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Original Article

Seasonal variation of microbial ecology in hemlock soil of Tatachia Mountain, Taiwan

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Background and Purpose: Forest soil microorganisms and fauna decompose the organic materials, and thus strongly influence the nutrient cycling of the ecosystem. Soil microorganisms also contribute to soil structure and soil fertility. In Taiwan, the microbial distributions of soils have only been determined in acidic soil, inorganic acidic soil, upland soil, alkaline soil and power plant areas. There are few data on the microbial populations of forest soils. Tatachia Mountain is located in the central part of Taiwan and is a typical high altitude protected ecosystem area, designated as a National Park. This study investigated the role of microorganisms in the ecology and nutrient transformation of forest soil in Taiwan.

Methods: As part of long-term ecological research in Taiwan, the environmental conditions, seasons, microbial populations, biomass and organic acid contents of hemlock soil were investigated. We also studied the effect of depth on microbial populations and biomass.

Results: The soil temperatures were between 5.5 and 15.6°C and the soil pH ranged from 3.3 to 4.4. Total organic carbon and total nitrogen contents ranged from 2.3 to 37.1% and from 0.3 to 1.7%, respectively. The carbon/nitrogen ratio was between 8.2 and 24.4. In topsoil, each gram of soil contained 10^5-10^7 colony-forming units (CFU) culturable bacteria, 10^2-10^5 CFU actinomycetes, 10^3-10^5 CFU fungi, 10^4-10^6 CFU cellulolytic microbes, 10^4-10^6 CFU phosphate-solubilizing microbes, and 10^3-10^6 CFU nitrogen-fixing microbes. Microbial populations were higher in topsoil compared with subsoil, but lower in topsoil than in organic layer. Microbial populations also decreased with the depth of soil. Microbial populations at 1E horizon were 0.6% to 9.4% of those at O horizon. The microbial biomass evaluated contained carbon 391-1013 μg, nitrogen 51-146 μg, malic acid 76-557 nM and succinic acid 37-527 nM per gram of soil. Summer season had higher microbial populations, biomass and organic content than winter season, but the differences were not significant.

Conclusion: Heavy coverage of organic matter was found in hemlock and spruce soils and was associated with acidic pH. Microbial populations decreased with increasing soil depth. Microbes play a very important role in organic matter decomposition and nutrition transformation in hemlock soil.

Key words: Biomass, ecosystems, seasons, soil microbiology, trees

Introduction

Forest soil microorganisms and fauna decompose organic materials, and thus they strongly influence the nutrient cycling of ecosystems. The numbers and species of microbes in soil vary directly with environmental conditions, nutrient availability, soil texture, and type of vegetation cover [1,2]. Soil microorganisms also contribute to soil structure and soil fertility [3,4]. The soil microflora has been extensively studied in different environments, such as high altitude [5-7], tundra [8], boreal forest [9], tropics [10], dessert mountain [11], and polluted and cultivated areas [12].

In Taiwan, the microbial distributions of soils have only been determined in acidic soil of Yang-Ming-Shan, inorganic acidic soil, upland soil, and alkaline soil of Tainan and power plant areas. There are few data on the microbial populations of forest soils [5,7,13]. Tatachia Mountain is located in the central part of Taiwan and is a typical high altitude protected ecosystem area,

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designated as a National Park, with 3 major vegetations: hemlock, spruce and grass. As part of long-term ecological research in Taiwan, we investigated the environmental conditions, seasons, microbial populations, biomass and organic acid contents of hemlock.

Methods

Sampling location

The study was conducted at Tatachia, in the saddle of Jade Mountain, central Taiwan (120°52' East, 23°28' North). Tatachia has an elevation from 1800 to 3952 m. Some environmental conditions and properties of the 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. Geologically, the study area consists of metamorphosed sedimentary rock (Miocene epoch), comprising sandstone and shale. The coniferous forest is dominated by Chinese hemlock (*Tsuga chinensis*). Less dominant species include Taiwan false cypress (*Chamaecyparis formosensis*), Armand's pine (*Pinus armandi*), spruce (*Picea morrisonicola*), dwarf bamboo (*Yushania niitakayamensis*), and alpine silver grass (*Miscanthus transmorrisonensis*).

Soil sampling

One soil core was collected randomly at the rhizosphere soil around large hemlock, small hemlock and dwarf bamboo in the organic layer, topsoil (0-20 cm) and subsoil (21-40 cm), respectively. The samples were sieved to 4 mm, homogenized, and stored at 4 ± 1°C for further studies. Soil samples were collected at different seasons from September 1996 to October 2000. Soil samples were collected during March to May, June to August, September to November and December to February, representing spring, summer, autumn and winter seasons, respectively.

