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Key Points:
- Precipitation was the major driver of topsoil CO_2 release rate in alpine ecosystems over a broad geographic scale
- Basal microbial respiration rate (*B*) was the best predictor of Q_{10} variability in alpine steppe soils
- Soil pH outweighed *B* as the key regulator of Q_{10} variation in alpine meadow soils

Supporting Information:
- Supporting Information S1
- Table S4

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Linking temperature sensitivity of soil CO_2 release to substrate, environmental, and microbial properties across alpine ecosystems

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Abstract Our knowledge of fundamental drivers of the temperature sensitivity (Q_{10}) of soil carbon dioxide (CO_2) release is crucial for improving the predictability of soil carbon dynamics in Earth System Models. However, patterns and determinants of Q_{10} over a broad geographic scale are not fully understood, especially in alpine ecosystems. Here we addressed this issue by incubating surface soils (0–10 cm) obtained from 156 sites across Tibetan alpine grasslands. Q_{10} was estimated from the dynamics of the soil CO_2 release rate under varying temperatures of 5–25°C. Structure equation modeling was performed to evaluate the relative importance of substrate, environmental, and microbial properties in regulating the soil CO_2 release rate and Q_{10}. Our results indicated that steppe soils had significantly lower CO_2 release rates but higher Q_{10} than meadow soils. The combination of substrate properties and environmental variables could predict 52% of the variation in soil CO_2 release rate across all grassland sites and explained 37% and 58% of the variation in Q_{10} across the steppe and meadow sites, respectively. Of these, precipitation was the best predictor of soil CO_2 release rate. Basal microbial respiration rate (*B*) was the most important predictor of Q_{10} in steppe soils, whereas soil pH outweighed *B* as the major regulator in meadow soils. These results demonstrate that carbon quality and environmental variables coregulate Q_{10} across alpine ecosystems, implying that modelers can rely on the "carbon-quality temperature" hypothesis for estimating apparent temperature sensitivities, but relevant environmental factors, especially soil pH, should be considered in higher-productivity alpine regions.

1. Introduction

As the largest pool of terrestrial organic carbon (C), soil stores at least three times as much C as the atmosphere [*Carvalhais et al*., 2014]. Even slight changes in the soil C stock may induce fluctuations in atmospheric carbon dioxide (CO_2) concentration, consequently resulting in dramatic climate change [*Heimann and Reichstein*, 2008]. It is well known that microbes utilize soil organic matter as both energy and nutrient sources and consequently transfer C to the atmosphere as CO_2, constituting an important component of the terrestrial C cycle [*Chapin et al*., 2011]. This process, to a large degree, may determine the direction and magnitude of C-climate feedback [*Davidson and Janssens*, 2006]. However, due to the large spatial heterogeneity, distinct molecular fractions, and the different inherent decomposability of soil C, there are still considerable uncertainties regarding the fate of soil C under the background of continuous climate warming [*Schmidt et al*., 2011]. In particular, large uncertainties in model predictions could be partly attributed to the inadequate parameterization of the drivers of soil CO_2 release and its temperature sensitivity (i.e., commonly described by Q_{10}, a factor by which CO_2 release increases for every 10°C increase in temperature) in Earth System Models. Therefore, our knowledge of the basic patterns and fundamental drivers of soil CO_2 release rate and its temperature sensitivity is crucial for improving the predictability of soil C dynamics in Earth System Models and for deepening the understanding of terrestrial C-climate feedback under a continuing warming scenario [*Schuur et al*., 2015].

During the past several decades, considerable efforts have been made to depict the spatial patterns and driving factors of soil CO_2 release rate at a regional scale [*Colman and Schimel*, 2013; *Doetterl et al*., 2015]. Previous

studies illustrated that the soil CO_2 release rate was closely associated with many factors, including substrate [*Franzluebbers et al*., 2001], environmental [*Doetterl et al*., 2015; *Schimel et al*., 1994], and microbial properties [*Colman and Schimel*, 2013; *Whitaker et al*., 2014]. However, these factors may interact with each other, making the relative importance of these driving factors still unclear. Based on large-scale soil incubation experiments, microbial biomass was observed to be the most important factor that shaped the spatial variability of the soil CO_2 release rate in North American regions, whereas environmental and substrate properties only exerted indirect effects through their alterations on microbial biomass [*Colman and Schimel*, 2013]. In contrast, it was suggested that the interactions of climatic and geochemical factors determined the spatial variation of the soil CO_2 release rate in South America [*Doetterl et al*., 2015]. These pioneering studies have greatly advanced our knowledge of the mechanisms regulating soil CO_2 release rate over a broad geographic scale. However, whether or not these findings, which were mostly derived from temperate and tropical regions, still holds true in alpine ecosystems remains an open question. Temperature may likely be more of a driving feature in alpine ecosystems due to the limitation of temperature on microbial physiological processes [*Nedwell*, 1999]. However, little is known about the relative importance of substrate, environmental, and microbial properties in regulating the regional variability of soil CO_2 release rate in alpine ecosystems. Without this knowledge, our understanding of the drivers of soil CO_2 release rate across diverse climate zones remains incomplete.

During the past decade, the temperature sensitivity of soil CO_2 release (hereafter referred to as apparent temperature sensitivity) has gained more attention [*Billings and Ballantyne*, 2013; *Conant et al*., 2011; *Davidson and Janssens*, 2006]. Two previous incubation studies demonstrated that the basal microbial respiration rate per unit organic C could predict Q_{10} variations over a regional scale [*Craine et al*., 2010a; *Fierer et al*., 2006]. These results supported the "carbon-quality temperature" hypothesis, which states that low-quality C substrates are more sensitive to changes in temperature than high-quality C substrates on the grounds that a higher resistance to decomposition is associated with greater activation energy [*Bosatta and Ågren*, 1999]. These two incubation studies significantly improved our understanding of the fundamental drivers of Q_{10} over a broad geographic scale. However, the effects of microbial properties have not yet been adequately addressed. Actually, either the composition of the microbial community [*Garcia-Pausas and Paterson*, 2011] or the stoichiometric imbalance between microbial decomposers and their soil resource ($S_{C:N}/M_{C:N}$) [*Mooshammer et al*., 2014a, 2014b] was deemed to have a significant impact on soil CO_2 release, but these microbial effects were ignored in previous studies. Moreover, the relative importance of the effects of substrate, environmental, and microbial properties on Q_{10} has not yet been quantified. Structural equation modeling (SEM) analysis offers the possibility to disentangle the direct and indirect effects of substrate, environmental, and microbial properties on Q_{10} and to evaluate the importance of these properties, but it has never been conducted at the regional scale.

