Increased precipitation accelerates soil organic matter turnover associated with microbial community composition in topsoil of the alpine grassland on the eastern Tibetan Plateau

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Increased precipitation accelerates soil organic matter turnover associated with microbial community composition in topsoil of the alpine grassland on the eastern Tibetan Plateau

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Abstract

Large quantities of carbon are stored in the alpine grassland of the Tibetan Plateau (TP), where is extremely sensitive to climate change. However, it remains unclear whether soil organic matter (SOM) in different layers responds to climate change analogously, and whether microbial communities play vital roles in SOM turnover of topsoil. In this study we measured and collected SOM turnover by ^{14}C method in the alpine grassland to test climatic effects on SOM turnover in soil profiles. Edaphic properties and microbial communities in the northwestern Qinghai Lake were investigated to explore microbial influence on SOM turnover. SOM turnover in surface soil (0-10 cm) was more sensitive than that in subsurface layers (10-40 cm) to precipitation. Precipitation also imposed stronger effects on the composition of microbial communities in surface layer than that in deeper soil. At the 5-10 cm depth, the SOM turnover rate was positively associated with the bacteria/fungi biomass ratio and the relative abundance of *Acidobacteria*, both of which related to precipitation. Partial correlation analysis suggested that increased precipitation could accelerate the SOM turnover rate in topsoil by structuring soil microbial communities. Conversely, carbon stored in deep soil would be barely affected by climate change. Our results provide valuable insights into the dynamic and storage of SOM in alpine grasslands under future climate scenarios.

Key words: alpine grassland, soil profile, SOM turnover, precipitation, microbial community

Introduction

The turnover of soil organic matter (SOM) is a critical parameter indicating carbon segregation, stabilization, and dynamics (Schmidt et al. 2011). Whether SOM turnover in soil profiles is affected by climate conditions and how microbial communities mediate SOM turnover under changing climates remain unclear. Addressing these issues could help us understand factors driving SOM turnover and provide a sound basis for predicting carbon storage and dynamics under future climate scenarios.

SOM turnover rates usually decline with soil depth (Lawrence et al. 2015). In topsoil, climate conditions have proven to impact SOM turnover at a global scale across various terrestrial ecosystems (Chen et al. 2013; Zhang et al. 2015a). However, the climatic dependence of SOM turnover is not consistent across all research studies. Jia et al. (2016) noted that SOM turnover was independent of temperature in the 400 mm isopleth of mean annual precipitation in China. In addition, Epstein et al. (2002) found an insignificant relationship between SOM turnover and precipitation in topsoil in the Great Plains of the United States. Therefore, the climatic influence on SOM turnover in surface soil is still unclear. In deeper soil, many researchers have identified the impact of fresh carbon input and physical protection on SOM turnover (Conant et al. 2011; Qiao et al. 2015). The relationship between climatic condition and SOM turnover in subsurface soil is rarely investigated. Although many efforts have been made to distinguish the driving factors of SOM in surface and subsurface soil in manual control experiments (Garcia-Pausas et al. 2008), field environments are far more complex than laboratory conditions. Considering the discrepancy of SOM turnover induced by variations in climate conditions under natural settings (Torn et al.

2008; Zhang et al. 2015a), the study of SOM turnover in soil under field conditions is an urgent issue since it could provide essential information regarding soil carbon dynamics and carbon stocks.

Climate change could affect the composition of microbial communities (Wang et al. 2014; Zhang et al. 2015c; Zhang et al. 2017). Soil microbes are the dominant decomposers in affecting SOM turnover due to their vital roles in decomposing organic matters (Bardgett et al. 2008). For example, Köster et al. (2014) found a strong correlation between SOM turnover rate and fungi biomass during the post-fire recovery of forest. Although some laboratory tests (Kemmitt et al. 2008) and mathematical models (Yoo et al. 2011) suggested that microbial communities were not momentous factors in mediating SOM turnover, microorganisms are highly recommended to be taken into account in soil carbon projections (Wieder et al. 2013). A recent study revealed that carbon decomposition dynamics were affected by both climate conditions and microbial communities in Korean pines forest (Zhou et al. 2015). A similar relationship is expected in the alpine grassland on the TP. The TP is unique in its high elevation and alpine climate conditions, with substantial amounts of carbon stored in alpine grassland (Ding et al. 2016). Substantial studies have reported SOM turnover influenced by land use (Tao et al. 2007), vegetation (Zhao et al. 2014), and waterlogging (Gao et al. 2015). However, further research is needed to investigate how the microorganisms mediate SOM turnover under changing climate in the alpine grassland of the TP.

We hypothesized that SOM turnover in different soil layers responds to climate conditions differently, and emerging climate change may alter SOM turnover by changing soil microbial communities involved in the process of organic matter decomposition. To verify our hypothesis, SOM turnover rate reflected by soil ^{14}C

activity were measured in this study and collected from published literatures to reveal climatic effects on SOM turnover in soil profiles in the alpine grassland of the eastern TP. Furthermore, the composition and biomass of microbial communities were measured to examine their functions in regulating SOM turnover in the surface soil, taking the alpine grassland of the northwestern Qinghai Lake as an example. Our study helps to shed light on the climatic and microbial effects on SOM turnover and prediction in carbon dynamics under varying climate conditions.