Culture media and conditions

Bacteria were counted at 25°C after 5 days' incubation on nutrient agar containing beef extract 3.0 g/L, peptone 5.0 g/L, and agar 15.0 g/L at pH 6.8 ± 0.1. Actinomycetes were cultivated at 25°C for 7 days on glycerol-yeast extract medium comprised of glycerol 5.0 g/L, yeast extract 2.0 g/L, dipotassium hydrogenphosphate 1.0 g/L, and agar 15.0 g/L 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 [14]. Fungi were grown at 25°C for 5 days on Rose Bengal medium containing glucose 10.0 g/L, peptone 5.0 g/L, dipotassium hydrogenphosphate 1.0 g/L, magnesium sulfate 0.5 g/L, Rose Bengal 0.033 g/L, and agar 15.0 g/L at pH 6.8 ± 0.1. Cellulolytic microbes were assayed at 25°C after 7 days' incubation on Mandels-Reese medium with carboxymethylcellulose (CMC) as the sole carbon source and sprayed with Congo red to show clear zone around the colonies [5]. Phosphate-solubilizing microbes were measured at 25°C after 5 days on rock phosphate medium by the clear zone around the colonies [15]. Nitrogen-fixing microbes were characterized at 25°C after 7 days' incubation on nitrogen-free mannitol medium [5]. All experiments were carried out in triplicate.

Effect of soil profile on microbial populations

Soil profiles were taken from the hemlock zone and classified into horizons according to the international soil classification system [16]. Soil samples were collected from each horizon from the top of soil to 130 cm depth. The microbial populations at each horizon were measured in order to study the effect of depth on microbial distribution.

Microbial biomass carbon and nitrogen

Microbial biomass carbon and nitrogen were determined by the chloroform fumigation extraction method [17, 18]. Fresh soil samples (25 g) at 40% water-holding capacity were placed in a 50 mL beaker and kept in a vacuum desiccator containing a 100 mL beaker with 50 mL chloroform (ethanol removed) for 72-h fumigation. The fumigated treatments were evacuated using a vacuum pump until the chloroform boiled rapidly. After fumigation, the soil samples were transferred to a 250 mL conical flask and extracted with 0.5 M potassium sulfate (100 mL) after shaking at 250 rpm for 30 min, and filtered through Whatman No. 42 filter paper.

Soil extracts (8.0 mL) and 0.066 M potassium dichromate 2 mL, mercuric oxide 70 mg, concentrated sulfuric acid 10.0 mL and 85% phosphoric acid 5.0 mL were mixed thoroughly. The mixture was digested at 150°C for 30 min. The digest was titrated with 0.033 M ferrous (II) ammonium sulfate using 1,10-phenanthroline-ferrous sulfate mixture as indicator. For microbial biomass nitrogen, the digested filtrate was distilled by steam distillation by a modified Kjeldahl method [19], and titrated against hydrochloric acid (0.01 N). 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 carbon from fumigated and non-fumigated samples and K_{EC} = 0.45 [20]. The microbial biomass nitrogen (B_N) was calculated as $B_N = E_N/K_{EN}$, where E_N is the difference between extractable nitrogen from fumigated and non-fumigated samples and K_{EN} = 0.54 [17].

Organic acid determination

Twenty g soil and 20 mL 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 a 0.45 μm Millipore filter, organic acid was determined with a Shimadzu LC-9A high-performance liquid chromatograph (Shimadzu Co., Kyoto, Japan). The operating conditions were a Supelcosil LC-18DB column (length 25 cm, inside diameter 4.6 mm, and particle size 5 μm) [Supelco Co., Bellefonte, 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 mL/min, and the organic acid was detected with a Shimadzu SPD-2A UV detector at wavelength 210 nm integrated with a Shimadzu CR-6A recorder [21]. Authentic organic acids were used as the standard for qualitative and quantitative determination in the range from 0-200 μM.

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 5 times volume of distilled water equilibrated with soil for 1 h with a 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. Total nitrogen was determined by a modified Kjeldahl method [20]. Total organic carbon was measured as following: soil sample 1.0 g, 1 N potassium dichromate 10 mL and concentrated sulfuric acid 20 mL were mixed thoroughly and left to stand for 30 min, after which distilled water 200 mL and 85% boric acid 10 mL were added. After cooling, diphenylamine 1 mL was added as indicator and the reaction mixture was titrated with 0.5 N ferrous ammonium sulfate [22]. Experiments were carried out to obtain 3 measurements and statistical analysis of the results was performed using analysis of variance and Duncan's multiple range tests (p=0.05) with the help of Statistical Analysis System as previously described [23].

Results

Soil properties

Tatachia Mountain is located in the central part of Taiwan and the altitude is 1800 to 3952 m above sea level. It can be divided into 3 parts according to the vegetation, hemlock, spruce and grassland. Dwarf bamboo and graminaceous ferr are also grown in this area. The hemlock soil of Tatachia Mountain is sandy loam. The pH of organic layer was the lowest, topsoil was the next, and subsoil was the highest, due to the abundant leaf litter on the surface and the organic acid accumulation during the decomposition of litter (Table 1). Hemlock soil belonged to the acidic type.

