Effects of Warming and Increased Precipitation on Soil Microbial Residues on the Qinghai–Tibet Alpine Meadows

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Abstract. Amino sugars, as the biomarkers of microbial residues, help explore soil microorganisms' response to global climate change. However, research on how microorganisms in the alpine meadows regulate the soil carbon cycle under the influence of climate change is limited. We hypothesized that climate change might cause different effects on soil microbial residues due to the impact on soil physical properties, chemical properties, and enzyme activities. Therefore, we conducted four-year continuous warming and increased precipitation experiments in the semi-arid grasslands to examine the differences in soil microbial residues at different depths under simulated changes. The results showed warming stimulated the accumulation of microbial residues, while increased precipitation led to their decline. The Glucosamine/Muramic Acid ratio trend indicates that the contribution of fungal residues was more significant than that of bacteria with increased precipitation, whereas that of bacterial residues exceeded that of fungi with warming. The increased precipitation had no significant effect on soil extracellular enzyme activities and amino sugar concentrations. In addition, changes in the different enzyme activities led to soil carbon loss and different amino sugar accumulation patterns under the warming treatment. These results may aid in assessing the responses of soil microorganisms to future climate change scenarios.

Keywords: Amino sugars; soil extracellular enzyme activity; warming; increased precipitation; alpine meadow; the Qinghai–Tibet Plateau.

1 Introduction

Amino sugars are derived from microbial metabolites and their cell wall residues, which are highly stable microbial sources, and their contents account for 5–10% of soil organic nitrogen (Glaser et al., 2004). They are essential components of the soil's active organic nitrogen and the primary source of soil mineral nitrogen (Pronk et al., 2015). Amino sugars are mainly derived from the cell walls of soil microorganisms so that they can be regarded as biomarkers of soil microorganisms (He et al., 2011). Up to 26 amino sugars have been identified in microorganisms, but only glucosamine (GluN), muramic acid (MurA), and galactosamine (GalN) are usually quantified (Wang et al., 2017; Zhang and Amelung, 1996). GluN is primarily derived from fungi and is the only component of fungal chitin and the major component of deacylated chitin. In contrast, MurA is exclusively derived from bacteria as a component of lipopolysaccharides and peptidoglycan in cell walls (Zhang

and Amelung, 1996). Bacterial MurA and fungal GluN are indicators of bacterial and fungal biomass (Joergensen, 2018). Therefore, amino sugars can be effective indicators for studying the different mechanisms of soils.

Amino sugars, extracellular enzymes, and decomposers may be affected by climate change (Henry, 2013; Liu et al., 2021). Due to various natural and human factors, the global climate has undergone significant changes (Immerzeel et al., 2010;

35   Kraaijenbrink et al., 2021), and the Qinghai-Tibet Plateau plays a role as a global climate amplifier (Krishnan et al., 2019). Until 2100, the mean global temperature and precipitation are expected to increase continuously by ~1.35–8.03 °C and 4.6– 10.1%, respectively (Du et al., 2022; Fan et al., 2020), which will significantly impact soil community structures, ecosystem, and biosphere. Soil microbes are crucial in how climate change affects ecosystems (Poll et al., 2013). Temperature and precipitation are important factors affecting soil microorganisms' growth and development. Increased precipitation affects

40   aboveground and underground biomass and soil microbial biomass (Zhang and Xi, 2021), while warming alters root production, mortality and turnover rates, and relative abundances of different microorganisms in different soil layers (Wang et al., 2017). Thus, the responses of these organisms to soil moisture and heat are critical links in ecological feedback. The impact of global warming on terrestrial ecosystem processes is evident across spatial and temporal scales, typically in high-altitude regions (Walther et al., 2002). Existing research on how the soil responds to climate change has primarily been based

45   on transect data, including soil respiration, carbon, nitrogen pools, and pH (Li et al., 2020; Na et al., 2011; Shen et al., 2015). Extracellular enzymes are essential indicators of soil fertility and are the main drivers of soil organic matter (SOM) decomposition in changing environments (Li et al., 2018). The higher surface temperature can increase the activity of extracellular enzymes, such as ligninase and cellulase activities (Chen et al., 2020a). But it may also reduce the rate of enzyme-substrate binding, slowing down enzyme-mediated reactions (Henry, 2013). This paradox occurs because not all

50   enzymes respond consistently to temperature increases. Likewise, the activities of extracellular soil enzymes don't change continuously with increasing precipitation (Sardans and Peñuelas, 2005). Previous studies have suggested that warming may significantly affect soil carbon content in alpine areas (Ding et al., 2019). Although warming leads to increases in microbial biomass and enzyme activity, the resultant enhancement of decomposition leads to decreases in soil organic carbon (SOC) (Li et al., 2019). Zhang et al. (2019a) demonstrated that SOC and precipitation correlate significantly and negatively. At the

55   same time, Chen et al. (2010) revealed that the changes in precipitation have little effect on SOC in forest areas.

Although how warming and increased precipitation affect soil microorganisms has been intensively studied, its effect on amino sugar in the increased soil temperature and moisture under climate change remains unknown. Clarifying the changes in soil microbial residues due to climate change will aid in assessing the response of soil microorganisms under future climate change scenarios. Herein, we conducted four-year continuous warming and increased precipitation experiment to

60   examine the changes in soil microbial residues at different depths under simulated changes We hypothesized that simulated changes (warming and increased precipitation) might cause different effects on soil microbial residues due to the impact on soil physical properties, chemical properties, and enzyme activities. To test the hypothesis, we examined GluN, MurA, GalN,

soil extracellular enzyme activity, biomass, and C and N concentrations for 4 scenarios in 4 fields. We applied these data to

structural equation modeling to identify key drivers of climate change impacts on soil microbial residues.

