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Effects of simulated warming on soil ammonia-oxidizing bacteria and archaea communities in an alpine forest of western Sichuan, China

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ABSTRACT

Ongoing climate change, characterized by winter warming, snow cover decline and extreme weather events, is changing terrestrial ecosystem processes in high altitude and latitude regions. Winter soil processes could be particularly sensitive to climate change. In fact, winter warming and snow cover decline are interdependent in cold biomes, and have a synergistic effect on soil processes. Soil microorganisms not only play crucial roles in material cycling and energy flow, but also act as sensitive bio-indicators of climate change. However, little information is available on the effect of winter warming on forest soil ammonia-oxidizing bacteria (AOB) and archaea (AOA). The alpine and subalpine forest ecosystems on the eastern Tibet Plateau have important roles in conserving soil, holding water, and maintaining biodiversity. To understand the changes in AOB and AOA communities under climate change scenarios, an altitudinal gradient experiment in combination with soil column transplanting was conducted at the Long-term Research Station of Alpine Forest Ecosystems, which is situated in the Bipeng Valley of Lixian County, Sichuan, China. Thirty intact soil columns under an alpine forest at an altitude of 3582 m were transplanted and incubated at 3298 m and 3023 m forest sites, respectively. Compared with the 3582 m, we expected air temperature increases of 2 °C and 4 °C at the 3298 m and 3023 m, respectively. However, the temperatures in the soil organic layer (OL) and mineral soil layer (ML) increased by 0.27 °C and 0.13 °C, respectively, at 3023 m and −0.36 °C and −0.35 °C at 3298 m. Based on a previous study and with simultaneous monitoring of soil temperature, the abundances of AOB and AOA communities in both the OL and ML were measured by qPCR in December 2010 (i.e., the onset of the frozen soil period) and March 2011 (i.e., the late frozen soil period). The soil columns incubated at 3023 m had relatively higher AOB abundances and lower AOA/AOB ratios than those at 3298 m, while higher AOA abundances and AOA/AOB ratios were observed at 3298 m. The abundance of the microbial community at the late frozen period was higher than that at the onset of frozen soil, and the changes in microbial community abundance at the late frozen period were more substantial. Furthermore, the nitrate nitrogen (N) concentrations in both the OL and ML were significantly higher than ammonia N concentrations, implying that soil nitrate N is the primary component of the inorganic N pool in the alpine forest ecosystem. Additionally, the responses of AOA and AOB in the soil OL to soil column transplanting were more sensitive than the responses of those in ML. In conclusion, climate warming alters the abundance of the ammonia-oxidizing microbial community in the alpine forest ecosystem, which, in turn, might affect N cycling.

1. Introduction

Nitrogen (N) cycle is one of the key factors in the soil ecological system. Ammonia oxidation is the first and rate-limiting step of nitrification, has its ecological significance in the global N cycle and environmental implications [1]. Numerous studies in recent years have demonstrated that except the ammonia-oxidizing bacteria (AOB), the ammonia-oxidizing archaea (AOA) was detected in extreme environments, such as deep marine areas, hot springs and soils, and participate in the ammonia oxidation [2–5]. Traditionally, low temperature or soil freezing will directly result in soil microbial death or dormant [6]. And the existing studies was pay more attention to the diversity of AOA and AOB characteristic in the growing season of agricultural soil, different ways of land use and N fertilization [7–10]. However, numerous researches over the last decade have demonstrated that ecologically significant levels of microbial activity occurs in soils of cold biomes [11–16], and takes part in the production of plant in next growing season. It is worth noting that the global warming has become an indisputable fact [17]. As the roof of the world, the Tibetan Plateau was suggested to be the most sensitive areas to respond to global climate

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change [17–18]. The warming at this area was obviously larger than that in low altitude regions [18]. The different altitude can lead to the continuous change of environment factors, such as temperature and moisture [6,19–20], may affect the composition of soil microbial community [5, 21], and further influence the soil N cycle. Therefore, research on the responded of simulate warming of ammonia-oxidizing microorganisms will contribute to further understanding soil N cycle in the alpine region.

The alpine forests located in the upper reaches of Yangtze River and the eastern Tibetan plateau, which play important role in conserving water resource, nursing biodiversity, sequestering atmospheric carbon dioxide, and indicating climate change [22–23]. And this region was suggested to be the most sensitive areas to respond to global climate change. There are obvious freeze-thaw cycles with temperature fluctuations over wintertime and soil frozen time was up to 5–6 months [23], which could have significant influences on microbial community structure and diversity [11–12]. However, little information is available on the characteristics of ammonia oxidization microbial in this area [13–14]. Therefore, an altitudinal gradient experiment was conducted in this study. Intact soil cores from alpine forest ecosystems were incubated three elevational sites (3582 m, 3298 m and 3023 m). Real-time qPCR was used to investigate the abundance of ammonia oxidization microbial *amoA* gene in an alpine forest in wintertime, in order to understand deeply the response of soil ammonia oxidization microbial to simulate warming and provide some scientific basis for N cycle in alpine forest.

