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ORIGINAL ARTICLE

# Effects of vegetation type on microbial biomass carbon and nitrogen in subalpine mountain forest soils

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*Background/purpose:* Microbial biomass plays an important role in nutrient transformation and conservation of forest and grassland ecosystems. The objective of this study was to determine the microbial biomass among three vegetation types in subalpine mountain forest soils of Taiwan.
*Methods:* Tatachia is a typical high-altitude subalpine temperate forest ecosystem in Taiwan with an elevation of 1800–3952 m and consists of three vegetation types: spruce, hemlock, and grassland. Three plots were selected in each vegetation type. Soil samples were collected from the organic layer, topsoil, and subsoil. Microbial biomass carbon ($C_{mic}$) was determined by the chloroform fumigation–extraction method, and microbial biomass nitrogen ($N_{mic}$) was determined from the total nitrogen ($N_{tot}$) released during fumigation–extraction. Bacteria, actinomycetes, fungi, cellulolytic microbes, phosphate-solubilizing microbes, and nitrogen-fixing microbes were also counted.
*Results:* The $C_{mic}$ and $N_{mic}$ were highest in the surface soil and declined with the soil depth. These were also highest in spruce soils, followed by in hemlock soils, and were lowest in grassland soils. $C_{mic}$ and $N_{mic}$ had the highest values in the spring season and the lowest values in the winter season. $C_{mic}$ and $N_{mic}$ had significantly positive correlations with total organic carbon ($C_{org}$) and $N_{tot}$. Contributions of $C_{mic}$ and $N_{mic}$, respectively, to $C_{org}$ and $N_{tot}$ indicated that the microbial biomass was immobilized more in spruce and hemlock soils than in grassland soils. Microbial populations of the tested vegetation types decreased with increasing soil depth.
*Conclusion:* $C_{mic}$ and $N_{mic}$ were high in the organic layer and decreased with the depth of layers. These values were higher for spruce and hemlock soils than for grassland soils. Positive correlations were observed between $C_{mic}$ and $N_{mic}$ and between $C_{org}$ and $N_{tot}$.


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## Introduction

Natural forests have gained recognition as the sites with high biodiversity. The complex relationships among fauna, flora, and microflora are due to the richness of habitat. Microbial biomass comprises about 2–3% of total organic carbon ($C_{org}$) and represents a major labile pool of nutrients in the soil.[1,2] It plays an important role in nutrient transformation and conservation of forest and grassland ecosystems, in both tropical and temperate climates.[3–8] The microbial biomass is influenced by soil organic matter content,[9,10] temperature,[4,11] moisture content,[12] and pH.[13] Soil microbial biomass is an important indicator of soil fertility in soil ecological studies[14,15] and sustainable environmental management.[16] The ratio of microbial biomass carbon to total organic carbon ($C_{mic}/C_{org}$) in soil may serve as a quantitative indicator of soil carbon dynamics.[17,18]

Forest vegetation affects the microbial processes of carbon and nitrogen cycles due to the differences in quality and quantity of litters, root exudates, and soil properties.[11,19–21] Tree species has an impact on soil fertility and microbial community composition, which in turn can affect the soil microbial biomass and microbial efficiency in carbon utilization.[3,9]

Microbial biomass varies with the seasonal patterns of soil temperature, moisture content, and substrate availability,[22,23] and also with seasons and soil depth.[24,25] Sarathchandra et al[22] showed that soil microbial biomass was greater in spring and autumn than in summer and winter; by contrast, Kaiser and Heinemeyer[26] observed higher microbial biomass in summer than in winter. Changes of microbial biomass with soil depth had been well documented.[24,25]

The size of soil microbial biomass is usually resource limitation, and the $C_{mic}$ and microbial biomass nitrogen ($N_{mic}$) are generally related to the soil organic matter content.[4,10] The different chemical compositions of roots and leaves can affect the compositions and functions of the microbial community. The amount of information on soil microbial biomass under different vegetation types in forest ecosystems is limited. Therefore, the objective of this study was to determine the microbial biomass under the three vegetation types in subalpine mountain forest soils of Taiwan. Seasonal variations of microbial biomass and microbial populations were also investigated.

## Materials and methods

### Site description and soil sampling

This study was conducted at Tatachia forest, in the saddle of Jade Mountain, central Taiwan (120° 52′ E, 23° 28′ N). Tatachia is a typical high-altitude subalpine temperate forest ecosystem in Taiwan with an elevation of 1800–3952 m. Tatachia forest (>400 ha) has been identified, by the Taiwan Long Term Ecological Study Network, as one of the four natural forest sites of subalpine forest ecosystems to study the long-term monitoring of environmental variables, plant successions, ecosystem phenomena and processes, dynamics of animal communities, soil nutrient movements, human impacts (recreational activities), ecological modeling, and data management. The study area geologically consists of metamorphosed sedimentary rock (*Miocene epoch*) comprising sandstone and shale.

