Effects of Long-Term Warming on Microbial Nutrient Limitation of Soil Aggregates on the Qinghai-Tibet Plateau

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Abstract

**Background and aims** Global warming has increasingly serious impacts on the structure and function of the Tibetan Plateau ecosystem. However, the mechanism by which warming affects the biogeochemical processes and consequently the microbial nutrient limitation in soil aggregates is not clear.

**Methods** In the present study, we used open-top chamber experiments to simulate warming in an alpine meadow and an alpine shrubland on the Qinghai-Tibet Plateau to understand how warming affects nutrient utilization and microorganism-limiting mechanisms in soil aggregates.

**Results** The results showed that long-term warming treatment had contrasting effects on soil organic carbon (SOC) content of the alpine meadow and that of the shrubland. This difference was more pronounced with the increase in soil aggregate size, and the SOC content in microaggregates (MIGA) was significantly higher than that in large macroaggregates (LMGA). Soil enzyme activity increased with the decrease in aggregate size and was not significantly affected by warming treatment. Enzyme stoichiometry demonstrated that microbial P limitation is widespread on the Tibetan Plateau, and the long-term warming treatment exacerbated it, which has significant differences in shrubland. At the same time, the long-term warming treatment had no significant effect on C limitation in the alpine shrubland and the alpine meadow, but soil aggregate size affected the C limitation patterns of microorganisms and showed strong limitations in MIGA.

**Conclusions** The microbial P limitation in shrubland is more sensitive to warming than that of grassland. Soil aggregates mediate the acquisition of carbon by microorganisms, and the carbon limitation in MIGA is the greatest. By providing a new perspective on this topic, our study increased our understanding of the effects of warming on microbial nutrient utilization and restriction patterns in soil aggregates.

Introduction

Starting with the Industrial Revolution, greenhouse gas emissions have been causing a continuous increase in global temperatures, and a temperature rise of 1.4–5.8°C has been predicted by the year 2100 (Haines, 2003). Global warming impacts not only the soil environment, including soil temperature and moisture (Brzostek et al., 2012; Nie et al., 2014), but also the length of the growing season (Post et al., 2009) and species composition of plant communities (Saxe et al., 2001). As the driving forces of the biogeochemical cycle and energy flow in ecosystems (Sinsabaugh and Biochemistry, 2010), soil microorganisms are very sensitive to changes in the living environment. Warming can promote the metabolic activities of soil microorganisms (Jiang et al., 2016) and the decomposition and mineralization of soil organic matter, but at the same time, it reduces soil moisture, which may limit soil organic matter decomposition by microorganisms (Luo et al., 2009). Moreover, warming has an impact on soil microbial community structure and function.

Microorganism growth and development are usually limited by nutrient resources (Ekblad and Nordgren, 2002). Changes in external conditions (such as land-use changes, nitrogen deposition, and global warming) often affect the nutrient limitation patterns of microorganisms (Chen et al., 2018; Chen et al., 2019; Wang et al., 2014). Microorganisms can accelerate the decomposition of soil organic matter by releasing the corresponding enzymes in order to meet their resource demands and decrease nutrient restrictions (Qiao et al., 2019). Soil enzyme activity can reflect the direction and intensity of material circulation and energy flow in soil
ecosystems (Kalbitz et al., 2000). In recent years, warming experiments have been extensively carried out to study their effect on the Qinghai-Tibet Plateau ecosystem (Jiang et al., 2016; Luo et al., 2009; Wang et al., 2014). Previous studies have shown that the soil enzyme activity responses to warming are diverse (Bell et al., 2010; Nie et al., 2013; Zhou et al., 2013). Soil enzyme stoichiometry can reveal the relative change in different enzyme activities, and it has been suggested to be an effective indicator of microorganism resource limitation patterns (Moorhead et al., 2013; Sinsabaugh et al., 2009). Zheng et al. (2020) found that short-term warming decreased microbial C limitation but increased microbial P limitation. However, to our knowledge, there are no studies on the effects of experimental warming on the relative C, N, and P limitations of microbial processes at the soil aggregate level. Soil aggregates are important factors influencing soil physical and chemical properties (such as soil porosity, soil bulk density, water-holding capacity, and soil erosion resistance), thus shaping the basic soil physical structure (Deng et al., 2018; Zhu et al., 2017). A stable aggregate structure provides microorganisms with a suitable living environment by adjusting the flow of water and oxygen (Six et al., 2004). Nie et al. (2014) suggested that soil aggregate size distribution mediates microbial climate change feedback. Therefore, studying the effects of warming on the enzyme stoichiometry of soil aggregates is beneficial to better understand the impact of warming on plateau ecosystems.