2. Materials and methods

2.1. Site description

This study was conducted in the Bipenggou Nature Reserve (E102°53′–102°57′, N31°14′–31°19′, 2458–4619 m *a.s.l.*) that is located in Li County, western Sichuan, China. This is a transitional area situated between the Tibetan Plateau and the Sichuan Basin. The mean annual temperature is 2–4 °C, with maximum and minimum temperatures of 23 °C (July) and −18 °C (January), respectively. Annual precipitation is about 850 mm. Forests consist of conifer forest and natural mixed hardwood depending on the elevation, which are mainly dominated by *Abies faxoniana*, *Picea purpurea*, and *Betula albo-sinensis*. Understory shrubs are mainly dominated by *Fargesia spathacea*, *Rhododendron delavayi* and *Berberis julianae*. The herbs are mainly *Cacalia* spp., *Cystopteris montana*, *Carex* spp. and *Cyperus* spp. [9,17].

2.2. Sampling and incubation methods

The typical alpine forest (3582 m) soil samples were collected in primary conifer forest, and plants and fresh litters were cleaned on the ground, then PVC cylinders (20 cm in length, 5 cm in diameter) were inserted into the soil to take undisturbed soil cores, the bottom of which sealed with nylon cloths and elastic belts, the soil organic layer (OL) and mineral soil layer (ML) were 0–10 cm and 10–20 cm, respectively. There were scored of 90 PVC cylinders (3 forest ecosystems × 3 replicates × 2 incubation times × 5 sampling plots). Thirty soil cores were incubated in the primary conifer alpine forest at 3582 m. The other sixty cores were transferred in the mixed forest with conifer and broad-leaf trees at 3298 m and secondary forest at 3023 m, which was simulated increase of air temperature 2 °C and 4 °C, respectively.

Soil sample was collected on the winter of 2010–2011. Based on early monitoring results [24–25], soil usually completely frozen at December, and thawing started at the next later March or early April in research area. Therefore, study samples were collected on December 23rd, 2010 (onset of the frozen soil period) and March 3rd, 2011 (late frozen soil period). In each sampling period, 15 PVC cylinders were collected in every altitude. Each PVC cylinders was collected by different layer, resulting in two samples per plot. Samples were transported to the laboratory within 24 h and stored in −80 °C.

2.3. DNA extraction

DNA was extracted from 0.5 g (dry weight) of soil using the modified bead-beating method [26], with a bead-beating time of 30 s at maximum speed in a mini-beadbater (Biospec) after a soil pre-lysis washing procedure. The extracted DNA was purified through agarose electrophoresis using an OMEGA E.Z.N.A™ Gel Extraction Kit.

2.4. Quantification of amoA genes by real-time PCR

Primer pair *amoA*-1F/*amoA*-2R [27] and Arch-amoAF/Arch-amoAR [28] were used to amplify archaeal and bacterial *amoA* gene fragments, respectively. Real-time PCR was performed using the CFX96 system (Bio-Rad) in 25 μL reactions containing 12.5 μL SYBR® Premix Ex Taq ™ (TaKaRa), 0.4 $mg \cdot mL^{-1}$ bovine serum albumin, 200 $nmol \cdot L^{-1}$ of each AOA primer or 400 $nmol \cdot L^{-1}$ of each AOB primer, and 1 μL of DNA (1–10 ng) as template. Three analytical replicates were performed for each soil sample. The product specificity was confirmed using a melting curve analysis (65–95 °C, 0.5 °C per read with a hold time of 5 s) at the end of each PCR run.

amoA gene clone and the creation method of standard curves were described previously [14]. The PCR efficiency and correlation coefficients for standard curves were 91.8% and $r^2 = 0.999$, respectively, for AOB, 92.1% and $r^2 = 0.997$ for AOA.

2.5. Ammonium and nitrate N measure

Ammonium and nitrate N in fresh soil were extracted with 2 M KCl at room temperature. Ammonium and nitrate concentrations in extract were measured by the indophenols-blue and phenol disulfonic acid colourimetry [29].

2.6. Statistical analyses

All statistical tests were performed using the Software Statistical Package for the Social Science (SPSS) version 16.0 (IBM, Armonk, NY, USA). Three-way analysis of variance (ANOVA) was used to examine the effects of altitude, soil layer, sampling stage and their interactions on the abundances of AOA and AOB, log ratio of AOA/AOB. One-way analysis of variance (ANOVA) was used to compare archaeal and bacterial *amoA* gene copy numbers and ammonium and nitrate N content in each sampling period. Differences of $P < 0.05$ were considered significant.

3. Results

3.1. The characteristic of temperature

Temperature dynamics of air and soil in three elevations from December 1st, 2010 to March 31st, 2011 were shown in Fig. 1. Air temperature was increased with the decrease of altitude, but the soil temperature was showed different characteristic between different altitudes. The temperatures in OL and ML increased by 0.27 °C and 0.13 °C, respectively, at 3023 m, and −0.36 °C and −0.35 °C at 3298 m.

3.2. Soil inorganic N content

As shown in Fig. 2, soil inorganic N displayed a significant change in OL and ML. Of which, the inorganic N content in OL was greater than ML. In OL, nitrate N content was decreased in 3298 m and then increased in 3023 m during two sampling stages. But ammonium N content has no significant difference among three altitudes. In ML, ammonium N content was increased in 3298 m and decreased in 3023 m during two sampling stages. Nitrate N content only showed significantly difference in the onset of the frozen soil period, the 3023 m had the highest nitrate N content among three altitudes.