Materials and methods

Study sites

Soil profiles in the alpine grassland of the TP were sampled from four soil layers (0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm) in the northwest of Qinghai Lake (QHL) and three layers (0-10 cm, 10-20 cm and 20-30 cm) in Yushu prefecture (YS) and Qamdo county (QD) in winter, 2009 (Fig. 1, Table S1). Five to six soil cores were mixed as one sample, and two replicates from each site were collected. Soils from YS, QD and the 5-10 cm layer in the northwest of QHL, regarding the disturbance of sand storm to topsoil in Qinghai Lake basin (Pullen et al. 2011), were measured SOM turnover rate by ^{14}C method (Table S1). To suppress the impact of geographical distance on the composition of soil microbial communities (King et al. 2010), samples in the northwestern QHL were measured regarding microbial biomass and soil physico-chemical properties. Replicates were mixed as one sample in each soil layer for the study of microbial community composition in four soil layers at site Q5 and the 5-10 cm layer at other sites.

Although radiocarbon (^{14}C) was applied as a tracer of SOM turnover rate (Feng et al. 2016; He et al. 2016), the expensive cost of ^{14}C detection and the hostile

environment often lead to a limited number of studies regarding SOM turnover in the alpine grassland of the TP. A literature review indicates that six sites in the region have published ^{14}C data in 0-40 cm soil depths (Table S1), including three sites at Nagqu station (NAQ3, NAQ7 and NAQ12, Kaiser et al. 2008) and three sites at Haibei station (ASC I, ASC II and JLM, Tao et al. 2007) respectively. The lack of data implies an urgent need to research SOM turnover on the TP using ^{14}C , a method more accurate than laboratory incubation and ^{13}C methods (Feng et al. 2016). The SOM turnover rate at Nagqu station (Kaiser et al. 2008) was calculated from the pMC data according to formulae (1), (2) and (3) below.

The mean annual temperature (MAT) and mean annual precipitation (MAP) at all the study sites were extracted from the WorldClim database (Hijmans et al. 2005). These sites are distributed across a wide range of latitude and longitude (Table S1). The combined effects of Westerlies, Indian monsoon and East Asian monsoon contribute to the declining precipitation from southeast to northeast on the TP (Yao et al. 2013). The large ranges of precipitation (135-539 mm) and temperature (-3.95-4.78 °C, Table S1) were found in our study region, which provides an accessibility for investigating the climatic effects on SOM turnover.

Measurement of edaphic properties

Soil moisture was gravimetrically determined with drying at 105 °C for 12 hours (Fierer et al. 2003). Soil pH was detected with a ratio of soil to water 1: 2.5. Total nitrogen (TN) was detected using a modified Kjeldahl method (Bremner 1960). Total organic carbon (TOC) was measured using TOC analyzer (TOC-VCPH, Shimadzu, Japan). Concentrations of ions were determined by ion chromatography (ICS2500,

Dionex Corporation). Soil texture (clay/sand/silt percentage) was measured with a Microtrac S3500 Particle Size Analyzer.

Measurement of soil ^{14}C activity

Soil ^{14}C activity was measured according to a previous study (Marzaioli et al. 2010). Briefly, samples were dried at 60 °C first, treated with 1 M HCl, washed to neutral pH, dried at 105 °C, combusted at 800 °C under vacuum, and finally were synthesized to be C_6H_6 . The liquid C_6H_6 was measured using a Wallac 1220 Quantulus ultralow level liquid scintillation spectrometer to obtain ^{14}C activity data, which was reported as pMC or $\Delta^{14}\text{C}$ ($\Delta^{14}\text{C} = \text{pMC} - 1$, Komada et al. 2012).

Calculation of SOM turnover rate

SOM turnover rate could be calculated according to iterative formula as follows (Zhang et al. 2013).

$$\text{pMC}_s(1955) = m / (m + \lambda) \quad (1)$$

$$\text{pMC}_s(t) = \text{pMC}_s(t-1) - (m + \lambda) \text{pMC}_s(t-1) + m \text{pMC}_0(t) \quad (2)$$

The pMC_s and pMC_0 stand for the pMC in soil and atmosphere respectively. The λ value is ^{14}C decay constant ($1/8268 \text{ yr}^{-1}$) and m is SOM turnover rate (yr^{-1}). Values of $\text{pMC}_0(t)$ in 1955-1958 and 1959-2003 were obtained from previous studies (Hua and Barbetti 2004; Levin and Kromer 2004). The $\text{pMC}_0(t)$ in 2004-2010 were calculated based on the formula established by Levin and Kromer (1997). We firstly assigned values to m and $\text{pMC}_s(1955)$ respectively in equation (1), and then put them into equation (2) and finally we got $\text{pMC}_s(2010)$ by interactive method. The m was changed with a precision of 0.0001 yr^{-1} until the final pMCs (2010) value was approximate or equal to the measured value, and thus we got the final SOM turnover rates.

