Variations in Soil Enzyme Activities and Microbial Communities along an Altitudinal Gradient on the Eastern Qinghai–Tibetan Plateau

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Abstract: The Qinghai–Tibetan Plateau is the highest plateau in the world and is sensitive to climate change. The dynamics of soil enzyme activities and microbial communities are good indicators of alpine biochemical processes during warming. We collected topsoil (0–10 cm) and subsoil (10–20 cm) samples at altitudes of 3200–4000 m; determined the activities of β-1,4-glucosidase (BG), cellobiohydrolase (CBH), β-1,4-N-acetyl-glucosaminidase (NAG) and acid phosphomonoesterase (PME); and performed Illumina 16S rRNA high-throughput sequencing. We found that the soil carbon (total organic carbon and dissolved organic carbon) and nitrogen (total nitrogen and dissolved organic nitrogen) fluctuated with altitude in both the topsoil and subsoil, whereas the dissolved phosphorus continuously decreased with the increasing altitude. BG and CBH decreased from 3200 to 3600 m and increased from 3800 to 4000 m, with the lowest levels occurring at 3600 m (topsoil) and 3800 m (subsoil). NAG and PME showed similar fluctuations with altitude, with the highest levels occurring at 3600 m and 4000 m in both the topsoil and subsoil. Generally, the altitudes from 3600 to 3800 m were an ecological transition belt where most of the nutrients and enzyme activities reached their lowest levels. All of the alpine soils shared similar dominant phyla, including Proteobacteria (32.7%), Acidobacteria (30.2%), Actinobacteria (7.7%), Bacteroidetes (4.4%), Planctomycetes (2.9%), Firmicutes (2.3%), Gemmatimonadetes (2.0%), Chloroflexi (1.2%) and Nitrospirae (1.2%); Gemmatimonadetes and Verrucomicrobia were significantly affected by soil depth and Planctomycetes, Firmicutes, Gemmatimonadetes, Nitrospirae, Latescibacteria and Armatimonadetes were significantly affected by altitude. In addition, nutrient availability, enzyme activity and microbial diversity were higher in the topsoil than in the subsoil, and they had more significant correlations in the subsoil than in the topsoil. Our results provide useful insights into the close linkages between soil nutrient cycling and microbial activities on the eastern Qinghai–Tibetan Plateau, and are of great significance for further assessing the long-term impact of environmental changes in the alpine ecosystems.

Keywords: Qinghai–Tibetan Plateau; climate change; nutrient availability; enzyme activity; microbial diversity; alpine soil; altitudinal gradient

1. Introduction

The Qinghai–Tibetan Plateau of China, the highest plateau in the world, represents a series of elevations and temperature gradients, and is thought to be sensitive to global climate change [1]. It is also an important global carbon pool [2], and increasing focus has been placed on the ecological processes of the alpine ecosystems in this area, including the impacts of climate change and human activities [3,4], carbon and nitrogen patterns [5–7], wetland and grassland degradation [7–11], variability in snow cover [12], and microbial community composition and diversity [4,13].
The alpine environment has special soil conditions with low temperatures, and the vertical zonation of the eastern Qinghai-Tibetan Plateau provides an optimal setting for explorations of the spatial characteristics of soil nutrient availability and microbial community composition, allowing insights into large-scale soil nutrient distribution, and the transformation and related microbial metabolisms in alpine ecosystems. Many scholars have investigated the altitudinal sensitivity of soil nutrient decomposition, enzyme activities and microbial communities in alpine ecosystems [14–19].

Soil enzymes participate in soil microbial metabolism and nutrient cycling processes, and they can be used as sensitive indicators of microbial nutrient demand and soil quality [20,21]. Cellulases, including β-1,4-glucosidase (BG) and cellobiohydrolase (CBH), are critical to the transformation of soil organic carbon [22,23]; β-1,4-N-acetyl-glucosaminidase (NAG) and acid phosphomonoesterase (PME) play significant roles in the mineralization of soil organic nitrogen and phosphorus by catalyzing the degradation of chitin and phosphate esters, respectively [20,24].

As the most active components of soils, microorganisms play an important role in nutrient biogeochemical cycling and the maintenance of the ecosystem function [25]. Recently developed molecular biology methods, such as high-throughput sequencing based on the 16S rRNA gene, have been highly accurate and can provide a large quantity of information regarding the soil microbial community [26]. Combined with diversity indices and other statistical methods, these approaches have been widely used to describe soil microbial behaviors in various environments [27–34].