Subsoil samples had higher pH values than those of organic layer or topsoil because of the lower organic matter content, microbial activity and organic acid

Table 1. Environmental conditions and properties of tested Tatachia hemlock soils

Variable	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		Hemlock, dwarf bamboo, ferr	
Color	Blackish brown	Grey to blackish grey	Grey to yellowish grey
Temperature (°C)			
Air	0.0-17.9	-	-
Soil	5.5-15.6	5.8-14.5	66.0-14.1
Light intensity (lux)	58-2333	-	-
pH	3.4-3.8	3.3-4.3	3.3-4.4
Moisture content (%, wet weight basis)	64.6-72.6	34.5-56.5	31.2-50.3
Water holding capacity (%)	379.7-467.3	145.0-357.2	107.5-125.7
Total organic carbon (%)	23.2-37.2	5.2-9.2	2.3-7.8
Total nitrogen (%)	1.0-1.7	0.9-1.7	0.3-0.9
Carbon/nitrogen ratio	21.5-24.4	8.2-14.3	8.3-11.5

accumulation. The pH values were slightly lower in summer season than in winter season as high temperature stimulates organic matter decomposition.

The moisture content of organic layer was the highest, while that of subsoil was the lowest because of the lack of water penetration into soil and the effect of precipitation. Seasonal variation of the moisture content of organic layer sample was higher than those of topsoil and subsoil due to the greater environmental change and the heavier organic matter covering. The soil moisture in summer season was also higher than those in winter season, consistent with the rainy season occurring during July to September. The water-holding capacities of organic layer in hemlock soil were higher than those of topsoil and subsoil because of the high organic matter content in organic layer. The total organic carbon, the total nitrogen and the carbon/nitrogen ratio of organic layer were also the highest, and were lowest in subsoil.

Environmental conditions

Annual average air temperature is around 12°C, with most months having cool weather. January and February have low temperature (2.2-7.8°C), while August and September have high temperature (12-17.9°C). The soil temperature in winter season (5.5-9.2°C) was higher than the air temperature, while in the summer season (10.3-15.6°C) it was lower than the air temperature.

The fluctuation of soil temperature was narrower than that of air temperature due to the high heat capacity of soil. The soil temperature was a major factor affecting the microbial population and nutrient cycling in forest soil. The annual precipitation of Tatachia Mountain was between 2800 and 3000 mm. The percentage of evaporation was between 9.35 and 23.9.

Microbial biomass carbon and nitrogen content

The microbial biomass carbon and nitrogen content are illustrated in Table 2. Each gram contained 891 to 1582, 684 to 1013 and 391 to 531 μg biomass carbon in organic layer, topsoil and subsoil, respectively.

Per-gram values were 69 to 113, 108 to 146 and 51 to 136 μg for biomass nitrogen in organic layer, topsoil and subsoil, respectively. Organic layer had high microbial biomass carbon and nitrogen because of high concentrations of oxygen, total organic carbon and total nitrogen. Summer season had high microbial biomass carbon and nitrogen due to the high temperature and the abundant leaf residue on the surface. However, the seasonal variation of microbial biomass carbon and nitrogen contents in all tested samples was not significant. The microbial biomass nitrogen content was also consistent with soil total nitrogen content.

Bacterial population

The bacterial populations of hemlock soil ranged from $(1.4 \pm 0.3) \times 10^6$ to $(5.9 \pm 0.8) \times 10^7$ CFU/g organic layer, from $(2.1 \pm 1.2) \times 10^5$ to $(1.1 \pm 0.1) \times 10^7$ CFU/g topsoil, and from $(1.1 \pm 1.0) \times 10^5$ to $(3.7 \pm 1.7) \times 10^6$ CFU/g subsoil (Fig. 1A). Organic layer of small hemlock had the highest bacterial populations, followed by large hemlock, and dwarf bamboo. Most organic layer and topsoil samples had higher bacterial populations than those of subsoil because of high organic matter content and high oxygen concentration. However, the bacterial populations in summer season (July to September) usually had higher values than those in winter season (December to February). Statistical analysis of bacterial populations showed no significant difference between summer season and winter season, but a significant (30.7%) difference was found between topsoil and subsoil samples.