If soil $\Delta^{14}\text{C} < 0$, SOM turnover rate was calculated according to Trumbore et al. (1996) as following,

$$m = -\lambda (1000 / \Delta^{14}\text{C} + 1) \quad (3)$$

where m is SOM turnover rate (yr^{-1}) and λ is ^{14}C decay constant ($1/8268 \text{ yr}^{-1}$).

Microbial community analysis

The microbial phospholipid fatty acid (PLFA) method was used to measure microbial biomass using a modified Bligh-Dyer method (Bligh and Dyer 1959). PLFAs were analyzed with an Agilent (6890/5973) GC-MS system. Microbial biomass was calculated according to PLFAs in each type of microorganism based on previous studies (Frostegård et al. 1993).

Microbial community composition was investigated based on the 16S rRNA gene clone library method (Lin et al. 2016a). Microbial genomic DNA was extracted from fresh soils using MP FastDNA[®] Spin Kits (MP Laboratories, USA) according to the manufacturer's instructions. Bacterial 16S rRNA genes were amplified with the primer pairs Bac27F/1492R (Lane 1991). Amplified genes were inserted into pGEM-T vectors (Promega Inc., Madison, WI), and the vectors were transformed into *E. coli* DH5 α competent cells. Plasmids carrying inserted 16S rRNA genes were sequenced with an ABI 3100 sequencer (Applied Biosystems, Warrington, UK). Sequences obtained were manually verified with Sequencher 4.5 software (Gene Codes, Ann Arbor, MI) and checked with the Bellerophon program (Huber et al. 2004). All qualified sequences were classified in RDP (<http://rdp.cme.msu.edu/>) and divided into OTUs using 97% similarity.

Data analysis

Correlations among the SOM turnover rate, environmental factors, and microbial communities were analyzed in SPSS 19.0 (IBM Corporation, Armonk, NY, USA). SOM turnover rates were grouped into three soil layers (0-10 cm, 10-20 cm and 20-40 cm respectively) based on the average sampling depths through Turkey HSD test in SPSS 19.0. Forward selection redundancy analysis (RDA) was used to estimate the environmental influence on the microbial communities in R software (version 2.12.1, <https://www.r-project.org/>). Variance inflation factors (VIFs) were controlled to be lower than 20 to decrease autocorrelations among factors in RDA, and significant values were determined using Monte Carlo permutation tests (Zhang et al. 2015b). Partial correlation was calculated in 19.0 SPSS to distinguish the impact of microbes on SOM turnover from climatic influence.

Nucleotide sequence accession numbers

All sequences detected in our study have been deposited into the GenBank database (<http://www.ncbi.nlm.nih.gov/Genbank/submit.html>) under accession numbers KU370502-KU371826.

Results

Climatic effects on SOM turnover in soil profiles

Across all sites in Table S1, the SOM turnover rate declined exponentially with soil depth ($R^2 = 0.58$, $P < 0.001$, Fig. 2a), ranging from approximately 0.02 yr^{-1} at the 0-2 cm depth to nearly 0.0003 yr^{-1} at the 35-40 cm depth. SOM turnover rates at various sampling depths were divided into three groups, including 0-10 cm, 10-20 cm and 20-40 cm based on ANOVA analysis (Turkey HSD method, Fig. 2b). The SOM turnover rate at the 0-10 cm depth was significantly higher than that at the 10-20 cm

and 20-40 cm depths (Fig. 2b). Below the 20 cm depth, the SOM turnover rate gradually decreased from 0.000294 yr^{-1} to 0.000985 yr^{-1} . MAP exclusively impacted the SOM turnover rates at the 0-10 cm soil depth ($r = 0.45$, $P = 0.03$, Table 1), whereas MAT had an indistinct effect on the SOM turnover rate within the 0-40 cm soil depth in our study area ($P > 0.05$, Table 1).

Factors influencing soil microbial communities

At the 0-10 cm soil depth, the biomass of bacteria and *Actinomycetes*, and the biomass ratio of bacteria to fungi (bacteria/fungi) greatly were correlated with MAP ($P < 0.05$, Table 2). At the 10-20 cm depth, the bacterial biomass, bacteria/fungi and the biomass ratio of G+/G- (G+/G-) were significantly affected by MAT, followed by MAP, TOC, TN and soil moisture ($P < 0.05$). However, at the deeper depths (20-40 cm), concentrations of saline ions greatly impacted soil microbial biomass. Therefore, MAP was a major driver controlling soil microbial biomass with a lesser role at deeper soil depths.

MAP showed the greatest influence ($F = 7.36$, $P = 0.002$) on microbial community composition based on the clone library of 16S rRNA genes (Fig. S1) according to RDA (Fig. 3), followed by NO_3^- ($F = 4.81$, $P = 0.008$), soil moisture ($F = 3.99$, $P = 0.026$), and concentrations of Cl^- and SO_4^{2-} ($\text{Cl}^- + \text{SO}_4^{2-}$, $F = 3.79$, $P = 0.023$). This result suggested the importance of water availability in structuring the microbial community in this arid/semiarid area.