Soil physiochemical conditions, microbial metabolism, and extracellular enzyme activities are highly correlated. The characteristics of enzyme activities change dramatically in different regions, and they can reflect the biogeochemical equilibrium between the microbial biomass and the organic elemental composition [35]. Environmental changes such as global warming will affect the substrate availability, enzyme synthesis and secretion, and microbial composition and diversity [36,37], and further affect the entire alpine ecosystem. Therefore, studies of microbial metabolism and enzyme activity on the eastern Qinghai–Tibetan Plateau are urgently needed and have considerable environmental significance.

The purpose of this study was to explore the dynamics of nutrient availability, enzyme activity and microbial diversity across an altitudinal gradient on the eastern Qinghai–Tibetan Plateau as indicators of crucial natural biochemical processes. We collected alpine soils at different depths and altitudes, determined the soil chemical properties and enzyme activities, and analyzed the microbial communities based on the 16S rRNA sequence to interpret the spatial variations and complex correlations of the soil microbial activity and nutrient cycling in alpine soils.

2. Materials and Methods

2.1. Study Area and Soil Sampling

The study area is located on Mengbi Mountain (31°32′–31°42′ N, 102°19′–102°25′ E), on the eastern margin of the Qinghai–Tibet Plateau in Southwestern China. This area has a unique ecological belt in which vegetation ranges from alpine forest to meadow, with abundant sunlight and a large daily temperature difference. The mean annual temperature is 8–9 °C, and the mean annual rainfall is approximately 750 mm. The frost-free period lasts for 220 d on average with early frost in November and late frost in March.

The plateau soils from five plots along the altitudinal range (A3200–A4000) were sampled in June 2016 and are shown in Table 1. At each sampling altitude, three replicates were collected from the topsoil (L1: 0–10 cm) and subsoil (L2: 10–20 cm). After visible plants and root residues were manually removed, the samples were air-dried and sieved through a 2-mm mesh and then stored at 4 °C (<10 days) until further analysis or experimentation.
Table 1. Basic information on sampling sites.

| Altitude ID | Community Type     | Sampling Altitude (m) | Longitude (E) | Latitude (N) |
|-------------|--------------------|-----------------------|---------------|--------------|
| A3200       | Alpine meadow      | 3237                  | 102°25'       | 31°32'       |
| A3400       | Treeline Ecotone   | 3430                  | 102°23'       | 31°36'       |
| A3600       | Alpine forest      | 3606                  | 102°20'       | 31°39'       |
| A3800       | Alpine forest      | 3810                  | 102°19'       | 31°41'       |
| A4000       | Alpine forest      | 4018                  | 102°19'       | 31°42'       |

2.2. Soil Chemical Properties

Soil pH was measured using a pHS-4C+ acidometer (Fangzhou Technology, Chengdu, China) in a 1:2.5 soil/water slurry (2.5 mL of water per gram of soil). Total organic carbon (TOC) and total nitrogen (TN) were analyzed using a Vario MACRO cube (Elementar, Langenselbold, Germany). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted following Jones et al. [38] and analyzed using a Vario TOC cube (Elementar, Langenselbold, Germany). Dissolved phosphorus (DP) was analyzed using an ICP-AES analyzer (Shimadzu, Kyoto, Japan).

2.3. Soil Enzyme Activity

The activities of enzymes were measured using microplate assays according to Dick [39]. Assays were incubated at 20 °C for 4.5 h in a modified universal buffer with a pH of 6.0 (BG, CBH and PME) or 5.5 (NAG) and measured fluorometrically under 365 nm excitation and 450 nm emission. The substrates of BG (EC3.2.1.21), CBH (EC3.2.1.91), NAG (EC3.2.1.52) and PME (EC3.1.3.2) were 4-methylumbelliferyl-β-D-glucopyranoside (M3633), 4-methylumbelliferyl-β-D-cellobioside (M6018), 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (M2133) and 4-methylumbelliferyl-phosphate (M8883), respectively. The enzyme activities were expressed as μmol methylumbelliferone (MUF) g⁻¹ soil h⁻¹.

2.4. DNA Extraction, PCR and High-Throughput Sequencing

Soil DNA was extracted using the FastDNA® spin kit for soil (MP Biomedicals, Solon, USA) and amplified with primers 515F (5′-GTGCCAGCMGCGCGG-3′) and 907R (5′-CCGTCATATTCTCTTTRAGTTT-3′) targeting the 16S rRNA gene (V4-V5). PCR was performed in a thermal cycler (Bio-Rad Laboratories, Emeryville, CA, USA). High-throughput sequencing was performed on a MiSeq sequencer (Illumina, San Diego, CA, USA). The sequence was submitted to the Sequence Read Archive (SRA) repository of the National Center for Biotechnology Information (NCBI) under BioProject number PRJNA661057. Paired reads were merged using Flash [40]. Quality filtering was performed using QIIME as described by Bokulich et al. [41]. Sequences were clustered into operational taxonomic units (OTUs) under 97% similarity using the UPARSE algorithm [42], and representative sequences were then annotated from the domain to the genus level using the RDP classifier [43].

