



## Microbial population of spruce soil in Tatachia mountain of Taiwan

Shang-Shyng Yang \*, Hsiao-Yun Fan, Chiun-Kai Yang, I-Chien Lin

*Department of Agricultural Chemistry, National Taiwan University, Taipei 10617, Taiwan, ROC*

### Abstract

To investigate the role of microorganisms in the ecology and the nutrient transformation of forest soil, soil property, microbial population, biomass and organic acid content of Spruce soil in Tatachia mountain were determined during January 1997 to November 1999. Soil temperatures were between 5.5 and 15.6 °C and soil pH ranged from 3.6 to 5.0. Total organic carbon and nitrogen contents ranged from 5.83% to 34.35% and from 0.90% to 3.19%, respectively. C/N ratio was between 7.07 and 18.24. Each gram of dry soil contained microbial biomass carbon 308–870 µg, microbial biomass nitrogen 107–240 µg, malic acid 74–211 nM and succinic acid 32–175 nM. In addition, each gram of dry organic layer contained microbial biomass carbon 216–653 µg, microbial biomass nitrogen 10.3–33.8 µg, formic acid 256–421 nM, acetic acid 301–435 nM, malic acid 795–1027 nM and succinic acid 204–670 nM. About 45.5–90.9% of topsoil samples had higher microbial population than those of subsoil especially in actinomycetes, cellulolytic and phosphate-solubilizing microorganisms. Although rhizosphere of Spruce had higher total organic carbon and total nitrogen content than non-rhizosphere and dwarf bamboo areas, the microbial population had no significant difference among them.

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### 1. Introduction

Forest soil microorganisms and fauna decompose organic materials, and thus they strongly influence the nutrient cycling of ecosystem. The numbers and species of microbes in soil vary directly with environmental conditions, nutrient availability, soil texture, and type of vegetation cover (Atlas and Bartha, 1998). Fields cropped continuously to oats contain more fungi, actinomycetes are greater in grassland and pasture soils than in cultivated land, and bacteria in grassland are greater than in arable land (Alexander, 1977).

In Taiwan, the microbial distributions of soils have only been determined in acidic soil of Yang-ming-san, inorganic acidic soil, upland soil, alkaline soil of Tainan and power plant areas (Chu, 1933; Yang et al., 1998a,b, 1999; Yang and Yang, 2001). There are few data in the microbial population of forest soil (Yang et al., 1998a; Imberger and Chiu, 2001). Tatachia mountain is located in the central part of Taiwan and has been designated as a National Park. There are three major vegetations, Spruce, Hemlock and grass, in this temperate area. It is a typical high altitude ecosystem protected area in Taiwan. For the long-term ecological research in Taiwan, the microbial populations, biomass and organic acid content of Spruce soil during January 1997 to November 1999 were investigated. The effect of season and depth on microbial population and biomass are also studied in this article.

\* Corresponding author. Tel.: +886-2-2362-1519; fax: +886-2-2367-9827.

E-mail address: [ssyang@ccms.ntu.edu.tw](mailto:ssyang@ccms.ntu.edu.tw) (S.-S. Yang).

## 2. Materials and methods

### 2.1. Sampling location

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 1800 to 3952 m. Some environmental conditions and properties of tested soils are listed in Table 1. The area has been selected as a representative long-term ecological study site of sub-alpine forest ecosystems in Taiwan. The study area geologically consists of metamorphosed sedimentary rock (Miocene epoch) comprising sandstone and shale. The coniferous forest is dominated by spruce (*Picea morrissonicola*). Less dominant species include Taiwan false cypress (*Chamaecyparis formosensis*), Armand's pine (*Pinus armandi*), Chinese hemlock (*Tsuga chinensis*), dwarf bamboo (*Yushania niitakayamensis*), and alpine silver grass (*Miscanthus transmorrisonensis*).

### 2.2. Culture media and conditions

Bacteria were counted at 25 °C after 5 days incubation on nutrient agar containing (g l<sup>-1</sup>) beef extract 3.0, peptone 5.0, and agar 15.0 at pH 6.8 ± 0.1. Actinomycetes were cultivated at 25 °C for 7 days on glycerol–yeast extract medium comprised of (g l<sup>-1</sup>) glycerol 5.0, yeast extract 2.0, K<sub>2</sub>HPO<sub>4</sub> 1.0, and agar 15.0 at pH 7.0 ± 0.1. Streptomycin and cycloheximide were added to inhibit the growth of bacteria and fungi at a final concentration of 10 µg cm<sup>-3</sup> (Yang et al., 1999). Fungi were grown at 25 °C for 5 days on Rose bengal medium containing (g l<sup>-1</sup>) glucose 10.0, peptone 5.0, K<sub>2</sub>HPO<sub>4</sub> 1.0, MgSO<sub>4</sub> · 7H<sub>2</sub>O 0.5, Rose bengal 0.033 and agar 15.0 at pH 6.8 ± 0.1. Cellulolytic microbes were counted at

25 °C after 7 days incubation on Mandels–Reese medium with carboxymethylcellulose (Sigma) as the sole carbon source and sprayed with Congo red to show clear zone around the colonies. Phosphate-solubilizing microbes were measured at 25 °C after 5 days on rock phosphate medium and had clear zone around the colonies. Nitrogen-fixing microbes were characterized at 25 °C after 7 days incubation on nitrogen-free mannitol medium (Yang et al., 1998a). All the experiments were carried out in triplicate.