Relationships among climate, microbes, and SOM turnover

The complex relationships among climate conditions, SOM turnover rates and microbial community composition at 5-10 cm soil depth in the northwest of QHL (Table 3) were found. The SOM turnover rate was significantly sensitive to MAP ($P <$

0.05, Table 3). In addition, SOM turnover rates were evidently correlated to some taxa of bacteria, including *Acidobacteria* ($r = 0.89$, $P < 0.05$) and its divisions *Gp1*, *Gp4* and *Gp6* ($P < 0.05$), and bacteria/fungi ($r = 0.82$, $P < 0.05$, Table 3). According to the partial correlation, most of the climatic effects on the SOM turnover rate in topsoil relied on the transformation of soil microbial community composition, such as the abundance of *Acidobacteria* ($r = 0.77$, $P < 0.05$) and bacteria/fungi ($r = 0.69$, $P < 0.05$, Table S2).

Discussion

There are three main methods commonly applied to estimate SOM turnover rate (Feng et al. 2016). The laboratory-based incubation method measures SOM turnover rate through the detection of soil respiration and decomposition rates of SOM under anthropogenic environment, which clearly deviates from natural ecosystems (Wei et al. 2017). The ^{13}C method is based on the natural difference of ^{13}C isotope during photosynthesis in the C3-C4 plant switch process (Hafner et al. 2012; Yang et al. 2015), which restricts its applications in cases of vegetation succession. In contrast, the ^{14}C bomb method is more accurate than the two methods mentioned above. This is mainly because it explicitly takes the annual atmospheric ^{14}C into account (Feng et al. 2016). Although great efforts have been made to examine SOM turnover rate using ^{14}C method in the alpine grassland of the TP (Wang et al. 2005; Tao et al. 2007; Kaiser et al. 2008; Tian et al. 2009), to the best of our knowledge, this study is the first to link SOM turnover rate in soil profiles based on ^{14}C method with climatic factors and microbial community composition in the alpine grassland of the TP.

In the soil profiles, SOM turnover rate decreased by approximately 7-15 times from the 0-5 cm to 30-40 cm soil depths in the alpine grassland (Fig. 2). This was

supported by studies on evergreen broadleaf forest soils (Ding et al. 2010) and alpine pasture (Budge et al. 2011). Our results confirmed that carbon cycles were faster in surface soil than in deeper soil of the TP (Li et al. 2004).

The fast SOM turnover rate in surface soil could be attributed to the priming effect of plant litter input, climate conditions, and microbial functions (Conant et al. 2011). In our study area, the SOM turnover rates at the 0-10 cm soil depth were greatly influenced by precipitation, rather than temperature (Table 1). This implies that precipitation overrode temperature in mediating soil organic pools in alpine grassland on the TP (Lin et al. 2016b). Although the sensitivity of SOM turnover to temperature was found in alpine grassland of Swiss Alps (Leifeld et al. 2009), a large difference in precipitation between the Swiss Alps (578-1230 mm) and our study area (115-580 mm) may explain the precipitation sensitivity of SOM turnover in our study. In addition, the faster SOM turnover rate in the Gongga Mountains (Wang et al. 2005), where is much colder with higher level of moisture than our study area, also indicates the importance of precipitation in mediating SOM turnover in arid/semiarid regions. Therefore, the response of SOM turnover to temperature could be constrained by limited amounts of precipitation in the arid/semiarid area (Epstein et al. 2002).

Climate effects on SOM turnover were not found at the 10-40 cm soil depth (Table 1), suggesting that surface soil was more sensitive to climate change than subsurface soil (Davidson and Janssens 2006). This result verified the inference from a global SOM turnover model for terrestrial ecosystems (Chen et al. 2013), which showed a strong correlation between climate conditions and SOM turnover in topsoil. In subsurface soil, soil texture, physical protection, carbon quality and fresh carbon supply may have a larger effect on SOM turnover than climate factors (Conant et al. 2011). In addition, the large amount of evapotranspiration (239-271 mm, 38°25'N,

98°19'E, Wu et al. 2015) may prevent precipitation (135-539 mm) from permeating into deep soils, which may also explain the insensitivity of SOM turnover to climate conditions in the deeper soil layers of our study (Table 1).

A better understanding of microbial ecology is crucial to assess terrestrial carbon-climate feedbacks. According to the partial correlation analysis (Table S2), it was inferred that shifts in microbial community composition were the primary mechanisms by which precipitation impacted the SOM turnover in topsoil. This result could be explained by the vital roles of microbial physiology in mediating responses of soil carbon to climate change (Allison et al. 2010). Furthermore, our study affirmed the necessity of taking microbial processes into consideration when predicting soil carbon dynamics in carbon-climate models (Wieder et al. 2013). The aggregation and sorption of mineral surfaces could also affect SOM turnover (Budge et al. 2010). However, as a constituent of mineral soil (Grandy et al. 2009), clay particles only accounted for a small proportion of 1.79% on average due to the weak weathering under the cold-dry climatic conditions in northwestern QHL (Wang et al. 2015). Therefore, the physical protection provided by the clay fractions might be a weak factor influencing SOM turnover in this area. In addition, soil pH was shown to affect SOM turnover (Leifeld et al. 2013). Nevertheless, soil pH showed a weak relationship with SOM turnover in our results ($r = -0.55$, $P = 0.063$, Table 3). These irrelevant phenomena highlighted the important influence of precipitation and microbial communities on SOM turnover in our study area.