2.5. Data Analysis

Analysis of variance (ANOVA) was performed and Pearson correlation coefficients were calculated using SPSS 21.0. Boxplots of soil enzyme activities and bar charts of the relative abundance of soil microbes were plotted using Origin 9.0. Sample clustering based on Bray–Curtis distances was performed in Past 4.01 using the UPGMA (unweighted pair group method with arithmetic mean) algorithm. Venn diagrams and diversity indices were implemented in R.3.5.2 with the VennDiagram and vegan packages, respectively. A redundancy analysis (RDA) was performed using CANOCO for Windows 4.5 with soil chemical factors as explanatory variables, and enzyme activities and microbial species as response variables.
3. Results
3.1. Characteristics of Soil Chemical Properties

The soil chemical properties are shown in Table 2. For all of the altitudes, the average soil pH was 6.60 ± 0.20 (mean ± SE) in the topsoil and 6.48 ± 0.23 in the subsoil. The average contents of TOC, TN, DOC, DON and DP were 61.67 ± 4.27, 6.23 ± 0.35, 0.67 ± 0.03, 0.18 ± 0.02 and 0.0095 ± 0.0006 g/kg in the topsoil and 51.35 ± 5.45, 5.35 ± 0.46, 0.58 ± 0.03, 0.15 ± 0.01 and 0.0084 ± 0.0006 g/kg in the subsoil, respectively. Two-way ANOVA showed that TOC, TN, DOC and DP were significantly affected by the soil depth and altitude (p < 0.05), and the pH was significantly affected by the altitude (p < 0.01), whereas the effects of the soil layer and altitude on DON did not reach significant levels. The average values of the soil pH and the nutrient contents (TOC, TN, DOC, DON and DP) in the topsoil were all higher than those in the subsoil. The soil pH reached a minimum at 3400 m in both of the soil layers, and the lowest contents of most nutrients in the topsoil (TOC, TN and DON) and all nutrients in the subsoil (TOC, TN, DOC, DON and DP) occurred at A3800. In general, when the altitude increased from A3200 to A4000, most of the soil nutrients decreased initially, but finally recovered from A3800 to A4000 with the altitude of A4000.

Table 2. Soil chemical properties of the sampling sites.

| Soil Layer | Altitude ID | pH (in Water) | TOC (g/kg) | TN (g/kg) | DOC (g/kg) | DON (g/kg) | DP (mg/kg) |
|------------|-------------|---------------|------------|-----------|------------|------------|------------|
| Topsoil (0–10 cm) | A3200 | 7.78 ± 0.02a | 65.57 ± 9.42b | 6.82 ± 1.04b | 0.81 ± 0.16 | 0.14 ± 0.02 | 13.33 ± 1.89a |
| | A3400 | 5.89 ± 0.48c | 65.29 ± 8.66b | 5.64 ± 0.82bc | 0.74 ± 0.10 | 0.21 ± 0.04 | 10.10 ± 0.95b |
| | A3600 | 6.72 ± 0.39b | 51.86 ± 4.98c | 5.55 ± 0.65bc | 0.56 ± 0.10 | 0.24 ± 0.20 | 8.33 ± 1.62bc |
| | A3800 | 6.66 ± 0.45b | 40.38 ± 4.27c | 4.92 ± 0.45c | 0.60 ± 0.06 | 0.14 ± 0.02 | 8.27 ± 0.60bc |
| | A4000 | 5.92 ± 0.11c | 85.24 ± 3.03a | 8.21 ± 0.34a | 0.64 ± 0.06 | 0.20 ± 0.02 | 7.63 ± 0.64c |
| Mean | 6.60 ± 0.20 | 61.67 ± 4.27 | 6.23 ± 0.35 | 0.67 ± 0.03 | 0.18 ± 0.02 | 9.53 ± 0.62 |
| Subsoil (10–20 cm) | A3200 | 7.80 ± 0.03A | 68.33 ± 5.59A | 6.80 ± 0.44AB | 0.67 ± 0.10A | 0.14 ± 0.02AB | 12.03 ± 2.34A |
| | A3400 | 5.40 ± 0.71C | 35.47 ± 14.54C | 3.71 ± 1.40C | 0.62 ± 0.11AB | 0.17 ± 0.04AB | 8.50 ± 1.10B |
| | A3600 | 6.64 ± 0.21B | 49.83 ± 2.13B | 5.49 ± 0.15B | 0.51 ± 0.04BC | 0.15 ± 0.07AB | 7.80 ± 0.89B |
| | A3800 | 6.80 ± 0.55B | 25.69 ± 7.85C | 3.39 ± 0.83C | 0.47 ± 0.06C | 0.10 ± 0.03B | 6.23 ± 0.21B |
| | A4000 | 5.76 ± 0.22C | 77.25 ± 0.44A | 7.38 ± 0.18A | 0.65 ± 0.02AB | 0.21 ± 0.01A | 7.50 ± 1.42B |
| Mean | 6.48 ± 0.23 | 51.35 ± 5.45 | 5.35 ± 0.46 | 0.58 ± 0.03 | 0.15 ± 0.01 | 8.41 ± 0.60 |

