Effects of Rainfall Manipulation on Ecosystem Respiration and Soil Respiration in an Alpine Steppe in Northern Tibet Plateau

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The response mechanism of ecosystem respiration (Re) and soil respiration (Rs) to different water conditions is of great significance for understanding the carbon cycle under future changes in the precipitation patterns. We used seven precipitation treatments to investigate the effects of precipitation on Re and Rs on a typical alpine steppe in Northern Tibet. Precipitation was captured and relocated to simulate the precipitation rates of −25, −50, −75, 0 (CK), +25, +50, and +75%. The soil moisture was influenced by all the precipitation treatments. There was a positive linear relationship between the soil moisture and Re, Rs in the study area during the experiment (July–October). Soil volumetric water content (VWC), absolute water content (AWC), soil temperature (ST), aboveground biomass (AGB), bulk density, soil total nitrogen (TN), and alkaline hydrolysis nitrogen (AHN) were the predictors of Re and Rs. The multiple linear regression analysis showed that ST and AWC could explain 90.6% of Rs, and ST, AWC, and AHN could explain 89.4% of Re. Ecosystem respiration was more sensitive to the increased precipitation (+29.5%) whereas Rs was more sensitive to the decreased precipitation (−23.8%). An appropriate increase in water (+25 and +50%) could improve the Re and Rs, but a greater increase (+75%) would not have a significant effect; it could have an effect even lower than those of the first two. Our study highlights the importance of increased precipitation and the disadvantage of decreased precipitation on Re and Rs in an arid region. The precipitation changes will lead to significant changes in the soil properties and AGB, and affect Re and Rs, to change the climate of the alpine steppe in Northern Tibet in the future. These findings contribute to our understanding of the regional patterns of environmental C exchange and soil C flux under the climate change scenarios and highlight the importance of water availability to the regulating ecosystem processes in semi-arid steppe ecosystems. In view of these findings, we urge future researchers to focus on manipulating the precipitation over longer time scales, seasonality, and incorporating more environmental factors to improve our ability to predict and model Re and Rs and feedback from climate change.

Keywords: ecosystem respiration, soil respiration, precipitation change, soil moisture, aboveground biomass
INTRODUCTION

Ecosystem respiration (Re), one of the major fluxes in the terrestrial ecosystems and the atmospheric carbon cycle, is the main way that carbon is removed from an ecosystem (Johnston et al., 2021). Therefore, Re is an important factor affecting the carbon matter and energy balance of ecosystems and has received widespread attention (Valentini et al., 2000; Janssens et al., 2001; Saleska et al., 2003; Fuchsueger et al., 2014; Sun et al., 2021). Many scholars found that Re is more representative of the difference in carbon sink flux observation points than Gross Ecosystem Production (Valentini et al., 2000; Pilegaard et al., 2001; Noormets et al., 2007; Walsh et al., 2017). Therefore, the dynamic patterns of Re and its response to the environmental factors have been the subject of considerable research in recent years. Many studies have shown that air temperature, relative air humidity, soil temperature (ST), soil moisture content, plant aboveground biomass (AGB), aboveground respiration, and groundwater level affect the monthly total change of Re and seasonal dynamics; however, different regions exhibited different responses (Huang et al., 2009; Fang et al., 2012).

Soil respiration (Rs) is the main component of Re, and its cycle process has been a focus of ecological research (Nie et al., 2019). Soil carbon flux is the main mechanism of soil carbon storage and the global carbon cycle (Gougoulias et al., 2014). Soil carbon flux can not only affect the carbon storage capacity of an ecosystem but also significantly affect the concentration of greenhouse gases in the atmosphere, which in turn affect the global climate (Dixon et al., 1994; Burton and Pregitzer, 2003; Dhaliwal et al., 2019). Soil contains approximately two times as much carbon as that stored in the atmosphere; therefore, even small soil carbon flux changes can have a significant impact on the global ecosystem carbon cycle (Lee et al., 2017). In addition, many predictions suggest that Rs will increase as temperatures rise due to global warming (Schuur et al., 2015). At the same time, the loss of carbon dioxide in the soil will induce the positive feedback that further exacerbates global warming (Woodwell et al., 1998; Rustad et al., 2000; Jeong et al., 2018). Therefore, quantifying Rs has become the initial task for predicting the future changes in the atmospheric CO₂ concentration (Hirano et al., 2003; Wang et al., 2015). Precipitation is usually the driving factor of soil moisture dynamics (Chen et al., 2016). Any changes in the root biomass, soil organic matter, or root and microbial activities due to the precipitation changes may influence Rs (Li et al., 2010). Considerable research has been conducted on the influence of precipitation on Rs (Liu et al., 2016; Du et al., 2020). Several studies have suggested that increasing precipitation can stimulate belowground productivity, resulting in a greater supply of C to Rs, particularly in dryland areas (Yan et al., 2010; Zhang et al., 2017). In hot and humid tropical forests, drought can stimulate Rs by reducing overall soil hypoxia (Cleveland et al., 2010). Soil respiration in a semi-arid steppe increased non-linearly with increased precipitation, and the effect of precipitation change on the soil heterotrophic respiration was greater than that of autotrophic respiration (Zhang et al., 2019). In summary, the precipitation changes affect Rs mainly through the changes in the ecosystem processes, such as plant growth (Yan et al., 2011; Zhou et al., 2016), soil microbial activity (Zhao et al., 2016a; Ren et al., 2018), and temperature sensitivity (Liu et al., 2016). Many previous studies on the response of Rs to precipitation in the alpine steppe have concluded that Rs increases with an increase in precipitation (Zhang et al., 2019). However, there is no consensus on the range of increase in Rs, the linear or non-linear trend, and whether the responses to water reduction and water increase are consistent (Chen et al., 2008; Wang et al., 2019). The effects of different precipitation rates in Rs and its relationship with soil moisture have not been well-investigated, particularly in the subtropics steppe. Therefore, it is imperative to improve our mechanistic understanding of Rs responses to the precipitation and soil moisture changes.