### 2.3. Seasonal variation of microbial ecology

Temperatures are high in summer and low in winter. Soil samples were collected at different seasons from January 1997 to November 1999. Organic matter content is high in the organic layer and the value is low in the subsoil. Cellulolytic, phosphate-solubilizing and nitrogen-fixing microbes are very important in grassland and forest soil for the elemental cycle and the nutritional supplement. Bacteria, actinomycetes, fungi, cellulolytic, phosphate-solubilizing and nitrogen-fixing microbes were counted in both the topsoil (0–20 cm depth) and the subsoil (21–40 cm depth). Microbes in the organic layer were also analyzed in December 1998, March and April 1999 for comparison.

### 2.4. Effect of soil profile on microbial populations

Soil profiles were taken from the spruce zone and classified into horizons according to the international soil classification system (Soil Survey Staff, 1994). Soil samples were collected from each horizon and transported to the laboratory from the top of soil to 100 cm depth in September 1998. Microbial populations at each

Table 1  
Environmental conditions and properties of tested Tatachia forest soils

Properties	Organic layer	Topsoil (0–20 cm)	Subsoil (21–40 cm)
Altitude (m)	2500	2500	2500
Annual precipitation (mm)	2800–3000	2800–3000	2800–3000
Soil texture	–	Sandy loam	Sandy loam
Vegetation	Picea, morrissonicola, spruce, dwarf bamboo		
Color	Blackish brown	Grey to blackish grey	Grey to yellowish grey
Temperature (°C)			
Air	0.0–17.9		
Soil	5.5–15.6	5.8–15.3	6.0–14.9
Light intensity (lx)	577–2667		
pH	3.58–4.19	3.77–4.83	3.83–4.99
Moisture content (%)			
Wet weight basis	61.0–73.7	33.9–55.8	29.0–45.9
Dry weight basis	89.7–270.5	46.7–191.9	36.5–141.8
Water holding capacity (%)	389.7–498.3	155.0–376.2	97.5–143.5
Total organic carbon (%)	23.66–34.35	11.09–24.51	5.83–11.20
Total nitrogen (%)	1.26–3.19	1.06–2.87	0.90–1.90
C/N ratio	7.42–18.24	8.22–14.31	7.07–9.31

horizon were measured for studying the effect of depth on microbial distribution.

### 2.5. Total nitrogen

Soil samples were air-dried and digested with concentrated  $\text{H}_2\text{SO}_4$ . Total nitrogen was determined with a modified Kjeldahl method (Yang et al., 1991).

### 2.6. Total organic carbon

Soil sample 1.0 g, 1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  10  $\text{cm}^3$  and concentrated  $\text{H}_2\text{SO}_4$  20  $\text{cm}^3$  were mixed thoroughly, left to stand for 30 min, after which distilled water 200  $\text{cm}^3$  and 85%  $\text{H}_3\text{BO}_3$  10  $\text{cm}^3$  were added. After cooling, diphenylamine 1  $\text{cm}^3$  was added as indicator and the reaction mixture was titrated with 0.5 N ferrous (II) ammonium sulfate (Nelson and Sommers, 1982).

### 2.7. Microbial biomass carbon

Microbial biomass carbon was determined by the fumigation–extraction method (Vance et al., 1987). Soil sample (25 g) at 40% water-holding capacity and boiled chloroform (ethanol removal) (50  $\text{cm}^3$ ) were put in a desiccators for 20–24 h. After fumigation, 0.5 M  $\text{K}_2\text{SO}_4$  100  $\text{cm}^3$  was added, shaken at 250 rpm for 30 min, and filtered through Whatman No. 42 filter paper. Soil extract 8.0  $\text{cm}^3$  and 0.066 M  $\text{K}_2\text{Cr}_2\text{O}_7$  2.0  $\text{cm}^3$ , HgO 70 mg, conc.  $\text{H}_2\text{SO}_4$  10.0  $\text{cm}^3$  and 85%  $\text{H}_3\text{PO}_4$  5.0  $\text{cm}^3$  were mixed thoroughly. The mixture was refluxed for 30 min. After cooling, distilled water 20  $\text{cm}^3$  was added from the top to recover the reaction mixture, 0.025 M 1,1-phenanthroline–ferrous sulfate mixture 0.1  $\text{cm}^3$  was added as the indicator. The reaction mixture was back-titrated with 0.033 M ferrous (II) ammonium sulfate. Microbial biomass carbon was calculated as the difference of carbon content between non-fumigated and fumigated soil extracts.

### 2.8. Microbial biomass nitrogen

Microbial biomass nitrogen was measured by a fumigation–extraction method (Raghubanshi, 1991). The soil fumigation extract 5  $\text{cm}^3$ ,  $\text{Na}_2\text{S}_2\text{O}_3$  0.5 g and salicylic acid 0.5 g were dried at 60 °C, and the nitrogen content of the dry sample was determined by a modified Kjeldahl method (Yang et al., 1991) and microbial biomass nitrogen was calculated by the difference of nitrogen content between non-fumigated and fumigated soil extracts.

### 2.9. Organic acid determination

About 20 g soil and 20  $\text{cm}^3$  deionized water were mixed thoroughly at 100 rpm on a shaker for 1 h, and

filtered through Whatman No. 42 filter paper. After filtration with 0.46  $\mu\text{m}$  Millipore filter, organic acid was determined with a Shimadzu LC-9A high performance liquid chromatograph (Shimadzu Co., Japan). The operating conditions were with a supelcosil LC-18DB column (length 25 cm, i.d. 4.6 mm and particle size 5  $\mu\text{m}$ ) (Supelco Co., USA), and a mixture of 0.01 M phosphate buffer pH 3 and methanol (5:1, v/v) as the mobile phase. The flow rate was 0.8  $\text{cm}^3 \text{min}^{-1}$ , and the organic acid was detected with a Shimadzu SPD-2A UV detector at wavelength 210 nm and integrated with a Shimadzu CR-6A recorder. Authentic organic acids were used as the standard for qualitative and quantitative determination in the range from 0.0 to 200  $\mu\text{M}$ .