2 Material and methods

2.1 Site selection and experiment design

Soil was collected from the Qinghai–Tibet Plateau (QTP), which is one of the most sensitive regions to global climate

change (Li et al., 2022; Xu et al., 2008). The Nagqu River watershed (NRW) is the source area of the Nu River (Yang et al.,

2021a; Yang et al., 2021b) and has the typical features of the QTP: rising air temperature and increased precipitation. The

geographical coordinates of the NRW are ~30°54′–32°43′N, ~91°12′–92°54′E, and it is at an elevation of 4140–5897 m a.s.l

(Figure 1a). From 1951 to 2020, the mean annual air temperature was 0.3 °C, and the mean annual precipitation was ~483.7

mm. The thawing period is from May to September, and 89.7% of the yearly precipitation falls during this period (Man et al.,

2019). The frozen soil area is ~1.53 × 104 km2, and the soils developed in the Kobresia meadows were Mat-Gryic Cambisol

(Chinese Soil Taxonomy Research Group, 1995), corresponding to Gelic Cambisol (The World Reference Base, 1998).

Based on the climate change simulation method recommended by the International Tundra Experiment, an open-top chamber

(OTC) made of glass fiber was laid out (Figure 1b-c). Four treatments were used in the experiment: warming (W) (~1.5–

2.0 °C), increased precipitation (P) (add 50 mm water in July and August, respectively), increased precipitation and warming

(P & W), and control group (C). Four replicate sites with flat terrain and even plant distribution were selected in the

watershed, and the sites were closed with net fences. Soil moisture and temperatures at 10, 20, and 30 cm depth in each

treatment were monitored every 30 min with 5TM sensors connected to EM-50 data loggers (Decagon Devices Inc., USA).

We chose this fields because the experiment has been under our protection and management since it was laid out in August

2018. Compared to other places, the QTP has presented the most significant trend of increasing temperature and

precipitation over the past few decades (Zhang et al., 2019b). Since we aimed at the effects of warming and increased

precipitation on the changes in soil microbial residues under simulated changes, this site was suitable places for our scientific

question.

2.2 Soil sampling measurement

2.2.1 Biomass measurement

The aboveground biomass (AGB) and underground biomass (UGB) of every treatment were measured in August. To avoid

the edge effects, samples were only measured in the central area of the OTC (Xu et al., 2010). To estimate the AGB, the

grass was cut and harvested with living plants, and litter was treated separately. More than 95% of the UGB on the QTP is

distributed at 0–30 cm (Zhao et al., 2022), so the UGB samples were divided into three layers (0–10 cm, 10–20 cm, and 20–

30 cm). After filtering the soil with a 1-mm mesh sieve, the AGB and UGB samples were transported to the laboratory,

drying to a constant weight at 70 °C (Liu et al., 2017).

2.2.2 Soil sample collection

95 Corresponding to the AGB and UGB, soil samples were triplicately collected in 3 layers (0–10 cm, 10–20 cm, and 20–30 cm)

by simple random sampling (Wadoux et al., 2019). After removing visible roots and gravels, samples were mixed and passed

through a 2 mm sieve. Sieved samples were stored in a refrigerator at 4 °C before being transported to the laboratory.

2.2.3 Soil chemical properties measurement

Soil pH was measured by mixing air-dried soil with the potentiometry method. Soil total carbon (TC) was analyzed using the

100 combustion oxidation-non-dispersive infrared method (TOC analyzer, Shimadzu, Japan). SOM was measured via the

potassium dichromate oxidation-external heating method. Soil total nitrogen (TN) was measured via the Kjeldahl method

(Nitrogen analyzer, Velp, Italy). Soil total phosphorus (TP) was measured via molybdenum antimony spectrophotometry

(Xipu, China). All samples were measured in triplicate.

2.2.4 Soil extracellular enzyme activity measurement

105 Since soil extracellular enzyme activity is a key factor affecting soil microbial residues (Chen et al., 2020a), we collected and

tested 6 potential soil enzyme activity indicators, including β-glucosidase (BG), acid phosphatase (AP), leucine

aminopeptidase (LAP), β-D-cellobiosidase (CB), β-N-acetylglucosaminidase (NAG), and β-xylosidase (XYL). The soil

enzyme activity was analyzed via microplate fluorescence in which 3 g of fresh soil was first weighed. Subsequently, 125

mL of Tris buffer was added, and the soil suspension was prepared. Then, 150 μL of the suspension and 50 μL of the

110 substrate were added to a 96-well microtiter plate to prepare the sample wells. Blank microwells (150 μL suspension and 50

μL Tris buffer), standard microwells (150 μL Tris buffer and 50 μL 4-methylumbelliferone), and negative control (150 μL

Tris buffer and 50 μL substrate) were measured in triplicate. All samples were incubated at 25 °C in the dark for 0.5 h (AP),

2 h (NAG, LAP), and 4 h (BG, CB, and XYL), respectively. After incubation, the microwell multifunctional microplate

reader was used for excitation at 365 nm, and the fluorescence of each sample was detected at 450 nm (MD SpectraMax 190,

115 USA).

2.2.5 Amino sugars measurement

The concentration of GluN, MurA, and GalN in the soil were measured through gas chromatography (Agilent, USA) derived

from the saccharonitrile acetyl ester employed by (Zhang and Amelung, 1996). In brief, soil samples were air-drying

equivalent to 0.3 mg N in hydrolysis bottles with hydrochloric acid at 105 °C for 8 h. after cooling them to room temperature