The Tibetan Plateau, known as the "third pole" of the Earth, provides an ideal field to explore large-scale patterns in soil CO_2 release rate and Q_{10} in alpine ecosystems. The climate is characterized as cold and dry for the main body of the plateau (Figure S1 in the supporting information), with a wide precipitation gradient on the plateau (Figure S2). Across this precipitation gradient, edaphic variables (e.g., texture and pH) [*Ji et al*., 2014], substrate (e.g., soil organic C and C:N ratio) [*Yang et al*., 2008], and microbial properties (e.g., microbial biomass C (MBC) and fungal-to-bacterial biomass ratio (F:B ratio)) [*Chen et al*., 2016] exhibit significant variability. The large variations among substrate, environmental, and microbial properties make it easier to detect their joint effects on soil CO_2 release rate and Q_{10} in alpine ecosystems. Moreover, the dominant limiting role of precipitation on both plant production [*Yang et al*., 2009] and soil respiration [*Geng et al*., 2012] on the plateau offers us a great opportunity to seek different patterns from arctic regions. For instance, it has been demonstrated that precipitation (or water availability) rather than temperature drives the variability in vegetation production [*Yang et al*., 2009] and soil organic C density (C stock per unit area) across the plateau [*Yang et al*., 2008]. In addition, there are two major vegetation types of the same growth form but different in terms of dominant species, i.e., the alpine steppe and the alpine meadow from the arid end to the moist end of the precipitation gradient. The alpine meadow is characterized by greater ecosystem productivity [*Yang et al*., 2009] and more labile soil C (water-soluble carbon fractions) [*Wu et al*., 2014] and microbial biomass [*Chen et al*., 2016] compared with the alpine steppe. This provides the opportunity to test the hypothesis that both soil CO_2 release rate and Q_{10} could differ between the two grassland types due to their differences in substrate, environmental, and microbial

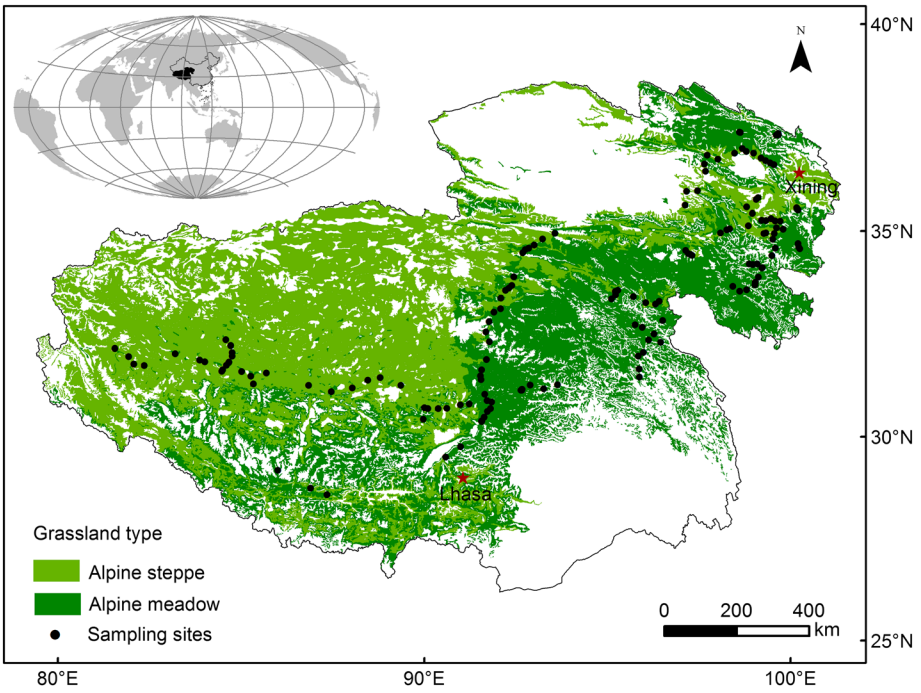

Figure 1. Sampling sites and vegetation map across alpine grasslands on the Tibetan Plateau. The vegetation map was obtained from China's vegetation atlas with a scale of 1:1,000,000 [*Chinese Academy of Sciences*, 2001].

properties. Overall, these characteristics of the plateau offer us a unique opportunity to fill the knowledge gaps involved in current studies regarding soil CO_2 release rate and its temperature sensitivity in alpine environments with cold and dry climate.

In this study, we provided the first large-scale investigation to explore the regional-scale patterns and potential drivers of soil CO_2 release rate and Q_{10} in Tibetan alpine grasslands by incubating soil samples obtained from 156 sites across the study area. We also determined a suite of accompanying soil properties and microbial characteristics, synthesized meteorological data, and then examined the relative importance of substrate supply (i.e., supply of compounds to enzymes or to microbes), physical environment, and microbial properties in regulating the spatial variation of soil CO_2 release rate and its temperature sensitivity. Specifically, we aimed to answer the following three questions: (i) Whether or not and how substrate, environmental, and microbial variables determine the spatial pattern of soil CO_2 release rate in alpine ecosystems. Which is the dominant driver? (ii) Whether or not and how other environmental and microbial properties, in addition to substrate properties, regulate Q_{10} variability over a regional scale. Which is the most important factor? (iii) To what degree does the difference in soil C chemical characteristics drive differences in CO_2 release rate and its temperature sensitivity between steppe soils and meadow soils? Our starting point hypotheses include the following: (i) precipitation was the major driver of soil CO_2 release rate, (ii) basal microbial respiration rate was the key regulator of the spatial pattern of Q_{10}, and (iii) meadow soils had higher soil CO_2 release rates but lower temperature sensitivity than steppe soils.

2. Materials and Methods

2.1. Study Area

This study was conducted on the Tibetan Plateau (Figure 1), which is a vast elevated plateau, with an average elevation of 4000 m above sea level [*Yang et al.*, 2008]. The mean annual temperature (MAT) ranges between −4.1 and 7.4°C, with the mean annual maximum and minimum temperature varying from 19.2 to 31.6°C and from −35.9 to −17.2°C, respectively. The mean annual precipitation (MAP) ranges from 99.9 to 549.2 mm. The precipitation decreases from southeast to northwest of the plateau, approximately 90% of which falls within the growing season from May to September [*Yang et al.*, 2008]. The alpine steppe and alpine meadow are the

two most representative of the vegetation types on the plateau, accounting for 34% and 27% of the area of the plateau, respectively. The aboveground and belowground biomass in alpine grasslands (steppe and meadow) on the plateau ranged from 10.2 to 215.1 g m^{-2} and from 51.7 to 2784.8 g m^{-2}, respectively [*Yang et al*., 2010]. The main soil parent material on the plateau includes glacial till and residuals [*Yu and Lu*, 2011], and the clay minerals primarily contain illite, montmorillonite, and chlorite [*Hong et al*., 2010].

The two grassland types are different in terms of climate, vegetation, soil, and microbial conditions. Specifically, the alpine steppe, characterized by dry climate conditions (the average MAP is 294 mm), is dominated by *Stipa purpurea* and *Carex moorcroftii* [*Yang et al*., 2015], with low species richness and aboveground biomass (Figure S3). The alpine meadow, characterized by relatively wet climate conditions (the average MAP is 460 mm), is dominated by *Kobresia pygmaea*, *K. humilis*, and *K. tibetica* [*Yang et al*., 2015], with relatively high species richness and aboveground biomass (Figure S3). According to the World Reference Base for Soil Resources (Food and Agriculture Organization of the United Nations), the main soil groups include xerosols for the alpine steppe and cambisol for the alpine meadow [*Lu et al*., 2004]. Compared with the alpine steppe, the alpine meadow is characterized by lower soil pH, sand content, and F:B ratio (Figure S3).