Table 2. Microbial biomass carbon and nitrogen content in hemlock soil (mean ± standard deviation; n = 3)

Sampling date	Organic layer (μg/g dry soil)		Topsoil (μg/g dry soil)		Subsoil (μg/g dry soil)	
	Biomass C	Biomass N	Biomass C	Biomass N	Biomass C	Biomass N
July 23, 1997			1013 ± 213	108.6 ± 32.3	494 ± 95	56.6 ± 42.1
October 10, 1997			912 ± 138	146.5 ± 58.3	402 ± 74	136.6 ± 32.5
January 18, 1998			781 ± 145	121.2 ± 35.3	531 ± 89	51.4 ± 32.5
April 30, 1998			684 ± 133	128.2 ± 46.3	512 ± 114	88.5 ± 29.7
March 10, 1999			771 ± 21	121.8 ± 29.2	391 ± 20	127.3 ± 11.8
January 5, 2000	891 ± 230	69.3 ± 27.2	355 ± 65	55.5 ± 4.3	100 ± 26	12.0 ± 5.3
April 23, 2000	1334 ± 446	69.4 ± 21.3	502 ± 18	64.6 ± 2.4	184 ± 19	11.5 ± 6.5
July 15, 2000	1484 ± 499	107.5 ± 69.3	492 ± 14	95.6 ± 3.6	178 ± 18	44.1 ± 10.1
October 6, 2000	1582 ± 489	113.1 ± 20.2	593 ± 11	113.7 ± 4.1	201 ± 11	51.4 ± 6.4

Abbreviations: C = carbon; N = nitrogen

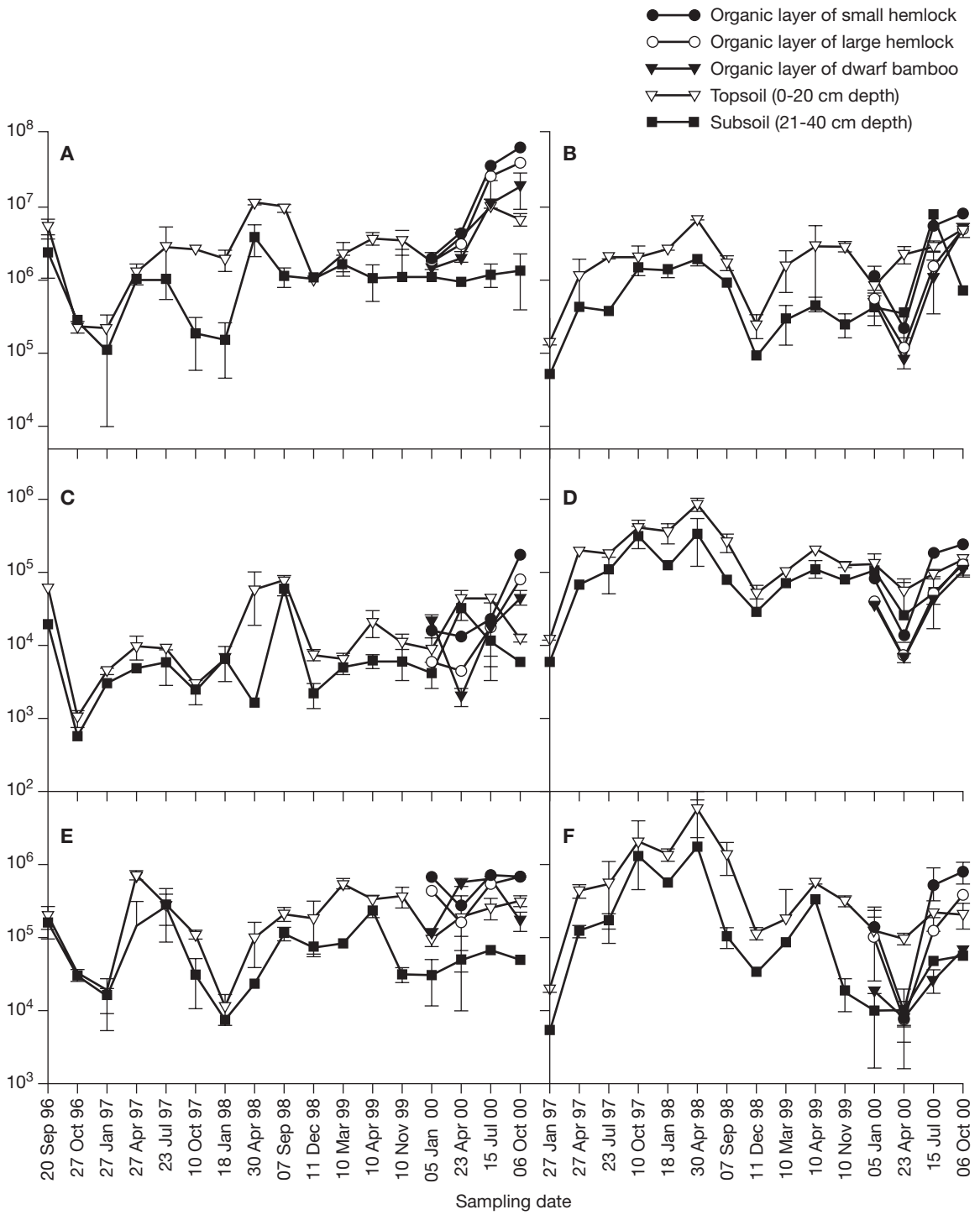

Fig. 1. Microbial populations of hemlock soil in Tatachia Mountain. (A) Bacteria; (B) actinomycetes; (C) fungi; (D) cellulolytic microbes; (E) phosphate-solubilizing microbes; (F) nitrogen-fixing microbes. Vertical bars represent standard deviation (n = 3).