120 (~20 ºC), 100 μL of inositol (internal standard 1) was added and filtered in heart-shaped bottles. Dried samples were

dissolved in centrifuge tubes with small amounts of distilled water, centrifuging at 3000 rev min⁻¹, and freezing the supernatant with a freeze dryer. The residues were then dissolved in anhydrous methanol and centrifuged again at 3000 rev min⁻¹. The supernatants were transferred to 5 mL derivatization flasks, dried with a nitrogen blower at 45 °C, and added to 1 mL of water and 100 µL of N-methylamino. Glucose (internal standard 2) was thoroughly mixed and freeze-dried for 8 h and

125   then derivatized with nitrile acetyl ester. The derivative was dissolved in 200 µL of ethylacetate-n-hexane (1:1) and then transferred to a chromatographic bottle equipped with an inner cannula for gas chromatography (GC-7890B, Agilent Technologies, USA) with an HP-5 capillary column (30 m × 0.25 mm × 0.25 µm). An internal standard was used to calculate the contents of each amino sugar (mg/kg) as follows:

$$\mathrm{m}_x = \frac{\mathrm{m}_i A_x}{A_i R_f},\tag{1}$$

130   where m_i is the quality of the added internal standard (inositol), A_i and A_x are the peak areas of the inositol and amino sugar, and R_f is the relative correction factor for each amino sugar, using the amino sugar and standard sample calculation of the alcohol correction factor.

2.3 Statistical analyses

To determine the relationship between different influencing factors, we used the structural equation model (SEM) to study

135   changes in soil amino sugars at different depths and under different treatments (Amos Development Corporation, Chicago, IL, USA) (See supplementary). To study the effects of different climate changes and different soil depths on soil amino sugars, we constructed two priori structural equations according to previous studies (Chen et al., 2016; Chen et al., 2020b; Ding et al., 2019; Joergensen, 2018; Ma et al., 2020; Ni et al., 2020) (Figure S1-S2) and selected the suitable indicator for further analysis according to the significance. Evaluation indicators in SEM include χ^2 test, comparative fit index (CFI), and

140   root mean square error of approximation (RMSEA).

3 Material and methods

3.1 Changes in soil amino sugars with different depth

The total amino sugars (TAS) among the three soil layers (0–10 cm, 10–20 cm, and 20–30 cm) were mainly consisted of GluN, with concentration ranging from 136.65–264.72 µg/ml, followed by GalN and MurA at 27.94–145.53 µg/ml and

145   12.22–15.75 µg/ml, respectively. As presented in Tables 1 and 2, the TAS and GluN concentration in the 20–30 cm soil layer was significantly higher than that in the 0–10 cm and 10–20 cm layers (p < 0.05), ranging from 210.76–423.60 µg/ml and 168.50–264.72 µg/ml, respectively (p < 0.05). The GalN and MurA were the most abundant in the 10–20 cm and 0–20 cm soil layers, respectively (p < 0.05). The ratio of GluN/MurA at 0–10 cm, 10–20 cm, and 20–30 cm gradually decreased, with

the value of 16.22, 15.97, and 15.46, respectively (Table 2). Besides, the amounts of ST, SM, BG, AP, LAP, NAG, XYL, and SOM varied significantly among three soil layers.

3.2 Changes in soil amino sugars with warming and increased precipitation

The correlations of soil physical properties, chemical properties, enzyme activities, and amino sugars revealed that GluN and GalN were positively correlated with ST, ABG, TP, TK, TC, and most of the enzyme activities. In contrast, MurA was negatively correlated with enzyme activities $(p < 0.05$, Figure 2).

3.2.1 Increased precipitation treatment

Under the increased precipitation treatment, AGB, UGB, BG, AP, LAP, CB, NAG, XYL, TC, TN, TP, and SM increased significantly. The results of the four-year continuous experiment showed that increased precipitation caused soil moisture to increase by 0.06 $(p < 0.001$, Tables 3 and Tables S2). The concentration of TAS, GluN, GalN, and MurA changed by 5.72 %, -1.65 %, 26.30 %, and 6.60 %, respectively, among which only the variation of GluN was not statistically significant. The ratio of GluN/MurA in the increased precipitation treatment was 16.26, which was the highest among all treatments (Table 3).

3.2.2 Soil extracellular enzyme and amino sugars

The warming treatment increased the soil temperature by 1.04 °C $(p < 0.001$, Tables 4 and S2). Among the amino sugars, the concentration of TAS, GluN, GalN, and MurA were reduced by 12.53 %, 7.69 %, 28.94 %, and -2.35 %, respectively. The ratio of GluN/MurA in the warming treatment was 13.90, which was the lowest among the four treatments (Table 3).

3.2.3 Both warming and increased precipitation treatment

Both warming and increased precipitation treatment increased the soil temperature by 0.69 °C $(p = 0.004$, Table 4 and S2) and soil moisture by 0.06 $(p < 0.001$, Table 4 and S2). Among them, AGB, CB, XYL, and TP increased significantly. TAS, GluN, and MurA increased by 1.94 %, 5.92 %, and 0.96 %, respectively, whereas GalN decreased by 9.08 %. The ratio of GluN/MurA in the soil was 15.50, which was second among the four treatments (Table 3).

3.3 The influence factors of soil amino sugars

3.3.1 Physicochemical properties and amino sugars

As the SEM of the turnover rate of GluN and soil physicochemical properties in Figure 3, TC and SOM directly affected GluN. The path from TC to GluN had a coefficient of -0.47, which indicated that 1 unit of increase in TC could cause 0.47 unit of decrease in GluN. Likewise, the path from TC to GluN had a coefficient of -0.47. P treatment and SOM concentration

had two ways to GluN, with standardized total effects (the sum of the direct and indirect effects by combining all path coefficients) of -0.041 and 0.171 (Table 4). W treatment had a total effect size of 0.163, suggesting that 1 unit of the increase in warming treatment could increase 0.163 unit of GluN.