2.2. Soil Sampling and Processing

We collected soil samples across 156 sites throughout the spatial span of the alpine grasslands on the Tibetan Plateau (Figure 1) [*Ding et al*., 2016]. The sampling sites were set along a 3000 km transect to cover broad climatic gradients and major grassland types across the study area. The average distance between adjacent sampling sites was approximately 30 km. Each sampling site was labeled by either the alpine steppe or alpine meadow according to the dominant species. Of the 156 sampling sites, 81 sites were from the alpine steppe and 75 sites were from the alpine meadow. At each site, we set up five 1×1 m^2 quadrats located at each corner and the center of a 10×10 m^2 square plot. For each quadrat, the aboveground biomass was clipped at the ground level and pooled. Three topsoil samples (0–10 cm) were collected within three quadrats along one diagonal line of the plot. Each thoroughly homogenized soil sample, weighing approximately 1000 g, was divided into two subsamples for different purposes. One set of the subsamples was passed through a 2 mm sieve to remove coarse roots and rocks, and then it was maintained at −20°C until used in the incubation experiments and the microbial measurements [*Doetterl et al*., 2015; *Gutierrez-Giron et al*., 2015; *Lavoie et al*., 2011]. The other set of the subsamples was air-dried and processed to determine its physical and chemical properties.

2.3. Microbial Respiration and Q_{10} Determination

In this study, we performed an incubation experiment under varying temperatures to quantify the soil CO_2 release rate and Q_{10} for 156 sampling sites. Compared with the "equal-time" method (soil CO_2 release rates from different samples were measured after an equal time of incubation under two constant temperatures) [*Hamdi et al*., 2013], this method is advantageous due to the alleviation of the bias introduced by the differential soil C concentration and microbial biomass due to different depletion rates under different incubation temperatures in equal-time incubation experiments [*Chen et al*., 2010], and thus, it has been increasingly used to estimate Q_{10} [*Curiel Yuste et al*., 2010; *Fang et al*., 2005; *Hamdi et al*., 2013; *Hartley et al*., 2008; *Koch et al*., 2007; *Xu et al*., 2010].

For each site, we evenly mixed the fresh soil from three replicates, and then we took out 60 g dry-weight soil samples and further divided them into three equal parts for watering. After adjustment to 60% of water holding capacity in 250 ml jars, the soils were then allowed to equilibrate at the new water potential for 10 days at 20°C to avoid pulses in microbial activities induced by the disturbance of mixing and adjusting moisture content [*Fierer et al*., 2006]. Three more empty jars were incubated simultaneously as blanks.

The incubation temperatures were set to range from 5 to 25°C by a 5°C step (Figure 2). That is to say, the temperature was initially increased from 5 to 25°C and subsequently decreased from 25 to 5°C stepwise to resemble the diurnal temperature dynamics during the growing season (Figure S4) and account for the possible inconsistency of microbial response with ascending and descending temperature [*Fang and Moncrieff*, 2001]. After each temperature changed, an equilibration period of 3 h was required on the incubation jars (equilibration time) to allow the soil samples to adapt to the altered temperature and to avoid possible hysteresis of CO_2 release in response to temperature changes [*Chen et al*., 2010]. Subsequently, the jars were sealed by using butyl rubber septa and flushed with ambient air for 4 min (flushing time) by using a pump

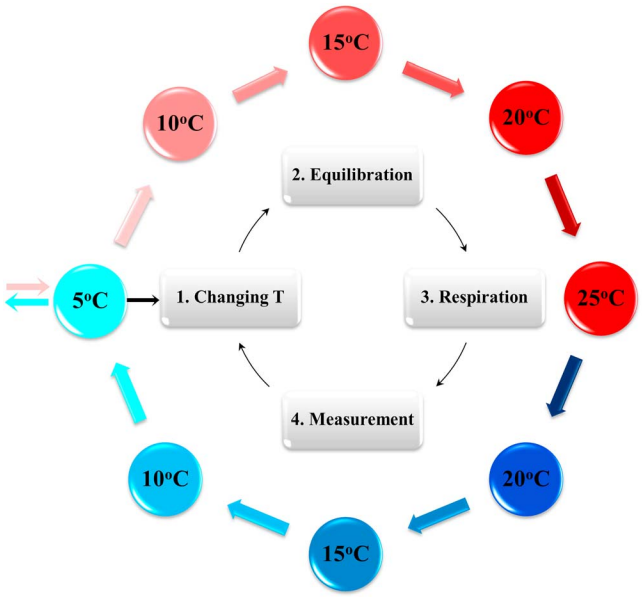

Figure 2. Flow diagram of the incubation experiment under varying temperatures in sequence. A complete measurement period was composed of soil incubation under ascending temperatures from 5 to 25°C and then decreasing temperatures from 25 to 5°C. That is to say, all soil samples were conducted by identical times of changing temperature, including 2 times at 5°C, 2 times at 10°C, 2 times at 15°C, 2 times at 15°C, and 2 times at 25°C. After each temperature change (1. Changing T), the soil samples first went through an equilibration period of 3 h (2. Equilibration) and a subsequent respiration period with varying time lengths according to the incubation temperatures (3. Respiration). Gas samples were then collected by using syringes and measured by using a gas chromatograph (4. Measurement).

to make equal initial gas conditions among all jars (Figure S5). During the equilibration period, the jars were covered with porous film to maintain the water content of the soils (water losses less than 0.2%). It should be noted that the little water loss from soil samples was regularly corrected by adding deionized water once the weight of water loss reached 0.1 g during the 7 day incubation period [*Chen et al.*, 2010; *Fang et al.*, 2005]. After preset time lengths of sealing incubation (respiration time: 35, 20, 10, 6, and 4 h for 5, 10, 15, 20, and 25°C, respectively; Table S1 in the supporting information), 10 ml gas samples were extracted from the headspace of each jar by using syringes and measured by using a gas chromatograph (Agilent 7890A, California, USA). Considering that the accumulation of CO_2 in the jars would increase the dissolution of CO_2 in the water phase of the samples under a wide alkaline range of pH (soil pH ranges from 6.2 to 10.1 in this study) [*Oren and Steinberger*, 2008], we kept headspace CO_2 concentrations below 0.1% in all jars to minimize the dissolution of CO_2 by controlling the time lengths of sealing incubation (Table S1). The soil CO_2 release rate was then calculated on the basis of the net rate of CO_2 accumulation in the headspace. All related incubation parameters, such as equilibration time, flushing time, and respiration time, were determined by a preliminary experiment.

A commonly used exponential function (equation (1)) was adopted to fit changes in soil CO_2 release rate with temperature (Figure S6), and it accurately described the trend for all soil samples ($r^2 > 0.85$ in all cases, data not shown). Based on equation (1), Q_{10} was then calculated to represent the temperature sensitivity of soil CO_2 release (equation (2)).

$$R = Be^{kT} \tag{1}$$

$$Q_{10} = \frac{R_{T+10}}{R_T} = \frac{Be^{k(T+10)}}{Be^{kT}} = e^{10k} \tag{2}$$

where R is the soil CO_2 release rate at a given temperature ($\mu g\ C\text{-}CO_2\ g^{-1}\ SOC\ d^{-1}$), T is temperature in°C, and B and k are model parameters. The parameter "B" represents the basal microbial respiration rate per unit organic C at 0°C, and it has been commonly used to represent soil C quality [*Fierer et al.*, 2003, 2006; *Koch et al.*, 2007]. Here we used the parameter B as an index of soil C decomposability, referring to the availability and lability of the C substrates [*Fierer et al.*, 2006]. Given the cold environment on the Tibetan Plateau, we used the rate of microbial respiration at 5°C (R_5) during the increasing temperature process to analyze the patterns and drivers of soil CO_2 release rate over a broad geographic scale.