The Qinghai-Tibet Plateau is one of the most sensitive and fragile ecosystems in the world. Understanding how microbial resource limitation changes under warming treatments would greatly improve our predictions of future nutrient utilization and dynamics of Qinghai-Tibet Plateau ecosystems in response to global warming. In the present study, we investigated the distribution characteristics of soil aggregate nutrients and enzyme activities in an alpine meadow and an alpine shrubland on the Qinghai-Tibet Plateau under a warming treatment, using chemometric knowledge to explore the resource limits of microorganisms at the aggregate level. We hypothesized that (1) long-term warming treatment would reduce soil nutrient content and increase soil enzyme activity, (2) microbial P limitation would increase as a consequence of warming, and (3) microbial feedback on warming would vary among different soil aggregate sizes.

Materials And Methods

Experimental site

The study site was located at the Haibei Alpine Meadow Ecosystem Research Station (101° 12’ E, 37° 30’ N, 3200 m a.s.l.), in the northeastern part of the Qinghai-Tibet Plateau (Fig. 1). The area has a typical continental monsoon-type climate. The summers are short and cool, and the winters are long and cold (Ji et al., 2017; Jiang et al., 2016). The average annual temperature is -1.7 °C, annual extreme maximum temperature is 27.6 °C, annual extreme minimum temperature is -37.1 °C, and annual precipitation range is 426–860 mm, 80% of which is distributed in the growing season from May to September. The average annual sunshine duration is 2462.7 hours, with 60.1% total available sunshine (Zhao and Zhou, 1999). The main soil type is Mollic-Cryic Cambisol (Zhao and Zhou, 1999). The dominant species of the alpine meadow and alpine shrubland are *Kobresia humilis* and *Potentilla fruticosa*, respectively (Li et al., 2016; Zhao and Zhou, 1999).

Controlled warming experiment
In 1997, six warmed plots and six control plots were set up within a fenced area of 30 × 30 m² in each of the alpine meadow site and the alpine shrubland site. The adjacent warm plots is 2m away. The warming plots were covered by open-top chambers (OTCs) to capture solar energy according to the ‘International Tundra Experiment’ (ITEX) approach (Marion et al., 1997). OTCs are 1 mm thick fiberglass truncated cones that are tilted 60 ° horizontally inward, with a height of 0.40 m, and bottom and top diameter of 1.48 and 1.08 m, respectively (Sun-Lite® HP, Solar Components Corporation, Manchester, NH, USA). The mean daily soil temperature increased (depth of 12 cm) by 0.3–1.9°C and had little impact on average soil moisture, only reducing it by 3% in the OCTs (Zhang et al., 2017).

Soil sampling and analysis

Samples of undisturbed soil were taken from warmed plots and control plots on August 10, 2018 by collecting the surface layer (0–5 cm) of bulk soils in quadruplicate from each plot using a soil core sampler. Since OTCs showed the warming effect at a depth of 0–12 cm (Zhang et al., 2017), the surface soil of 0-5cm was beneficial to better explore the warming response of the soil ecosystem. Afterwards, the samples from each plot were mixed to form a composite sample. Litter and debris were removed before sampling. We carefully transported the samples back to the laboratory to avoid damaging the soil physical structure. In all soil samples, we used the ‘optimal moisture’ method to separate the aggregates (Bach and Hofmockel, 2014; Dorodnikov et al., 2009; Mendes et al., 1999). First, soil samples were placed in a 4°C constant-temperature environment to dry until reaching a 10% gravimetric water content. The samples were then gently sorted by hand to below 8 mm in size and shaken three times for 2 min using a mechanical shaker to partition the aggregate sizes. Soil samples were sieved into three grades using stacking sieves (2 and 0.25 mm): > 2 mm (large macroaggregates, LMGA), 2–0.25 mm (small macroaggregates, SMGA), and < 0.25 mm (microaggregates, MIGA) (Nie et al., 2014). A part of each composite soil sample was placed in a refrigerator at 4°C to determine soil enzyme activity, and the rest was air-dried to measure other physicochemical properties.