Northern Tibet is located at the hinterland of the Qinghai-Tibet Plateau. Under global warming, the precipitation has shown a significant increasing trend in the past 50 years in this region (Li, 2018). The Qinghai-Tibet Plateau has exhibited a trend of “warm and humid” (Zhao et al., 2018); however, extreme precipitation events have also become more frequent in the period of 1961–2017 (Ma et al., 2020). It is not clear how Re and Rs will respond under future changes, and how they may differ. We propose two hypotheses, as follows, to investigate this problem. Hypothesis 1: the increase in precipitation will increase Re and Rs, and as precipitation increases in the alpine grassland area, respiration would become stronger. Hypothesis 2: Re and Rs may be different in sensitivity or response to water increase or decrease due to dry climate. Water is the critical factor of environmental change in the region of the semi-arid climate. All aspects of the alpine grassland ecosystem would be implicated in precipitation change. For specific performance, variation in precipitation could contribute to the alterations in the process of Re and Rs, and lead to changes in the CO₂ concentration. Ultimately, the carbon cycle of terrestrial ecosystem and the process of carbon balance would be changed (Phillips et al., 2017). If the extent and direction of responses to increased or decreased precipitation for Re and Rs are discrepant, then it is helpful for us to predict the trend of variation of Re and Rs by means of precipitation change and evaluate accurately the alpine grassland carbon pool in the future. Here, we selected the northern Tibet alpine grassland at Xainza Experimental Station, as the study area. We simulated different precipitation conditions through the field experiments and observed changes in Re and Rs rates. This study provides insight into the effects of Re and Rs on the ST and moisture, and the responses of Re and Rs to the changes in external factors, such as AGB and soil physicochemical properties. It is expected that this study can provide theoretical support for future research on the Re and Rs changes in grassland ecosystem.

MATERIALS AND METHODS

Study Site
The experiment was conducted in Xainza Alpine grassland and the Wetland Ecosystem Observation and Experimental Station (30°57′N, 88°42′E, 4,675 m elevation) in Xainza County, Nagqu City, Tibet Autonomous Region (Figure 1). The region belongs...
to the semi-arid climate of the plateau subfrigid zone, with thin air and a cold and dry climate. The mean annual temperature is 0.4°C and mean annual precipitation is 298.6 mm which is concentrated from May to September. The area has an annual average wind speed of 3.8 m/s. The annual mean wind speed is more than eight reaches 104.3 days, the frost period lasts 279.1 days, and there are 2915.5 h of sunshine annually. The soil type in this area is mainly alpine grassland soil with a thin soil layer and is easily eroded. The alpine grasslands are the main vegetation types and the floral community is relatively simple, mainly \textit{Stipa purpurea} and \textit{Carex Moorcroftii}. Accompanying species are \textit{Leontopodium}, \textit{Oxytropis}, \textit{Artemisia capillaris}, \textit{Bulegrass}, and \textit{Stellera Chamaejasme}.