2.4. Soil and Microbial Analyses

We determined a suite of accompanying soil physical and chemical properties to explore the effects of edaphic variables on soil CO_2 release rate and Q_{10}. Soil texture and pH represent soil environmental conditions, and soil organic C concentration and soil carbon:nitrogen ratio (C:N ratio) reflect the substrate properties. These

variables have been widely used to analyze potential drivers of soil C dynamics [*Colman and Schimel*, 2013; *Craine et al*., 2010a, 2010b; *Fierer et al*., 2006]. Soil texture was measured by using a particle size analyzer (Malvern Masterizer 2000, Malvern, Worcestershire, UK) after removal of organic matter and carbonates by using 30% hydrogen peroxide and 30% hydrochloric acid, respectively. Soil pH was determined in a 1:2.5 soil-to-deionized water mixture and then analyzed by using a pH electrode (PB-10, Sartorius, Germany). Total C and nitrogen (N) concentrations were measured by using an elemental analyzer (Vario EL Ш, Elementar, Germany). Soil inorganic C concentration was determined with a carbonate analyzer (Eijkelkamp 08.53, Netherlands). The soil organic C concentration was obtained by subtracting the soil inorganic C concentration from the total C concentration. The soil C:N ratio was then calculated as the quotient of the soil organic C concentration and total N concentration.

In view of the microbial controls over soil C cycling [*Schimel and Schaeffer*, 2012], we also measured microbial properties to assess their effects on soil CO_2 release rate and Q_{10}. Specifically, microbial biomass was demonstrated to be the most direct and most important driver of soil CO_2 release [*Colman and Schimel*, 2013], whereas microbial community composition may also be important for the rate of soil CO_2 release [*Schimel and Schaeffer*, 2012]. MBC and microbial biomass N were determined by the chloroform ($CHCl_3$) fumigation-extraction method, using 0.5 M K_2SO_4 in a soil:solution ratio of 1:5 [*Vance et al*., 1987], after all frozen soil samples were thawed at 5°C for 24 h and incubated at 20°C for 10 days [*Gutierrez-Giron et al*., 2015; *Koch et al*., 2007; *Lavoie et al*., 2011]. The C and N concentrations in the extracts were measured with a multi-NC-analyzer (Analytik Jena, Thuringia, Germany). We also calculated the stoichiometric imbalance between microbial decomposers and their resource ($S_{C:N}/M_{C:N}$) by soil resource C:N ($S_{C:N}$) normalized to microbial biomass C:N ($M_{C:N}$), which could alter microbial element use efficiencies and thus have a significant impact on soil CO_2 release [*Mooshammer et al*., 2014a, 2014b]. In addition, the microbial community composition was characterized by using phospholipid fatty acid analysis [*Bossio and Scow*, 1998]. The F:B ratio was used to analyze the effects of microbial community composition on soil CO_2 release and its temperature sensitivity.

2.5. Climate Synthesis

We interpolated the climate data (MAT and MAP) by using the Cokriging method with altitude as a covariant to account for the topographic effects, and then we retrieved the interpolated climate data for each sampling site at a spatial resolution of $10 \times 10\,km^2$. The original data, including records from 73 weather stations on the plateau, were obtained from the China Meteorological Data Sharing Service System (http://cdc.nmic.cn/home.do). The interpolation analyses were performed by using ArcMap 10.0 (Environmental Systems Research Institute, Inc., Redlands, CA, USA).

2.6. Statistical Analyses

We used the following statistical approaches to discern patterns and drivers of soil CO_2 release rate and its temperature sensitivity. First, to examine differences in the two target variables (i.e., CO_2 release rate and Q_{10}) between the two grassland types, data were analyzed by using the *t* test with the group-wise $\alpha = 0.05$. Correlation analyses were then conducted to examine the relationships between soil CO_2 release rate and substrate, environmental, and microbial properties, as well as the relationships of Q_{10} with the above-mentioned variables. We further used structural equation modeling (SEM) to explore the direct and indirect factors regulating soil CO_2 release rate and its temperature sensitivity, as well as to evaluate the contributions of these factors by assessing the degree of the standardized total effect (direct effect plus indirect effect). SEM is a multivariate statistical analysis technique that is based on a collection of simultaneous procedures that test the hypothetical pathways of influence (direct and indirect) among many variables using covariance among those variables [*Grace*, 2006; *Miao et al*., 2009; *Shipley*, 2000]. This technique goes beyond traditional multivariate techniques that relate predictors directly to the responsor, ignoring the overall effects derived from the interactions among variables [*Grace*, 2006; *Miao et al*., 2009; *Shipley*, 2000].

We constructed SEMs for the two grassland types separately in the consideration of potential differences in the mechanisms underlying soil CO_2 release rate and Q_{10}. To obtain the final SEM, the following two steps involving base model construction and model optimization were specified. First, we established a base model on the basis of empirical knowledge. Specifically, based on the correlation analysis between forcing variables and response variables (soil CO_2 release rate and Q_{10}), we included all variables that were significantly correlated with the response variables in the base model. We then built causal relationships between

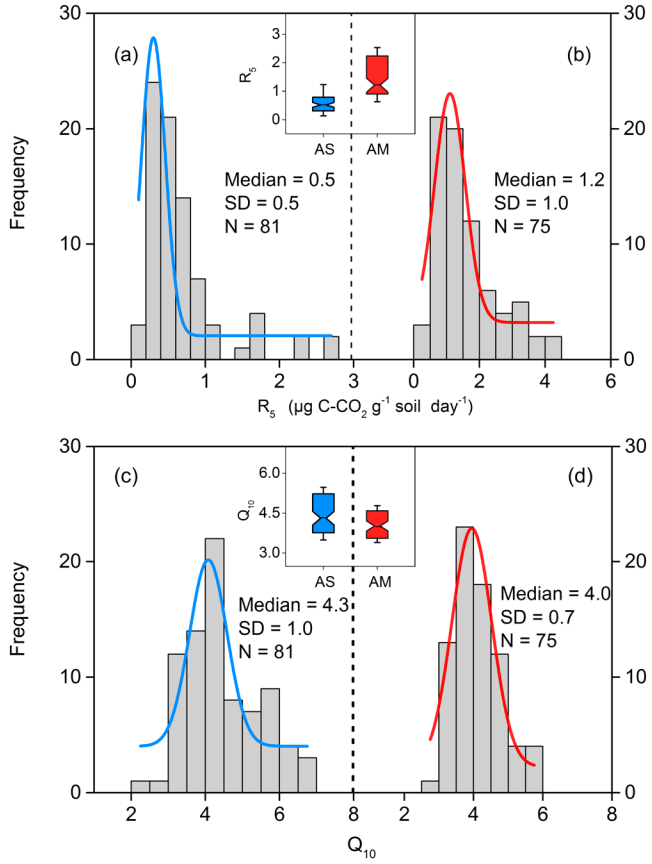

Figure 3. Histogram plots and box-whisker plots showing soil CO_2 release rate at 5°C (R_5) and its temperature sensitivity (Q_{10}) across (a and c) the alpine steppe (AS) and (b and d) alpine meadow (AM) on the Tibetan Plateau. The whiskers illustrate the standard deviation, and the box ends indicate the 25th and the 75th quartiles. The horizontal lines inside each box show the medians, and the notches represent the 95% confidence intervals. Nonoverlapped notches indicate significant differences between groups (Kruskal-Wallis test, $P < 0.05$).

these variables and response variables. As the carrier of the decomposition process, microbes should have a direct effect on the process. Thus, we assumed that microbial variables could affect the soil CO_2 release rate and its temperature sensitivity directly. Moreover, as the objects of the decomposition process, substrate properties were supposed to play direct roles in soil CO_2 release and indirect roles through regulating microbial properties. In addition, the decomposition process is also modified indirectly by the complex soil properties and climate background simultaneously. We thus assumed that soil properties were directly and indirectly connected with soil CO_2 release through other substrate and microbial properties. Given that soil samples were incubated under controlled temperature and moisture conditions, the direct effects of climatic factors were eliminated. Consequently, we assumed that the climate conditions affected the soil CO_2 release rate and its temperature sensitivity indirectly through their effects on soil, substrate, and microbial properties. These assumptions led us to the base model (Figure S7).