Soil physicochemical properties and enzyme analysis

Soil organic C (SOC) was assayed by dichromate oxidation (Kalembasa et al., 2010), total N (TN) was assayed using the Kjeldahl method (Bremner and Mulvaney, 1982), and total P (TP) was measured colorimetrically using the ammonium molybdate method (Schade and Levine, 2003).

We determined the activity of β-1,4-glucosidase (BG), leucine aminopeptidase (LAP), β-N-acetylglucosaminidase (NAG), and alkaline phosphatase (AP) by using a modified version of standard fluorometric techniques (Chen et al., 2020; Wu et al., 2020) (Table 1). In brief, 3 g soil samples were placed in 125 mL of Tris buffer (50 mM, pH = 8.0) and homogenized. The samples were placed in a 96-well microplate with eight repeating groups in each column. After the microplate was loaded, it was shaken, mixed well, and placed in a 25°C incubator for 0.5, 2 or 4 h. We then used a microplate reader at 365 nm for excitation and 450 nm for emission to measure the amount of fluorescence, and enzyme activity was expressed in nmol·g⁻¹·h⁻¹.
Table 1
Enzyme introduction of this study

| Group                | Enzyme name            | Abbrev | Function                                      |
|----------------------|------------------------|--------|-----------------------------------------------|
| C-acquiring enzyme   | β-1, 4-glucosidase     | BG     | Cellulose degradation                         |
| N-acquiring enzymes  | leucine aminopeptidase | LAP    | Proteolysis                                   |
|                      | β-N-acetylglucosaminidase | NAG     | Chitin and peptidoglycan degradation         |
| P-acquiring enzyme   | alkali phosphatase     | AP     | Hydrolysis of organic P                       |

Data analysis

SOC, TN, TP, and enzyme activities were analyzed by one-way analysis of variance (ANOVA) to assess the effect of long-term warming treatment on their distribution in soil aggregates. Duncan's tests at p < 0.05 were performed for multiple comparisons. All statistical analyses were performed using SPSS 20.0 (IBM SPSS, Chicago, IL, USA), and Origin 9.0 (Origin Lab Corporation, Northampton, MA, USA) was used to prepare graphs.

Vector analysis of enzymatic stoichiometry was used to measure resource limitation (Chen et al., 2018; Moorhead et al., 2013). This method is based on the relative levels of C, N, and P-acquiring enzyme activity to explore the limitations of microbial resources by using the following equations:

\[
Vector\ L = \sqrt{(\ln BG/\ln (LAP + NAG))^2 + (\ln BG/\ln AP)^2}
\]

\[
Vector\ A = Degrees (ATAN2((\ln BG/\ln AP), (\ln BG/\ln (NAG + LAP))))
\]

Relatively longer vector L indicated greater C limitation; the vector A < 45° and > 45° indicated N and P limitation, respectively. When the vector A < 45°, smaller angle indicates stronger N limitation of microbial resources. Similarly, when the vector A > 45°, greater angle indicates stronger P limitation of microbial resources.

Results

Soil nutrients and enzyme activities

In the alpine meadow, the SOC content under the warming treatment was lower than that under the control treatment, and there was a significant difference in LMGA and SMGA between the two treatments (Fig. 2). However, in the alpine shrubland, the SOC content under the warming treatment was higher than that under the control treatment, and there was a significant difference in LMGA between the two treatments. In the alpine meadow, the TN content under the warming treatment was lower than that under the control treatment, and there was a significant difference in LMGA and SMGA between the two treatments. In the alpine shrubland, there was no significant difference in TN content between the warming treatment and the control treatment. In the alpine meadow, the content of SOC and TN decreased with the increase in soil aggregate size, and the content of MIGA was significantly higher than that of LMGA. In the alpine shrubland, SOC and TN contents did not change with the changes in soil aggregate size. Compared to the soil nutrient contents,
TP content in both the alpine meadow and alpine shrubland was less affected by the long-term warming treatment.