Three permanent plots, each for a major vegetation type, were established in Tatachia subalpine ecosystem. The hemlock area is a thick forest floor dominated by Chinese hemlock (*Tsuga chinensis*) and dwarf bamboo (*Yushania niitakayamensis)*. The spruce area is a deep soil ecosystem dominated by spruce (*Picea morrisonicola*). Less dominant species include Taiwan false cypress (*Chamaecyparis formosensis*) and Armands pine (*Pinus armandi*). The grassland area is dominated by alpine silver grass (*Miscanthus transmorrisonensis*). The spruce and hemlock areas have the greatest canopy patches of woody plants, whereas the grassland area has the greatest canopy gaps and the shallowest soil formation due to erosion. Sampling was done after the selection of representative plots under three vegetation types. In each vegetation type, three 2 m × 2 m plots were selected; three replicate soil samples were collected from each plot at the organic layer, topsoil, and subsoil by digging vertically from the surface. The organic layer was collected from 0–5 cm soil layer after removal of the above-ground plant debris. Soils underneath the organic layer were then sampled at topsoil (6–20 cm depth) and subsoil (21–40 cm depth). The soils were blackish–brown (organic layer), grey–blackish grey (topsoil), and grey–yellowish grey (subsoil) in color. Soil samples were collected during March–May, June–August, September–November, and December–February, representing spring, summer, autumn, and winter seasons, respectively. The sampling sites were the same in all sampling periods. The collected soils were packed separately in plastic bags and rapidly transported to the laboratory. Visible materials such as roots and litters were manually removed prior to sieving through a 2 mm sieve. Soil samples were stored at 4°C in the dark until use.

### Microbial biomass carbon and nitrogen

The $C_{mic}$ was determined by the chloroform fumigation–extraction method.[27] Fresh soil was adjusted to 55% of water-holding capacity and preincubated at 25°C for 7 days prior to the measurement. Soils were fumigated for 24 hours with alcohol-free chloroform ($CHCl_3$) vapors. After the fumigant was removed, soils were extracted with 0.5 M $K_2SO_4$. The nonfumigated control soils were extracted under the same conditions at the time fumigation commenced. Fumigated soil extract (8.0 mL), 0.066 M $K_2Cr_2O_7$ (2 mL), HgO (70 mg), concentrated (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 minutes and titrated with 0.033 M ferrous (II) ammonium sulfate, using 1,10-phenanthroline–ferrous sulfate mixture as an indicator. The $C_{mic}$ was calculated according to the method described by Wu et al.[28] $C_{mic} = E_C/K_{EC}$, where $E_C$ is the difference between the C extracted from the fumigated and nonfumigated samples and $K_{EC} = 0.45$. The $N_{mic}$ was determined by analyzing total nitrogen ($N_{tot}$) in the 0.5 M $K_2SO_4$ extract obtained from fumigation–extraction.[29] The $N_{tot}$ of the extract was measured by the modified Kjeldahl method.[30] The $N_{mic}$ was calculated following the method described by Brookes et al.[29] $N_{mic} = E_N/K_{EN,}$ where $E_N$ is the difference between the N extracted from the fumigated and nonfumigated samples and $K_{EN} = 0.54$.

## Microbial populations

Bacteria were counted at 25°C for 5 days on nutrient agar (Merck, Darmstadt, Germany). Actinomycetes were cultivated at 25°C for 7 days on a glycerol—yeast extract medium consisting of (g/L) glycerol 5.0, yeast extract 2.0, $K_2HPO_4$ 1.0, and agar 15.0 at pH 7.0 ± 0.1. Streptomycin and cycloheximide were added to inhibit the growth of bacteria and fungi at a final concentration of 10 μg/mL.[31] Fungi were grown at 25°C for 5 days on the Rose Bengal medium containing (g/L) glucose 10.0, peptone 5.0, $K_2HPO_4$ 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, Rose Bengal 0.033, and agar 15.0 at pH 6.8 ± 0.1. Cellulolytic microbes were assayed at 25°C after 7 days of incubation on a modified Mandels—Reese medium with carboxymethylcellulose (Sigma-Aldrich, St. Louis, MO, USA) as the sole carbon source and sprayed with Congo red to show a clear zone around the colonies.[32] Phosphate-solubilizing microbes were measured at 25°C after 5 days on a rock phosphate medium from the clear zone around the colonies.[33] Nitrogen-fixing microbes were counted after incubation at 25°C for 7 days on a nitrogen-free mannitol medium.[24] All experiments were carried out in triplicate.