Different from the pattern for GalN (Figure S4), the concentration of MurA was only related to TN (path coefficient = 0.34,

180   P = 0.035, Figure 4), and other physical and chemical properties indirectly affected MurA by affecting TN (Figure 4). The cross-influence of other elements on TN resulted in different effects of TN on the three aminosugar components in the two scenarios. Similar to the pattern of MurA, TAS increased the negative effect of warming on TN (Figure S3).

3.3.2. Soil extracellular enzyme and amino sugars

Among the various soil extracellular enzymes, β-D-cellobiosidase (CB) had the most significant positive total effect pathway on GluN, while XYL had the most significant negative total effect pathway on GluN (Table 5). Unlike other amino sugars'

185   turnover process, during the turnover of GluN, the activity of NAG had almost no effect on it. BG no longer has a strong pointing effect on NAG, but is replaced by a circular conversion mechanism of BG, CB and XYL (Figure 5). Dep had three paths to affect GluN concentration, directly or indirectly through the action of CB with XYL. However, due to the variation of CB and BG in vertical, the standardized total effect of Dep was just -0.023.

In the P treatment, increased precipitation had no significant effect on soil extracellular enzyme activities and amino sugar

190   concentrations. After eliminating insignificant paths and variables, the SEM of GalN and soil extracellular enzyme activities in the warming treatment were shown in Figure 6. PH and Acid Phosphatase (ACP) had direct effects on the GalN, with path coefficients of -0.23 and -0.22, respectively. The pH of the soil environment had a significant negative effect on ACP activity (path coefficient = -0.31, P = 0.01, Figure 6). In addition, warming treatment, NAG, and XYL activity had highly

195   significant effects on ACP activity, with path coefficient of -0.39 (P=0.003), 0.41 (P=0.001) and 0.44 (P<0.001).

4 Discussion

4.1 Distribution and turnover patterns of amino sugars in alpine meadows

Numerous studies have found that GluN was the highest components among TAS in all types of landuse scenarios and soil layers, while the concentration of MurA was the lowest (Joergensen, 2018; Liang et al., 2021). This may be due to GluN is

200   more resistant to microbial breakdown and builds up in the soil (He et al., 2011). Under the warming treatment, the concentration of GluN decreased while the proporrtion of it in TAS increased. The most reason is due to the sharp decrease in GalN, which may be related to the significant decline in microbial community diversity under warming treatment (Sheik et al., 2011).

GluN acted as markers of fungal dominance in soil microbial communities over extended periods (Schulten and Schnizter,

205   1997). However, previous studies have shown that GluN can be obtained from decomposing fungal and bacterial residues

(Engelking et al., 2007). So the ratio of GalN/MurA can better explain the population characteristics of soil microorganisms

than the GluN/MurA. Decreases in the GluN/MurA indicate that the contribution of the transformation and accumulation of

bacterial residues is more significant than that of fungi. On the contrary, increases in the proportion suggest that the relative

contribution of fungal residues in the alpine meadows is more critical than that of bacteria (Zhang et al., 2013). The

210   GluN/MurA ratio in this study are within the range of amino sugar characteristics of mineral soils summarized by Amelung

et al. (2001). It indicates that the contribution of fungal residues was higher than that of bacteria with increased precipitation

treatment, whereas that of bacterial residues was greater than that of fungi with enhanced warming. It also indicates that the

TAS accumulation pattern in the central Tibetan area is that actinomycetes and bacteria have a higher contribution to the

microbial community, and bacteria are more dominant (Joergensen, 2018). The decrease in the ratio of GluN/MurA in the

215   warming treatment was due to the changes in the fungal and bacterial community structures, as well as their different rates of

decomposition. Similar to previous study (Ding et al., 2017), the ratio of GluN/MurA decreased with soil depth. However,

this phenomenon is not universal, and may be caused by a single microbial community structure in alpine meadows.

It is generally considered that fungal residues are more difficult to decompose than bacterial residues; therefore, the primary

reason for the decrease in the ratio of GluN/MurA in the warming treatment was the changes in microbial community

220   structure (Yang et al., 2019). Besides, increased precipitation can change the community structure of microorganisms, and

the increases of soil moisture can make the bacterial-dominated soil microbial community structure evolve toward the

fungus-dominated structure. (Yang et al., 2021c). This may be that the increase in soil moisture inhibits the enzyme activity

of bacteria. Therefore, bacterial demand for soil moisture is less than that of fungi. When soil temperatures are high, the

microbial community is dominated by bacteria, which causes the bacteria to contribute more to converting TN than fungi.

225   Combining amino sugars with the biomarkers of living microorganisms, such as phospholipid fatty acids, would help

improve our understanding of the changes in soil microbial community structure in alpine meadows caused by warming and

increased precipitation.

4.2 Influencing factors of amino sugars accumulation under warming and increased precipitation treatments

Soil warming and increased precipitation will change the soil's active microbial biomass, increasing or decreasing the

230   residues after the death of the respective microorganisms (Chen et al., 2020a). In this study, the four TAS and GluN

concentrations at 0–10 cm under each treatment exceeded those at 10–20 cm and 20–30 cm, which is consistent with the

results of Zhang et al. (2014). It is because the root systems of herbaceous plants are mainly distributed near the soil surface,

and these systems are the primary organs through which the plants can absorb nutrients and are the most active sites for

microorganisms. There was a large amount of litter accumulated on the soil surface. The nutrient source was sufficient, and

235   the moisture, heat, and ventilation conditions were improved, which was more conducive to the growth and reproduction of

various microorganisms (Zhu et al., 2019).