Actinomycete population

Actinomycetes are known to have a diverse set of enzymes, enabling these organisms to degrade complex compounds for energy and biomass production. Organic layer and topsoil samples also had higher actinomycete populations than those of subsoil samples due to relatively high organic matter content and oxygen concentration (Fig. 1B). Summer season had higher actinomycete populations than winter season. From statistical analysis, it was also found that actinomycete

populations did not differ significantly between summer and winter seasons, and 38.4% of actinomycete populations differed significantly between topsoil and subsoil samples.

Fungal population

The pH of hemlock soil was acidic, which is favorable for fungal propagation. The fungal populations were found to be high in summer season and low in winter season, consistent with warmer temperatures favoring fungal growth. The fungal populations were high in organic layer and topsoil samples due to the abundant amounts of organic matter (Fig. 1C). From statistical analysis of fungal populations, it was found that: 1) 30.7% of topsoil samples had significantly higher populations than those in subsoil samples; and 2) the seasonal variation was not statistically significant.

Cellulolytic, phosphate-solubilizing and nitrogen-fixing microbes

Cellulolytic, phosphate-solubilizing and nitrogen-fixing microbes are very important in the elemental cycle and in the plant nutrition of forest soils. The populations varied with sampling season and soil depth. All organic layer and topsoil samples had higher counts of cellulolytic microbes than subsoil samples because of the abundant amount of leaf litter on soil surface and the high contents of total organic carbon and total nitrogen (Fig. 1D).

The populations of tested samples were higher in summer season and lower in winter season. The ratios of these microbes to the total viable count could be used to assay the change of microbial ecology. The percentages were higher in topsoil and lower in organic layer due to the differing carbon/nitrogen ratio between these layers. The values were between 3.7% and 59.7% (mean, 18.7%) in organic layer, between 18.0% and 89.7% (mean, 65.4%) in topsoil, and between 9.0% and 86.8% (mean, 47.2%) in subsoil samples. The values of topsoil ranged from 18.0% to 72.8% (mean, 45.4%) in summer season, and from 24.1% to 82.2% (mean, 57.5%) in winter season. The seasonal variation of microbial populations in the total microbial count was not significant in all tested samples, but was consistent with total organic carbon content. Thus, the populations of cellulolytic microbes and the ratio of cellulolytic microbes to the total viable count correlated with the total organic carbon content in nutrition cycles.

The content of phosphate-solubilizing microbes ranged from $(1.1 \pm 0.1) \times 10^4$ to $(3.3 \pm 0.8) \times 10^6$ CFU/g (Fig. 1E). Most samples had high numbers of phosphate-solubilizing microbes in all tested samples, and 50.0% and 45.5% of them showed significant differences between organic layer and topsoil, and between topsoil and subsoil, respectively. The seasonal variation was not significant. The percentage of phosphate-solubilizing microbes in the total count ranged from 0.4% to 12.2% (mean, 2.9%) in organic layer, from 9.2% to 66.5% (mean, 27.3%) in topsoil and from 6.8% to 60.7% (mean, 24.2%) in subsoil. The percentages of phosphate-solubilizing microbes in the total microbial count of organic layer were significantly lower than values for other organisms. However, the percentages were not significantly different between topsoil and subsoil samples or between summer season and winter season samples.

The content of nitrogen-fixing microbes ranged from $(1.2 \pm 0.3) \times 10^4$ to $(5.8 \pm 1.6) \times 10^5$ CFU/g in organic layer, from $(2.5 \pm 0.2) \times 10^4$ to $(3.2 \pm 1.7) \times 10^6$ CFU/g in topsoil, and from $(8.0 \pm 1.0) \times 10^3$ to $(1.2 \pm 2.8) \times 10^6$ CFU/g in subsoil (Fig. 1F). From statistical analysis, 50.0% and 63.6% of topsoil samples had significantly higher populations than organic layer and subsoil, respectively. The percentages of nitrogen-fixing microbes in the total count ranged from 0.3% to 7.2% (mean, 1.7%), from 7.4% to 51.8% (mean, 21.2%) and from 2.2% to 49.7% (mean, 17.4%) in tested organic layer, topsoil and subsoil samples, respectively. The percentages of nitrogen-fixing microbes in the total microbial count of organic layer were significantly lower than topsoil and subsoil. Although the percentages of nitrogen-fixing microbes in the total microbial count of topsoil were higher than those of subsoil, it was not a significant difference. Seasonal variations of the microbial populations were also not significant.