**Experimental Design**

The precipitation treatment experiment commenced in early June 2020. The experiment used seven levels and three blocks with a total of 21 plots, each $3 \times 2$ m in size (Figure 1C). The seven levels of precipitation gradients were a 25% decrease (D1), 50% decrease (D2), 75% decrease (D3), control (CK), 25% increase (W1), 50% increase (W2), and 75% increase (W3). The spacing between the two adjacent decreased precipitation treatments was 2 m, and the spacing between the increased and decreased precipitation treatments was 1.5 m to avoid a marginal effect between the plants (Figure 1C). Rain shelter were set up on each decreased precipitation treatments. The rain shutters were fixed to the steel pillars by lag spike; the polyethylene plates were fixed in “V” shapes to intercept the precipitation and allow rainfall to flow along the PVC pipe into the water storage bucket on the right to decrease precipitation (Figure 1A). Within 12 h of precipitation, the rainwater in the water storage bucket was sprayed equally to the corresponding quadrat on the right to increase precipitation (Figure 1D). The angle of the polyethylene rain shield was 120° and the width was 15 cm. The water storage bucket could accommodate rainwater trapped by rainfall with radius of 20 cm and height of 60 cm. The area-specific gravity method was used to regulate the water gradient. In terms of the rain shutter setup, five rain shutters were uniformly spread out to cover 25% of the plot in D1 to retain 25% of the precipitation, and 10 and 15 rain shutters were used in D2 and D3 treatments, respectively, to intercept 50 and 75% precipitation. CK was a control treatment for ambient rainfall conditions (Figure 1A).

**Index Measurements**

**Respiration, ST, and Volumetric Water Content Measurements**

Ecosystem respiration and Rs were measured using the Li-8100 connected to a soil chamber (LI-8100, LI-COOR Inc., Lincoln, NE, USA). Ecosystem respiration and Rs were measured in two polyvinyl chloride (PVC) soil collars (20 cm diameter, 15 cm height, 0.5 cm thickness) which were permanently inserted in each plot with 5 cm of the collars remaining above the soil surface. The PVC collars were inserted into the places in each plot where the vegetation coverage was uniform to allow measurement of Re. To reduce the influence of soil disturbance on the measurement results, the surrounding areas were compacted with soil and were not removed during the experiment (Wu et al., 2013). The plants inside the collars were clipped to the ground level after the insertion of the collars and whenever they were found in the collars, to eliminate the aboveground plant respiration during the...
measurement of Rs (Yan et al., 2011). The litter that had fallen into the collars was retained in the collars to account for CO₂ released from litter decomposition (Ren et al., 2014; Wei et al., 2014). The measurements commenced on July 16, 2020, and were conducted every 5 days from 10:00 to 12:00 a.m. (Figure 1B). The interval time could be adjusted appropriately according to the weather conditions in the test area. To reduce the error in the Rs rate caused by long observations and large temperature changes, the measurements were completed in the sunny conditions.

Soil temperature and moisture sensors (Oset HOBO U30-NRC-SYS-ADV, America) were buried at a 10-cm depth in each plot near the soil collars (see below) to continuously monitor the ST and volumetric water content (VWC). The data were recorded every hour.

**AGB and Soil Physicochemical Properties**

Aboveground biomass was obtained by the harvesting method on July 24 and August 26, 2020, and the biomass was measured by randomly selecting a 0.5 × 0.5 m plot in each precipitation treatment plot. The aboveground parts of the harvested plants were taken back to the laboratory and dried at 65°C to constant weight after drying in an oven at 105°C, their weights were then recorded. After cutting the plants at ground level, the soil at a 0–10 cm depth was removed using the soil drill method. Three soil samples were taken from each sample square and were placed into sealed bags, transported to the laboratory, and were naturally air-dried for measuring the soil physicochemical properties. The total nitrogen (TN) in the soil was determined using the Kjeldahl method (Song, 2019) and the alkaline hydrolysis nitrogen (AHN) was measured using the alkali-diffusion method (Na, 2014). Soil bulk density was determined using the valve bag method (Zhang et al., 2012). Soil absolute water content (AWC) was determined by sampling drying method because this method was relatively accurate and the operation process was simple (Wang and Shu, 2017).

\[
\text{Soil bulk density} = \frac{D_{SW}}{SV} \\
\text{Absolute soil moisture content} = (W_{SW} - D_{SW})/D_{SD}
\]

In Equations (1) and (2), \(D_{SW}\) denotes the dry weight of soil; \(SV\) denotes the volume of soil; and \(W_{SW}\) denotes the wet weight of soil; and \(D_{SD}\) denotes the dry weight of soil.