Note: Values presented are the mean ± SE (n = 3). Different lowercase letters (topsoil) and capital letters (subsoil) indicate significant (p < 0.05) differences at different altitudes in one-way analysis of variance (ANOVA) based on Duncan’s comparison procedure. TOC = total organic carbon, TN = total nitrogen, DOC = dissolved organic carbon, DON = dissolved organic nitrogen, and DP = dissolved phosphorus (the same below). Differences in DON and DP along the altitudinal gradients in topsoil were not significant (p > 0.05).

3.2. Spatial Variations in Soil Enzyme Activities

The soil enzyme activities in the topsoil were significantly (p < 0.05) higher than those in the subsoil. The Activities of BG CBH, NAG and PME were 3.63 ± 0.13 (mean ± SE), 0.56 ± 0.03, 1.08 ± 0.06 and 4.30 ± 0.36 μmol g⁻¹ soil h⁻¹ MUF in the topsoil and 1.91 ± 0.15, 0.28 ± 0.03, 0.76 ± 0.06 and 2.96 ± 0.27 μmol g⁻¹ soil h⁻¹ MUF, respectively, in the subsoil. As the altitude increased, the activities of BG and CBH decreased from A3200 to A3600, and then increased from A3600 to A4000 in both the topsoil (Figure 1) and subsoil (Figure 2); the activities of NAG and PME increased from A3200 to A3400, decreased from A3400 to A3600, and increased from A3600 to A4000 in the topsoil. The lowest levels of BG, CBH and NAG occurred at A3600 or A3800, whereas the lowest value of PME was at A3200 in both of the soil layers. Two-way ANOVA showed that soil layer and altitude both had significant effects on the activities of BG, CBH, NAG and PME.
Figure 1. Soil enzyme activity in the topsoil (0–10 cm) along the altitudinal gradient of Mengbi Mountain: (a) BG = β-glucosidase, (b) CBH = cellobiohydrolase, (c) NAG = β-N-acetyl-glucosaminidase, and (d) PME = phosphomonoesterase. Different capital letters indicate significant (p < 0.05) differences at different altitudes in one-way analysis of variance (ANOVA) based on Duncan’s comparison procedure (the same in Figure 2).

Figure 2. Soil enzyme activity in the subsoil (10–20 cm) along the altitudinal gradient of Mengbi Mountain: (a) BG = β-glucosidase, (b) CBH = cellobiohydrolase, (c) NAG = β-N-acetyl-glucosaminidase, and (d) PME = phosphomonoesterase.
3.3. Composition of Soil Microbial Communities

The Venn diagrams (Figure 3) showed that the number of OTUs shared among the five altitudes was 2501 in the topsoil (accounting for 42.8%–46.8%) and 2,076 in the subsoil (accounting for 36.9%–44.6%). In the topsoil, the proportion of specific OTUs (only appearing at a given altitude) was highest at A3400 (12.4%), followed by A3600 (9.1%), A3800 (7.8%), A4000 (7.7%), and A3200 (7.0%). In the subsoil, the proportion of specific OTUs was highest at A3200 (11.6%), followed by A3600 (10.7%), A3400 (10.7%), A4000 (9.8%), and A3800 (7.9%). Sample clustering (Figure 4) based on the Bray–Curtis distance indicated that the soils at the same altitude were more analogous than the soils from the same soil layer. The soils from different layers at A3400, A3600 and A3800 clustered together. However, the distances between the topsoil and the subsoil at A3200 and A4000 were relatively large, indicating differences in microbial composition.

![Figure 3](Image)

**Figure 3.** Shared and specific OTUs in the (a) topsoil (0–10 cm) and (b) subsoil (10–20 cm).

![Figure 4](Image)

**Figure 4.** Relative abundance of soil microbes at each altitude and in each soil layer at the phylum level.

At the phylum level, the dominant phyla among all of the soil samples were Proteobacteria (32.7%), Acidobacteria (30.2%) and Actinobacteria (7.7%), followed by Bacteroidetes (4.4%), Planctomycetes (2.9%), Firmicutes (2.3%), Gemmatimonadetes (2.0%), Chloroflexi (1.2%), and Ni-
trospirae (1.2%). The sum of the relative abundances of other identified phyla was 2.5%, and the remaining 12.8% was unclassified. Two-way ANOVA showed that soil depth had significant ($p < 0.05$) impacts on the relative abundances of Gemmatimonadetes and Verrucomicrobia, and altitude had significant ($p < 0.05$) impacts on the relative abundances of Planctomycetes, Firmicutes, Gemmatimonadetes, Nitrospirae, Latescibacteria and Armamatimonadetes.