Second, we optimized the base model on the basis of actual measurements. Specifically, we first examined modification indices to ensure that no important paths were left out of the model, and then we removed paths with coefficients that were not significant at $P < 0.05$ [*Colman and Schimel*, 2013]. The iterative model optimization was then performed to improve model fit. This iterative process continued until the model predictions fit well with the observed values. The chi-square (χ^2) statistic, whole-model *P* value, the root-mean-square error of approximation (*RMSEA*), and Akaike information criterion (*AIC*) were used to assess the overall goodness of model fit [*Grace*, 2006]. Low values of χ^2, *RMSEA*, and *AIC* and a high *P* value (>0.05) suggest that there is small difference between the modeled and observed values [*Grace*, 2006; *Shipley*, 2000]. When comparing alternative models, the model with the lower *AIC* value was chosen if the difference in *AIC* between the two models was >7 [*Colman and Schimel*, 2013]. All data were tested for normality using the Kolmogorov-Smirnov test, and nonnormal data were log-transformed (e.g., MBC, R_5, and *B*). The "sem" and "stats" packages in the software R version 3.2.1 were used to perform SEM and other statistical analyses [*R Development Core Team*, 2015].

3. Results

3.1. Variations of Soil CO_2 Release Rate and Q_{10} Along the Transect

Soil CO_2 release rate exhibited large variations across 156 sampling sites, ranging between 0.1 and 4.2 $\mu g\ C\text{-}CO_2\ g^{-1}\ soil\ d^{-1}$. It differed by grassland type, with a median rate of 0.5 $\mu g\ C\text{-}CO_2\ g^{-1}\ soil\ d^{-1}$ in

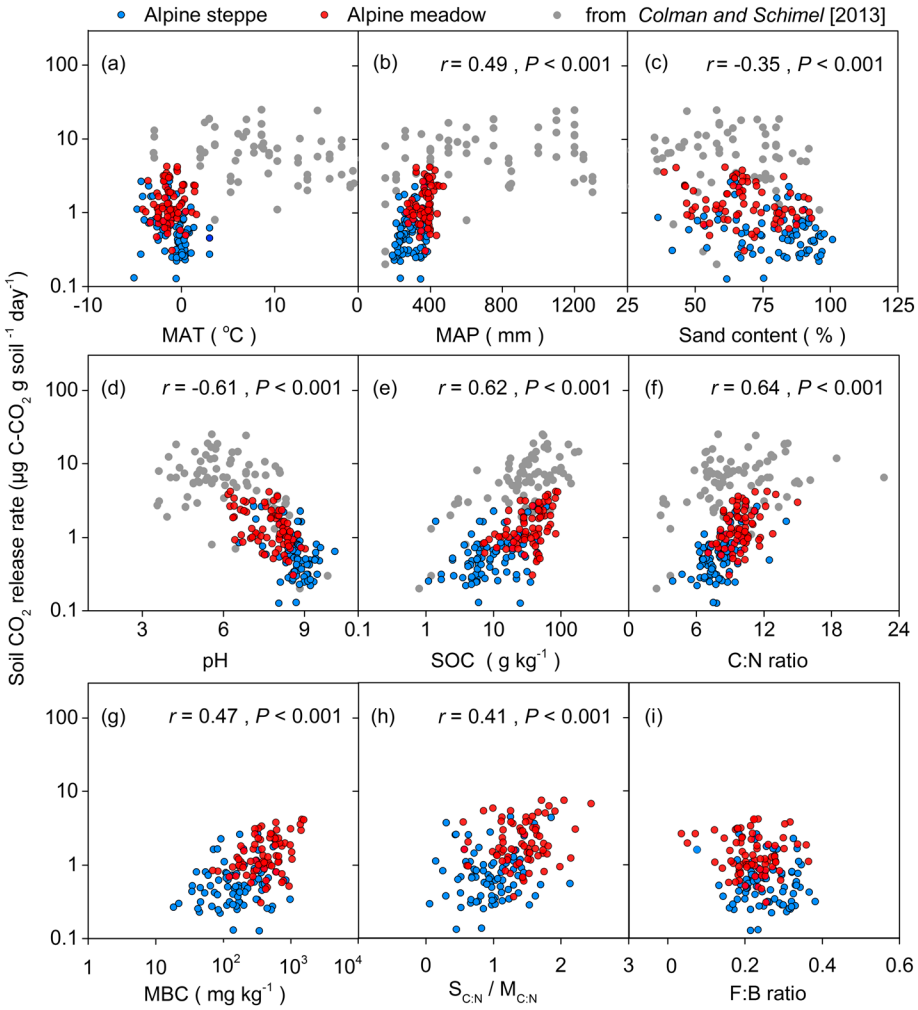

Figure 4. Relationships of soil CO_2 release rate at 5°C (R_5) with environmental (MAT, MAP, sand content, and pH), substrate (SOC and C:N ratio), and microbial (MBC, $S_{C:N}/M_{C:N}$, and F:B ratio) properties in the alpine steppe and alpine meadow. The grey solid circles denote the reference data obtained from *Colman and Schimel* [2013]. Both correlation coefficients (r) and associated P values were for this study. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon concentration; C:N ratio, the ratio of SOC to total N concentrations; MBC, microbial biomass carbon; $S_{C:N}/M_{C:N}$, soil resource C:N ($S_{C:N}$) normalized to microbial biomass C:N ($M_{C:N}$); F:B ratio, the ratio of fungal to bacterial biomass.

the alpine steppe and 1.2 μg C-CO_2 g^{-1} soil d^{-1} in the alpine meadow ($P < 0.05$; Figures 3a and 3b). Similar to the release rate, significant variations were detected in Q_{10} among 156 sampling sites, ranging between 2.3 and 6.8, with a median of 4.1 (Figures 3c and 3d). Significant differences were also observed between the two grassland types. However, the results were the opposite of those of the release rate: steppe soils had higher Q_{10} than meadow soils (4.3 versus 4.0, $P < 0.05$; Figures 3c and 3d).

