Kuninaga 2006). Members of Acidobacteria were the most abundant in the clone libraries from Arizona pinyon pine rhizosphere and bulk soils (Dunbar et al. 1999), and in desert, prairie, and forest soils (Fierer et al. 2005).

Tatachia grassland soil has an acidic pH, also implying the existence of certain acidophilic bacteria. Despite the effectiveness of culture-independent methods, such as SSU rRNA or rDNA analyzes, microorganisms from these natural habitats must be cultivated to understand their roles in environmental processes and for novel commercially important

microbes. Moreover, identification of these unclassified group may result in complex diversity. Bacterial species can be identified by generating clone libraries of the 16S rDNA followed by sequencing and comparison with databases containing thousands of ribosomal sequences to allow a phylogenetic affiliation to cultured, as well as uncultured microorganisms (Maidak et al. 1999; Ranjard et al. 2000). These techniques have proven to be very suitable for comparative fingerprinting of soil samples (Watanabe et al. 2004). The library of diverse 16S rRNA genes from Tatachia grassland soils can be used to construct special DNA primers and probes to target bacterial groups of interest, which gives an opportunity to investigate the relationship between soil bacterial communities and grassland ecosystem in Tatachia forest.

In conclusion, Tatachia grassland soils were weakly acidic and the microbial populations were characterized by high numbers of bacteria, followed by fungi and actinomycetes. The microbial communities were significantly different between topsoil and subsoil. The microbial populations, microbial biomass, and DNA contents were higher in topsoil than those in subsoil. The ratios of cellulolytic microbes to total culturable bacteria count were high due to the heavy coverage of organic matter lying on the soil surface. There was a positive correlation between microbial populations and microbial biomass carbon. The microbial biomass varied with the soil properties and depths. Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, candidate division TM1, candidate division TM7, Gammatimonadetes, and Verrucomicrobia were identified in grassland soils. Members of Proteobacteria (44.4%) and Acidobacteria (33.3%) dominated the clone libraries.

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