Although temperature and precipitation were both likely the main determination of soil microbial community on the whole TP (Chen et al. 2016), precipitation was the primary driver of bacterial biomass and bacteria/fungi biomass ratio on the northeastern TP (Table 2) where is arid and semiarid (Qin et al. 2015). This result was

consistent with findings on topsoil (0-20 cm) of the Mongolian Plateau (Chen et al. 2015a, 2015b) which was adjacent to the TP. In addition, the crucial roles of precipitation were also verified in structuring microbial community in artificial experiments (Zhang et al. 2016). These findings together could give sufficient supports for the microbial distribution patterns driven by precipitation on the northeastern TP (Table 2, Fig. 3). Increasing precipitation could positively influence vegetation types and ecosystem production (Piao et al. 2012), which could bring in much more fresh carbon input and thus accelerate SOM turnover rate through the priming effect (Rousk et al. 2015). Moreover, precipitation could mediate SOM turnover by altering soil microbial community biomass and composition (Bardgett et al. 2008; Zhang et al. 2016). Consequently, the bacteria/fungi ratios were found to be significantly related to both precipitation and the SOM turnover rates (Table 2, Table 3). This suggested that different microbial communities had specific capabilities in mineralizing organic carbon. Despite the effects of bacteria/fungi on SOM turnover were challenged in a manual experiment in a temperate forest by Rousk and Frey (2015), the input and removal of plant carbon in the study may cover up the relative importance of bacteria and fungi in carbon turnover. Moreover, another artificial experiment controlling levels of carbon addition recently found a significant correlation between bacteria/fungi and soil carbon storage (Malik et al. 2016), which confirmed the impact of bacteria/fungi on SOM turnover in our study. This phenomenon could also be due to the physiological and ecological difference between bacteria and fungi. First, bacteria (0.15-4 μm) are usually smaller than fungi (3-8 μm) in size (Wallstedt et al. 2011), which allows bacteria to diffuse and obtain organic matters more easily. Second, fungal products are more chemically resistant to decomposition than that of bacteria (Jastrow et al. 2007). Third, bacterial membranes

primarily consist of labile decomposition molecules such as phospholipids. Comparably, melanin and chitin on fungal cell walls could be retained for a longer time than phospholipids (Guggenberger et al. 1999). Therefore, a large bacteria/fungi ratio could help to explain the accelerating of SOM turnover rate.

Considering the functional differences of microbial species, microbial species involved in carbon cycling process may also contribute more to SOM turnover than irrelevant microbes. In our study, we found that the abundance of *Acidobacteria* and its subdivisions *Gp1*, *Gp4*, *Gp6* showed significant correlations with SOM turnover rate (Table 3), which could be explained by the substantial decomposition capacity of *Acidobacteria* (Schneider et al. 2012). There are plentiful functional genes relating to carbon degradation and organic remediation in *Acidobacteria*, such as *cda*, *linB*, *hmgA* and *AceA* (Rawat et al. 2014). Furthermore, *Acidobacteria* can survive in a wide range of carbon sources including labile and recalcitrant carbon (de Castro et al. 2013), thus they contribute much more to SOM turnover than other kinds of bacteria. Consequently, increases in *Acidobacteria* proportions in microbial communities could accelerate carbon turnover rate through their potential carbon decomposition ability. Therefore, the major roles of microbial communities in SOM turnover could be confirmed (Table S2). Despite an insignificant correlation of precipitation with SOM turnover rate was drawn from partial correlation (Table S2), the direct influence of precipitation could not be excluded absolutely due to lack in measurement of physical protection and other important abiotic variables.

Conclusion

The relationship between climatic conditions, microbial community composition and SOM turnover in soil profiles based on the ^{14}C method was examined for the first

time in the alpine grassland of the eastern TP. The SOM turnover in topsoil (0-10 cm) was sensitive to precipitation rather than temperature. In subsurface layers (10-40 cm), SOM turnover rate showed insignificant correlation with climate conditions. This result indicated the importance of precipitation as a limiting factor in arid/semiarid regions and the vulnerability of carbon in surface soil. Furthermore, the SOM turnover rate was related to microbial community composition. In the context of climate change, elevated precipitation may cause a faster carbon cycling by altering the microbial community composition in the topsoil of alpine grasslands. However, SOM turnover was a complex process controlled by various biotic and abiotic factors. Further research is required to address the combined impacts of abiotic factors, such as physical protection and substrate quality, and biotic factors on SOM turnover using more advance techniques with larger sampling size to better understand the SOM dynamics on the TP.

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Tables

Table 1 Relationship among SOM turnover rates and climate factors in alpine grassland on the Tibetan Plateau based on Spearman correlation.