## Chemical analysis

Moisture content was determined by drying the sample overnight at 105°C to a constant weight. Soil pH was measured in five times volume of distilled water equilibrated with soil for 1 hour by a pH meter (Good digital pH meter model 2002, Plasma Equipment, Taiwan). Air temperatures were determined directly and under 5 cm depth of soil, respectively, with a thermometer. The $N_{tot}$ was measured by the modified Kjeldahl method[30] and $C_{org}$ was estimated by the modified Walkey—Black method, as described by Nelson and Sommers.[34] Soil sample (0.1 g) was mixed vigorously with 1 N $K_2Cr_2O_7$ (10 mL) and conc. $H_2SO_4$ (20 mL) for 30 minutes, and then 85% $H_3PO_4$ (10 mL) and distilled water (200 mL) were added to the mixture. After cooling, it was titrated with 0.5 N $Fe(NH_4)_2(SO_4)_2$ using diphenylamine as an indicator.

$$C_{org}\ (\%) = [(1 - S/B) \times 3.896]/\text{weight of soil sample} \times 100,$$

where $S$ and $B$ are the titer values of the soil and blank samples, respectively.

## Statistical analysis

Analyses were carried out using triplicate samples, and the results were reported on a dry weight basis. Effects of vegetation type, soil horizon, and sampling date on soil properties were tested by the one-way analysis of variance and the Tukey multiple range tests using the statistical package Sigma Stat for Windows version 2.0.[35] Linear regression analyses were carried out to find out the relationship between $C_{mic}$ and $N_{mic}$ and other soil variables ($C_{org}$, $N_{tot}$, C/N ratio, pH, and moisture content), and it was considered significant at $p < 0.05$.

# Results

## Environmental conditions and soil properties

Mean air and soil temperatures of spruce, hemlock, and grassland vegetation types ranged from 5.40 ± 0.28°C to 20.72 ± 0.65°C and from 6.22 ± 0.22°C to 16.05 ± 0.63°C, respectively (Table 1). The air temperatures were low in the winter season and high in the summer season. Soil temperatures were higher than air temperatures in the winter season and the reverse trend was observed in the summer season. Soil properties of spruce, hemlock, and grassland vegetation types are presented in Fig. 1. Tatachia soils are acidic (pH ranging from 3.40 ± 0.09 to 4.70 ± 0.11), and the pH of deeper horizons were higher than those of the organic layer. The pH of grassland soils was highest, whereas that of hemlock soils was lowest. Moisture contents were high in the organic layer and low in the subsoil. Hemlock soils had the highest moisture content, whereas grassland soils had the lowest. The $C_{org}$ and $N_{tot}$ of spruce and hemlock soils were higher than those of grassland soils. Furthermore, $C_{org}$, $N_{tot}$, and C/N ratio were high in the organic layer and low in deeper layers.

Based on the seasonal variation, $C_{org}$ and $N_{tot}$ were high in the spring season and low in the winter season; pH and moisture content were high in the autumn season and low in the summer season. Analysis of variance showed that vegetation types, soil horizons, and sampling seasons had significant to very significant effects on $C_{mic}$ ($p < 0.01$—$0.001$) and $N_{mic}$ ($p < 0.001$). Vegetation types had significant to very