**Statistical Analysis**

The statistical analysis was conducted using R version 4.0.3 (R Core Team 2020) with the packages “vegan” and “ggplot2” (Wickham, 2016; Oksanen et al., 2020) as well as SPSS (IBM, Chicago, IL USA). Before the analysis, the Shapiro–Wilk test was used to check the normality of all data \((P < 0.05)\) and Bartlett's test was used to check the homogeneity of variances \((P > 0.05)\). The one-way ANOVA test was used when the values for both normality of data and the homogeneity of the variances passed their tests. The non-parametric tests were used when both normality and homogeneity of variance did not pass their tests. In this study, the Kruskal–Wallis test method was used for Re and Rs and the one-way ANOVA test was used to analyze ST, VWC, AGB, AWC, TN, and AHN under different precipitation treatments. Duncan's new multiple-range test was used for post-hoc multiple comparisons (Harter, 2008). In addition, to compare the differences among the increased precipitation treatment, decreased precipitation treatment, and overall ambient treatment, the Rs and Re under three increased precipitation treatment gradients were integrated into wet treatments (W) while the Rs and Re under three decreased precipitation treatment gradients were integrated into drought treatments (D). The linear model was used to determine the relationship between Re and Rs and VWC, AWC, AGB, TN, AHN, and bulk density. The quadratic function model was used to analyze the relationship among Re, Rs, and ST. After conducting the correlation analyses for the environmental factors and Rs and Re, we also constructed a multiple linear regression model to establish the relationships between each factor and Rs and Re to allow us to determine which factor had the greatest influence on Rs and Re. We used stepwise regression analysis and set the probability of F as 0.05 and 0.1 for entry and removal, respectively. During this analysis, we eliminated independent variables that were not significant in the model test and were finally left with the three independent variables of ST, AWC, and AHN. The \(R^2_{adj}\) of the mode of Rs and Re reached 0.906 and 0.894, indicating a strong goodness of fit (Table 2). Furthermore, all P-values in the variance tests were <0.001, indicating that the variance tests and the overall model passed significance tests. Finally, we were able to express the multiple linear regression from the results of parameter tests as follows (as shown in Tables 2, 3):

\[
Rs = 2.95 - 0.113*ST + 2.321*AWC
\]

\[
Re = 6.382 - 0.242*ST - 0.018*AHN + 7.28*AWC
\]
Table 2: Summary of the results of multiple linear stepwise regression analysis of the effects of environmental factors on soil respiration (Rs).

| Variable | Estimate | SE  | t-value | p-value | VIF  | Tolerance |
|----------|----------|-----|---------|---------|------|-----------|
| Interception | 2.950 | 0.446 | 6.615 | 0.000 | 3.958 | 0.253 |
| ST       | −0.113 | 0.023 | −4.797 | 0.000 | 3.958 | 0.253 |
| AWC      | 2.321 | 0.946 | 2.454 | 0.025 | 3.958 | 0.253 |

ST: soil temperature; AWC: absolute water content.

Table 3: Summary of the results of multiple linear stepwise regression analysis of the effects of environmental factors on ecosystem respiration (Re).

| Variable | Estimate | SE  | t-value | p-value | VIF  | Tolerance |
|----------|----------|-----|---------|---------|------|-----------|
| Interception | 6.382 | 1.363 | 4.681 | 0.000 | 3.958 | 0.253 |
| ST       | −0.242 | 0.071 | −3.394 | 0.003 | 3.958 | 0.253 |
| AHN      | −0.018 | 0.005 | −3.347 | 0.004 | 3.958 | 0.253 |
| AWC      | 7.281 | 2.886 | 2.523 | 0.022 | 3.958 | 0.253 |

ST: soil temperature; AWC: absolute water content; AHN: alkaline hydrolysis nitrogen.

RESULTS

Rs and Re Under Precipitation Change

There were significant differences in the Rs ($\chi^2 = 47.079, P < 0.01$) and Re ($\chi^2 = 97.027, P < 0.01$) among the seven precipitation treatments during the experiment. For Re, W2 had the highest mean rate (i.e., 2.46) and D3 had the lowest mean rate (i.e., 1.23). However, Re was significantly higher in W1, W2, and W3 than in CK, D1, D2, and D3. Among the three decreased precipitation treatments, the Re and Rs in D3 were the lowest. For Rs, the mean rate was the highest in the W3 treatment (i.e., 1.32), there was no significant difference from that of W2, and that in D3 was the lowest (i.e., 0.82). The Rs was significantly higher in W1 and CK than in D2, and there was no significant difference between D1 and D2 (Figures 2A, B). In general, the mean Rs and Re values under drought treatment were 0.96 and 1.52 $\mu$mol·m$^{-2}$·s$^{-1}$, respectively, which were significantly lower than those under increased precipitation (1.29 and 2.28 $\mu$mol·m$^{-2}$·s$^{-1}$, respectively) and ambient treatment (1.26 and 1.76 $\mu$mol·m$^{-2}$·s$^{-1}$, respectively) (Figure 3). We found that Re and Rs decreased by 13.6 and 23.8%, respectively, under decreased precipitation compared with the ambient treatment. At the same time, Re and Rs increased by 29.5 and 24%, respectively, under increased precipitation compared with ambient precipitation. This indicated that Re and Rs were more sensitive to the increases and decreases, respectively, in precipitation.

Environmental Characteristics Under Precipitation Change

Aboveground Biomass

In general, the AGB of W2 was the highest (89.3 g·m$^{-2}$) and was significantly higher than that of D1, D2, and D3 ($F = 5.21, P < 0.01$). The AGB of D3 was the lowest (27.16 g·m$^{-2}$). There was no significant difference in AGB between CK and W3, D2, or D1 (Figure 4). The mean AGB from July to August decreased as follows: W2 > W1 > W3 > D2 > CK > D1 > D3. Aboveground biomass of D1, D2, and D3 decreased by 23.6, 17.3, and 59.3%, respectively, compared with the control. D3 was extremely short of water due to the 75% reduction in water; therefore, the AGB levels were extremely low. However, both a 50 and 25 reduction in water reduced aboveground productivity due to the decrease in precipitation. The AGB in W1 and W2 increased by 30.7 and 33.5%, respectively, while AGB in W3 decreased by 0.07%.