Variables	Spearman	Soil depth (cm)		
	correlation	0-10	10-20	20-40
	<i>r</i>	0.40	-0.01	-0.20
MAP	<i>P</i>	0.048	0.964	0.341
	<i>N</i>	25	21	24
	<i>r</i>	-0.34	-0.05	0.14
MAT	<i>P</i>	0.092	0.824	0.505
	<i>N</i>	25	21	24

Abbreviations: SOM: soil organic matter; MAP, mean annual precipitation; MAT, mean annual temperature. *N* means sample size.

Note: All study sites in Table S1 were analyzed. Sites with an average sampling depth within 0-10 cm (10-20 cm or 20-40 cm) would be grouped into “0-10 cm” group (“10-20 cm” or “20-40 cm” group) based on the ANOVA analysis.

Table 2 Pearson correlation between microbial biomass and environmental variables in four soil layers in the northwest of Qinghai Lake on the Tibetan Plateau.

Variables	Soil depth (cm)	Bacteria	Fungi	G+	G-	Actinomycete	bacteria/fungi	G+/G-
MAP	0-5	0.89***	0.04	0.82**	0.80**	0.75**	0.81**	-0.30
	5-10	0.94***	-0.25	-0.02	0.97***	0.78**	0.84**	-0.44
	10-20	0.70*	-0.34	0.68*	0.57	0.42	0.65*	-0.56
	20-40	0.04	-0.4	0.22	0.11	-0.23	0.52	0.15
MAT	0-5	-0.57	0.03	-0.23	-0.53	-0.44	-0.44	0.68*
	5-10	-0.55	0.22	0.26	-0.72**	-0.47	-0.37	0.57
	10-20	-0.90**	0.43	-0.86**	-0.81**	-0.81**	-0.89**	0.70*
	20-40	0.01	0.50	-0.01	-0.03	0.32	-0.32	0.26
TOC	0-5	0.12	-0.32	-0.16	0.06	0.18	0.23	-0.41
	5-10	0.11	-0.04	-0.17	0.32	0.21	0.06	-0.3
	10-20	0.67*	-0.25	0.63*	0.61*	0.69*	0.70*	-0.54

TN	20-40	-0.16	-0.32	-0.14	-0.15	-0.26	0.12	-0.22
	0-5	0.14	-0.24	-0.15	0.09	0.19	0.18	-0.38
	5-10	-0.03	0.17	-0.06	0.17	0.15	-0.15	-0.15
	10-20	0.69*	-0.29	0.65*	0.62*	0.73**	0.66*	-0.5
C/N	20-40	-0.19	-0.21	-0.21	-0.19	-0.25	0.05	-0.32
	0-5	-0.08	-0.38	-0.02	-0.11	0.13	0.19	0.04
	5-10	0.06	-0.45	-0.46	-0.04	-0.15	0.28	-0.3
	10-20	-0.19	0.42	-0.35	-0.19	-0.27	-0.18	-0.21
Moisture	20-40	-0.38	-0.42	0.01	-0.27	-0.33	-0.13	0.83**
	0-5	0.3	0.31	-0.04	0.36	0.02	-0.16	-0.38
	5-10	0.26	0.38	0.06	0.37	0.34	-0.14	-0.17
	10-20	0.88**	-0.11	0.86**	0.89**	0.94**	0.85**	-0.59*
pH	20-40	-0.33	-0.16	-0.4	-0.27	-0.60*	-0.09	-0.45
	0-5	-0.08	0.36	-0.05	0.04	-0.04	-0.38	-0.11
	5-10	-0.06	-0.1	-0.43	-0.31	-0.2	-0.25	-0.25
	10-20	-0.03	0.48	-0.18	0.03	-0.1	-0.13	-0.26
Sand	20-40	-0.34	-0.49	0.03	-0.17	-0.51	-0.06	0.64*
	0-5	-0.62*	-0.2	-0.39	-0.42	-0.28	-0.27	-0.05
	5-10	-0.64*	-0.37	-0.51	-0.58*	-0.55	-0.23	-0.14
	10-20	-0.73*	0.47	-0.75**	-0.58	-0.64*	-0.72*	0.14
Cl ⁻	20-40	0.17	-0.44	0.46	0.28	0.12	0.14	0.77**
	0-5	-0.44	0.26	-0.28	-0.35	-0.25	-0.47	0.26
	5-10	-0.54	0.16	0.29	-0.56	-0.35	-0.43	0.45
	10-20	-0.59*	0.16	-0.46	-0.48	-0.36	-0.59*	0.56
NO ₃ ⁻	20-40	0.41	0.61*	0.06	0.33	0.61*	-0.17	-0.47
	0-5	-0.35	0.09	-0.03	-0.35	-0.23	-0.27	0.89**
	5-10	-0.41	0.74**	0.86**	-0.4	-0.03	-0.29	0.90**
	10-20	-0.48	0.19	-0.31	-0.39	-0.32	-0.45	0.44
SO ₄ ²⁻	20-40	-0.25	0.71**	-0.4	-0.37	0.14	-0.45	-0.13
	0-5	-0.54	0.12	-0.29	-0.49	-0.27	-0.44	0.59*
	5-10	-0.5	0.3	0.4	-0.53	-0.28	-0.45	0.52
	10-20	-0.51	0.14	-0.39	-0.41	-0.29	-0.51	0.44
	20-40	0.66*	0.22	0.37	0.66*	0.62*	0.1	-0.41

Note: Data in table was r value calculated from Pearson correlation.