In this study, the amino sugar concentrations under the warming treatment were lower those under the increased precipitation

treatment had the opposite effects. It indicates that warming and precipitation can affect the abundance of soil

microorganisms, likely because they can change the structure and activity of bacterial communities, transforming the

240   community into one which more adaptable to high temperatures and high growth rates. OTC warming was more effective

than infrared heating and could significantly increase microbial abundance by 15.1% (Chen et al., 2016). The specific

performance of the warming experiment in this study was increased by 1°C. Temperature changes can directly affect the

stability of amino sugars and accelerate their decomposition (Shao et al., 2018). According to the SEM, the warming

treatment has a negative impact on the TC. Chen et al. (2020b) believed this effect is mainly due to the increased activity of

245   cellulase enzymes such as BG, which causes soil microorganisms to decompose soil carbon with more complex and stable

chemical properties to obtain energy. However, in the alpine meadows, this directivity is not significant. Under the warming

and humidifying process of the QTP (Bibi et al., 2018), soil carbon loss caused by warming may lead to the release of old

carbon from the QTP carbon pool (Patoine et al., 2022), weakening carbon sinks, forming new carbon sources, and further

accelerating the process of global warming (Chen et al., 2016; Mu et al., 2020).

250   Precipitation is the primary factor that restricts the growth and development of soil microorganisms in alpine meadows, and

therefore, the changes in precipitation will significantly impact the abundance of microorganisms (Wu et al., 2020).

Increased precipitation only positively affected the migration and transformation of MurA. This effect mainly affects amino

sugars by promoting subsurface biomass, TC, and TN. The increased precipitation treatment did not appear to elevate the

mineralization of SOC (Jia et al., 2020). The effect of the increased precipitation treatment on soil extracellular enzyme

255   activities was insignificant, and this effect also weakened with depth. It may be due to the more significant potential

evaporation in the central Tibetan area (Zhang et al., 2018), exceeding the contribution of the increased precipitation. The

process of carbon and nitrogen cycle has been an important research object in the turnover process of soil amino sugars (Li

et al., 2019; Xie et al., 2022). ACP activity was highest at pH=5, while in the sampling data. The experimental results show

that the pH range of all samples is 6.3-7.5, which was more suitable for bacterial amino sugar accumulation (Turrión et al.,

260   2002). Similar to previous results (Ni et al., 2020), only pH has direct and indirect effects on GalN, with the negative

standardized total effects. In this study, a more meticulous conclusion can be obtained in the SEM using the two variables of

TC and TN instead of the TC/TN ratio. TC plays a different role in converting three amino sugars. The TC concentration in

the soil has a strong inhibition effect on the synthesis of GluN. For GalN and MurA, TN inhibits and promotes the two

amino sugars. TN is only involved in the SEM of GalN and MurA and plays a different role. The enrichment of TN will

265   adversely affect GalN, but it has a solid promoting effect on MurA. It may be since increased nitrogen input can attenuate

soil respiration (Xing et al., 2022), affecting different microbiota evolution.

5 Conclusions

Amino sugars, as the biomarkers of microbial residues, help in exploring the response of soil microorganisms to global climate change. However, its response to the increased soil temperature and moisture under climate change remains

270   unknown. Therefore, we set up a four-year experiment to simulate warming and increased precipitation treatments at four experimental stations of the Qinghai-Tibet Plateau to analyze the composition and changes in soil amino sugars. All soil samples were triple collected from depths of 0–10 cm, 10–20 cm, and 20–30 cm. With increasing soil depth, the contribution of the transformation and accumulation of fungal residues decreased. Warming treatment stimulated the accumulation of microbial residues, while increased precipitation had the opposite effects. The increased precipitation had no significant

275   effect on soil extracellular enzyme activities and amino sugar concentrations. The application of structural equation modeling also showed the specificity of the different components of aminosaccharides in the soil evolution. Glucosamine, unlike other aminosaccharide fractions, did not have a significant effect of total nitrogen on its turnover, while the specificity of galactosamine was reflected in the specific effects of environmental PH and ACP activity on it. Overall, our results suggest that changes in different enzyme activities are the main reason for the different patterns of soil carbon loss and soil

280   amino sugar accumulation under warming treatment. Our work has additional implications for predictions of the impacts of warming and increased precipitation on soil microbial residues in alpine meadows.

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Data availability

These data will be archived in an appropriate public repository (Figshare) and the data DOI will be included at the end of the article if the manuscript is accepted for publication.

Author contributions

290   Conceptualization, Baisha Weng, Zhaoyu Dong and Yuheng Yang; data curation, Mengyu Li and Yuhang Zhang; methodology, Baisha Weng, Zhaoyu Dong and Yuheng Yang; writing-orginal draft preparation, Baisha Weng and Yuheng Yang; writing-review and editing, Denghua Yan, and Zhaoyu Dong; supervision, Denghua Yan; project administration, Zhaoyu Dong and Yuheng Yang; funding acquisition, Baisha Weng and Denghua Yan.

Declaration of competing interest

295   Authors declare that they have no conflict of interest.

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Tables

Table 1 Changes of Amino Sugars and Related Indexes in Soils at Different Depths

Variable	10 cm	20 cm	30 cm
ST (℃)	10.82±1.96	10.59±1.49	9.53±2.16
SM	0.16±0.08	0.17±0.09	0.15±0.06
AGB (g/m^2)	259.33±75.94	259.33±75.94	259.33±75.94
AP (nmol/g)	1.8±0.73	1.67±0.67	1.62±0.97
BG (nmol/g)	258.88±150.02	156.58±54.74	113.56±72.04
CB (nmol/g)	13.08±10.6	10.28±6.79	8.12±6.92
GalN (mg/kg)	74.46±46.28	86.73±58.8	86.42±56.8
GluN (mg/kg)	211.61±43.51	199.88±63.22	216.61±48.11
MurA (mg/kg)	14.37±1.38	13.89±1.67	14.15±1.51
LAP (nmol/g)	6.94±4.34	6.15±2.05	4.72±2.65
NAG (nmol/g)	7.84±4.55	5.06±2.33	4.24±2.48
SOM (g/kg)	15.05±7.05	12.96±5.49	11.75±6.75
TAS (mg/kg)	300.44±91.17	300.5±123.69	317.18±106.42
TC (g/kg)	10.39±3.19	8.81±3.37	8.6±4.76
TN (mg/kg)	0.92±0.34	0.84±0.33	0.81±0.38
TP (mg/kg)	0.03±0	0.03±0	0.03±0.01
UGB (g/m^2)	1693.5±785.16	1884.52±663.52	1713.01±726.79
XYL (nmol/g)	8.45±5.17	7.72±4.23	8.23±6.71