Soil Physicochemical Properties

There were significant differences in soil bulk density among the different moisture gradients, which increased with a decrease in soil moisture. The bulk density of W3 was the lowest (0.95 g·cm$^{-3}$), and that of D3 was the highest (1.50 g·cm$^{-3}$) ($F = 97.447, P < 0.01$). The bulk density decreased from D3 > D2 > D1 > CK, W1 > W2 > W3. The difference in the soil bulk density between CK and W1 was not significant. The soil AWC ranged from 3.43 to 10.46%, and the difference between the precipitation treatments was significant. The AWC in W2 and W3 were significantly higher than those in CK and W1, which were significantly higher than those in D1 and D2; D3 had the lowest AWC (3.43%; $F = 26.891, P < 0.01$). The highest soil TN content in D3 was 0.23%, followed by D1 and CK, which were 0.15 and 0.16%, respectively. D2, W1, W2, and W3 were significantly lower than D3, which were 0.1, 0.09, 0.12, and 0.11%, respectively. There was no significant difference in soil AHN, which was between 38.22 and 50.05 mg·kg$^{-1}$, under each treatment; it was clear that the average content of AHN in water-reducing treatments were slightly higher than those in water-increasing treatments. Soil AHN in CK was the highest (50.51 mg·kg$^{-1}$), and the average content of AHN in water reduction treatments were slightly higher than that in water increase treatments, but the difference was not significant.
**FIGURE 2** | (A) Ecosystem respiration rate and (B) Rs rate under different precipitation treatments; 25% reduction (D1), 50% reduction (D2), 75% reduction (D3), control (CK), 25% increase (W1), 50% increase (W2), and 75% increase (W3). "n" denotes the number of measurements. Different letters (a, b, c, d, and e) represent the significant differences at $p < 0.05$. 
ST and VWC

The variation trend of ST and VWC under precipitation treatments was consistent during the experiment period (Figure 5). The ST increased gradually from July 16 to August 21, reached the maximum value (D3; 21.15°C) on August 21, and then, showed a decreasing trend and dropped to the minimum value (W1; 9.25°C) on October 17 (Figure 5A). Soil VWC showed two peaks in July–August, i.e., 18 and 17% on July 31 and August 31, respectively. Subsequently, soil VWC gradually decreased and reached its minimum value (D2; 4%) on October 17 (Figure 5B). The mean ST and VWC in CK were 15.86°C and 11.7%, respectively. The soil VWCs in D1, D2, and D3 decreased by approximately 15, 51, and 45% compared with the CK plot, respectively. The mean STs of D2 and D3 increased by approximately 2.4 and 12.5%, respectively. The soil VWC in W1, W2, and W3 plots increased by approximately 6.2, 14.3, and 8.6%, respectively. Compared with CK, the mean STs of W1, W2, and W3 decreased by approximately 6, 5.4, and 4.9%, respectively.

Relationship Between Environmental Factors and Re and Rs

Correlations Between Environmental Factors and Re and Rs

Aboveground biomass was positively correlated with Re and Rs (Figure 6) and accounted for 69.8% of the variations in Re; however, the positive correlation between AGB and Rs was not significant. The linear fitting results showed a significant linear relationship between the VWC and Re and Rs, with $R^2_{adj}$ of 0.4 and 0.152, respectively (Figure 7A). Soil temperature and Re and Rs—the latter two of which first increased with the increase of ST—were fit by a quadratic linear model. When the ST reached about 15°C, Re and Rs began to decrease (Figure 7B). In addition, soil bulk density was significantly negatively correlated with Re and Rs, with $R^2_{adj}$ reaching 0.694 and 0.728, respectively (Figure 8A). Absolute water content was significantly positively correlated with Re and Rs, the $R^2_{adj}$ reached 0.767 and 0.798, respectively (Figure 8B). The TN had a weak negative correlation with Re and Rs significantly (Figure 8C). Alkaline hydrolysis nitrogen had a weak negative correlation with Re significantly, and AHN had no significant negative correlation with Rs (Figure 8D).

Multiple Linear Regression Analysis

Based on the multiple linear regression results, expressions (3) and (4) showed that the main factors affecting Rs were ST and AWC, the regression coefficient showed that ST and Rs had an inverse growth relationship and increases in ST may have inhibited Rs. Absolute water content and Rs grew in the same direction. The main factors affecting Re were ST, AHN, and AWC. Soil temperature and AHN showed negative growth with Re while AWC showed positive growth with Re.
**FIGURE 4** | Aboveground biomass (AGB) in the precipitation treatments from July to August; 25% increase (W1), 50% increase (W2), 75% increase (W3), control (CK), 25% reduction (D1), 50% reduction (D2), and 75% reduction (D3). Different letters (a, b, c, and d) indicate significant differences at $p < 0.05$.