Although the variation of culturable microbes was high during the tested periods, the microbial populations were low in subsoil samples due to the low total organic carbon, total nitrogen and moisture content. The microbial populations had higher values in summer season than winter season because of the higher temperatures in summer season. The microbial populations of hemlock in subsoil samples showed 11.6% to 112.0% (mean, 57.9%) of bacteria, 2.6% to 97.0% (mean, 53.9%) of actinomycetes, 8.5% to 97.1% (mean, 52.4%) of fungi, 8.9% to 86.2% (mean, 33.8%) of cellulolytic microbes, 24.7% to

Table 3. Properties and microbial populations at different horizons in hemlock soil

Variable	O horizon	1A horizon	2A1 horizon	2B horizon	2B2 horizon	1E horizon
Depth (cm)	0-13	14-24	25-64	65-81	82-125	>125
pH[a]	4.1 ± 0.1	3.9 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
CEC (cmol/kg)	17	15	19	16	15	-
Potassium(cmol/kg soil)	0.5	0.2	0.1	0.1	0.1	-
Sodium (cmol/kg soil)	ND	0.1	ND	0.1	0.1	-
Calcium (cmol/kg soil)	0.4	ND	ND	ND	ND	ND
Magnesium (cmol/kg soil)	0.2	0.1	ND	ND	ND	ND
Moisture content (%)[a]	63.2 ± 1.4	41.7 ± 2.9	39.4 ± 1.7	38.2 ± 1.5	37.0 ± 1.9	37.5 ± 3.2
Total organic carbon (%)	6.7	4.3	2.2	1.0	1.6	-
Total nitrogen (%)	0.8	0.4	0.2	0.2	0.3	-
Carbon/nitrogen ratio	8.1	10.5	9.0	5.4	5.5	-
Bacteria (CFU/g dry soil)[a]	(3.2 ± 0.4) × 10^5	(7.7 ± 2.2) × 10^4	(6.8 ± 2.2) × 10^4	(7.3 ± 2.3) × 10^4	(5.8 ± 0.1) × 10^4	(1.1 ± 0.1) × 10^4
Fungi (CFU/g dry soil)[a]	(8.0 ± 2.2) × 10^4	(6.9 ± 1.7) × 10^3	(1.7 ± 0.2) × 10^3	(1.9 ± 0.4) × 10^3	(1.4 ± 0.2) × 10^2	(5.1 ± 0.8) × 10^2
Actinomycetes (CFU/g dry soil)[a]	(8.1 ± 0.3) × 10^4	(3.1 ± 0.5) × 10^4	(1.4 ± 0.2) × 10^4	(1.0 ± 0.1) × 10^4	(1.1 ± 0.1) × 10^4	(7.7 ± 0.0) × 10^3

Abbreviations: O = organic layer; 1A = 14-24 cm; 2A1 = 25-64 cm; 2B = 65-81 cm; 2B2 = 82-125 cm; 1E = >125 cm; CEC = cation exchange capacity; CFU = colony-forming units; ND = not detected

[a]Mean ± standard deviation (n = 3).

76.1% (mean, 46.7%) of phosphate-solubilizing microbes and 8.8% to 67.6% (mean, 38.3%) of nitrogen-fixing microbes in topsoil samples. However, the percentage of microbial population in subsoil samples relative to organic layer samples was less than the above data.

Effect of soil depth on microbial populations

The soil properties and the microbial populations at different horizons of hemlock soil are shown in Table 3. The soil pH increased slightly with depth, whereas the moisture content decreased with increasing depth. The cation exchange capacity decreased slightly with depth and had high value at 2A1 horizon. Potassium, calcium and magnesium concentrations also decreased with increasing depth. The total organic carbon, total nitrogen and carbon/nitrogen ratio decreased gradually with depth. Microbial populations decreased with depth of soil. The bacterial, actinomycete and fungal counts at 1E horizon were 3.4%, 9.4% and 0.6% of those at O horizon, respectively.

Organic acid content

The organic acid content of hemlock soil is illustrated in Table 4. pH of organic layer in hemlock soil was the lowest, while the pH of subsoil was the highest. pH differed significantly between organic layer and soil samples. The organic acid content was relatively high in organic layer and low in subsoil; organic acid content also decreased with increasing depth. Heavy coverage of organic matter was found in hemlock and spruce soils. Much organic acid was accumulated in soil, thus maintaining the acidic nature of Tatachia forest soils.

