Soil available nitrogen, dissolved organic carbon and microbial biomass content along altitudinal gradient of the eastern slope of Gongga Mountain

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A B S T R A C T

Gongga Mountain is a unique mountain in western China which has not only modern low-latitude glaciers, but also an integrated vertical vegetation distribution from subtropical forests to tundra. Our study aimed to understand the soil fertility status of subalpine and alpine ecosystems in this region through measuring the soil available nitrogen (SAN), dissolved organic carbon (DOC) and soil microbial biomass (SMB) along the eastern slope of Gongga Mountain. We found that the SAN, DOC and SMB varied along the altitudinal gradient, and decreased from the soil surface to subsurface, probably due to the different return plant residue, decomposition rate, as well as temperature and moisture in different elevations. The range of NH4+–N content was from 1.7 mg kg–1 to 134.2 mg kg–1; NO3–N+NO2–N was from 2.6 mg kg–1 to 202.0 mg kg–1; DOC was from 30.6 mg kg–1 to 610.2 mg kg–1; soil microbial biomass carbon (SMBC) was from 41.4 mg kg–1 to 2538.5 mg kg–1; and soil microbial biomass nitrogen (SMBN) was from 0.6 mg kg–1 to 410.7 mg kg–1. SAN, DOC and SMB were all significantly related to each other, indicating that all these three indexes are dependent on soil organic matter. At last, the ratio of SMB to SMBC ranged from 2.4 to 65.3, mostly less than 6.0, which meant the bacteria dominated the soil microbial community in our study sites.

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1. Introduction

Soil available nitrogen (SAN) is the nitrogen form which can be uptaken and utilized directly by vegetation [1], mainly including ammonium nitrogen (NH4+–N), nitrate and nitrite nitrogen (NO3–+NO2––N). SAN also is the essential nutrient for plant growth [2], and it’s content level can significantly affect terrestrial ecosystem productivity, and has feedback relationship with species diversity, community succession and sustainability of forestry ecosystem [3].

Dissolved organic carbon (DOC), soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) are the small part of soil organic carbon pool, but the most important and active parts in soil ecosystem [4]. They participate in soil C and N biogeochemical cycle and play a critical role in nutrient retention and soil fertility in terrestrial ecosystems [5].

The Gongga Mountain of eastern Qinghai–Tibetan Plateau is a typical alpine region with high peaks, deep valleys, transitional climate and integrated vertical vegetative zone. It is a glacier succession slush with a integrated natural vertical band of plants from subtropical to frigid zone [6], with complicated geology, different landforms, rich natural resources and obvious transitional climate. At different latitudes of the mountain, soil temperature, precipitation, plant and soil animal diversity vary greatly.

Previous studies on Gongga Mountain mainly focused on plant floristics [6], soil development [7], caliloid lichens and fungi [8,9], greenhouse gas fluxes [10], soil organic matter dynamics [11], succession features of subalpine forest [12], mountain ecosystem [13], geoeology [14], glacier [15,16] and climate [17], with little attention given to the natural spatial distribution of SAN, DOC, SMBC and SMBN along an altitudinal gradient of the Gongga Mountain. The present study aimed to ascertain the changes, concentrations and the relationship among SAN, DOC, SMB and SMBN under vertical vegetation zones along the elevation of Gongga Mountain. The results are expected to fill up the gap of knowledge about the soil C, N cycle and soil fertility status of Gongga Mountain, and to provide reference for further studies.