**FIGURE 5** | Daily mean (A) soil temperature (ST) and (B) moisture in each precipitation treatment; 25% increase (W1), 50% increase (W2), 75% increase (W3), control (CK), 25% reduction (D1), 50% reduction (D2), and 75% reduction (D3).
FIGURE 6 | Relationships between AGB and Re and Rs. Each point is the average value through time. *p < 0.05 and **p < 0.01.

FIGURE 7 | Relationships between volumetric water content (VWC), Rs and Re (A); Relationships between ST and Rs and Re (B). **p < 0.01.
DISCUSSION

Effects of Precipitation Changes on Re and Rs Characteristics in the Growing Season

Moisture is often the most important limiting factor in the alpine regions (Green et al., 2019; Wang Y. et al., 2020). When the precipitation changes, the environment in the study area will change accordingly, thus affecting the release of carbon (Liu et al., 2009; Carbone et al., 2011; Matías et al., 2011; Correia et al., 2012). Many previous studies have indicated highly variable effects of precipitation manipulation experiments on Rs (Beier et al., 2012). It is widely assumed that the precipitation can influence Rs by altering the soil moisture, which directly influences the production and breakdown of the organic matter that forms the substrate for heterotrophic respiration, as well as the physiological processes of roots and microorganisms (Borken et al., 2006). Thus, a decrease in the precipitation is expected to lead to low soil CO$_2$ emissions, and several precipitation exclusion experiments have indeed shown that Rs decreased under decreased precipitation (Miao et al., 2017; Li et al., 2020; Wang J. et al., 2020). In this paper, Re and Rs were between 1.23–2.46 and 0.82–1.32 μmol·m$^{-2}$·s$^{-1}$, respectively, in the growing season (Figures 2A,B). In addition, Re and Rs increased significantly under the increased precipitation treatment (Figure 2), which reflected the difference between the semi-arid ecosystems and forest ecosystems. It is reported that Rs in the subtropical forests showed little response to precipitation increase, even when the precipitation was doubled (Deng et al., 2012). But a large increase of 31% in Rs was reported in the arid and semi-arid grassland with 30% increase in annual precipitation (Liu et al., 2009). The different responses might be attributed to the differences in climate type (temperature and precipitation), soil condition (e.g., infiltration rates, slopes, textures, depths, and impermeable layers), and vegetation (types and covers) at these study sites (Daniels, 2016; Ma et al., 2018; Shi et al., 2020). In turn, reducing the precipitation reduced the Rs, which was consistent with a study in the Mediterranean that decreased the Rs by 11% through 3-year rainfall exclusion experiment (Misson et al., 2010). The study area in this paper was located in the arid and semi-arid alpine region, which was characterized...
by high altitude, scarce year-round precipitation, and sparse vegetation (Green et al., 2019). Water is the main limiting factor of environmental change. So, Re and Rs were very sensitive to the changes in precipitation. Increased precipitation increases the soil moisture, enhances substrate supply, and stimulates increased root and plant biomass and microbial activity, which releases more nutrients from soil organic matter and increases Re and Rs (Liu et al., 2009, 2016; Deng et al., 2017; Song et al., 2019). This is consistent with the response of Re and Rs of many grassland ecosystems to precipitation change (Chen et al., 2013; Wang et al., 2019). Differing from a study on a subtropical forest (Deng et al., 2012), the effect of precipitation on Rs was offset by the decrease of ST sensitivity, and the slight increase of soil microbial biomass and AGB had no significant effect on Rs and Re. In addition, combining the precipitation treatment with the increase and decrease in Re and Rs rates, it could be concluded that Re was more sensitive to the increased precipitation, and Rs was more sensitive to the reduced precipitation (Figure 3). This is because there are many strong winds weather in the study area, which make the evaporation strong (Gao, 2016). Therefore, when the precipitation increases slightly, the evaporation of soil surface water is more vigorous, and only a little part of it infiltrates, so the Rs will not be greatly improved. As for Rs in this study area, reduced precipitation increased the scarcity of water in the arid soil, which would seriously inhibit microorganism and root activities and the transformation of soil organic matter, leading to a significant decline in Rs (Wani et al., 2013; Wang and Wang, 2017). When the increments of water reached 50 and 75%, the sufficient soil moisture stimulated the increase of AGB and captured soil water; therefore, Re increased significantly (Flanagan and Johnson, 2005; Wang et al., 2021). Our results suggest that if the precipitation increases in the future, the Re in the study area may significantly increase, and Rs may not notably increase. Whereas, if less precipitation occurs in the future, Rs may significantly decrease.