* $P < 0.05$, ** $P < 0.01$. Abbr.: MAP, mean annual precipitation; MAT, mean annual temperature; TOC, total organic carbon; TN, total nitrogen. Bacteria/fungi: the biomass ratio of bacteria to fungi. G+: gram positive bacteria. G-: gram negative bacteria. G+/G-: the biomass ratio of gram-positive bacteria to gram-negative bacteria.

Table 3 Pearson correlations between SOM turnover rates and variables in environment, soil bacterial community composition and soil microbial biomass at 5-10 cm soil depth in the northwest of Qinghai Lake in alpine grassland on the Tibetan Plateau.

Environmental variables		Bacteria community composition	
MAP	0.67 [*]	<i>Proteobacteria</i>	-0.78
MAT	-0.46	<i>Acidobacteria</i>	0.89 [*]
TOC	0.36	<i>Actinobacteria</i>	0.68
TN	0.16	<i>Gemmatimonadetes</i>	-0.21
C/N	0.19	<i>Verrucomicrobia</i>	0.5
Moisture	-0.06	<i>Firmicutes</i>	-0.4
pH	-0.55	<i>Planctomycetes</i>	0.36
Clay	-0.24	<i>Acidobacteria_Gp1</i>	0.86 [*]
Cl ⁻	-0.37	<i>Acidobacteria_Gp4</i>	0.96 [*]
NO ₃ ⁻	-0.11	<i>Acidobacteria_Gp6</i>	0.93 [*]
SO ₄ ²⁻	-0.41		
Microbial biomass			
Bacteria	0.49	Actinomycete	0.64
Fungi	-0.45	Bacteria/Fungi	0.82 [*]
G+	0.07	G+/G-	-0.37
G-	0.65		

Note: Data shown in table was r value from Pearson correlation test. ^{*} P < 0.05.

Abbreviation: G+, gram-positive bacteria; G-, gram-negative bacteria; MAP, mean annual precipitation; MAT, mean annual temperature; TOC, total organic carbon; TN, total nitrogen.

Figure captions

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Supplements

Table S1 Information of study sites, including geographic location, altitude, climate conditions, vegetation, soil ^{14}C activity and SOM turnover rate.

Study sites	Sites ID	Longitude	Latitude	Altitude (m)	MAP [†] (mm)	MAT [†] (°C)	Vegetation	Sampling depth (cm)	pMC (%)	$\Delta^{14}\text{C}$ (‰)	SOM turnover rate (yr ⁻¹)	Data source
Nagqu station	NAQ3	92.63°E	31.765°N	4572	436	-1.5	Kobresia pygmaea	16-18	79.11	-208.9	0.000471	Kaiser et al. 2008
	NAQ7	93.78°E	31.86°N	4484	539	0.5		13-15	91.39	-86.1	0.001321	
	NAQ22	92.06°E	30.32°N	4446	334	0.9		13-15	95.2	-48	0.002469	
Haibei station	ASC I	101.19°E	37.37°N	3220	479	-3.95	Kobresia humilis	0-2	114.57	145.7	0.021277	Tao et al. 2007
								2-4	115.08	150.76	0.022222	
								4-6	107.75	77.53	0.009804	
								6-8	98.41	-15.91	0.005988	
								8-10	89.95	-100.51	0.001114	
								10-12	86.79	-132.07	0.000784	
								12-14	91.21	-87.88	0.001264	
								14-16	88.31	-116.92	0.000925	
								16-18	86.92	-130.81	0.000827	
								18-20	83.38	-166.16	0.000624	
								20-22	86.79	-132.07	0.000796	
								22-24	84.65	-153.54	0.000683	
								24-26	86.04	-139.65	0.000758	
								26-28	84.14	-158.59	0.000659	
								28-30	80.1	-198.99	0.000492	

ASC II	101.25°E	37.37°N	3230	484	-3.75	Kobresia humilis	30-35	79.22	-207.83	0.000476
							35-40	70.88	-291.16	0.000294
							0-2	111.07	110.69	0.013699
							2-4	113.84	138.36	0.018868
							4-6	113.59	135.85	0.018519
							6-8	111.32	113.21	0.014085
							8-10	105.16	51.57	0.006711
							10-12	97.86	-21.38	0.004525
							12-14	91.45	-85.53	0.001355
							14-16	94.21	-57.86	0.002024
							16-18	98.74	-12.58	0.007634
							18-20	94.59	-54.09	0.002101
							20-22	88.68	-113.21	0.000976
							22-24	86.54	-134.59	0.000802
							24-26	79.25	-207.55	0.000484
							26-28	82.14	-178.62	0.000564
							28-30	75.6	-244.03	0.000381
30-35	73.33	-266.67	0.000345							
35-40	70.31	-296.86	0.000296							
JLM	101.19°E	37.4°N	3352	475	-3.2	Dasiphora fruticosa shrub meadow	0-2	104.25	42.46	0.005848
							2-4	101.61	16.08	0.004184
							4-6	101.53	15.33	0.004065
							6-8	94.37	-56.28	0.002110
							8-10	95.65	-43.47	0.002591
							10-12	92.26	-77.39	0.001466
							12-14	89.55	-104.52	0.001070