### 2.10. Chemical analysis

Moisture contents of soils were determined by drying the sample at 105 °C overnight to a constant weight. Soil pH was measured in five times volume of distilled water equilibrated with soil for 1 h with pH meter (Good digital pH meter model 2002, Taiwan). Air and soil temperatures were determined directly or under 5 cm depth of soil with a thermometer. Experiments were carried out to obtain three measurements and statistical analysis of the results was performed using analysis of variance and Duncan's multiple range test ( $p = 0.05$ ) with the help of the Statistical Analysis System as previously described (SAS Institute, 1988).

## 3. Results

### 3.1. Soil properties

Spruce soil of Tatchia mountain is sandy loam. The pH of the organic layer was the lowest, topsoil was the next, and subsoil was the highest, due to the deposition of organic layer on the surface and the incomplete decomposition of organic matter (Table 1). Spruce soil belonged to the acidic type. Moisture content of the organic layer was the highest, while subsoil was the lowest because of the lack of water penetration into soil and the effect of precipitation. Total organic carbon and nitrogen contents of the organic layer were also the highest.

### 3.2. Environmental conditions

Tatchia mountain is located in the central part of Taiwan, Nantou County. Daily air mean temperature was in the range of 4.0–17.5 °C. Air temperatures were higher than those of soil temperatures in the summer season, while the phenomenon was reversed in the winter season. The fluctuation of soil temperature was narrower than that of air temperature due to the high

heat capacity of soil. Annual average air temperature is around 12 °C, most months having cool weather. January and February have low temperature (2.2–7.8 °C), while August and September have high temperature (12–17 °C). The annual precipitation of Tatachia mountain was between 2800 and 3000 mm, and the annual evaporation ranged from 289.4 to 432.5 mm. The percentage of evaporation was between 9.35 and 23.9.

### 3.3. Bacterial population

Bacterial populations of Spruce soil ranged from  $1.38 \pm 0.26 \times 10^6$  to  $6.37 \pm 0.21 \times 10^6$  CFU g<sup>-1</sup> dry topsoil, and from  $8.07 \pm 0.55 \times 10^5$  to  $4.70 \pm 0.96 \times 10^6$  CFU

g<sup>-1</sup> dry subsoil (Fig. 1). Topsoil sample had higher population than that of subsoil because of the higher organic matter contents and higher oxygen concentration in the topsoil. From the statistical analysis, bacterial populations between topsoil and subsoil samples differed by 63.6%.

### 3.4. Actinomycetes population

Actinomycetes are known to have a diverse set of enzymes to degrade complex compounds for energy and biomass production. Actinomycete populations were between  $1.12 \pm 0.21 \times 10^4$  and  $7.33 \pm 0.64 \times 10^4$  CFU g<sup>-1</sup> dry topsoil, and between  $1.90 \pm 0.16 \times 10^3$  and  $4.96 \pm$

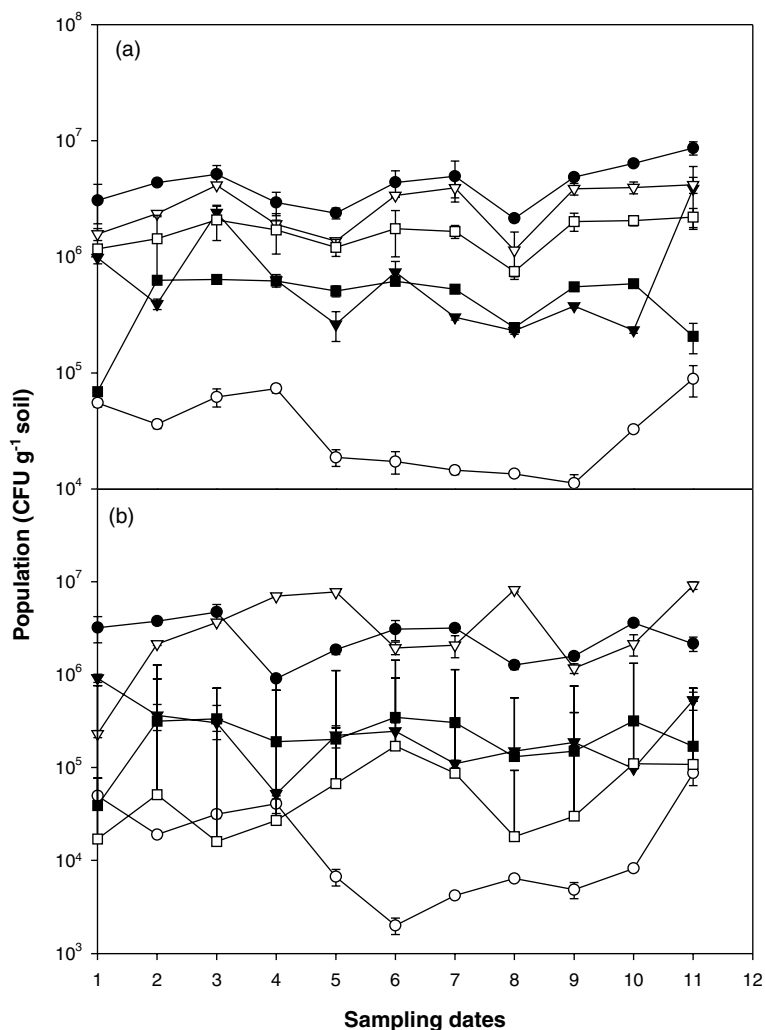


Fig. 1. Microbial population of Spruce soil in Tatachia mountain. Sampling dates were: (1) January 27, 1997; (2) April 27, 1997; (3) July 23, 1997; (4) October 10, 1997; (5) January 18, 1998; (6) April 30, 1998; (7) September 7, 1998; (8) December 11, 1998; (9) March 10, 1999; (10) April 10, 1999 and (11) November 10, 1999. (a) Topsoil (0–20 cm depth) (b) Subsoil (21–40 cm depth) (●): bacteria; (○): actinomycetes; (▼): fungi; (▽): cellulolytic; (■): phosphate-solubilizing; (□): nitrogen-fixing microbes).