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2. Materials and methods

2.1. Study site

Gongga Mountain is located in the junction (29°20′–30°20′N, 101°30′–102°15′E) of four counties in Sichuan Province, Luding, Kangding, Jiulong and Shimian counties (Fig. 1). It is a famous alpine region of Hengduan Mountain with an area of 955876.8 hm² and a highest peak of 7556 m. Geomorphologically, Gongga Mountain is an alpine valley landscape situated in transitional zone of the Qinghai–Tibetan Plateau (4000 m) and Sichuan Basin (500 m). Gongga Mountain is also on the transitional zone of warm moist subtropical monsoon climate in eastern China and temperate sub-humid climate in Eastern Qinghai–Tibetan plateau (Fig. 2). Along the eastern slope of Gongga Mountain, it has integrated vertical vegetative zone from subtropical zone to frigid zone along the altitudinal gradient from 1000 m to 5000 m [12]. The vertical vegetation zones can be divided into the evergreen broad-leaved forest (1000–2200 m), coniferous broad-leaved mixed forest (2200–2500 m), subalpine coniferous forest (2500–3600 m), alpine bush meadow (3600–4600 m) and alpine gravel vegetation zone (4600–5000 m) [47]. Our five research sites (Fig. 3) were set along the altitudinal gradient, with detailed information of each site listed in Table 1.

2.2. Sample collection and analyses

Soil samples (0–5 cm, 5–10 cm, 10–20 cm) were collected in November 2011 from three random sampling quadrats (10 m × 10 m) at each altitudinal site described above. In each of sampling plots, the soil at different sampling depth was collected from three sites randomly after clearing the litter layer on the ground and, and then mixed into a composite samples. Because of big snow and high elevation, we did not collect 10–20 cm soil sample of the highest elevation (alpine shrub meadows). Therefore, we collected 14 mixed soil samples in all. All soil
samples were stored in freezer boxes, and transported to the laboratory within 24 h. A sample of fresh soil was immediately sieved through a 2 mm mesh after excluding the visible fragments and debris of blocks, plants, roots and animals, and stored at 4 °C. The samples were used to determine the microbial biomass and dissolved organic carbon. The remained air-dried soil was allowed to pass through a 0.25 mm sieve for determination of soil available nitrogen.

Soil samples used to analyze NH$_4^+$–N and NO$_3^−$–N were shaken with 2 M KCl on an orbital shaker for 1 h and the suspension was then filtered. Soil samples used to analyze DOC was also shaken with 0.5 M K$_2$SO$_4$ in a centrifuge tube by shaking the mixture for 1 h on a reciprocal shaker, and then centrifuging at 13,000 rpm for 30 min at 4 °C. The supernatant was filtered through a 0.45 μm glass fiber filter. At last, all extracts were analyzed by continuous flow analyzer of San++ (SKALAR, Netherlands). SMBC and SMBN were determined by the chloroform fumigation extraction method after 14 days of conditioning at 25 °C and at 50% of their total water holding capacity, followed by 0.5 M K$_2$SO$_4$ extraction method of both unfumigated and fumigated samples [18–20]. Extraction for SMBC was analyzed by TOC-V WP (SHIMADZU, Japan); extraction for SMBN was digested and then analyzed by continuous flow analyzer of San++ (SKALAR, Netherlands).

2.3. Statistical analyses

The altitudinal gradients and soil sampling depths are the main factors for analyzing soil properties and soil microbial biomass. Repeated measures Kruskal–Wallis were used to test the main effects of altitude, soil sampling depth on variables. Mann–whitney was used to analyze the difference of SAN, DOC, SMBC, SMBN and the ratio of SMBC to SMBN between different elevations and different sampling depths. We also calculated Spearman correlations between soil chemical properties and soil microbial biomass. All analyses were performed using the SPSS 12.0 statistical software package (SPSS Inc., USA).