**Table 1** Environmental conditions of spruce, hemlock, and grassland soils in Tatachia forest

| Properties | Spruce | Hemlock | Grassland |
|---|---|---|---|
| Characteristic plant | *Picea morrisonicola* | *Tsuga chinensis*<br>*Y. niitakayamensis* | *Miscanthus transmorrisonensi* |
| Mean air temperature (°C)[a] | | | |
| Autumn | 13.35 ± 0.19—13.77 ± 0.21 | 14.21 ± 0.57—15.03 ± 0.60 | 14.97 ± 0.20—15.24 ± 0.24 |
| Winter | 5.40 ± 0.28—5.83 ± 0.31 | 6.70 ± 0.20—6.94 ± 0.23 | 7.50 ± 0.29—7.97 ± 0.33 |
| Spring | 14.88 ± 0.61—15.60 ± 0.70 | 16.32 ± 0.35—16.93 ± 0.40 | 16.82 ± 0.46—17.21 ± 0.51 |
| Summer | 16.14 ± 0.48—16.78 ± 0.52 | 16.89 ± 0.51—17.51 ± 0.67 | 20.01 ± 0.53—20.72 ± 0.65 |
| Mean soil temperature (°C)[a] | | | |
| Autumn | 12.13 ± 0.08—12.31 ± 0.10 | 12.72 ± 0.13—12.91 ± 0.16 | 14.57 ± 0.12—14.72 ± 0.13 |
| Winter | 6.22 ± 0.22—6.51 ± 0.28 | 10.52 ± 0.42—11.11 ± 0.63 | 11.22 ± 0.31—11.53 ± 0.36 |
| Spring | 13.01 ± 0.21—13.40 ± 0.30 | 13.11 ± 0.42—13.82 ± 0.51 | 13.47 ± 0.44—14.06 ± 0.56 |
| Summer | 14.31 ± 0.44—14.75 ± 0.51 | 14.47 ± 0.40—14.91 ± 0.50 | 15.23 ± 0.58—16.05 ± 0.63 |

[a] Means of temperature measured during the sampling period ($n = 3$).