In most cases, the warming treatment had no significant effect on enzyme activity, except for AP and LAP of MIGA and BG of SMGA in the alpine shrubland (Fig. 3). In the alpine meadow, BG activity was significantly higher in MIGA than in LMGA and SMGA. In the shrubland, BG, NAG, and AP activities in MIGA were significantly higher than those in LMGA and SMGA. BG, LAP, NAG, and AP activities increased as aggregate size decreased and were higher in the alpine meadow than those in the shrubland.

**Ecological stoichiometry and indicators of resource limitation**

In the alpine meadow, compared to the control treatment, the long-term warming treatment significantly reduced the C:P ratios of LMGA and SMGA and the N:P ratios of SMGA and MIGA, whereas it had no significant effect on the C:N ratios (Table 2). In this habitat, the C:P and N:P ratios increased as the soil aggregate size decreased, and MIGA size was significantly larger than LMGA size. In the shrubland, compared to the control treatment, the warming treatment significantly increased the soil aggregate C:N ratios and the C:P ratios of LMGA and SMGA. The long-term warming treatment had no significant impact on enzyme stoichiometry in the alpine meadow (Table 3). The enzymatic C:P and N:P activity ratios were decreased in the alpine shrubland under the warming treatment compared to those under the control treatment, and there were significant differences in LMGA between the warming and control treatment. Soil aggregate size had no significant effect on enzyme stoichiometry in both habitats.

### Table 2
Effects of different aggregate size categories on topsoil stoichiometry of the alpine meadow and shrubland under the long-term warming treatment.

| Warming treatment | C:N ratio | C:P ratio | N:P ratio |
|------------------|-----------|-----------|-----------|
|                  | LMGA      | SMGA      | MIGA      | LMGA    | SMGA    | MIGA      | LMGA    | SMGA    | MIGA    |
| W-AM             | 10.58 ± 0.18b | 10.74 ± 0.17ab | 11.17 ± 0.08a | 66.13 ± 2.05Bc | 73.12 ± 2.01Bb | 87.35 ± 1.81a | 6.27 ± 0.25b | 6.82 ± 0.21Bb | 7.82 ± 0.13Ba |
| CK-AM            | 11.08 ± 0.26 | 10.62 ± 0.14 | 10.85 ± 0.16 | 79.81 ± 4.22Ab | 88.03 ± 5.17Aab | 93.50 ± 2.2a | 7.22 ± 0.41b | 8.30 ± 0.52Aab | 8.63 ± 0.24Aa |
| W-AS             | 15.95 ± 0.29Ab | 14.1 ± 0.32Ab | 13.17 ± 0.19Ac | 113.06 ± 6.53A | 114.94 ± 7.5A | 103.49 ± 5.05 | 7.14 ± 0.55 | 8.16 ± 0.54 | 7.86 ± 0.39 |
| CK-AS            | 12.19 ± 0.7B | 13.04 ± 0.2B | 12.21 ± 0.3B | 88.34 ± 5.5B | 91.01 ± 7.06B | 88.68 ± 6.35 | 7.31 ± 0.41 | 6.96 ± 0.48 | 7.25 ± 0.44 |

Note: W-AM: Warming-alpine meadow; CK-AM: Control-alpine meadow; W-AS: Warming-alpine shrubland; CK-AS: Control-warming-alpine shrubland. Values are shown as mean ± SE. Different uppercase letters indicate significant differences among different treatments in the same vegetation type and soil aggregate size at p < 0.05. Different lowercase letters indicate significant differences among different aggregates size categories in the same vegetation type and treatment at p < 0.05, n = 6.
Table 3
Effects of different aggregate size categories on topsoil enzymatic stoichiometry of the alpine meadow and shrubland under the long-term warming treatment.