$0.41 \times 10^4$  CFU  $g^{-1}$  dry subsoil (Fig. 1). Topsoil sample also had higher population than that of subsoil due to the higher organic matter content and higher oxygen concentration in the topsoil. From the statistical analysis, it was also shown that 90.9% of actinomycete populations had significant difference between tested topsoil and subsoil samples.

### 3.5. Fungal population

The pH of Spruce soil was acidic, which is favorable for fungal propagation. The fungal population was found to be high in the summer season as a result of warm temperature that favors fungal growth. The fungal population was high in topsoil sample due to the abundant amount of organic matter. Each gram of dry topsoil contained fungi  $2.31 \pm 0.16 \times 10^5$  to  $2.42 \pm 0.33 \times 10^6$  CFU, and each gram of dry subsoil had fungi between  $5.20 \pm 0.61 \times 10^4$  and  $9.16 \pm 0.90 \times 10^5$  CFU (Fig. 1). From the statistical analysis of fungal population, it was found that: (1) 45.5% of topsoil samples had significant higher population than those in subsoil and (2) seasonal variation showed no significant difference.

### 3.6. Cellulolytic, phosphate-solubilizing and nitrogen-fixing microbes

Cellulolytic, phosphate-solubilizing and nitrogen-fixing microbes are very important in the elemental cycle and in plant nutrition of grass and forest soils. The populations varied with the sampling season and the soil depth. Each gram of dry topsoil contained cellulolytic microbes  $1.57 \pm 0.19 \times 10^5$ – $4.13 \pm 0.07 \times 10^6$  CFU, while subsoil had  $2.30 \pm 0.20 \times 10^5$ – $3.65 \pm 0.06 \times 10^6$  CFU (Fig. 1). About 90.9% of topsoil samples had higher cellulolytic microbes than those of subsoil. Although, the populations of cellulolytic microbes varied at different seasons, the percentage of cellulolytic microbes in the total microbial count was very consistent. The values were between 42.43% and 80.19% (average was 62.28%), and between 41.84% and 86.37% (average was 65.19%) in topsoil and subsoil samples, respectively. The difference between the percentage of cellulolytic microbes in the total count of topsoil and subsoil samples was not significant. The seasonal variation of microbial populations and percentage of cellulolytic microbes in the total microbial count were also not significant.

Each gram of dry topsoil had phosphate-solubilizing microbes ranging from  $6.89 \pm 0.21 \times 10^4$  to  $6.40 \pm 0.17 \times 10^5$  CFU, while subsoil contained  $3.90 \pm 0.02 \times 10^4$ – $3.47 \pm 0.19 \times 10^5$  CFU (Fig. 1). Topsoil sample had higher number of phosphate-solubilizing microbes than subsoil in all tested samples, and 90.9% of them had significant difference. The percentage of phosphate-

solubilizing microbes in the total count ranged from 9.22% to 21.30% (average was 13.37%) in the topsoil and it ranged from 7.11% to 20.95% (average was 10.63%) in the subsoil. The percent of phosphate-solubilizing microbes in the total microbial count were not significantly different between topsoil and subsoil samples. Seasonal variation of microbial populations also showed no significant difference between topsoil and subsoil samples.

Each gram of dry topsoil contained nitrogen-fixing microbes  $7.48 \pm 0.75 \times 10^5$ – $2.08 \pm 0.70 \times 10^6$  CFU, and the values were between  $4.33 \pm 0.18 \times 10^5$  and  $1.34 \pm 0.16 \times 10^6$  CFU in subsoil (Fig. 1). From the statistical analysis, 54.5% of topsoil samples had significantly higher populations than those of subsoil. The percentage of nitrogen-fixing microbes in the total count ranged from 31.62% to 58.89% (average was 40.49%) and from 22.53% to 68.72% (average was 35.55%) in tested topsoil and subsoil samples, respectively. The percent of nitrogen-fixing microbes in the total microbial count was not significantly different between topsoil and subsoil samples. Seasonal variation of the microbial populations and the percentage of nitrogen-fixing microbes were also not significant.

### 3.7. Microbes in organic layer

There is a heavy layer of organic matter on the surface of Spruce soil. Three locations, rhizosphere, non-rhizosphere and dwarf bamboo, were chosen to compare the effect of rhizosphere and microbial populations in each location are presented in Table 2. Rhizosphere soil had the highest microbial population due to the abundance of root secretion. But the difference among them was not significant. Percentage of cellulolytic, phosphate-solubilizing and nitrogen-fixing microbes in the total microbial count was lower than that of topsoil (Table 3).

### 3.8. Microbial biomass carbon and nitrogen content

Microbial biomass carbon and nitrogen contents in the organic layer, topsoil and subsoil samples are illustrated in Tables 2 and 4. Topsoil had the highest microbial biomass carbon, followed by organic layer, and subsoil was the least. In the case of microbial biomass nitrogen, topsoil had the highest, followed by subsoil, and organic layer was the least. In organic layer, rhizosphere of Spruce had the highest microbial biomass carbon and nitrogen, while non-rhizosphere area was the lowest. There were significant differences among organic layer, topsoil and subsoil; or the organic layer among rhizosphere, dwarf bamboo and non-rhizosphere areas. The seasonal variation of microbial biomass carbon and nitrogen contents in all tested samples was not significant (Table 4).