At the genus level, 44 genera had an average relative abundance of more than 0.1%, and the relative abundance of all of the unclassified OTUs was 80.7%. The top 10 genera in relative abundance were Gemmatimonas (2.0%), Gaiella (1.4%), Bradyrhizobium (1.3%), Nitrospira (1.0%), Variorovax (1.0%), Steroidobacter (0.9%), Arthrobacter (0.5%), Pedamicribium (0.4%), Solirubrobacter (0.4%), and Arenimonas (0.3%). Two-way ANOVA showed that soil depth had significant ($p < 0.01$) impacts on the relative abundance of Gemmatimonas, and altitude had significant ($p < 0.05$) impacts on the relative abundances of Gemmatimonas, Gaiella, Bradyrhizobium, Nitrospira, and Steroidobacter.

3.4. Diversity Indices of Soil Microbial Communities

The total reads, number of OTU taxa, and diversity indices of the soil microbial communities are shown in Table 3. The mean values of all of the indices in the topsoil were higher than those in the subsoil, although two-way ANOVA showed that only the differences in the number of OTU taxa between the two soil layers reached a significant level ($p < 0.05$). The altitude had different impacts on the soil microbial communities in the two soil layers. The total reads, the number of OTU taxa, and the Chao1 at A3200 were highest in the topsoil but lowest in the subsoil. In the topsoil, microbial evenness (Pielou) and diversity (Shannon and Simpson) were moderate at A3200, relatively low at A3400 and A3800, and relatively high at A3600 and A4000. In the subsoil, the microbial evenness and diversity were relatively low at A3200 and A3600, highest at A3400, and moderate at A3800 and A4000.

Table 3. Total reads, number of OTU taxa and diversity indices of soil microbial communities.

| Soil Layer | Altitude ID | Total Reads | OTU Taxa | Richness Chao1 | Evenness Pielou | Diversity Shannon | Simpson |
|------------|-------------|-------------|----------|---------------|----------------|-------------------|---------|
| Topsoil (0–10 cm) | A3200 | 48,612 | 3946 | 5687 | 0.789 | 6.529 | 0.993 |
| | A3400 | 37,415 | 3393 | 4979 | 0.814 | 6.342 | 0.992 |
| | A3600 | 38,965 | 3476 | 5083 | 0.786 | 6.632 | 0.995 |
| | A3800 | 37,291 | 3198 | 4741 | 0.811 | 6.340 | 0.992 |
| | A4000 | 37,684 | 3473 | 5055 | 0.898 | 6.509 | 0.995 |
| Mean ± SE | 39,163 ± 2298 | 3465 ± 113 | 5068 ± 141 | 0.797 ± 0.008 | 6.487 ± 0.075 | 0.994 ± 0.001 |
| Subsoil (10–20 cm) | A3200 | 29,441 | 2577 | 3983 | 0.763 | 5.930 | 0.987 |
| | A3400 | 35,860 | 3360 | 4976 | 0.797 | 6.467 | 0.994 |
| | A3600 | 41,197 | 2904 | 4522 | 0.744 | 5.931 | 0.989 |
| | A3800 | 34,259 | 2998 | 4514 | 0.793 | 6.338 | 0.993 |
| | A4000 | 38,054 | 3341 | 4868 | 0.791 | 6.420 | 0.993 |
| Mean ± SE | 35,762 ± 2802 | 3036 ± 170 | 4572 ± 211 | 0.778 ± 0.001 | 6.217 ± 0.107 | 0.991 ± 0.001 |

Note: One-way ANOVA showed that the difference in these parameters along the altitudinal gradient did not reach the significance level ($p > 0.05$).

3.5. Correlations between Soil Chemical Properties, Enzyme Activities and Microbial Communities

Pearson correlation coefficients showed that TOC and TN, as well as DOC and DP, were significantly ($p < 0.05$) positively correlated in both of the soil layers (Table 4). BG, NAG and PME in the topsoil, and BG, CBH and NAG in the subsoil were significantly positively correlated with each other. The soil enzyme activities had more significant ($p < 0.05$) correlations with soil nutrients in the subsoil than in the topsoil. Only in the subsoil were BG and CBH significantly correlated with DOC and DP, and NAG was significantly correlated with TOC, TN and DOC. Similarly, the soil microbial richness (OTU taxa and Chao1) and diversity (Shannon and Simpson) had more significant ($p < 0.05$) positive
correlations in the subsoil than in the topsoil. In addition, the soil pH was significantly negatively correlated with PME only in the topsoil ($p < 0.01$), and significantly negatively correlated with OTU taxa, ChaoI and Simpson only in the subsoil ($p < 0.05$).