Tatachia hemlock soil is weakly acidic and its microbial population is characterized by high numbers of bacteria, followed by fungi and actinomycetes. Populations of organisms with cellulolytic, phosphate-solubilizing and nitrogen-fixing activities play important roles in nutrient cycling to this ecosystem. The microbial populations and organic acid content decreased with increasing soil depth. Positive correlations between microbial populations and microbial biomass carbon and between microbial populations and organic acid content were detected.

Discussion

Berg et al [24] indicated that Wekerom forest soil in the Netherlands had a pH of 3.8 due to the high organic matter content. Yang and Yang [25] reported that the nuclear and thermal power plants in northern Taiwan had soil pH ranging from 4.3 to 4.5, because of the organic matter covering surface and Inceptisol. In spruce soil of Tatachia Mountain, pH was lowest for organic layer, and increased progressively moving to topsoil and then subsoil; these results are attributable to surface leaf residue and the accumulation of organic acids during

Table 4. Organic acid content of hemlock soil (nM/g dry soil) [mean ± standard deviation; n = 3]

Soil depth	Sampling date	Formic acid	Acetic acid	Malic acid	Succinic acid
Organic layer	September 7, 1998	127 ± 10	149 ± 11	380 ± 15	210 ± 11
	December 11, 1998	147 ± 11	131 ± 12	402 ± 21	264 ± 14
	March 10, 1999	146 ± 12	171 ± 14	431 ± 18	257 ± 15
	April 10, 1999	218 ± 14	235 ± 17	557 ± 31	527 ± 22
Topsoil	September 7, 1998	UD	UD	165 ± 11	115 ± 13
	December 11, 1998	UD	UD	218 ± 17	86 ± 7
	March 10, 1999	UD	UD	255 ± 18	175 ± 9
	April 10, 1999	UD	UD	267 ± 12	198 ± 19
Subsoil	September 7, 1998	UD	UD	76 ± 6	37 ± 3
	December 11, 1998	UD	UD	117 ± 10	48 ± 5
	March 10, 1999	UD	UD	138 ± 16	78 ± 7
	April 10, 1999	UD	UD	127 ± 11	87 ± 8
O horizon	April 10, 1999	UD	UD	167 ± 21	84 ± 10
1A horizon		UD	UD	1157 ± 9	71 ± 9
2A1 horizon		UD	UD	45 ± 5	37 ± 4
2B horizon		UD	UD	27 ± 2	22 ± 2
2B2 horizon		UD	UD	17 ± 2	3 ± 1
1E horizon		UD	UD	11 ± 2	2 ± 1

Abbreviations: O = organic layer; 1A = 14-24 cm; 2A1 = 25-64 cm; 2B = 65-81 cm; 2B2 = 82-125 cm; 1E = >125 cm; UD = under detection

leaf residue decomposition [7]. The soil moisture was also higher in summer season than in winter season, the rainy season being July to September. The same phenomena were found in power plant areas and spruce soil of Tatachia Mountain in Taiwan [7,25].

The water-holding capacity of organic layer in hemlock soil was higher than that of topsoil and subsoil because of the high organic matter content in organic layer. Srivastava and Singh [26] also showed that the water-holding capacity of forest soil was higher than that of grassland in India. The microbial biomass of carbon was higher than that in tropical soils in India (149-667 μg carbon/g soil), due to the high temperature and the low organic matter content of the latter [26], but lower than spruce soil in Tatachia Mountain of Taiwan (893-2801 μg carbon/g organic layer, 644-2018 μg carbon/g topsoil and 438-832 μg carbon/g subsoil) [7], due to the low organic matter content in hemlock soil.

The fumigation-extraction biomass carbon of pine and spruce forest soils in Finland was also higher in 0 to 3 cm depth than in 0 to 6 cm depth of mineral soil [27]; the values for biomass nitrogen of organic layer, topsoil and subsoil were in the range 69 to 113, 108 to 146 and 51 to 136 μg/g, respectively. The values were higher than those in tropical forest soil in India (38-78 μg nitrogen/g soil) [19], slightly less than that of spruce soil in Tatachia Mountain (75-139 μg nitrogen/g organic layer, 83-210 μg nitrogen/g topsoil and 43-138 μg nitrogen/g subsoil) [7] and lower than that of forest soil in Germany (30-347 μg nitrogen/g soil) [28]. Organic layer had high microbial biomass carbon and nitrogen because of high concentrations of oxygen, total organic carbon and total nitrogen. Summer season was associated with high microbial biomass carbon and nitrogen due to the higher temperatures and the abundant surface leaf residue. However, the seasonal variation of microbial biomass carbon and nitrogen contents in all tested samples was not significant. The microbial biomass nitrogen content was also consistent with soil total nitrogen content.