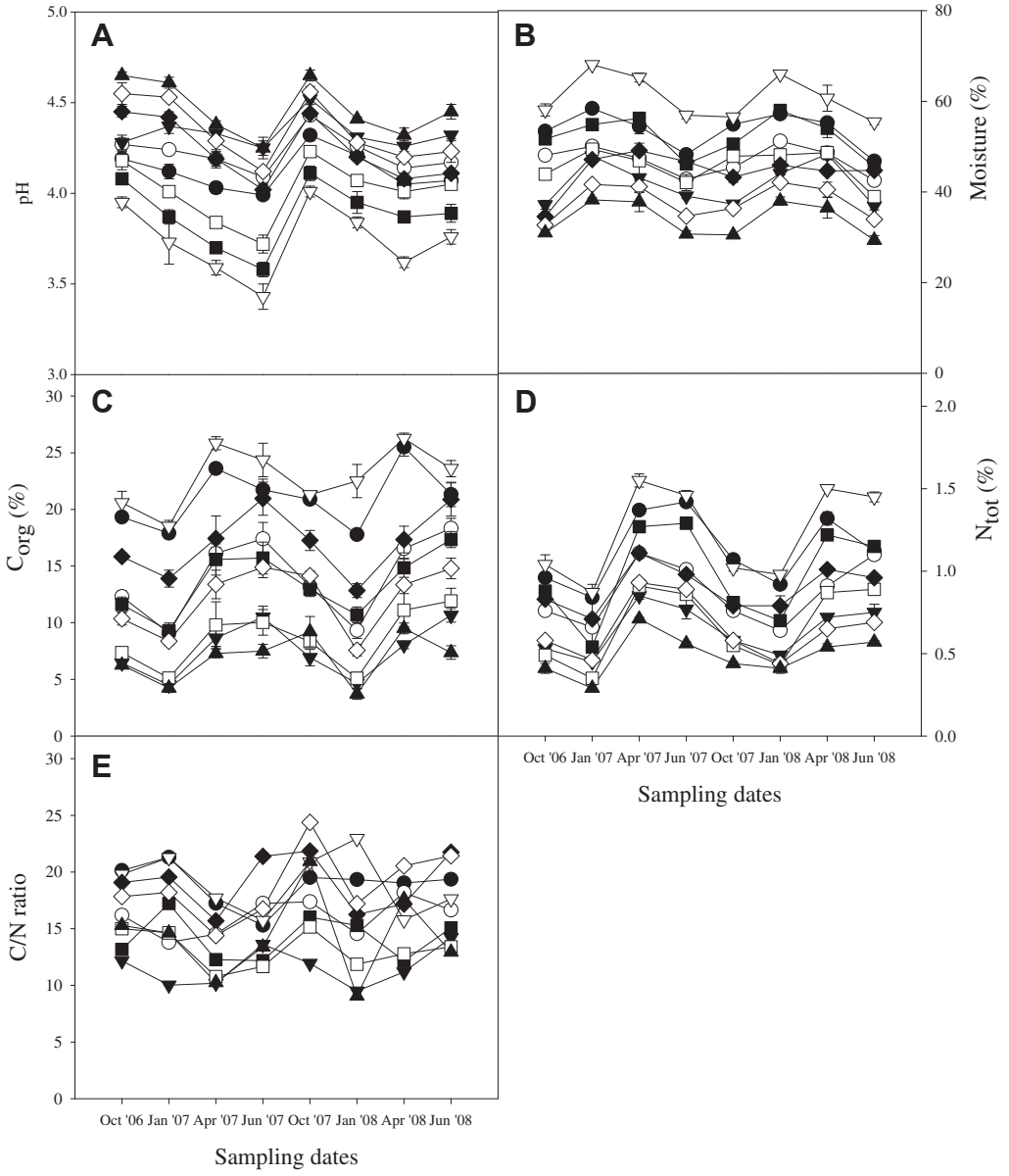


**Figure 1.** Physicochemical properties of spruce, hemlock, and grassland soils: (A) pH, (B) moisture content, (C) $C_{org}$, (D) $N_{tot}$, and (E) C/N ratio. Vertical bars represent the standard deviations ($n = 9$). Symbols used in the figure represent the following: ● = spruce organic layer; ○ = spruce topsoil; ▼ = spruce subsoil; ▽ = hemlock organic layer; ■ = hemlock topsoil; □ = hemlock subsoil; ◆ = grassland organic layer; ◇ = grassland topsoil; ▲ = grassland subsoil. Vertical bars represent the standard deviations ($n = 9$). $C_{mic}$ = microbial biomass carbon; $C_{org}$ = total organic carbon; $N_{mic}$ = microbial biomass nitrogen; $N_{tot}$ = total nitrogen.

significant effects on pH ($p < 0.001$), moisture content ($p < 0.001$), $C_{org}$ ($p < 0.001$), $C_{mic}/N_{mic}$ ($p < 0.001$), and $N_{tot}$ ($p < 0.05$). Soil horizons had significant to very significant effects on moisture content ($p < 0.001$), $C_{org}$ ($p < 0.001$), $N_{tot}$ ($p < 0.001$), $C_{org}/N_{tot}$ ($p < 0.001$), and pH ($p < 0.05$). Sampling seasons had very significant effects on pH ($p < 0.01$) and $N_{tot}$ ($p < 0.01$).

## Microbial biomass carbon and nitrogen

The $C_{mic}$ and $N_{mic}$ in three different vegetation types are presented in Fig. 2. In spruce soils, the $C_{mic}$ and $N_{mic}$ ranged from 380 ± 10 μg/g dry soil to 1320 ± 25 μg/g dry soil and from 74 ± 5 μg/g dry soil to 233 ± 8 μg/g dry soil, respectively. In hemlock soils, the values ranged from 405 ± 9 μg/g dry soil to 1305 ± 24 μg/g dry soil and from 65 ± 5 μg/g dry soil to 275 ± 10 μg/g dry soil, respectively. In grassland soils, the $C_{mic}$ and $N_{mic}$ ranged from 140 ± 7 μg/g dry soil to 855 ± 15 μg/g dry soil and from 48 ± 4 μg/g dry soil to 176 ± 8 μg/g dry soil, respectively. Grassland soils had the lowest $C_{mic}$ and $N_{mic}$ among all tested sites. The $C_{mic}$ and $N_{mic}$ declined substantially with soil depth in all three vegetation types. The organic layer had the highest $C_{mic}$ and $N_{mic}$, and the values decreased significantly with soil depth ($p < 0.001$). The maximal $C_{mic}$ and $N_{mic}$ were obtained in the spring season and the minimal values in the