The first two RDA axes explained 64.6% of the variance in the soil enzyme activities (Figure 5a) and 17.4% of the variance in the microbial communities (Figure 5b). With respect to the enzyme activities, BG and CBH had a strong positive relationship and were affected by TOC and TN. The PME activity had a strong negative association with pH, which is consistent with the results reported in Table 4. With respect to the soil microbes, the abundances of Firmicutes, Nitrospirae, Armatimonadetes and Acidobacteria were affected by soil chemical properties, particularly soil pH, DP, TOC and TN. In addition, the soil samples from similar altitudes clustered together; the samples at A3200 were mainly affected by soil pH and DP, and the samples at A4000 were mainly affected by TOC and TN.

**Figure 5.** Redundancy analysis (RDA) of (a) enzyme activities and (b) microbial communities constrained by soil chemical parameters.
Table 4. Pearson correlation coefficients between soil chemical properties, enzyme activities and microbial communities.

| Soil Layer | Parameter | pH  | TOC  | TN   | DOC  | DON  | DP   | BG   | CBH  | NAG  | PME  | Total Reads | OTU Taxa | Chao1 | Pielou | Shannon |
|------------|-----------|-----|------|------|------|------|------|------|------|------|------|-------------|----------|-------|--------|---------|
| Topsoil    | TOC       | −0.295 |      |      |      |      |      |      |      |      |      |             |          |       |        |         |
|            | TN        | −0.103 | 0.933 ** |      |      |      |      |      |      |      |      |             |          |       |        |         |
|            | DOC       | 0.251 | 0.472 | 0.441 |      |      |      |      |      |      |      |             |          |       |        |         |
|            | DON       | −0.187 | 0.251 | 0.242 | 0.231 |      |      |      |      |      |      |             |          |       |        |         |
|            | DP        | 0.489 | 0.047 | −0.014 | 0.636 * | 0.123 |      |      |      |      |      |             |          |       |        |         |
|            | BG        | −0.285 | 0.585 * | 0.670 ** | 0.087 | −0.101 | −0.376 |      |      |      |      |             |          |       |        |         |
|            | CBH       | 0.054 | 0.440 | 0.638 * | 0.228 | −0.147 | −0.174 | 0.734 ** |      |      |      |      |             |          |       |        |         |
|            | NAG       | −0.530 | 0.484 | 0.426 | 0.209 | 0.032 | −0.137 | 0.626 * | 0.347 |      |      |             |          |       |        |         |
|            | PME       | −0.691 ** | 0.583 * | 0.551 * | −0.130 | 0.030 | −0.483 | 0.766 ** | 0.465 | 0.771 ** |      |      |             |          |       |        |         |
|            | Total Reads | 0.282 | 0.028 | 0.041 | 0.212 | 0.218 | 0.670 ** | −0.179 | 0.062 | −0.227 | −0.337 |      |             |          |       |        |         |
|            | Chao1      | 0.394 | 0.106 | 0.085 | 0.058 | −0.050 | 0.420 | −0.197 | 0.018 | −0.532 | −0.433 | 0.699 ** |      |      | 0.995 ** |         |
|            | Pielou     | 0.125 | 0.061 | 0.121 | −0.397 | −0.192 | −0.529 | 0.119 | 0.039 | −0.414 | 0.005 | −0.458 | 0.221 | 0.237 |      |         |
|            | Shannon    | 0.218 | 0.105 | 0.150 | −0.317 | −0.159 | −0.328 | 0.052 | 0.051 | −0.529 | −0.130 | −0.157 | 0.530 | 0.541 * | 0.944 ** |         |
|            | Simpson    | 0.148 | 0.123 | 0.179 | −0.370 | −0.081 | −0.441 | 0.102 | 0.080 | −0.465 | −0.017 | −0.299 | 0.361 | 0.373 | 0.975 ** | 0.970 ** |
| Subsoil    | TOC       | 0.185 |      |      |      |      |      |      |      |      |      |             |          |       |        |         |
|            | TN        | 0.264 | 0.990 ** |      |      |      |      |      |      |      |      |             |          |       |        |         |
|            | DOC       | −0.058 | 0.527 * | 0.460 |      |      |      |      |      |      |      |             |          |       |        |         |
|            | DON       | −0.484 | 0.461 | 0.400 | 0.560 * |      |      |      |      |      |      |             |          |       |        |         |
|            | DP        | 0.503 | 0.449 | 0.440 | 0.661 ** | 0.106 |      |      |      |      |      |             |          |       |        |         |
|            | BG        | 0.316 | 0.831 ** | 0.816 ** | 0.588 * | 0.325 | 0.576 * |      |      |      |      |             |          |       |        |         |
|            | CBH       | 0.455 | 0.793 ** | 0.778 ** | 0.592 * | 0.192 | 0.728 ** | 0.930 ** |      |      |      |      |             |          |       |        |         |
|            | NAG       | −0.030 | 0.602 * | 0.585 * | 0.550 * | 0.369 | 0.312 | 0.719 ** | 0.556 * |      |      |      |             |          |       |        |         |
|            | PME       | −0.481 | 0.542 * | 0.516 * | 0.260 | 0.549 * | −0.215 | 0.468 | 0.210 | 0.639 * |      |      |      |             |          |       |        |         |
|            | Total Reads | −0.292 | −0.026 | −0.005 | 0.217 | 0.277 | −0.219 | −0.154 | −0.313 | 0.003 | 0.178 |      |      |      |      |         |
|            | OTU Taxa  | −0.528 * | −0.131 | −0.165 | 0.096 | 0.092 | −0.401 | −0.160 | −0.354 | 0.046 | 0.319 | 0.581 * |      |      |      |         |
|            | Chao1     | −0.520 * | −0.171 | −0.199 | 0.059 | 0.032 | −0.393 | −0.197 | −0.377 | −0.002 | 0.293 | 0.547 * | 0.989 ** |      |      |      |         |
|            | Pielou    | −0.338 | −0.193 | −0.252 | −0.264 | −0.130 | −0.439 | −0.082 | −0.150 | −0.001 | 0.167 | −0.422 | 0.427 | 0.426 |      |         |
|            | Shannon   | −0.512 | −0.223 | −0.281 | −0.148 | −0.053 | −0.519 * | −0.163 | −0.308 | 0.020 | 0.282 | −0.001 | 0.787 ** | 0.782 ** | 0.891 ** |      |         |
|            | Simpson   | −0.584 * | −0.357 | −0.406 | −0.284 | −0.067 | −0.715 ** | −0.320 | −0.486 | −0.042 | 0.262 | 0.089 | 0.742 ** | 0.733 ** | 0.832 ** | 0.945 ** |      |         |