| Warming treatment | InBG: In(NAG + LAP) | InBG: InAP | In(NAG + LAP): InAP |
|-------------------|---------------------|------------|---------------------|
|                   | LMGA | SMGA | MIGA | LMGA | SMGA | MIGA | LMGA | SMGA | MIGA |
| W-AM              | 1.02 ± 0.02 | 1.04 ± 0.02 | 1.03 ± 0.04 | 0.94 ± 0.01 | 0.97 ± 0.02 | 0.96 ± 0.03 | 0.92 ± 0.01 | 0.94 ± 0.01 | 0.93 ± 0.02 |
| CK-AM             | 1.01 ± 0.04 | 1.01 ± 0.04 | 1.02 ± 0.02 | 0.95 ± 0.01 | 0.97 ± 0.01 | 0.97 ± 0.04 | 0.94 ± 0.03 | 0.96 ± 0.04 | 0.95 ± 0.03 |
| W-AS              | 1.00 ± 0.05 | 1.01 ± 0.05 | 1.06 ± 0.06 | 0.90 ± 0.03Bb | 0.93 ± 0.02Bab | 0.95 ± 0.05a | 0.91 ± 0.04B | 0.92 ± 0.04 | 0.90 ± 0.02B |
| CK-AS             | 1.01 ± 0.03b | 1.03 ± 0.03ab | 1.05 ± 0.03a | 0.96 ± 0.04A | 0.97 ± 0.03A | 1.00 ± 0.04 | 0.95 ± 0.01A | 0.94 ± 0.01 | 0.95 ± 0.02A |

Note: W-AM: Warming-alpine meadow; CK-AM: Control-alpine meadow; W-AS: Warming-alpine shrubland; CK-AS: Control-warming-alpine shrubland. Values are shown as mean ± SE. Different uppercase letters indicate significant differences among different treatments in the same vegetation type and soil aggregate size at p < 0.05. Different lowercase letters indicate significant differences among different aggregate size categories in the same vegetation type and treatment at p < 0.05, n = 6.

In both alpine meadow and shrubland, the warming treatment had no significant effect on the vector L, but it decreased with increase in soil aggregate size (Table 4). In the alpine shrubland, the vector L of MIGA was significantly greater than that of LMGA. The mean vector A was greater than 45° across all observations in the control group. The vector A of the alpine meadow and shrubland increased under the long-term warming treatment compared to those under the control treatment, and it had a significant difference between LMGA and MIGA in shrubland.
Table 4

Effects of different aggregate size categories on topsoil enzymatic stoichiometry of the alpine meadow and shrubland under the long-term warming treatment.

| Warming treatment | Vector L | Vector A |
|-------------------|----------|----------|
|                   | LMGA     | SMGA     | MIGA     | LMGA     | SMGA     | MIGA     |
| W-AM              | 1.39 ± 0.01 | 1.42 ± 0.01 | 1.41 ± 0.02 | 47.24 ± 0.14 | 46.89 ± 0.13 | 47.02 ± 0.31 |
| CK-AM             | 1.39 ± 0.01 | 1.40 ± 0.01 | 1.41 ± 0.01 | 46.76 ± 0.42 | 46.34 ± 0.49 | 46.40 ± 0.37 |
| W-AS              | 1.35 ± 0.02b | 1.37 ± 0.01ab | 1.43 ± 0.03a | 47.73 ± 0.46A | 47.35 ± 0.45 | 48.06 ± 0.21A |
| CK-AS             | 1.39 ± 0.02b | 1.42 ± 0.02ab | 1.45 ± 0.02a | 46.39 ± 0.16B | 46.86 ± 0.15 | 46.38 ± 0.23B |

Note: W-AM: Warming-alpine meadow; CK-AM: Control-alpine meadow; W-AS: Warming-alpine shrubland; CK-AS: Control-warming-alpine shrubland. Values are shown as mean ± SE. Different uppercase letters indicate significant differences among different treatments in the same vegetation type and soil aggregate size at p < 0.05. Different lowercase letters indicate significant differences among different aggregates size categories in the same vegetation type and treatment at p < 0.05, n = 6.