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Table 2 GluN/MurA ratio changes in different depths and different treatments

ID	P	W	P & W	C	Average
10 cm	17.39	15.31	17.12	15.06	16.22
20 cm	16.51	15.11	16.43	15.84	15.97
30 cm	15.58	15.44	15.77	15.04	15.46
Average	16.49	15.29	16.44	15.32	-

Table 3 Changes of soil amino sugars and related indexes under the treatment of warming and precipitation

ID	P	W	P & W	C
ST (℃)	10.27 ± 1.32	11.33 ± 1.46	11.09 ± 1.64	10.32 ± 1.96
SM	0.19 ± 0.08	0.13 ± 0.05	0.17 ± 0.09	0.13 ± 0.05
AGB (g/m^2)	276.04 ± 68.53	197.73 ± 24.4	246.3 ± 79.11	214.97 ± 40.66
UGB (g/m^2)	1819.61 ± 780.91	1261.96 ± 442.78	1492.84 ± 884.79	1335.4 ± 567.66
BG (nmol/g)	165.52 ± 109.57	147.45 ± 69.35	160.34 ± 176.67	139.59 ± 92.96
AP (nmol/g)	2.02 ± 0.73	1.87 ± 0.55	1.73 ± 0.71	1.81 ± 0.86
S-LAP (nmol/g)	6.91 ± 5.53	5.02 ± 3.1	5.52 ± 3	5.34 ± 3.39
CBH (nmol/g)	10.12 ± 7.13	8.15 ± 6.58	10.5 ± 10.6	5.88 ± 6.16
S-NAG (nmol/g)	4.45 ± 3.19	4.11 ± 2.99	4.68 ± 5.7	4.27 ± 2.98
XYL (nmol/g)	8.14 ± 6.86	5.55 ± 4	7.22 ± 5.34	5.19 ± 7.07
TC (g/kg)	10.08 ± 3.18	9.4 ± 2.86	8.85 ± 4.45	8.75 ± 3.91
TN (mg/kg)	0.98 ± 0.32	0.94 ± 0.33	0.84 ± 0.36	0.86 ± 0.37
TP (mg/kg)	0.03 ± 0.01	0.03 ± 0	0.03 ± 0.01	0.03 ± 0.01
SOM (g/kg)	15.12 ± 4.35	14.73 ± 3.8	13.95 ± 7.2	13.02 ± 5.68
TAS (mg/kg)	1829.77 ± 315.63	1607.52 ± 229.81	1721.22 ± 375.15	1645.42 ± 231.99
GlcN (mg/kg)	1208.31 ± 220.05	1056.38 ± 153.03	1172.58 ± 248.68	1077.74 ± 132.48
GlcN (mg/kg)	73.24 ± 6.78	69.1 ± 3.76	71.3 ± 6.26	70.37 ± 4.19
MurA (mg/kg)	548.21 ± 258.14	482.04 ± 208.15	477.35 ± 223.4	497.31 ± 215.34

475 **Table 4** Standardized total effects of different scenarios and soil physicochemical properties on the conversion of different

amino sugar components. All effects are significant (P < 0.1).

Factors	Dep	W	P	SOM	TC	TN	UGB
TAS	0.011	0.031	-0.07	-0.088	-0.075	-0.132	-0.024
GluN	-0.047	0.163	-0.041	0.171	-0.477	\	\
GalN	0.025	0.042	-0.044	-0.125	-0.106	-0.175	\
MurA	-0.042	-0.125	0.161	0.223	0.124	0.331	0.057

Table 5 Standardized total effects of different scenarios and soil extracellular enzyme activities on the conversion of different amino sugar components.

Factors	Dep	W	XYL	BG	NAG	pH	ACP	CB
TAS	-0.046	-0.041	-0.195	0.209	-0.138	\	\	\
GluN	-0.023	0.24	-0.449	0.185	\	\	\	0.254
GalN	0.035	0.001	-0.117	-0.031	-0.086	-0.16	-0.227	\
MurA	-0.219	-0.094	0.376	0.06	0.164	\	\	\

480   All effects are significant ($P < 0.1$).

Table 6 Standardized indirect effects of different scenarios and soil physicochemical properties on the conversion of different amino sugar components.