Note: ** indicates significant correlations at $p < 0.01$ and * indicates significant correlations at $p < 0.05$. 
4. Discussion

An altitudinal gradient alters sunlight, water and temperature, and changes in microclimate, which affect soil physical properties, nutrient availability, microbial biomass and vegetation types, lead to further functional changes in the ecosystems of alpine areas [44]. Because the response of soil nutrient transformation to altitude changes is limited by the soil environment and altitudinal range, nutrient transformation varies greatly in different habitats [17]. Withington and Sanford [17] found that substrate decomposition rates increased or remained constant as elevation increased, and soil moisture was the primary factor affecting cellulose decomposition in the alpine tundra.

In our study, as the altitude increased from A3200 to A4000, DOC and DON fluctuated with the altitude; the lowest levels occurred at 3600–3800 m, whereas DP showed continuous decreases in both of the soil layers. The different trends in DOC, DON and DP across the altitudinal gradient were due to the different sensitivities of the dissolved C, N, and P to soil moisture, temperature, and organic content [45,46]. TOC and TN had similar dynamics, indicating that the soil total C and N were highly correlated and shared similar driving effects. In addition, there was no significant difference in pH between the topsoil and subsoil, indicating that altitudinal changes had synchronous effects on pH with soil depth.

The activities of the soil enzymes depend on the soil biochemical processes and in turn affect the existence, transformation and availability of soil nutrients. CBH and BG play important roles in the early and late stages of cellulose degradation, which accounts for a large proportion of terrestrial ecosystem productivity. NAG and PME contribute to the degradation of chitin and ester phosphates, and play important roles in soil N and P cycling [20]. In our study, BG and CBH synchronously decreased from A3200 to A3600, and increased from A3800 to A4000 in both of the soil layers due to their functional similarities. NAG and PME showed similar fluctuations as elevation increased (the highest levels occurred at A3600 and A4000), indicating that they may be limited by the same factors, and soil N and P cycling through microbial metabolism was tightly interlinked [24,47].

The soil is a multienzyme system with a variety of enzymatic reactions, and there are close relationships between enzyme activities and the soil environment [36,37]. TOC and TN were closely related to BG and CBG in the topsoil and all of the enzymes (PME, BG, CBH and NAG) in the subsoil, supporting the contention that carbon and nitrogen are important factors mediating the ecosystem productivity in alpine areas. The activity of PME in the topsoil was negatively correlated with soil DP, indicating that soil microbes increased the production of enzymes involved in P cycling when soil P availability was low [48]. The activity of PME was significantly negatively correlated with pH. This is because PME is an acid phospho-esterase and the optimum pH for PME (4–5) is lower than the pH range (5.40–7.80) of the soils in this study, and it also indicates that soil pH, the key factor limiting soil biochemical processes and nutrient availability, had a strong impact on enzyme activities related to P transformation.