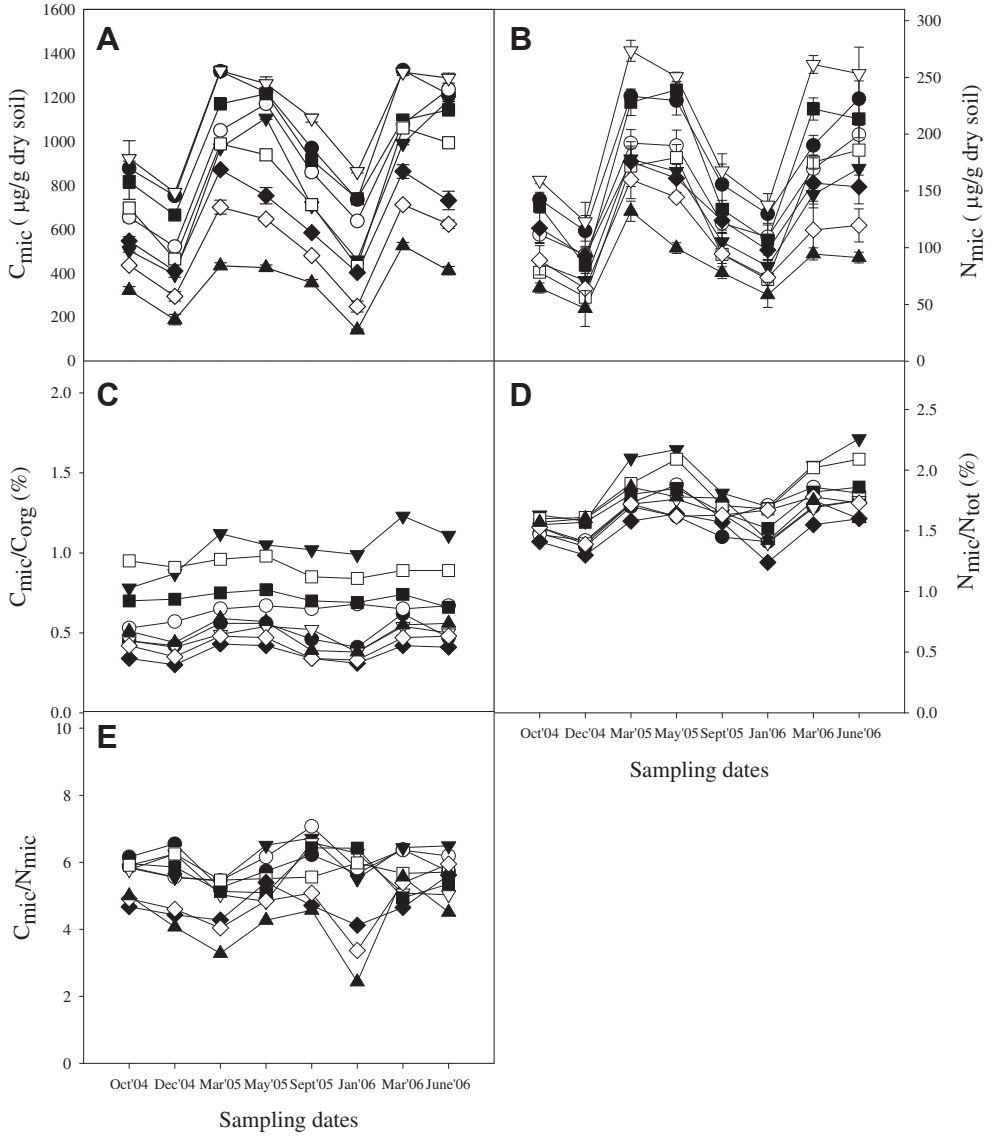


**Figure 2.** Values of $C_{mic}$ and $N_{mic}$ of spruce, hemlock, and grassland soils: (A) $C_{mic}$, (B) $N_{mic}$, (C) $C_{mic}/C_{org}$ (D) $N_{mic}/N_{tot}$, and (E) $C_{mic}/N_{mic}$. Vertical bars represent the standard deviations ($n=9$). Symbols used in the figure represent the following: ● = spruce organic layer; ○ = spruce topsoil; ▼ = spruce subsoil; ▽ = hemlock organic layer; ■ = hemlock topsoil; □ = hemlock subsoil; ◆ = grassland organic layer; ◇ = grassland topsoil; ▲ = grassland subsoil. Vertical bars represent the standard deviations ($n=9$). $C_{mic}$ = microbial biomass carbon; $C_{org}$ = total organic carbon; $N_{mic}$ = microbial biomass nitrogen; $N_{tot}$ = total nitrogen.

winter season. The $C_{mic}/C_{org}$, $N_{mic}/N_{tot}$, and $C_{mic}/N_{mic}$ ratios increased with soil depth. The highest ratios were observed in spruce soils, followed by for hemlock soils, and the lowest ratios were reported in grassland soils. The $C_{mic}$ and $N_{mic}$ had significant positive correlations with $C_{org}$ ($p < 0.05-0.001$) and $N_{tot}$ ($p < 0.01-0.001$) in all the tested vegetation types.

## Microbial populations

Microbial populations of the three tested sites are presented in Fig. 3. Grassland soils had the lowest microbial populations among the tested sites. However, the differences of microbial populations among the three vegetation types are more significant in the organic layer. The organic layer contained the highest populations, which decreased gradually with depth. Microbial populations were highest in the spring season and lowest in the winter season. Bacterial population was highest among the microbial populations. The ratios of cellulolytic microbes to total microbial populations in organic layers were high due to the roles of carbon cycle.