Table 2  
Properties of organic layer in Spruce soil

Item	Rhizosphere of Spruce	Non-rhizosphere	Dwarf bamboo
<i>(a) December 11, 1998</i>			
pH	3.58 ± 0.10 <sup>c</sup>	4.19 ± 0.11 <sup>a</sup>	3.88 ± 0.12 <sup>b</sup>
Moisture content (%)	61.03 ± 2.71 <sup>b</sup>	67.10 ± 2.30 <sup>a</sup>	63.69 ± 2.43 <sup>a,b</sup>
Total carbon content (%)	25.54 ± 0.96 <sup>a</sup>	21.06 ± 0.99 <sup>b</sup>	19.09 ± 0.35 <sup>b</sup>
Total nitrogen content (%)	2.27 ± 0.01 <sup>b</sup>	2.51 ± 0.03 <sup>a</sup>	2.24 ± 0.03 <sup>b</sup>
C/N ratio	11.27 <sup>a</sup>	8.38 <sup>b</sup>	8.53 <sup>b</sup>
Microbial biomass C (μg g <sup>-1</sup> )	513.53 ± 1.05 <sup>a</sup>	215.86 ± 1.02 <sup>c</sup>	432.67 ± 1.38 <sup>b</sup>
Microbial biomass N (μg g <sup>-1</sup> )	24.76 ± 0.84 <sup>a</sup>	10.32 ± 0.52 <sup>b</sup>	26.73 ± 1.06 <sup>a</sup>
<i>(b) March 10, 1999</i>			
Microbial biomass C (μg g <sup>-1</sup> )	624.44 ± 1.56 <sup>a</sup>	243.33 ± 2.89 <sup>c</sup>	391.11 ± 1.32 <sup>b</sup>
Microbial biomass N (μg g <sup>-1</sup> )	32.44 ± 1.21 <sup>a</sup>	12.15 ± 0.95 <sup>c</sup>	23.70 ± 0.74 <sup>b</sup>
<i>(c) April 10, 1999</i>			
pH	5.00 ± 0.07 <sup>a</sup>	4.91 ± 0.07 <sup>a</sup>	4.82 ± 0.02 <sup>a</sup>
Moisture content (%)	73.70 ± 3.51 <sup>a</sup>	63.30 ± 4.04 <sup>b</sup>	56.70 ± 2.08 <sup>c</sup>
Total carbon content (%)	32.37 ± 1.12 <sup>a</sup>	30.05 ± 1.21 <sup>a</sup>	23.67 ± 1.04 <sup>b</sup>
Total nitrogen content (%)	2.19 ± 0.05 <sup>a</sup>	2.17 ± 0.05 <sup>a</sup>	2.10 ± 0.04 <sup>a</sup>
C/N ratio	14.79 <sup>a</sup>	13.87 <sup>a</sup>	11.27 <sup>b</sup>
Microbial biomass C (μg g <sup>-1</sup> )	653.33 ± 0.05 <sup>a</sup>	258.89 ± 0.04 <sup>c</sup>	422.20 ± 1.02 <sup>b</sup>
Microbial biomass N (μg g <sup>-1</sup> )	33.77 ± 0.56 <sup>a</sup>	13.12 ± 0.88 <sup>c</sup>	24.72 ± 0.09 <sup>b</sup>

Means ± SD ( $n = 3$ ). Means in the same row that do not share the same alphabetic superscript are significantly different at 5% levels according to Duncan's multiple range tests.

### 3.9. Effect of soil depth on microbial population

Soil property and microbial population at different horizons of Spruce soil are shown in Table 5. Soil pH increased gradually with the depth and had the highest pH at 2B2 horizon, then decreased sharply at 1E horizon, whereas soil moisture content decreased with increasing depth. Microbial populations also decreased stepwise with the depth of soil. The bacterial, actinomycete and fungal counts at 2B2 horizon were only 0.632%, 0.018% and 0.337% of those at O horizon, respectively.

### 3.10. Organic acid content

Organic acid content of Spruce soil is illustrated in Table 6. pH of organic layer in Spruce soil was the lowest, while pH of subsoil was the highest. pH value had a significant difference between organic layer and soil sample. As is expected, organic matter was higher in organic layer than that in topsoil and subsoil. Therefore, organic acid content was also high in organic layer, and low in subsoil. Each gram of dry organic layer contained formic acid 256–421 nM, acetic acid 301–435 nM, malic acid 795–1027 nM and succinic acid 204–670 nM, while subsoil had only malic acid 74–103 nM and succinic acid 32–142 nM. Two undetermined peaks of organic acid with  $3383 \pm 263$  and  $1322 \pm 142$  units were also detected in organic layer.

## 4. Discussion

Tatachia mountain is located in the central part of Taiwan and the altitude is from 1800 to 3952 m height. It can be divided into three parts according to the vegetation, Spruce (*P. morrisonicola*), Hemlock (*T. chinensis*) and grassland. Dwarf bamboo (*Y. niitakayamensis*) and graminaceous fern are also grown in this area. There is much leaf deposit on the surface of Spruce soil. pH of Spruce soil and organic layer were weakly acidic due to the abundant leaf litter on the surface, the incomplete decomposition of the litter and the accumulation of organic acids. Subsoil samples had higher pH values than those of organic layer or topsoil because of the lower organic matter and oxygen contents, and lower microbial activity and organic acid accumulation. Berg et al. (1998) indicated that Wekerom forest soil in the Netherlands had a soil pH of 3.8 due to the high organic matter content. Yang and Yang (2001) also reported that 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.