Specific pedological processes, land use changes and agricultural practices influence the spatially heterogeneous distribution of soil microbes [49]. Based on 16S rRNA sequencing, we identified the dominant taxa for all of the alpine soils. There were significant changes in the relative abundances of the dominant taxa along the altitudinal gradient, including Planctomycetes, Firmicutes, Gemmatimonadetes, Nitrospirae, Latescibacteria and Armatimonadetes at the phylum level and Gemmatimonas, Gaiella, Bradyrhizobium, Nitrospira and Steroidobacter at the genus level. However, the richness and diversity of the soil microbial communities did not significantly change with altitude. Previous studies have also shown that global microbial communities shared a common diversity structure and have high environmental resilience [50,51].

Alpine soils with low temperatures may limit soil microbial and extracellular enzyme activities and play important roles in the cycling of soil nutrients. Cui et al. [52] found that warmer temperatures increased methane emissions in alpine areas, whereas the methanogenic community composition was associated with vegetation but not related to
temperature. This also implies that the effects of environmental changes (temperature, water, air and vegetation) on soil microbes are comprehensive and may not be simply linked to a single environmental factor. Soil enzyme activities reflect microbial nutrient limitations and can be used as indicators of nutrient allocation and microbial nutrient demand [53,54]. In our study, most of the nutrients and enzyme activities reached their lowest levels at an altitude of A3600 or A3800, indicating that the treeline ecotone (A3800) was an important ecological transition belt of nutrient availability and enzyme activity.

Spatial variability and relationships between nutrient availability and microbial activities can reveal the ecological processes in alpine soil ecosystems. In our results, the altitudinal variations in pH, DOC, DON and DP in the subsoil were generally consistent with those in the topsoil. However, TOC and TN at A3400 were significantly lower in the subsoil than in the topsoil. The activity of NAG at A3400 was significantly higher than that at A3200 in the topsoil, but was relatively similar in the subsoil. This trend presumably occurs because environmental conditions at A3400 in the subsoil limited the microbial activities and enzymatic reactions, and resulted in a decrease in TOC and TN. In addition, our sample clustering and RDA results showed that the microbial communities at A3200 and A4000 were different from the other samples and were mainly affected by soil pH and DP, TOC and TN.

The transformation of soil nutrients and microbial metabolism showed different patterns with soil depth [55–57]. In our study, soil nutrients (TOC, TN, DOC, DON and DP) and enzyme activities (BG, CBH, NAG and PME) in the topsoil were significantly higher than those in the subsoil, which is consistent with previous studies in other areas [55,58]. This was presumably because unfavorable soil temperature, nutrient availability and oxygen content limit the accumulation of nutrients and enzymatic reactions in the subsoil.

Our results also showed that soil nutrients had more significant correlations with enzyme activities in the subsoil than in the topsoil. This result is related to more microbial-derived compounds and less plant materials in the soil organic matter of the subsoil than the topsoil [55,59]. Jia et al. [60] found that microbial metabolism prioritizes the use of labile substrates and that microorganisms showed a higher priming effect in deeper soils. In addition, the microbial carbon accumulation in the subsoil of alpine grasslands is more vulnerable to warming conditions than that in the topsoil [60].

Generally, our study provides useful insights into the internal relations between microbial metabolism and nutrient cycling in alpine ecosystems, and reveals the necessity of incorporating soil enzyme activity and microbial composition into earth system models when predicting the response of ecosystem nutrient cycling to global environmental changes.

5. Conclusions

We investigated the variations in soil nutrient availability, enzyme activity and microbial community across the eastern Qinghai–Tibetan Plateau and found that (1) the variations in the enzyme activities across the altitudinal gradient were tightly linked to the soil nutrient availability; an altitudinal range of 3600–3800 m corresponded to an ecological transition zone where most of the nutrients and enzyme activities reached their lowest levels, whereas the diversity of the microbial communities was relatively stable along the altitudinal gradient. (2) Soil nutrient availability and enzyme activity were significantly higher in the topsoil, but there were more significant correlations between soil nutrients, enzyme activity and the microbial community in the subsoil. Our results provide useful insights into the characteristics of soil microbial activity and nutrient cycling on the eastern Qinghai–Tibetan Plateau. Further research is needed to determine whether the ecological transition zone occurs in other parts of the vast area of the Qinghai–Tibetan Plateau, and the temporal and spatial dimensions of nutrient availability, enzyme activity and microbial metabolism still need to be explored.
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