								14-16	95.43	-45.73	0.002481	
								16-18	89.17	-108.29	0.001026	
								18-20	89.47	-105.28	0.001028	
								20-22	88.79	-112.06	0.000985	
								22-24	87.29	-127.14	0.000838	
								24-26	83.07	-169.35	0.000605	
								26-28	76.73	-232.66	0.000408	
								28-30	83.74	-162.56	0.000632	
								30-35	86.16	-138.44	0.000748	
								35-40	79.98	-200.25	0.000497	
The northwest of Qinghai Lake	Q2	100.55°E	37.15°N	3272	385	0.31	Alpine grassland	5-10	107.91	79.1	0.009001	This paper
	Q4	99.81°E	37.20°N	3199	313	0.37		5-10	98.92	-10.8	0.002662	
	Q5	98.87°E	37.18°N	3804	260	-2.33		5-10	104.78	47.8	0.005410	
	Q6	98.14°E	37.00°N	2955	170	4.38	Alpine shrub	5-10	102.15	21.5	0.004130	
	Q7	97.60°E	37.13°N	2861	175	4.56		5-10	100.2	2	0.003131	
Yushu prefecture	Q8	96.60°E	37.40°N	3002	135	3.18		5-10	100.77	7.7	0.003400	
		96.33°E	31.96°N	4235	369	-2.57	Alpine meadow	0-10	104.32	43.2	0.005617	
	YS	96.33°E	31.96°N	4235	369	-2.57		10-20	95.38	-46.2	0.001657	
		96.33°E	31.96°N	4235	369	-2.57		20-30	85.7	-143	0.000674	
		30.62°E	97.06°N	4320	524	1.82	Alpine steppe	0-10	92	-80	0.0011494	
Qamdo county	QD1	30.62°E	97.06°N	4320	524	1.82		10-20	87.42	-125.8	0.0007664	
		30.62°E	97.06°N	4320	524	1.82		20-30	68.58	-314.2	0.0002598	
		31.26°E	96.60°N	3809	538	4.78	Alpine meadow	0-10	109.24	92.4	0.0108768	
	QD2	31.26°E	96.60°N	3809	538	4.78		10-20	98.03	-19.7	0.0023208	
		31.26°E	96.60°N	3809	538	4.78		20-30	84.11	-158.9	0.0006028	

Abbr.: MAP, mean annual precipitation; MAT, mean annual temperature.

Note: * The $\Delta^{14}\text{C}$ and SOM turnover rate were calculated from pMC data shown by Kaiser et al. (2008). † MAP and MAT was extracted from WorlfClim Database according to Hijmans et al. (2005).

Draft

Table S2 Individual effects of precipitation and microbial community composition on SOM turnover rate examined by a partial correlation in 5-10 cm soil layer.

Control variables	Variables	SOM turnover rate	
		r	P
MAP	<i>Acidobacteria</i>	0.77	0.005
MAP	Bacteria/Fungi	0.69	0.017
<i>Acidobacteria</i>	MAP	-0.32	0.335
Bacteria/Fungi	MAP	-0.11	0.735

Abbreviation: MAP, mean annual precipitation.

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Supplement figure captions

Fig. S1 Bacterial community composition at the phylum level. The phylum *Proteobacteria* was represented by *Alpha*-, *Beta*-, *Gamma*- and *Delta*- divisions.

Draft

Fig. 1 Locations of study sites on the Tibetan Plateau. Information of these sites was shown in Table 1. Three points (ASC I, ASC II and JLM) in Haihei station were too close to each other and were shown as one spot.

84x59mm (300 x 300 DPI)

Fig. 2 Turnover rates of soil organic matter (SOM) decreased exponentially with soil depths in alpine grassland on the Tibetan Plateau (A) with the highest turnover rates in 0-10 cm depth than 10-20 and 20-40 cm depths (B).

114x89mm (300 x 300 DPI)

Fig. 3 Relationship between environmental factors and soil bacterial community composition at class level based on the forward selection redundancy analysis (RDA) in the northwest of Qinghai Lake basin. The model passed Monte Carlo permutation test ($p < 0.05$) and the significant variables were labeled by asterisk ($p < 0.05^*$, $p < 0.01^{**}$). Concentrations of Cl^- and SO_4^{2-} were summed up and shown as one variable ($\text{Cl}^- + \text{SO}_4^{2-}$) due to their similar properties.

129x116mm (300 x 300 DPI)

203x118mm (300 x 300 DPI)