3.2. Linking Soil CO_2 Release Rate and Q_{10} to Substrate, Environmental, and Microbial Properties

The soil CO_2 release rate was correlated with environmental (MAP, pH, and sand content), substrate (SOC and C:N ratio), and microbial properties (MBC and $S_{C:N}/M_{C:N}$) (Figure 4). It did not show any significant correlation with MAT (Figure 4a), but it increased with MAP ($r = 0.49$, $P < 0.001$; Figure 4b). Both sand content ($r = -0.35$, $P < 0.001$; Figure 4c) and soil pH ($r = -0.61$, $P < 0.001$; Figure 4d) were negatively correlated with the release rate. The soil CO_2 process was also dependent on SOC and the C:N ratio: it exhibited significant increases with both SOC ($r = 0.62$, $P < 0.001$; Figure 4e) and the C:N ratio ($r = 0.64$, $P < 0.001$; Figure 4f). In addition, soil CO_2 release rate was positively correlated with both MBC ($r = 0.47$, $P < 0.001$; Figure 4g) and $S_{C:N}/M_{C:N}$ ($r = 0.41$, $P < 0.001$; Figure 4h), which reflected the microbial characteristics. However, there was no significant correlation between soil CO_2 release rate and the F:B ratio (Figure 4i).

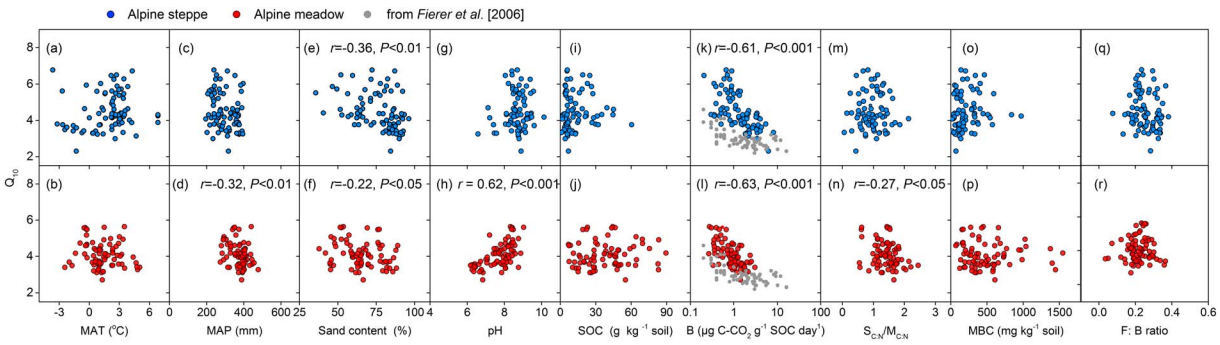

Figure 5. Relationships of temperature sensitivity (Q_{10}) with environmental (MAT, MAP, sand content, and pH), substrate (SOC and *B*), and microbial (MBC, $S_{C:N}/M_{C:N}$ and F:B ratio) properties in the alpine steppe and alpine meadow. The grey solid circles represent the reference data obtained from *Fierer et al.* [2006]. Both correlation coefficients (*r*) and associated *P* values were for this study. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon concentration; *B*, basal microbial respiration rate per unit organic C (from equation (1)); MBC, microbial biomass carbon; $S_{C:N}/M_{C:N}$, soil resource C:N ($S_{C:N}$) normalized to microbial biomass C:N ($M_{C:N}$); F:B ratio, the ratio of fungal to bacterial biomass.

Likewise, Q_{10} was also associated with environmental (MAP, pH, and sand content), substrate (*B*), and microbial properties ($S_{C:N}/M_{C:N}$) (Figure 5). Of all of the parameters examined, only two variables were significantly associated with Q_{10} for both the alpine steppe and alpine meadow: sand content (Figures 5e and 5f) and basal CO_2 release rate (*B* from equation (1); Figures 5k and 5l). Q_{10} was related to soil texture, exhibiting negative correlations with sand content (alpine steppe: $r = -0.36$, $P < 0.01$; alpine meadow: $r = -0.22$, $P < 0.05$; Figures 5e and 5f). Q_{10} was also negatively correlated with *B* (alpine steppe: $r = -0.61$, $P < 0.001$; alpine meadow: $r = -0.63$, $P < 0.001$; Figures 5k and 5l). Apart from *B* and soil texture, there were also other environmental and microbial factors including MAP, pH, and $S_{C:N}/M_{C:N}$ contributing to the spatial variation in Q_{10} in the alpine meadow. Q_{10} increased linearly with pH ($r = 0.62$, $P < 0.001$; Figure 5h) but decreased linearly with both MAP ($r = -0.32$, $P < 0.01$; Figure 5d) and $S_{C:N}/M_{C:N}$ ($r = -0.27$, $P < 0.05$; Figure 5n). In addition, there were no significant correlations between Q_{10} and other microbial factors including MBC and F:B ratio for both the alpine steppe and the alpine meadow (Figures 5o–5r).

3.3. Modeling Drivers of Soil CO_2 Release Rate and Q_{10}

The final SEM of soil CO_2 release rate was constructed for the alpine grasslands (including both the alpine steppe and alpine meadow) due to similar correlation patterns with forcing variables between the two grassland types (Figure 4). SEM analysis showed that SOC, soil C:N ratio, and pH had direct effects, whereas sand content and MAP exerted indirect effects on soil CO_2 release rate. Together, these variables predicted 52% of the variance in the release rate (Figure 6a). Specifically, substrate factors, including SOC and C:N ratio, had direct positive effects on soil CO_2 release rate, whereas pH exerted a direct negative effect. Environmental factors, including MAP and sand content, had indirect effects by mediating substrate and pH. Microbial variables could not enter the final SEM of soil CO_2 release rate (standardized path coefficient was 0.05, $P = 0.58$ for MBC; standardized path coefficient was 0.01, $P = 0.87$ for $S_{C:N}/M_{C:N}$; Table S2). Taking the direct and indirect effects together, MAP was the most important predictor shaping the spatial pattern of soil CO_2 release in Tibetan alpine grasslands (Figure 7a).

For the alpine steppe, *B*'s direct effect and sand content's indirect effect through *B* explained 37% of the variance in Q_{10} (Figure 6b). *B* had a higher prediction power for Q_{10} than soil texture (Figure 7b). For the alpine meadow, the revised model reserved MAP, sand content, *B*, and pH, and these factors explained 58% of the variance in Q_{10} (Figure 6c). The SEM demonstrated that *B* had direct controls on Q_{10}, whereas sand content and MAP had indirect effects through regulating substrate and soil pH, respectively. In addition, the SEM detected the direct and indirect effects (through substrate) of soil pH on spatial variation in Q_{10} (Figure 6c). The SEM analysis also revealed that pH had the greatest prediction power among the investigated variables for Q_{10} in the alpine meadow (Figure 7c). In addition, the microbial variable (i.e., $S_{C:N}/M_{C:N}$) was of minor importance in the SEM of Q_{10} (standardized path coefficient was 0.03, $P = 0.74$ for $S_{C:N}/M_{C:N}$; Table S3). Overall, these results demonstrated that *B* exerted a considerable but not exclusive effect on Q_{10} in alpine grasslands, and other environmental variables also had notable influence.