The ratios of cellulolytic microbes to total viable count in topsoil and subsoil were higher than in soils in nuclear and thermal power plant areas (17.2-60.2%; mean, 33.9%) and grassland in Tatachia (11.5-25.2%; mean, 18.3%), but lower than in spruce soil in Tatachia (41.8-86.4%; mean, 63.7%) [7,25]. The tendency was consistent with the total organic carbon content. Therefore, the populations of cellulolytic microbes and ratios of cellulolytic microbes to the total viable count are correlated with total organic carbon content in nutrition cycles. Tietema and Wessel [29] also reported that oak leaf decomposition microbes were very active in forest soil for the elemental cycle and the nutritional supplement. The chemical composition of litter and root exudates influenced the availability of carbon sources for different tree species [30,31].

In Tatachia, the ratios of phosphate-solubilizing microbes to total viable count in topsoil and subsoil

were higher than in soils in nuclear and thermal power plants areas (2.1-40.7%; mean, 22.3%), spruce soil (7.1-21.3%; mean, 12.0%) and grassland (1.9-5.7%; mean, 3.8%) [5,7,25].

The percentages for total viable count in topsoil and subsoil were higher than grassland in Tatachia (2.8-7.3%; mean, 5.1%), the same as in soils in nuclear and thermal power plant areas (1.2-60.7%; mean, 23.9%), but lower than in spruce soil in Tatachia (22.5-68.7%; mean, 38.0 %) [5,7,25].

Srivastava and Singh [26] showed that tropical soils had low carbon (0.6%) and nitrogen (0.01%) contents due to the relatively dry conditions and the lack of vegetation. Fisk et al [32] reported that microbial activity of peatland was greater in surface than subsurface soil due to the high organic matter content. Yang et al [7] also investigated spruce soil of Tatachia Mountain, and reported that total organic carbon (23.7-34.4%) and total nitrogen (1.3-3.2%) was highest in organic layer, followed by topsoil (11.1-24.5% and 1.1-2.9%) and subsoil (5.8-11.2% and 0.9-1.9%). Tatachia forest soil had a high humidity and heavy vegetation on the surface. This would account for the microbial populations of Tatachia hemlock and spruce soils being higher than those of tropical soils in India. Hemlock soil had slightly lower total organic carbon and total nitrogen contents than spruce soil, and hence also lower microbial populations.

The percentages for subsoil-to-topsoil ratios of microbial populations of spruce soil in Tatachia Mountain were 24.9-91.3% (bacteria), 11.6-98.0% (actinomycetes), 12.6-93.2% (fungi), 14.7-90.6% (cellulolytic microbes), 27.2-81.6% (phosphate-solubilizing microbes) and 24.9-74.6% (nitrogen-fixing microbes) [7]. The ranges of hemlock soil were slightly larger than those of spruce soil due to the loose soil structures in hemlock soil. In contrast, a power plant area had only 19.2-71.6% of the bacteria, 21.6-87.3% of the actinomycetes and 15.4-72.9% of the fungi found in topsoil; these findings were attributed to the 'stickiness' of tested soils nutritionally and the prevailing water penetration from topsoil to subsoil [25].

2B2 horizon had only 0.6%, 0.1% and 0.3% of the bacteria, actinomycetes and fungi, respectively, present at O horizon in spruce soil [7]. Yang et al [5] also reported that 2A1 horizon of grassland soil (21-40 cm depth) in Tatachia had only 15.0-40.0%, 2.1-17.4% and 0.6-2.1% of bacteria, actinomycetes and fungi, respectively, present at O horizon (0-20 cm depth). Similarly, Imberger and Chiu [13] found that Bt horizon (39-52 cm depth) had only 3.3% of the respiration and 1.8% of the ergosterol content of Oe1 horizon (0-15 cm depth) in hemlock soil of Tatachia.

Humus layer of *Vaccinium vitisidaea*, a fertile site of Finland forest, had higher numbers of culturable bacteria, pseudomonads, fungi and yeasts than mineral soil (0-3 cm depth) consistent with the former's high organic carbon content [27]. The microbial activity, biomass, adenosine triphosphate content, respiration and relative abundance of denitrifying and dinitrogen-fixing bacteria decreased with increasing depth in forest soil or peatland [32-35], and the decomposition of glycine-^{13}carbon declined in forest soil of southern Appalachian Mountains with increasing altitude [36]. The bacterial population and the mycelial content decreased with increasing depth in pine forest soil [24], and the nitrifying bacterial count also decreased with increasing soil depth in acidic forest soil of Douglas [37].

In summary, heavy coverage of organic matter was found in hemlock and spruce soils. Much organic acid was accumulated in soil, and kept the Tatachia forest soils acidic. Summer season had higher microbial populations, biomass and organic content than winter season, but the differences were not significant. Microbial populations were higher in topsoil compared with subsoil, but lower in topsoil than in organic layer. Microbial populations also decreased with the depth of soil. Microbes play a very important role in organic matter decomposition and nutrition transformation in hemlock soil.

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