Discussion

**Effects of warming treatment on nutrients and enzyme activities in soil aggregates**

Previous studies have reported inconsistent results of the analyses of soil carbon and nitrogen dynamics in response to warming treatments (Bai et al., 2013; Rui et al., 2011). Warming changed the storage of soil nutrients by mediating the relationship between nutrient input by litter and nutrient mineralization output (Guan et al., 2018; Wang et al., 2014). In the present study, warming decreased the SOC and TN content in the alpine meadow, which also differed significantly between LMGA and SMGA. A previous study showed that a warming treatment increased soil temperature, which improved soil microbial activity and promoted the decomposition and utilization of organic matter, leading to a decrease in SOC and TN (Rustad et al., 2001). Aggregate size fractionation has shown that the nutrient turnover rate in large aggregates is higher than that in small aggregates (Jastrow, 1996; Six et al., 2000). In the present study, we found that nutrient loss under warming treatment was significant in large aggregates, indicating that the carbon and nitrogen mineralization rates of large aggregates was more sensitive to warming than those of microaggregates. However, in the shrubland, we found that SOC in the warming treatment group was higher than that in the control group, and there was a significant difference in LMGA between the two groups. During sampling, we found less herbaceous plants in the warming shrubland than in the control shrubland. It is possible that the warming treatment reduced the soil water content (Nie et al., 2014), and the strong competition with shrubs resulted in the lower abundance of herbaceous plants. The roots of shrub species can penetrate deep into the soil in order to obtain nutrients from deeper soil layers, which is beneficial for the preservation of surface organic matter. In the present study, as TP was mainly affected by the soil parent material, warming treatment had no
significant effect. In the alpine meadow, SOC and TN contents decreased as soil aggregate size increased. Compared to LMGA, MIGA may provide better physical protection and has more stable SOC and TN contents (Six et al., 2001).

Soil extracellular enzyme activity can reflect the response of microorganisms to their nutrient requirements (Sinsabaugh et al., 2009). Previous studies have shown that long-term warming treatments significantly influence soil temperature and moisture content (Wang and Wu, 2013), which in turn affect the biogeochemical processes of soil microorganisms (Bell et al., 2010). Previous studies showed that the effects of warming on enzyme activity can be positive (Sardans et al., 2008), negative (Zhou et al., 2013), and neutral (Jing et al., 2014; Meng et al., 2020; Wang et al., 2014). Our study showed that warming had no significant effect on enzyme activity, except for AP and LAP activity in MIGA and BG activity in SMGA in the shrubland. This may be attributed to the coordinated change in biotic and abiotic factors, which maintains system balance under warming conditions (Wang et al., 2014). Nie et al. (2014) found that soil aggregate size distribution mediates the microbial feedback to climate change. Our research showed that enzyme activities increased as aggregate size decreased, which may be a consequence of a higher contribution of fast-growing microorganisms in MIGA than in LMGA (Dorodnikov et al., 2009; Wang et al., 2015). The greater specific surface area of smaller aggregates than that of larger ones facilitates the attachment of microorganisms on their surface (Amato and Ladd, 1992; van Gestel et al., 1996). In addition, smaller pore sizes in MIGA than those in LMGA provide better physical protection for microorganisms, thereby enabling them to stay in the soil for a longer time (Zhang et al., 2013).

**Effects of warming treatment on microbial resource limitation**

Some studies have indicated that warming can increase dissolved organic carbon concentration in the soil solution, which decreases the microbial C limitation (Luo et al., 2009). However, our results demonstrated that the warming treatment had no significant effect on microbial C limitation in the alpine meadow and shrubland. This may be because of the balance between microorganism carbon demand and the input of soil organic carbon during the long-term warming treatment. At the same time, enzyme analysis results showed that warming had no significant effect on BG activity, except in SMGA in the shrubland. The vector L decreased with increase in soil aggregate size, and MIGA was significantly higher than LMGA in the alpine shrubland. Soil aggregate structure affects microbial activities as fluxes of water and oxygen (Six et al., 2004), which can affect the accumulation and distribution of soil nutrients (Jastrow et al., 2007). It can also provide physical protection to prevent the rapid decomposition of soil organic carbon, and the level of protection depends on aggregate size (Pulleman and Marinissen, 2004). Jastrow et al. (2007) reported that the protection of SOC by MIGA is greater than the protection of SOC by LMGA, and soil organic matter (SOM) is more recalcitrant in MIGA than in LMGA. Researchers have also found that the water-soluble carbon and active carbon in macroaggregates were significantly higher than those in micro-aggregates (Jha et al., 2012).