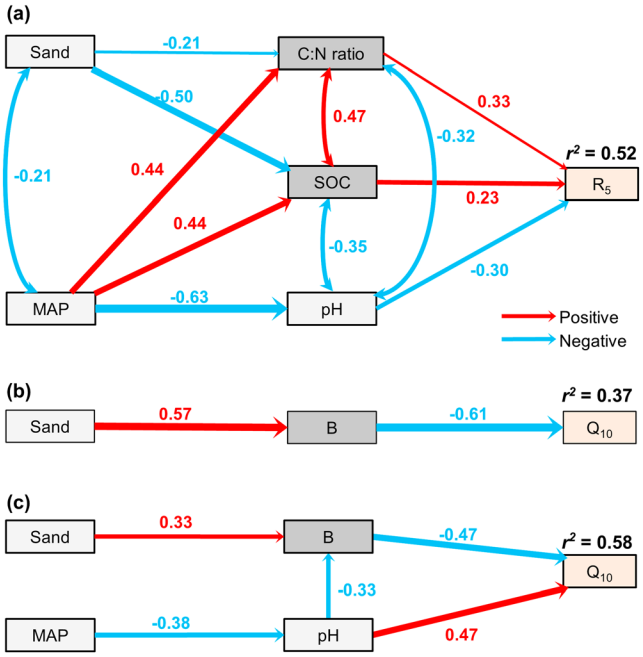

Figure 6. Structural equation modeling (SEM) with variables (boxes) and potential causal relationships (arrows) for soil CO_2 release rate at 5°C (R_5) (a) across all grassland sites and for temperature sensitivity (Q_{10}) (b) across steppe sties and (c) meadow sites. The double-headed arrows represent the covariance between related variables. The single-headed arrows indicate the hypothesized direction of causation. The arrow width is proportional to the strength of path coefficients. The numbers are standardized path coefficients, which can reflect the importance of the variables within the model [*Colman and Schimel*, 2013]. The model for R_5 had $\chi^2 = 7.03$, $P = 0.07$, $RMSEA = 0.09$, and $AIC = 55.03$, whereas the models for Q_{10} had $\chi^2 = 0.03$, $P = 0.87$, $RMSEA = 0.00$, and $AIC = 0.00$ in the alpine steppe and $\chi^2 = 4.78$, $P = 0.44$, $RMSEA = 0.00$, and $AIC = 24.78$ in the alpine meadow. MAP, mean annual precipitation; SOC, soil organic carbon concentration; C:N ratio, the ratio of SOC to total N concentrations; *B*, basal microbial respiration rate per unit organic C (from equation (1)).

4. Discussion

4.1. Precipitation-Induced Spatial Variation in Soil CO_2 Release Rate in Alpine Grasslands

The SEM revealed that MAP was the major driver of the spatial variation of soil CO_2 release rate in Tibetan alpine grasslands (Figure 7a), which was consistent with our first hypothesis. This finding revealed a unique pattern in Tibetan grasslands, as compared with the previous observations derived from temperate and tropical ecosystems of North America, where microbial biomass was the most important driver of soil CO_2 release rate [*Colman and Schimel*, 2013]. Such a difference indicated that the key regulator of soil CO_2 release would vary with various climatic conditions. In dry environments such as the Tibetan alpine grasslands (Figure S1), the limiting role of water availability in regulating vegetation C inputs and microbial properties may be responsible for the strong effects of precipitation on soil CO_2 release. It has been demonstrated that precipitation was a key limiting factor regulating vegetation production [*Yang et al.*, 2009] and soil organic C density [*Yang et al.*, 2008]. The spatial variations of microbial biomass and microbial community composition in Tibetan alpine grasslands were also reported to be regulated by the precipitation [*Chen et al.*, 2016]. Our additional analyses found significant effects of precipitation on soil C:N ratio (Figure S8a) and the

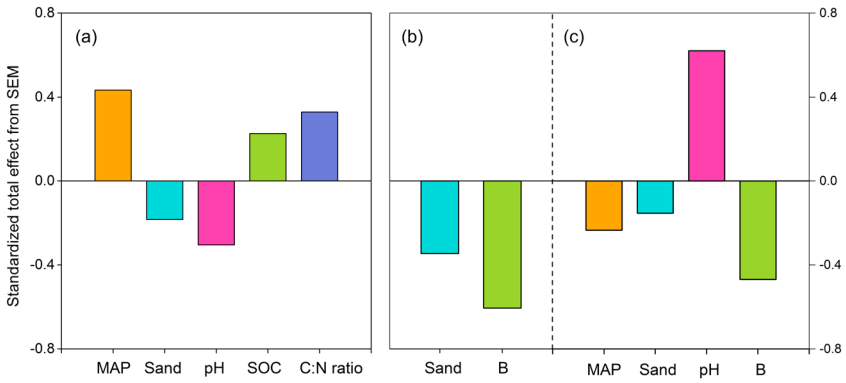

Figure 7. Standardized total effects (direct effect plus indirect effect) on soil CO_2 release rate at (a) 5°C (R_5) across all alpine grassland sites and (b) Q_{10} across alpine steppe sites and (c) alpine meadow sites derived from the structural equation modeling (SEM). MAP, mean annual precipitation; SOC, soil organic carbon concentration; C:N ratio, the ratio of SOC to total N concentrations; *B*, basal microbial respiration rate per unit organic C (from equation (1)).

stoichiometric imbalance between microbial decomposers and their resources ($S_{C:N}/M_{C:N}$; Figure S8b). It is widely accepted that wetter climate conditions are usually associated with greater vegetation production and thus sufficient substrate inputs to soils, and they lead to relieved pressure of C availability for soil microbes [*Orchard and Cook*, 1983; *Wang et al*., 2003]. Consequently, microbial effects on soil CO_2 release rates in arid regions could be enhanced when aridity is alleviated. This deduction was supported by the stronger microbial effects on soil CO_2 release in the alpine meadow (Figure S9), where the climate was significantly wetter than in the alpine steppe and more labile soil C was available for soil microbes [*Wu et al*., 2014]. In contrast, under wet conditions such as the temperate and tropical ecosystems of North America (Figure S1), biotic effects could become stronger because climatic limitations on both soil C supply and microbial properties are alleviated.

Other environmental factors, such as soil pH and sand content, were negatively linked with soil CO_2 release rate in Tibetan grasslands (Figures 4c and 4d). On the one hand, soil pH could directly regulate the activities of microorganisms and C-acquiring enzymes (e.g., b-glucosidase), and either high- or low-pH conditions would slow down the activities of microorganisms and enzymes [*Min et al*., 2014; *Turner*, 2010]. In acid soils, soil CO_2 release rate tends to increase with soil pH [*Andersson and Nilsson*, 2001; *Niklinska et al*., 1999]. In contrast, a negative correlation often occurs in alkaline soils [*Colman and Schimel*, 2013], which also holds true in alpine grasslands. On the other hand, soil texture regulated soil CO_2 release rate through mediation of soil C concentration and soil C:N ratio (Figure 6a). It has been reported that fine-textured soils would increase soil water holding capability, fuel plant growth, and then add more fresh C into soils [*Luo and Zhou*, 2006], especially in regions with yearly or seasonal water deficits, such as Tibetan alpine grasslands [*Yang et al*., 2009]. As a result, finer soils tend to have higher soil C concentrations (Figure S10a) and higher C:N ratios (Figure S10b), which could then lead to larger soil CO_2 release rates. It should be noted that fine-textured soils associated with aggregate formation would protect soil C from microbial decomposition [*Schimel et al*., 1994]. However, in our case, the fueling effects on vegetation C inputs clearly outweighed the protection effects.