Factors	Dep	W	P	SOM	TC	TN	UGB
TAS	0.011	0.031	-0.07	-0.088	-0.075	0	-0.024
GluN	-0.047	0.163	-0.041	-0.332	0	\	\
GalN	0.025	0.043	-0.044	-0.125	-0.106	0	\
MurA	-0.042	-0.125	0.161	0.223	0.124	0	0.057

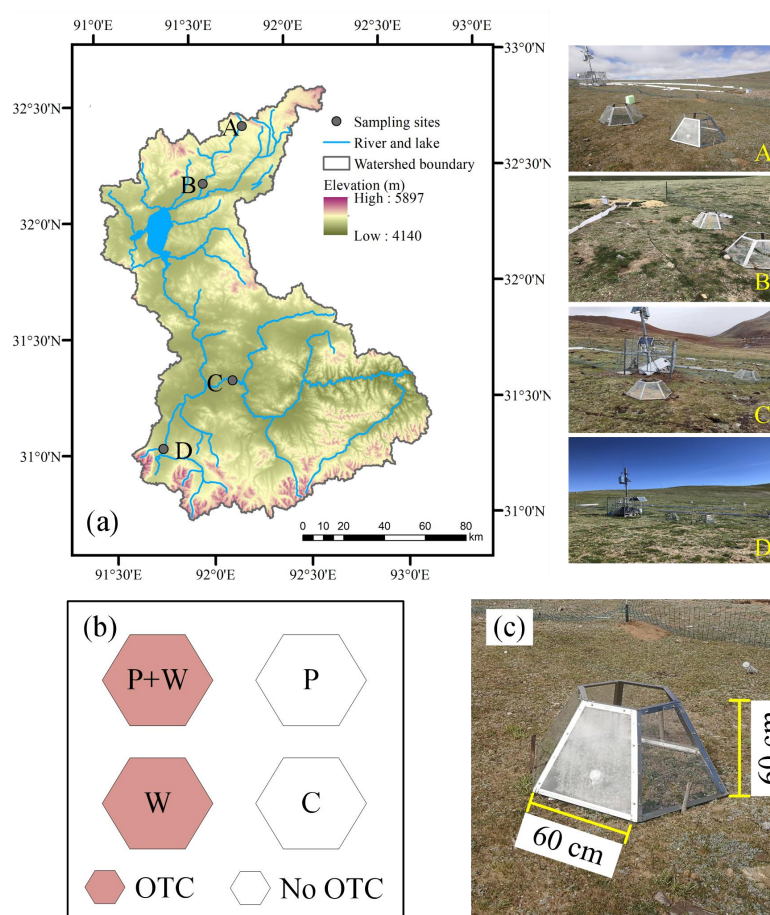
All effects are significant ($P < 0.1$).

Table 7 Standardized indirect effects of different scenarios and soil extracellular enzyme activities on converting

485   **different amino sugar components.**

Factors	Dep	W	XYL	BG	NAG	pH	ACP	CB
TAS	-0.046	-0.041	0.137	-0.05	0	\	\	\
GluN	-0.024	0	0.102	0.189	\	\	\	-0.143
GalN	0.035	0.001	-0.117	-0.031	-0.086	0.068	0	\
MurA	-0.067	0.049	0.039	0.06	0	\	\	\

All effects are significant (P < 0.1).

Figures

490   **Figure 1:** The (a) distribution of sampling points, (b) layout of the experimental site, and (c) size of the open-top chamber device. Note:

OTC: open-top chamber, P: increased precipitation, W: warming, C control group.

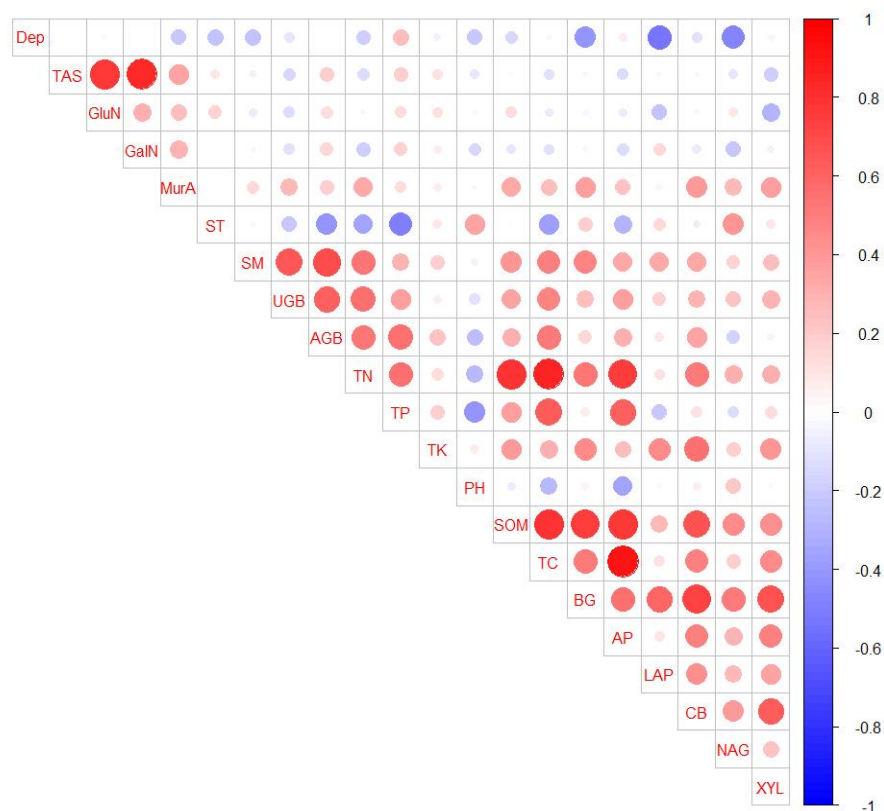



Figure 2: Correlation of soil physical properties, chemical properties, enzyme activities, and amino sugars. Note: Dep, Different Depth;

495   TAS, Total Amino Sugar; GluN, Glucosamine; GalN, Galactosamine; MurA, Muramic Acid; ST, Soil Temperature; SM, Soil Moisture;

UGB, Underground biomass; AGB, Aboveground Biomass; TN, Total Nitrogen; TP, Total Phosphorus; TK, Total Potassium; SOM, Soil

Organic Matter; TC, Total Carbon; BG, β-Glucosidase; AP, Acid Phosphatase; LAP, Leucine Aminopeptidase; CB, β-D-cellobiosidase;

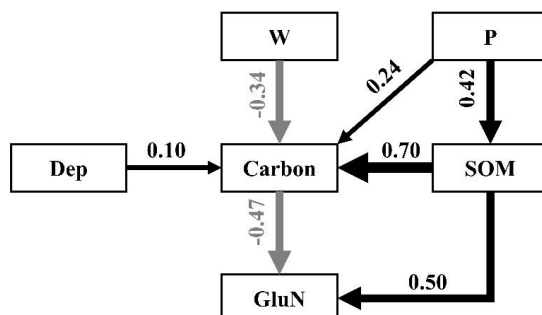
NAG, β-N-acetylglucosaminidase; XYL, β-xylosidase.