Our results showed that the vector A was greater than 45°, suggesting that microbial P limitation was widespread in the investigated alpine meadow and shrubland. We also found that InBG:InAP and ln(NAG + LAP):InAP were slightly below 1.0, which also demonstrated that the P acquisition enzyme activity was relatively higher than the C and N acquisition enzyme activities. The analysis of the second national soil survey showed that the available phosphorus content in the Qinghai-Tibet Plateau is generally lower than the
national average (Wang et al., 2008). This may be related to the cold climate of the Qinghai-Tibet Plateau and low rates of P-containing primary mineral weathering (Rui et al., 2012). Compared to the control treatment, the long-term warming treatment significantly exacerbated the microbial P limitation in the alpine shrubland, which was indicated by higher vector A. It shows that soil microbial P limitation in shrubland is more sensitive to warming treatment. It is possible that decreased soil moisture content caused by warming promoted the formation of solids with calcium and reduced the available P content (Wang et al., 2008). Warming can increase the accumulation of P in plant biomass (Rinnan et al., 2008) and strengthen the P competition between soil microorganisms and vegetation. In addition, we found that the long-term warming treatment intensified resource competition among plants and led to a decrease in herb biomass. Branches and leaves of shrubs are more difficult to decompose than herbs (Brigham et al., 2018), resulting in weakened nutrient cycling and low available P content.

**Conclusions**

In the present study, we investigated the distribution characteristics of nutrients, enzyme activities, and ecological stoichiometry of soil aggregates in an alpine meadow and alpine shrubland, and we explored the resource limits of soil aggregate microorganisms on the Qinghai-Tibet Plateau under a long-term warming treatment. We found that the long-term warming treatment had contrasting effects on the SOC content of the alpine meadow and that of the shrubland. This difference was more pronounced with the increase in soil aggregate size, and the SOC content in MIGA was significantly higher than that in LMGA. Enzyme activity increased as aggregate size decreased and was not significantly affected by the warming treatment. Enzyme stoichiometry demonstrated that microbial P limitation is widespread on the Tibetan Plateau, and the long-term warming treatment exacerbated this limitation, which has significant differences in shrubland. At the same time, the long-term warming treatment had no significant effect on the C limitation in the alpine shrubland and alpine meadow, but the soil aggregate size affected the C limitation patterns of microorganisms, showing strong limitations in MIGA. Our study can be used as a basis for better predictions of the impact of future global warming on the Qinghai-Tibet Plateau ecosystem.

**Declarations**

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Figures

**Figure 1**

Location of the study site (Haibei Alpine Meadow Ecosystem Research Station) on the Qinghai-Tibet Plateau, China. DEM, digital elevation model Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

Changes in soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) among different soil aggregate size categories in the alpine meadow and shrubland under the long-term warming treatment. Note: W-AM: Warming-alpine meadow; CK-AM: Control-alpine meadow; W-AS: Warming-alpine shrubland; CK-AS: Control-warming-alpine shrubland. MIGA: microaggregates, SMGA: small macroaggregates, LMGA: large macroaggregates. Error bars indicate standard errors. Different uppercase letters above the bars indicate significant differences in soil nutrients between different treatments in the same aggregate size category, and different lowercase letters indicate significant differences in soil nutrients under the same treatment among different aggregate size categories. The bars with the same letters are not significantly different at p=0.05, n=6.
Figure 3

Changes in the activities of β-1,4-glucosidase (BG), leucine aminopeptidase (LAP), β-N-acetylglucosaminidase (NAG), and alkaline phosphatase (AP) among different aggregate size categories in the alpine meadow and shrubland under the long-term warming treatment. Note: W-AM: Warming-alpine meadow; CK-AM: Control-alpine meadow; W-AS: Warming-alpine shrubland; CK-AS: Control-warming-alpine shrubland. MIGA: microaggregates, SMGA: small macroaggregates, LMGA: large macroaggregates. Error bars indicate standard errors. Different uppercase letters above the bars indicate significant differences in enzyme activities between different treatments in the same aggregate size category, and different lowercase letters indicate significant
differences in enzyme activities under the same treatment among different aggregate size categories. The bars with the same letters are not significantly different at p=0.05, n=6.