Soil moisture content depends on the environmental conditions and soil texture. Seasonal variation of moisture content of organic layer sample was higher than that of topsoil and subsoil due to the larger environmental change and the heavier organic matter covering. The same phenomena were also found in the power plant areas in Taiwan (Yang and Yang, 2001). In

Table 3  
Microbial populations in organic layer of Spruce soil

Sampling date	Rhizosphere of Spruce	Non-rhizosphere	Dwarf bamboo
<i>(a) Bacteria (CFU g<sup>-1</sup> dry soil)</i>			
December 11, 1998	1.72 ± 0.14 × 10 <sup>7a</sup>	1.51 ± 0.46 × 10 <sup>7a</sup>	1.67 ± 0.09 × 10 <sup>7a</sup>
March 10, 1999	4.48 ± 0.84 × 10 <sup>6a</sup>	3.47 ± 1.50 × 10 <sup>6a</sup>	3.29 ± 0.57 × 10 <sup>6a</sup>
April 10, 1999	1.28 ± 0.26 × 10 <sup>7a</sup>	8.32 ± 0.32 × 10 <sup>6b</sup>	7.70 ± 0.52 × 10 <sup>6b</sup>
<i>(b) Actinomycetes (CFU g<sup>-1</sup> dry soil)</i>			
December 11, 1998	4.76 ± 0.21 × 10 <sup>4a</sup>	3.75 ± 0.95 × 10 <sup>4a</sup>	1.23 ± 0.42 × 10 <sup>4b</sup>
March 10, 1999	2.45 ± 0.19 × 10 <sup>4a</sup>	1.63 ± 1.03 × 10 <sup>4a</sup>	1.47 ± 0.40 × 10 <sup>4a</sup>
April 10, 1999	6.68 ± 0.53 × 10 <sup>4a</sup>	4.19 ± 0.10 × 10 <sup>4a</sup>	4.75 ± 0.65 × 10 <sup>4a</sup>
<i>(c) Fungi (CFU g<sup>-1</sup> dry soil)</i>			
December 11, 1998	2.79 ± 0.84 × 10 <sup>6a</sup>	1.10 ± 0.11 × 10 <sup>6b</sup>	7.80 ± 1.10 × 10 <sup>5b</sup>
March 10, 1999	1.05 ± 0.01 × 10 <sup>6a</sup>	7.84 ± 1.10 × 10 <sup>5a</sup>	1.31 ± 0.13 × 10 <sup>6a</sup>
April 10, 2000	1.18 ± 0.26 × 10 <sup>6a</sup>	2.47 ± 0.11 × 10 <sup>5b</sup>	4.11 ± 0.78 × 10 <sup>5b</sup>
<i>(d) Cellulolytic microbes (CFU g<sup>-1</sup> dry soil)</i>			
December 11, 1998	4.22 ± 0.32 × 10 <sup>6a</sup>	3.74 ± 0.52 × 10 <sup>6a</sup>	1.08 ± 0.41 × 10 <sup>6b</sup>
	24.54 ± 1.25%*	24.97 ± 1.22%*	6.46 ± 0.42%*
March 10, 1999	8.91 ± 0.90 × 10 <sup>5a</sup>	4.09 ± 0.24 × 10 <sup>5a</sup>	1.92 ± 0.22 × 10 <sup>5b</sup>
	19.89 ± 1.23%*	11.79 ± 0.85%*	5.84 ± 0.30%*
April 10, 1999	3.28 ± 0.29 × 10 <sup>6a</sup>	1.19 ± 0.21 × 10 <sup>6b</sup>	1.05 ± 0.37 × 10 <sup>6b</sup>
	25.53 ± 1.31%*	14.30 ± 1.02%*	13.64 ± 1.09%*
<i>(e) Phosphate-solubilizing microbes (CFU g<sup>-1</sup> dry soil)</i>			
December 11, 1998	1.14 ± 0.33 × 10 <sup>6a</sup>	4.02 ± 0.54 × 10 <sup>5b</sup>	1.69 ± 0.17 × 10 <sup>6a</sup>
	6.63 ± 0.35%*	2.66 ± 0.26%*	10.12 ± 0.52%*
March 10, 1999	7.31 ± 0.14 × 10 <sup>5a</sup>	3.97 ± 0.24 × 10 <sup>5a</sup>	1.61 ± 0.08 × 10 <sup>5b</sup>
	16.32 ± 1.37%*	11.44 ± 1.01%*	4.89 ± 0.43%*
April 10, 1999	1.17 ± 0.25 × 10 <sup>6a</sup>	9.33 ± 0.16 × 10 <sup>5a</sup>	1.07 ± 0.40 × 10 <sup>6a</sup>
	9.14 ± 0.87%*	11.21 ± 1.00%*	13.90 ± 1.28%*
<i>(f) Nitrogen-fixing microbes (CFU g<sup>-1</sup> dry soil)</i>			
December 11, 1998	2.56 ± 0.35 × 10 <sup>5a</sup>	2.08 ± 0.07 × 10 <sup>5a</sup>	1.37 ± 0.90 × 10 <sup>5a</sup>
	1.49 ± 0.10%*	1.38 ± 0.15%*	0.82 ± 0.06%*
March 10, 1999	1.81 ± 0.44 × 10 <sup>5a</sup>	2.79 ± 0.52 × 10 <sup>5a</sup>	3.67 ± 0.16 × 10 <sup>5a</sup>
	4.04 ± 0.51%*	8.04 ± 0.72%*	11.15 ± 0.84%*
April 10, 1999	1.65 ± 0.51 × 10 <sup>6a</sup>	6.10 ± 0.11 × 10 <sup>5b</sup>	4.02 ± 0.43 × 10 <sup>5b</sup>
	12.89 ± 1.03%*	7.33 ± 0.61%*	5.22 ± 0.57%*

\* Percent of tested microbes in total viable count. Means ± SD ( $n = 3$ ). Means in the same row that do not share the same alphabetic superscript are significantly different at 5% levels according to Duncan's multiple range tests.