## Discussion

The $C_{org}$ and $N_{tot}$ were high in the organic layer and decreased in the deeper layers, which indicated that the major nutrient pool in the three vegetation types was the organic layer. The $C_{mic}$ and $N_{mic}$ are generally related to the soil organic matter content in forest soils.[4,7,8] The higher $C_{mic}$ and $N_{mic}$ in the surface soil than in the deeper layers were due to their positive correlations with organic matter content and oxygen availability.[36] The $C_{mic}$ and $N_{mic}$ were

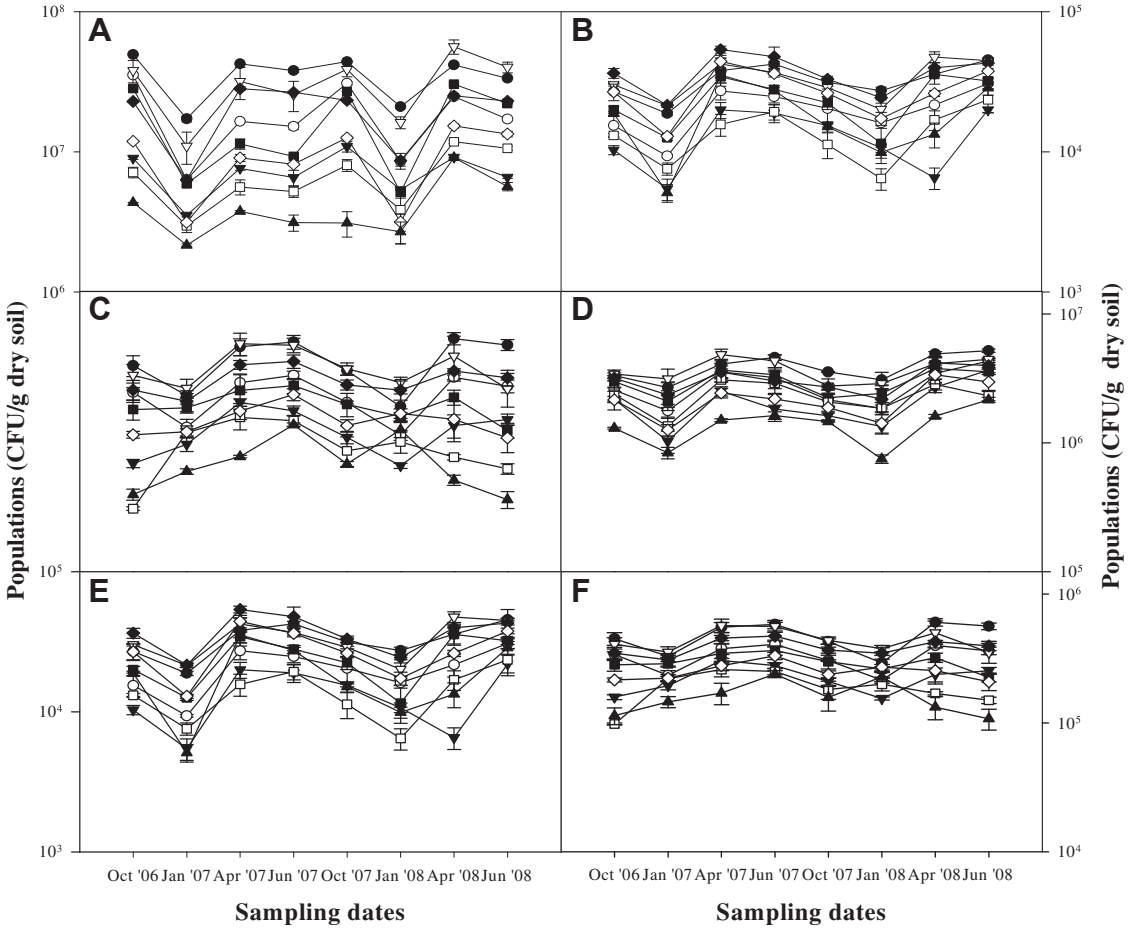


**Figure 3.** Microbial populations (CFU/g dry soil) of spruce, hemlock, and grassland soils: (A) bacteria, (B) actinomycetes, (C) fungi, (D) cellulolytic microbes, (E) phosphate-solubilizing microbes, and (F) nitrogen-fixing microbes. Symbols used in the figure represent the following: ● = spruce organic layer; ○ = spruce topsoil; ▼ = spruce subsoil; ▽ = hemlock organic layer; ■ = hemlock topsoil; □ = hemlock subsoil; ◆ = grassland organic layer; ◇ = grassland topsoil; ▲ = grassland subsoil. Vertical bars represent the standard deviations ($n$ = 9).