**Relationships Among Re, Rs, and Environmental Factors Under Precipitation Changes**

The precipitation changes affect the soil CO₂ flux by changing the soil micrometeorological conditions, such as soil moisture and ST (Yang et al., 2017). Generally, with the increase of precipitation, soil moisture will increase, and the availability and mobility of soil soluble organic matter will be enhanced. The increase of organic matter will promote microbial activity and provide sufficient substrate for microbial reproduction, thus increasing Rs (Chen et al., 2014). At the same time, the higher soil water content is conducive to the growth of plant roots and stems, promotes photosynthesis of aboveground plants, and allocates photosynthates to the carbon emission in the rhizosphere. Microbial populations will use this carbon source and the microbial respiration rate will be higher, thus enhancing the Re (Smith et al., 2014). Therefore, soil VWC and AWC were significantly positively correlated with Re and Rs (Figures 7A, 8B). Our study found that increases in ST do not always increase CO₂ emissions. When the ST exceeded 15° C, Re and Rs tended to decrease (Figure 7B). The response of Rs to temperature is not invariable. The sensitivity of Rs to temperature increase will reduce when the temperature further increases or prolongs the high temperature (Oechel et al., 2000; Tang et al., 2018), which leads to the decrease of the amount of Rs with the rising of temperature, showing the adaptation of Rs to temperature. Previous scholars also called this phenomenon the temperature saturation of Rs (Qi, 1994). The polyethylene plates used in this study prevented heat loss, resulting in STs in the decreased precipitation being higher than that in the increased precipitation. In addition, the soil moisture of decreased precipitation was lower, so Rs and Re were inhibited. This was particularly evident in D3, in which the ST was high but respiration was weak (Figures 5A, 7B).

The TN content in soil reflects the status of soil nitrogen cycle and is an important index to measure soil fertility and evaluate soil resources (Buondonno et al., 1997). Soil alkali-hydrolyzed nitrogen, also known as soil available nitrogen (Wang, 2020), is the sum of ammonium nitrogen, nitrate nitrogen, amino acids, amides, and easily hydrolyzed protein nitrogen (Zhang, 2020). Compared with soil TN, AHN can better reflect the seasonal or recent nitrogen supply capacity of soil (Liu and Huang, 2005), and is one of the important indexes to measure the soil fertility (Meng et al., 2021). It is reported that the net nitrification rate and nitrate leaching in the surface soil increased significantly under summer rainfall enhancement treatment, while the total organic nitrogen content in the soil decreased significantly (Schaeffer et al., 2013). Increasing rainfall will increase soil moisture, promote inorganic nitrogen leaching, denitrification, plant and microbial nitrogen uptake, accelerate soil nitrogen mineralization rate, and reduce soil inorganic nitrogen content. On the contrary, the decrease of precipitation will lead to the decrease of soil moisture, the inhibition of plant and microbial activities, the decrease of soil nitrogen mineralization rate and N₂O emission, and the increase of soil inorganic nitrogen content (Cregger et al., 2014; Ju et al., 2016). This is consistent with the results of this study. We found that the TN content of soil under water increasing treatment was lower, while that under water reducing treatment was higher, especially D3. The mean value of alkali-hydrolyzed nitrogen under water reduction treatment was slightly higher than that under water increase treatment (Table 1). In addition, carbon and nitrogen cycles are easily decoupled under the drought conditions, which affect the ecological environment and productivity. A study showed that the soil inorganic nitrogen content increased by five times compared with the control after summer drought treatment in the Grassland of the United States. However, due to the limitation of soil water, the above-ground and underground biomass and soil CO₂ emission were significantly lower than the control, indicating that the decoupling of carbon and nitrogen cycle may occur (Evans and Burke, 2013). Additionally, there are short-term experiments that show that the precipitation pulses lead to carbon and nitrogen decoupling. In arid ecosystems, the precipitation pulses stimulate the microbial activity and AGB synchronously, but stimulate nitrogen loss (Dijkstra et al., 2012). In our study, it was found that the soil nitrogen (TN and AHN) content was lower under increased precipitation...
and was negatively correlated with Re and Rs (Figures 8C–D). The soil nitrogen content was higher, but the AGB, Re and Rs were smaller under decreased precipitation. This may be due to the decoupling of carbon and nitrogen cycle under drought conditions that reduces Re and Rs, which may also represent a trend of decoupling of carbon and nitrogen.

The AGB could indicate Re and Rs rates to a certain extent, and precipitation played an important role in the change in the AGB (Ji et al., 2016; Zhao et al., 2016b). The higher AGB and canopy coverage of the increased precipitation treatment may have inhibited surface evaporation, so the Re and Rs rates corresponding to the more abundant AGB were also larger (Gang et al., 2018). At the same time, Re and Rs were strong, which reflected the strong activity of plants and microorganisms and the more efficient use of water and nutrients. Abundant AGB and strong plant metabolic capacity increase the rates of Re and Rs (Tiwari et al., 2021). Therefore, the AGB was positively correlated with Re and Rs (Figure 6).