Table 4  
Microbial biomass carbon and nitrogen contents in Spruce soil

Sampling date	Topsoil		Subsoil	
	Biomass C ( $\mu\text{g g}^{-1}$ dry soil)	Biomass N ( $\mu\text{g g}^{-1}$ dry soil)	Biomass C ( $\mu\text{g g}^{-1}$ dry soil)	Biomass N ( $\mu\text{g g}^{-1}$ dry soil)
July 23, 1997	735 ± 32	172 ± 11	425 ± 22	132 ± 9
October 10, 1997	713 ± 27	165 ± 12	371 ± 18	125 ± 10
January 18, 1998	604 ± 21	128 ± 10	308 ± 20	107 ± 11
April 30, 1998	852 ± 33	214 ± 15	455 ± 30	156 ± 23
December 11, 1998	684 ± 24	153 ± 9	321 ± 17	115 ± 8
March 10, 1999	822 ± 39	218 ± 29	434 ± 27	131 ± 11
April 10, 1999	870 ± 42	240 ± 11	652 ± 23	173 ± 10
November 10, 1999	612 ± 20	183 ± 13	327 ± 12	122 ± 11

Means ± SD ( $n = 3$ ).

Table 5  
Microbial population at different profiles of Spruce soil

Profile	Depth (cm)	pH	Moisture content (%)	Bacteria (CFU g <sup>-1</sup> dry soil)	Fungi (CFU g <sup>-1</sup> dry soil)	Actinomycetes (CFU g <sup>-1</sup> dry soil)
O horizon	0–13	4.6 ± 0.1	65.0 ± 2.4	8.55 ± 0.05 × 10 <sup>6</sup>	8.55 ± 0.35 × 10 <sup>5</sup>	6.41 ± 0.82 × 10 <sup>5</sup>
1A horizon	14–31	5.3 ± 0.1	23.4 ± 0.1	8.10 ± 0.73 × 10 <sup>5</sup>	3.00 ± 1.25 × 10 <sup>4</sup>	7.58 ± 0.47 × 10 <sup>4</sup>
2A horizon	32–46	7.2 ± 0.1	19.2 ± 0.8	3.00 ± 0.15 × 10 <sup>5</sup>	4.94 ± 1.23 × 10 <sup>3</sup>	1.37 ± 0.09 × 10 <sup>4</sup>
2B horizon	47–60	7.6 ± 0.1	18.0 ± 0.8	1.92 ± 0.32 × 10 <sup>5</sup>	2.27 ± 0.39 × 10 <sup>2</sup>	4.87 ± 0.21 × 10 <sup>3</sup>
2B2 horizon	61–91	8.0 ± 0.1	13.8 ± 0.1	5.41 ± 1.77 × 10 <sup>4</sup>	1.51 ± 0.31 × 10 <sup>2</sup>	2.16 ± 0.16 × 10 <sup>3</sup>
1E horizon	>91	6.4 ± 0.1	20.4 ± 0.6	2.35 ± 0.57 × 10 <sup>4</sup>	3.52 ± 0.54 × 10 <sup>2</sup>	9.30 ± 1.01 × 10 <sup>3</sup>

Means ± SD (n = 3).

Table 6  
Organic acid content of Spruce soil (nM g<sup>-1</sup> dry soil)

Soil depth	Sampling date	Formic acid	Acetic acid	Malic acid	Succinic acid
Organic layer	September 7, 1998	256 ± 9	309 ± 10	795 ± 12	310 ± 12
	December 11, 1998	284 ± 12	301 ± 13	905 ± 32	204 ± 13
	March 10, 1999	330 ± 17	350 ± 14	850 ± 25	270 ± 10
	April 10, 1999	421 ± 15	435 ± 21	1027 ± 41	670 ± 23
Topsoil	September 7, 1998	–	–	155 ± 7	51 ± 3
	December 11, 1998	–	–	108 ± 7	106 ± 7
	March 10, 1999	–	–	155 ± 11	175 ± 9
	April 10, 1999	–	–	211 ± 12	168 ± 9
Subsoil	September 7, 1998	–	–	85 ± 6	32 ± 3
	December 11, 1998	–	–	74 ± 5	53 ± 5
	March 10, 1999	–	–	78 ± 6	60 ± 6
	April 10, 1999	–	–	103 ± 7	142 ± 11

–: Under detection; Means ± SD (n = 3).

addition, the water-holding capacity of organic layer was higher than that of topsoil and subsoil because of the high organic matter content. Srivastava and Singh (1988) also showed that the water-holding capacity of forest soil was higher than that of grassland in India.