3. Results

3.1. Soil chemical properties

The effect of elevation was found to be significant on NH$_4^+$–N and NO$_3^−$–N (P = 0.003, Table 2) but not significant on DOC along the elevation (P = 0.055). Soil sampling depth also had significant influence on NH$_4^+$–N, NO$_3^−$–N and DOC (P = 0.003, P = 0.000, P = 0.001). NH$_4^+$–N and NO$_3^−$–N concentration increased with elevation at first, reaching maximum at the fourth elevation (3000 m) and the third elevation (2800 m) respectively, then began to decrease along increased elevation (Fig. 4). NO$_3^−$–N concentration was higher than NH$_4^+$–N concentration in the first three elevations, but lower in the last two elevations (Fig. 4). The concentration of DOC at first decreased with increase of elevation, then increased and kept a stable trend (Fig. 4). For sampling depths, the concentration of three indexes increased from the deep soil sampling layers to shallow layers except the NH$_4^+$–N at the highest elevation, 0–5 cm > 5–10 cm > 10–20 cm (Fig. 4). The range of NH$_4^+$–N content was from 1.7 mg kg$^{-1}$ to 134.2 mg kg$^{-1}$; NO$_3^−$–N was from 2.6 mg kg$^{-1}$ to 202.0 mg kg$^{-1}$; and DOC was from 30.6 mg kg$^{-1}$ to 610.2 mg kg$^{-1}$.

Through correlation analysis, we found there was high positive relationship between NH$_4^+$–N and NO$_3^−$–N (R = 0.702, P < 0.01), NH$_4^+$–N and DOC (R = 0.819, P < 0.01), NO$_3^−$–N and DOC (R = 0.647, P < 0.01).

### Table 1

<table>
<thead>
<tr>
<th>Elevation</th>
<th>Soil type</th>
<th>Vegetation belts</th>
<th>Major tree species</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 1600 m</td>
<td>Mountain yellow–brown soil</td>
<td>Subtropical mountain evergreen broad-leaved forest zone</td>
<td>Cyclobalanopsis glaucoidea, Quercus engleiana, Castanea mollissima, Phoebe chinensis, Lithocarpus clusoricarpus</td>
</tr>
<tr>
<td>II 2300 m</td>
<td>Mountain brown soil</td>
<td>Subtropical mountain evergreen broad-leaved and deciduous broad-leaved mixed forest zone</td>
<td>L. cleist, Cinnamomum longepani, Osmanthus yunnanensis, Tsuga dumosa, Eupolea pleiospermum</td>
</tr>
<tr>
<td>III 2800 m</td>
<td>Mountain dark brown soil</td>
<td>Warm temperate subalpine deciduous broad-leaved and mixed coniferous forest zone</td>
<td>Rhododendron cephalanthum, T. chinensis, Picea brachytyla</td>
</tr>
<tr>
<td>IV 3000 m</td>
<td>Brown coniferous forest soil</td>
<td>Frigid temperate subalpine coniferous forest zone</td>
<td>Abies fabri, Betula utilis, B. cylindristachya, Sorbus spp.</td>
</tr>
<tr>
<td>V 3900 m</td>
<td>Dark felt soil</td>
<td>Subfrigid alpine shrub and meadow zone</td>
<td>R. villosum, Salis spp., Kobresia cunenta, K. pygmaea, Festuca ovina</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Factor</th>
<th>NH$_4^+$–N</th>
<th>NO$_3^−$–N</th>
<th>DOC</th>
<th>SMBC</th>
<th>SMBN</th>
<th>$E_{max}/N_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude</td>
<td>0.003$^a$</td>
<td>0.015$^a$</td>
<td>0.055</td>
<td>0.588</td>
<td>0.682</td>
<td>0.322</td>
</tr>
<tr>
<td>Sampling depth</td>
<td>0.003$^b$</td>
<td>0.000$^b$</td>
<td>0.001$^a$</td>
<td>0.000$^a$</td>
<td>0.000$^a$</td>
<td>0.001$^a$</td>
</tr>
</tbody>
</table>

$^a$ P values (P < 0.01) are indicated with.