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Figure 3: Structural equation model of the turnover rate of GluN and soil physicochemical properties in different scenarios. An arrow

represents a causal relationship ($p < 0.1$). Arrow direction indicates the direction of effect. Arrow width indicates effect size. A black

arrow denotes a positive relationship, and a grey arrow a negative relationship. Numbers beside arrows are standardized path coefficients.

CMIN/DF = 0.575, CFI = 1.000, GFI = 0.959, AGFI = 0.893, RMSEA = 0.000, PCLOSE = 0.837. Note: Dep, Different Depth; GluN,

505   Glucosamine; SOM, Soil Organic Matter; W, Warming; P, increased precipitation.
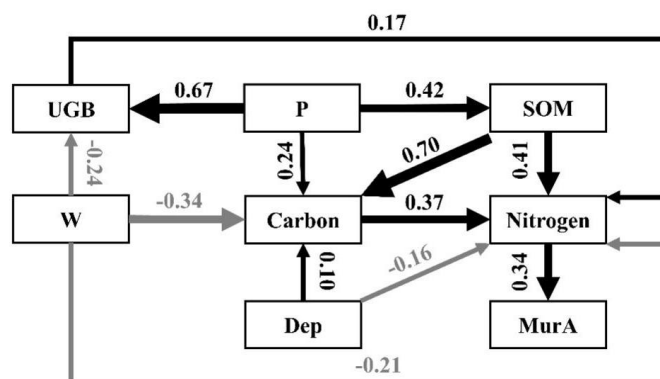
Figure 4: Structural equation model of the turnover rate of MurA and soil physicochemical properties in different scenarios. An arrow represents a causal relationship (p < 0.1). Arrow direction indicates the direction of effect. Arrow width indicates effect size. A black arrow denotes a positive relationship, and a grey arrow a negative relationship. Numbers beside arrows are standardized path coefficients. CMIN/DF = 0.561, CFI = 1.000, GFI = 0.943, AGFI = 0.864, RMSEA = 0.000, PCLOSE = 0.934. Note: Dep, Different Depth; SOM, Soil Organic Matter; W, Warming; P, increased precipitation; UGB, Underground biomass; MurA, Muramic Acid.
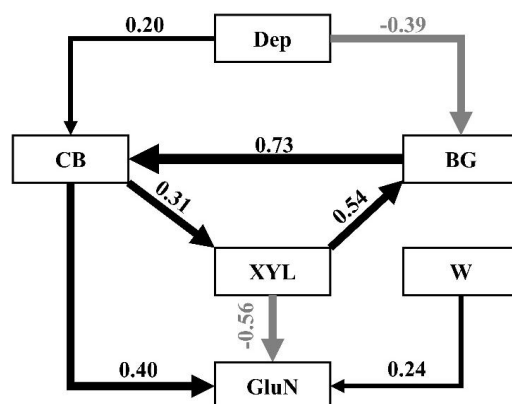
Figure 5: Structural equation model of GluN turnover rate and soil extracellular enzyme activities in different scenarios. An arrow represents a causal relationship (p < 0.1). Arrow direction indicates the direction of effect. Arrow width indicates effect size. A black arrow denotes a positive relationship, and a grey arrow a negative relationship. Numbers beside arrows are standardized path coefficients. CMIN/DF = 0.760, CFI = 1.000, GFI = 0.954, AGFI = 0.863, RMSEA = 0.000, PCLOSE = 0.674. Note: Dep, Different Depth; W, Warming; CB, β-D-cellobiosidase; BG, β-Glucosidase; GluN, Glucosamine; XYL, β-xylosidase.
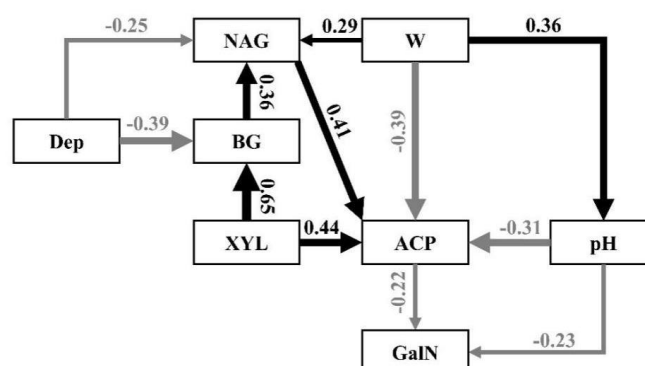
Figure 6: Structural equation model of GalN turnover rate and soil extracellular enzyme activities in different scenarios. An arrow

represents a causal relationship (p < 0.1). Arrow direction indicates the direction of effect. Arrow width indicates effect size. A black

arrow denotes a positive relationship, and a grey arrow a negative relationship. Numbers beside arrows are standardized path coefficients.

525   CMIN/DF = 0.841, CFI = 1.000, GFI = 0.920, AGFI = 0.819, RMSEA = 0.000, PCLOSE = 0.719. Note: Dep, Different Depth; W,

Warming; NAG, β-N-acetylglucosaminidase; GalN, Galactosamine; XYL, β-xylosidase; BG, β-Glucosidase; ACP, Acid Phosph.