Substrate properties such as soil C concentration and soil C:N ratio could directly regulate spatial variation of the soil CO_2 release rate in Tibetan alpine grasslands (Figure 6a). Specifically, soil CO_2 release rate increased with soil C concentration and soil C:N ratio (Figures 4e and 4f). This finding was supported by previous observations across North America [*Colman and Schimel*, 2013], reflecting the effects of C availability and lability on soil CO_2 release [*Haynes*, 2000; *Kadono et al*., 2008]. Consistent with this deduction, our data revealed positive associations between SOC and soil C:N ratio and dissolved organic C, which is an indicator of the labile C fraction [*Dannenmann et al*., 2009; *Fang et al*., 2005], in Tibetan alpine grasslands (Figure S11). Higher soil C concentrations and larger C:N ratios would be associated with a larger labile C pool, consequently resulting in a higher soil CO_2 release rate. Furthermore, higher activities of microbial communities and extracellular enzymes under labile C-rich conditions could also induce higher soil CO_2 release rates [*Hernández and Hobbie*, 2010].

4.2. Basal Microbial Respiration Rate Governed Q_{10} in Steppe Soils and pH in Meadow Soils

Basal microbial respiration rate (B) significantly and negatively affected Q_{10} in both the alpine steppe and alpine meadow on the Tibetan Plateau (Figures 5k and 5l), which was consistent with our second hypothesis. This finding was supported by previous observations in temperate grasslands [*Fierer et al*., 2006]. As mentioned above, similar to *Fierer et al.* [2006], we used B as an index of decomposability (the availability and the lability) of the substrates. The usage of B has been criticized due to the potential inherent negative correlation between Q_{10} and B in the model [*Reichstein et al*., 2005]. To test whether the observed Q_{10}-B relationship simply reflects an autocorrelation, we reanalyzed this relationship by synthesizing 386 paired data sets of Q_{10} and B from 55 independent papers (Table S4). Surprisingly, neither a positive nor a negative correlation was detected ($P = 0.12$; Figure S12). These additional analyses demonstrated that B could serve as an effective indicator of soil C decomposability. Moreover, molecular investigations on permafrost C quality using ^{13}C nuclear magnetic resonance spectroscopy showed consistent results with soil microbial respiration rate per unit soil C [*Waldrop et al*., 2010], indicating that substrates with high microbial respiration rates per unit soil C had high amounts of labile carbon [*Waldrop et al*., 2010]. This finding was also supported by our investigation of soil C quality by using biomarker analysis in Tibetan alpine grasslands (Chen et al., unpublished data). That is to say, the lower quality C substrate was more sensitive to changes in temperature in our case.

Notably, soil pH, which outweighed *B*, was most correlated with Q_{10} in the alpine meadow (Figure 7c). Such a pattern could be explained by the effects of soil pH on activities of C-acquiring extracellular enzymes (e.g., b-glucosidase) [*Min et al*., 2014]. In alkaline soils (pH above 6), such as Tibetan grasslands, the decline in enzyme activities with pH could result in lower mineralization rates [*Min et al*., 2014]. Lower mineralization rates could be linked to higher temperature sensitivity in general because of the linkage between higher activation energies for substrate-enzyme pairings and lower reaction rates [*Fierer et al*., 2006]. Given a wide alkaline range of pH in Tibetan grassland soils, the effect of pH on Q_{10} could be confounded by carbonate-CO_2-H_2O equilibria [*Oren and Steinberger*, 2008]. Specifically, accumulation of CO_2 in the jars would increase the dissolution of CO_2 in the water phase. However, in our case, the CO_2 dissolution would not affect our results very much because the headspace CO_2 concentrations were kept below 0.1% (500–800 ppm). To further illustrate this point, we reanalyzed the relationship between pH and Q_{10} by extracting sampling sites with pH less than 8, and we found that the positive association between them still existed (Figure S13). The pH still had the highest total effect on Q_{10} among the investigated variables in the alpine meadow (Figure S14). Such a pattern was also reported in previous studies, where soil pH explained 67% of the variation in the temperature sensitivity of microbial respiration in North American temperate grasslands [*Craine et al*., 2010b] and 25% in Mediterranean high mountain soils [*Gutierrez-Giron et al*., 2015].

4.3. Lower Soil CO_2 Release Rate but Higher Q_{10} in Steppe Soils Compared With Meadow Soils

The soil CO_2 release rate in the alpine meadow was significantly higher than that in the alpine steppe, whereas the opposite pattern occurred for Q_{10} (Figure 3), supporting our third hypothesis. This pattern may be mainly attributed to the difference in precipitation [*Yang et al*., 2015] and the associated vegetation C inputs between the two grassland types. It has been suggested that the alpine meadow had significantly higher precipitation [*Yang et al*., 2015], resulting in higher vegetation production [*Yang et al*., 2009] and soil C concentration [*Yang et al*., 2008]. Our data also revealed a larger labile C pool in alpine meadow soils than in alpine steppe soils (Figures S3h and S3i). Consequently, more high-quality soil C substrates would result in a higher soil CO_2 release rate and a lower Q_{10} in the alpine meadow.

5. Conclusions and Implications

In summary, this study analyzed variations of soil CO_2 release rate and its temperature sensitivity across broad substrate, environmental, and microbial gradients in alpine ecosystems. Our results revealed that MAP was the key regulator of the spatial variability of soil CO_2 release rate. Basal microbial respiration rate (*B*) was the most important predictor of Q_{10} in steppe soils, whereas soil pH outweighed *B* as the dominant control over Q_{10} in meadow soils. Microbial properties (e.g., MBC and $S_{C:N}/M_{C:N}$) were of minor importance in SEMs of soil CO_2 release and Q_{10}. Nevertheless, this does not mean that the importance of microbial properties for soil C dynamics can be dismissed. We observed that MBC was positively correlated with soil CO_2 release rate in Tibetan alpine grasslands (Figure 4g). We also found that $S_{C:N}/M_{C:N}$ had positive influences on soil CO_2 release rates across all alpine grasslands (Figure 4h). The increasing $S_{C:N}/M_{C:N}$ was consistent with decreasing C limitation, which resulted in decreasing microbial C use efficiency and consequently more soil CO_2 release [*Mooshammer et al*., 2014a, 2014b]. In addition, we also detected the effect of $S_{C:N}/M_{C:N}$ on Q_{10} in the alpine meadow (Figure 5n). Overall, these results demonstrated that soil CO_2 release was a complex process that was regulated by both direct and indirect controls from substrate, environmental, and microbial properties.

Our findings have the following two implications. First, precipitation determined the spatial pattern of the soil CO_2 release rate in the alpine ecosystems, which is different from temperate and tropical ecosystems, where microbial biomass was usually a key factor regulating the spatial variability of the soil CO_2 release rate [*Colman and Schimel*, 2013]. Such a difference implies that the major predictors of microbial respiration could vary with different climatic zones. Second, the basal microbial respiration rate governed Q_{10} in steppe soils and pH in meadow soils. Our finding demonstrates that substrate properties and environmental conditions jointly determined the spatial variation of Q_{10} in alpine ecosystems, suggesting relevant environmental factors, in particular, soil pH should also be considered in higher-productivity alpine regions to estimate the temperature response of soil CO_2 release. This finding is also different from previous results derived from

temperate and tropical regions, where the basal microbial respiration rate was the only dominating driver of Q_{10} variation [*Fierer et al*., 2006]. These regional differences in drivers of soil CO_2 release and Q_{10} should be considered when predicting soil C dynamics at the global scale.

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