Although the variation of culturable microbes was large during the tested periods, microbial populations were low in subsoil samples due to the low total organic carbon (23.66–34.35% in organic layer, 11.09–24.51% in topsoil and 5.83–11.20% in subsoil) and total nitrogen contents (1.26–3.19% in organic layer, 1.06–2.87% in topsoil and 0.90–1.90% in subsoil). 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. However, Tatachia forest soil had high humidity environment and heavy vegetation on the surface. Therefore, the microbial populations of Tatachia forest soils were higher than those of tropical soils in India. The microbial populations in subsoil samples showed 24.86–91.26% of bacteria, 11.63–97.98% of actinomycetes and 12.63–93.18% of fungi, 14.65–90.64% of cellulolytic microbes, 27.22–81.64% of phosphate-solubilizing microbes and 24.86–74.55% of nitrogen-fixing microbes as percentages of the values in topsoil samples. However, the percent of microbial population

in subsoil samples relative to organic layer samples was less than the above data. In contrast, the microbial populations in subsoil of power plant area 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 for the nutrition and moisture penetration from topsoil to subsoil (Yang and Yang, 2001). Microbial populations also decreased with increasing soil depth. 2B2 horizon had only 0.633%, 0.018% and 0.337% of bacteria, actinomycetes and fungi at O horizon, respectively. The same result was also found in grassland of Tatachia, 2A1 horizon (21–40 cm depth) had only 15–40%, 2.1–17.4% and 0.6–2.1% of bacteria, actinomycetes and fungi at O horizon (0–20 cm depth), respectively (Yang et al., 1998a). Bt horizon (39–52 cm depth) was only 3.32% of respiration and 1.75% of ergosterol content as Oe1 horizon (0–15 cm depth) in Hemlock soil of Tatachia (Imberger and Chiu, 2001). The humus layer of *Vaccinium vitisidaea* fertile site of Finland forest had higher numbers of culturable bacteria, pseudomonads, fungi and yeasts than those in the mineral soil (0–3 cm depth) (Priha et al., 2001). Microbial activity, biomass, ATP content and respiration decreased with increasing depth in beech forest soil (Stockfish et al., 1995; Lavahun et al., 1996). Bacterial



population and mycelial content decreased with increasing depth in pine forest soil (Berg et al., 1998). Nitrifying bacterial count also decreased with increasing soil depth in acidic forest soil of Douglas (de Boer et al., 1992).

Cellulolytic, phosphate-solubilizing and nitrogen-fixing microbes are very important in grassland and forest soil for the elemental cycle and the nutritional supplement. Although these populations varied with season and location, the ratios of these microbes to the total viable count could be used to assay the change of microbial ecology. The ratios of cellulolytic microbes to the total viable count were between 41.84% and 86.37% in Spruce soil, which were higher than 17.21–60.20% in nuclear and thermal power plants areas (Yang and Yang, 2001) and 11.53–25.15% in grassland of Tatachia (Yang et al., 1998a) due to the heavy coverage of organic matter lying on the surface of Spruce soil. Bradley and Fyles (1995) suggested that basal respiration was 2–3 times high in birch due to the high amount of root-labile C in this area. Tietema and Wessel (1994) also reported that oak leaf decomposition microbes were very active in forest soil for the elemental cycle and the nutritional supplement.

Microbial biomass carbon was high in topsoil sample and low in subsoil. The value was higher than that in tropical soils (149–667  $\mu\text{g C g}^{-1}$  dry soil), due to high temperature and low organic matter content in India (Srivastava and Singh, 1988). However, it was at the same level as the tropical forest soil in India (341–872  $\mu\text{g C g}^{-1}$  dry soil) (Raghubanshi, 1991), but lower than forest soil in Germany (317–2116  $\mu\text{g C g}^{-1}$  dry soil) (Joergensen et al., 1990). In microbial biomass nitrogen, topsoil of Spruce had a value higher than that in tropical forest soil in India (38–78  $\mu\text{g N g}^{-1}$  dry soil) (Raghubanshi, 1991), and at the same level as forest soil in Germany (30–347  $\mu\text{g N g}^{-1}$  dry soil) (Joergensen et al., 1990). Fumigation–extraction 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). Rhizosphere soil of Spruce had high microbial biomass carbon and nitrogen contents because of the abundance of root secretion. In addition, microbial populations in organic layer of Spruce rhizosphere were also higher than that of non-rhizosphere or dwarf bamboo. The differences in the chemical composition of litter and root exudates would be expected to result in differential availability of various carbon sources among stands of different tree species (Johansson, 1995; Grayston et al., 1996). Different mycorrhizal symbionts on the root of these trees can also significantly alter the chemical, physical and microbial composition of rhizosphere (Linderman, 1988).

Heavy coverage of organic matter was found in Spruce soil. Much organic acid was accumulated in the soil. Acetic acid and formic acid were only found in

organic layer, while the concentration of them in soil was below detection. Acetic acid and short chain fatty acids were found in anaerobic hog waste treatment (Nyns, 1989). Acetic acid, citric acid, fumaric acid, 2-ketogluconic acid, lactic acid, oxalic acid, and succinic acid were accumulated in microbially contaminated fuel oil (Yang et al., 1996), rhizosphere (Duff et al., 1963; Halder et al., 1991), and phosphate-solubilizing microbes (Gyaneshwar et al., 1998). Chen and Yang (2000) reported that 2B2 horizon (>56 cm depth) of Hemlock soil had only 2.17–14.28% of organic acid at O horizon (0–13 cm depth) in Tatachia.

## 5. Conclusion

In conclusion, Tatachia soil was weakly acidic. Bacteria were most abundant, followed by cellulolytic microbes, phosphate-solubilizing microbes, nitrogen-fixing microbes, fungi, and actinomycetes. Total organic carbon and nitrogen contents were high in Spruce soil of Tatachia. The microbial populations, microbial biomass carbon and nitrogen contents were also high in topsoil samples and low in subsoil. Microbial populations were significantly different between topsoil and subsoil samples. The ratios of cellulolytic microbes to the total viable count were high due to the heavy coverage of organic matter lying on the soil surface. Malic acid and succinic acid were the major organic acid in soil, while acetic acid and formic acid were also found in organic layer. They had positive correlation between microbial populations and microbial biomass carbon, between microbial populations and organic acid content.

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