$^b$ P < 0.05 are indicated with.
3.2. Soil microbial biomass

The effect of elevation on SMBC \( (P = 0.588) \), SMBN \( (P = 0.682) \) and the ratio of SMBC to SMBN \( (P = 0.322) \) was not significantly along the elevation (Table 2). Soil sampling depths had a highly significant influence on SMBC \( (P = 0.000) \), SMBN \( (P = 0.000) \) and the ratio of SMBC to SMBN \( (P = 0.001) \). Both SMBC and SMBN got maximum at the third elevation (2800 m), with SMBC increasing at first and then decreasing at the fourth elevation (3000 m); SMBN decreased at first, then began to increase at the fourth elevation (3000 m). The concentration of both SMBC and SMBN increased from the deep soil sampling layers to shallow layers (Fig. 5). The range of SMBC was from \( 41.4 \text{ mg kg}^{-1} \) to \( 2538.5 \text{ mg kg}^{-1} \), and SMBN was from \( 0.63 \text{ mg kg}^{-1} \) to \( 410.7 \text{ mg kg}^{-1} \). The ratio of SMBC to SMBN showed no regular trend along elevation, with the maximum value at the deepest soil layer (10–20 cm) at the fourth elevation (3000 m). It ranged from 2.4 to 65.3 (Fig. 5).

Through correlation analysis, we found a high positive relationship between DOC and SMBC \( (R = 0.696, P < 0.01) \), between SMBN and \( \text{NH}_4^+ / C_0 \text{N} \) \( (R = 0.564, P < 0.01) \) and \( \text{NO}_3^- / C_0 \text{N} \) \( (R = 0.743, P < 0.01) \). The correlation between SMBC and SMBN was also significant \( (R = 0.922, P < 0.01) \) (Table 3).

4. Discussion

In our study we quantified the natural distribution concentration and assessed the relationship among SAN, DOC and SMB in subalpine and alpine forest along the latitude gradient. The species composition, richness, structure, productivity, litter layer, soil animal and environmental factors, such as temperature and

![Fig. 4. NH\(_4^+\)–N, \( \text{NO}_3^- + \text{NO}_2^- \)–N and DOC concentrations at three soil sampling depths along five altitudinal gradients of the eastern slope of Gongga Mountain. Data are the mean values, and error bars represent standard deviations. Means with different capital and lowercase letters are significantly different between same depth in different elevation and different depth in same elevation respectively, at \( P < 0.05 \).](image4)

![Fig. 5. SMBC, SMBN and the ratio of SMBC to SMBN contents at three soil sampling depths along five altitudinal gradients of the eastern slope of Gongga Mountain. Data are the mean values, and error bars represent standard deviations. Means with different capital and lowercase letters are significantly different between same depth in different elevation and different depth in same elevation respectively, at \( P < 0.05 \).](image5)
precipitation changed greatly with increasing latitude along the eastern slope of Gongga Mountain [6,21–23]. Our results supported the conclusions that available N, DOC, and SMB were different along the latitudinal and vegetal zone, and such difference would influence the decomposition of soil organic matter and nutrient cycling.

\[ \text{NH}_4^+ - \text{N and NO}_3^- + \text{NO}_2^- - \text{N are soil available N which can be absorbed by plant directly. Their concentration change influence the process of soil nitrogen migration and conversion markedly, since the soil nutrient status and availability were deep affected by forest plant composition, forest productivity, stability and health of forest ecosystem [24]. In our study the concentration change of NH}_4^- - \text{N and NO}_3^- + \text{NO}_2^- - \text{N along the altitudinal gradient showed no regular trend (Fig. 4) with the maximum of NH}_4^- - \text{N concentration at 3000 m. This was probably due to the coniferous forest zone at this elevation. First, coniferous forest has acid soil [25], whose low pH has restricted effect on the growth of nitrifier [26], and the nitrification rate decrease with pH when pH < 6 [27]. On the other hand, many conifers like NH}_4^- - \text{N, and in order to meet their demand they maybe form mechanism to restrict the transformation of NH}_4^- - \text{N to NO}_3^- + \text{NO}_2^- - \text{N [28].}}\n
We also found the concentration of NO}_3^- + \text{NO}_2^- - \text{N got the maximum at 2800 m where the water table is close to the surface and soils had very high water content. This is inconsistent with others, they found that NH}_4^- - \text{N concentration was large than NO}_3^- + \text{NO}_2^- - \text{N under the flooded conditions due to nitrification limited by low O}_2^- - \text{availability [29,30]. However, in the present study, the highest NO}_3^- + \text{NO}_2^- - \text{N concentration was found and the reason may not be due to lower nitrification but due to higher input of NO}_3^- + \text{NO}_2^- - \text{N from circumstance, which is easy to leach with runoffs [43–46]. Our 2800 m sampling site was a flat wetland in the valley and surroundings were dense forests. Therefore, leaching NO}_3^- + \text{NO}_2^- - \text{N from dense forests nearby probably greatly enlarged NO}_3^- + \text{NO}_2^- - \text{N concentrations in the lowland wetland.}}\n
SMBC and SMNB had almost the same trend along the elevation, with the peak value of both occurring at the mixed forest of 2800 m (Fig. 5). The reasons can be listed as follows: (1) mixed forest has high content of soil organic matter (SOM) [31], and SMB as a fraction of SOM has positive relationship with SOM; (2) the microbial biomass content is associated with the return of plant residues to the soils [32], so compared with another mixed forest of alpine shrub and meadow zone of 3900 m, 2800 m has high return quality and quantity of the plant residues and more surface litter; (3) temperature decrease with the elevation [11], and the high temperature can improve the activity of microbe. The close relationship between SOM and SMB was in agreement with other researches [33,34].