higher in the spruce and hemlock soils than in the grassland soils, due to a high tree density and a greater quantity of litters in these soils. In addition, there are large areas of canopy gaps in the grassland patches, and the effective soil layer was shallow due to erosion. Therefore, leaf litter was lowest and soil pH was highest in the three tested soils. Apparently, the differences in substrate quantities between the vegetation types contributed to the differences in $C_{mic}$ and $N_{mic}$, as indicated by the positive correlations between $C_{mic}$ and $N_{mic}$ and between $C_{org}$ and $N_{tot}$. Sparling et al[37] had also reported positive correlations between $C_{mic}$ and $N_{mic}$ and between $C_{org}$ and $N_{tot}$ in native forest. The $C_{mic}$ values obtained in the present study were between 140 ± 7 μg/g and 1320 ± 25 μg/g. It was within the ranges reported by Vance et al[38] and Henrot and Robertson[5] in various temperate and tropical forest soils, by Arunachalam and Arunachalam[39] in subtropical forests (978–2088 μg/g), and by Devi and Yadava[40] in the mixed oak forest ecosystem (71–1412 μg/g). The values of $N_{mic}$ in Tatachia forest ranged from 48 ± 4 μg/g to 275 ± 10 μg/g, which were also comparable to those in evergreen forests soils (42–242 μg/g), broad-leaved deciduous forest soils (132–240 μg/g),[41] and forest soils in Germany (317–2116 μg/g).[42] The $C_{mic}$ and $N_{mic}$ of three tested vegetation types were significantly high in the spring season and low in the winter season. Similar phenomenon was also reported in tropical dry deciduous forests by Saratchandra et al[43] and Singh et al.[44] Low values of $C_{mic}$ and $N_{mic}$ in the winter season may be due to the low activities of microorganisms and slow rates of decomposition of litters in a dry and cool period.[40]

The $C_{mic}/C_{org}$ ratios in spruce soils (0.4–1.2%) and hemlock soils (0.4–0.96%) were higher than that in grassland soils (0.3–0.6%); the $N_{mic}/N_{tot}$ ratios in spruce soils (1.4–2.3%) and hemlock soils (1.4–2.1%) were also higher than that in grassland soils (1.2–1.7%). These indicated higher C and N immobilization in the spruce and hemlock soils than in the grassland soils. The $C_{mic}/C_{org}$ values obtained in the present study were lower than those of the tropical forest soils (1.5–5.3%)[45] and tropical wet evergreen forest soils (4–6%),[46] and similar to those of the subtropical humid forest soils (0.7–1.7%)[47] and Fushan forest soils (0.3–2.7%).[25] The $N_{mic}/N_{tot}$ ratios of the present study were comparable to those of the tropical wet evergreen forest soils (1.3–1.7%),[46] mixed oak forest soils (0.93–1.8%),[41] and Fushan forest soils (1.5–2.6%);[25] but lower than those of the agricultural soils (2–6%),[29] forest

soils (3.4–5.9%),[48] and forest regrowth soils (7.3–8.3%).[49] These may be due to the low $N_{mic}$ and $N_{tot}$ in the Tatachia forest soils, which indicate that the soil is poor in nitrogen. Joergensen et al[42] reported that most forest soils had lower $N_{mic}$ than agricultural soils. The $C_{mic}/C_{org}$ and $N_{mic}/N_{tot}$ ratios were high in the spring season and low in the winter season, which indicated high immobilization of $C_{mic}$ and $N_{mic}$ in the spring season.

The $C_{mic}/N_{mic}$ ratio is often used to describe the structure and state of the microbial community. A high $C_{mic}/N_{mic}$ ratio indicates that the microbial biomass contains a high proportion of fungi, whereas a low value suggests that bacteria predominate in the microbial populations.[42] Paul and Clark[50] reported that bacterial dominant soil had a C/N ratio between 3 and 5, whereas a C/N ratio between 10 and 15 indicated the dominancy of fungi. In the present study, the $C_{mic}/N_{mic}$ ratios of spruce, hemlock, and grassland soils were 5.2–6.5, 4.8–6.6, and 4.1–5.6, respectively, showing the dominancy of bacteria.

Microbial growth and metabolism in soils are limited by the availability and types of organic substrates.[51] Among the three tested vegetation types, grassland soils had the lowest microbial populations, which may be due to less $C_{org}$ and $N_{tot}$. The different plant species have different nutrient demands and produce different qualities and quantities of litter, which affect microbial populations and diversities.[52] Microbial populations were significantly high in the organic layers and decreased gradually with the depth of the layers in the studied vegetation types. Similar patterns of microbial abundance had been reported in the profiles of agricultural fields[53] and Fushan forest soils.[25] Miethling et al[49] reported that same soil type with different plant species also affected microbial populations.

## Conflicts of interest

The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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