The microbial C to N ratio reflects the relative proportion of fungi and bacteria in soil, and it can be used as an indication of the relative proportion of fungi to bacteria [35]. A report suggested that the wide microbial C to N ratio will indicate a greater proportion of fungal compared to bacterial biomass [36–38], and it range from the wide microbial C to N ratio will indicate a greater proportion of fungi to bacteria [35]. A report suggested that the wide microbial C to N ratio will indicate a greater proportion of fungi compared to bacterial biomass [36–38], and it range from 2.4 to 65.3, and the peak value occurred at the deep soil depth of 3000 m (Fig. 5). So we concluded that microbial biomass contained a higher proportion of bacteria in our study because almost all ratios were from 3 to 6. The peak value indicated that fungi predominated in the microbial population in the deep soil depth of 3000 m. The different ratios along the elevations might be affected by soil properties, such as SAN, SOM.

In addition, we found that SAN, DOC and SMB all increased from the deep soil sampling layers to shallow layers except the NO}_3^- + \text{NO}_2^- - \text{N on the highest elevation (Figs. 4 and 5). The result was consistent with other researches [40–42]. It was due to the fact that surface soil has return of plant residue, litter, and more population of microbe compared to deep soil. NO}_3^- + \text{NO}_2^- - \text{N increased with the soil depth probably because of its property to leach and runoff [43–46].}}\n
In conclusion, the content of SAN, DOC and SMB varied along the altitudinal gradient of eastern slope of Gongga Mountain. The reasons were listed as follow: (1) the different types and quantity of return plant residue. It cause different decomposition rate of different return plant residue and influence the accumulation of SOM which affect the concentration of SAN, DOC and SMB; (2) different temperature and moisture along the altitudinal gradient. Litter decomposition and microorganism living depend on appropriate soil temperature and moisture, and they can indirectly affect the content of SAN, DOC and SMB.

As we all know, Gongga Mountain has integrated vertical vegetation distribution from subtropical forests to tundra, where alpine ecosystems are sensitive to climate change. So the differences of SAN, DOC and SMB content between different vegetation zones along altitudinal gradient can reflect climate change impacts to some extent, and we can understand the response of alpine ecosystem of Gongga Mountain to climate change by studying soil C content, Soil N content and key microorganism involved in greenhouse gases (\text{CH}_4, \text{N}_2\text{O}) emission and absorbtion. Studies about microbes are limited in Gongga Mountain, and even no study is to analyze the diversity and phylogeny of key microorganism involved in methane oxidation and nitrous oxide production, such as Methanotroph, ammonia-oxidizing archaea and ammonia-oxidizing bacteria. Therefore, to understand well about spatial variations of SAN, DOC, and SMB, their determinants and their response to climate change, we should pay more attention to the microorganism diversity and abundance under different plant zones along the elevations, and analyze the relationship between microorganism abundance and environmental factors.

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