Soil bulk density reflects the smoothness of Rs discharge channels. Soil is porous and CO$_2$ released by roots and soil microorganisms gathers in the pores and is then gradually released into the atmosphere in accordance with the physical diffusion principle (Ryan and Law, 2005; Fang and Wang, 2007). Due to the lack of water in the soil under the decreased precipitation, the water-stable aggregates were reduced, resulting in decreased water and air permeability which hindered the smooth discharge of gas. The soil bulk density was lower with increased precipitation than in the D1, D2, especially D3 (Table 1); however, Re and Rs were higher under increased precipitation treatment (Figure 8A). This is so because the soil under the increased precipitation treatment was more conducive to the entry of O$_2$ required by heterotrophic respiration and the discharge of CO$_2$ due to respiration (Cao et al., 2004; Dong et al., 2005; Xu et al., 2012). Low soil bulk density was associated with higher porosity, resulting in higher availability of soil oxygen to improve the respiration rate, which was consistent with the previous findings (Chen et al., 2010; Wani et al., 2013). Multiple linear regression analysis showed that ST and AWC could explain 90.6% of Rs, while ST, AWC, and AHN could explain 89.4% of Re (Tables 2, 3), which indeed indicated that the soil moisture and temperature were the most important factors in the alpine and cold areas (Tang et al., 2018; Green et al., 2019). Factors, such as AGB and soil bulk density may also be induced by precipitation change. The significant effect of AHN on Re may be related to the internal mechanism of carbon and nitrogen cycle decoupling, which is an interesting and novel discovery, but it needs further research to be more clear (Evans and Burke, 2013).

LIMITATION OF THE STUDY

In this study, we selected an alpine grassland ecosystem in the north of Qinghai–Tibet and tested the effects of increased and decreased precipitation on Re and Rs. One shortcoming of the experimental design was that Re and Rs were only measured during July–October 2020 (i.e., in the peak growing season and at the end of the growing season). If a complete precipitation control experiment for a year or more is conducted, the variation characteristics and differences of Re and Rs in each gradient under the difference between precipitation and snowfall can be analyzed simultaneously (Chen et al., 2017), as well as the effects of freeze-thaw cycle and plant growth stages on Re and Rs (Lu and Liao, 2015; Gao et al., 2021). Soil respiration includes root respiration, soil microbial respiration, and soil animal respiration, but not aboveground plants. However, the respiration of the plants includes aboveground plants and belowground root respiration different from that Rs. Another limitation was that the chosen plot with PVC collars was too small in area to represent the overall field plot, in spite of choosing the plot of relative uniform conditions. Otherwise, due to the frequent rainfall in study site, we had to delay the measurement schedule resulted in irregular interval days for the 16 times. It might influence the evaluation of mean Re and Rs (Zhang et al., 2014). Although we tried to minimize the influence of clipped aboveground plants respiration by removing grass in advance and correcting the influence of changed soil environments on Rs in the PVC collars, there were still some biases of the dynamic chamber method should be noted in partitioning Rs (Subke et al., 2006; Zhang et al., 2016). The microbial activity, litter, and roots also play a crucial role in the changes in Re and Rs. To acquire the changes of microbial activity, litter, and roots respiration, it is necessary to divide detailed components of Re and Rs to compare. In the future research, we will include these indicators in the next step of our research and focus on analyzing their potential influence mechanisms on Re and Rs. Therefore, the inferences regarding the differences between Re and Rs in response to the precipitation changes in alpine steppe ecosystems should be carefully interpreted. The studies in multiple growing seasons are needed to verify the response characteristics of the Re and Rs to provide a theoretical basis for further predicting carbon emissions under the trend of precipitation change.

CONCLUSION

In summary, our research showed that the increased precipitation treatments increased Re, Rs, and AGB. Furthermore, when precipitation was increased by 50% compared with ambient precipitation, Re and Rs were the highest, whereas, when precipitation was decreased, the Re and Rs in D3 (75% decrease) were the smallest. This proved that hypothesis 1 was not valid. The Rs and Re were stronger with moderate increase in the water levels than with excessive increase, but respiration was the weakest with excessive decrease in the water levels. Ecosystem respiration was more sensitive to the increased precipitation and Rs was more sensitive to the decreased precipitation, which indicated that hypothesis 2 was applicable in our study area. Soil temperature and AWC were the main factors differentiating Re and Rs responses under different precipitation gradients. Alkaline hydrolysis nitrogen was also the dominant factor causing Re differences and may be involved in the decoupling of carbon and nitrogen cycles. The precipitation changes caused significant changes in ST, soil moisture, and soil physicochemical properties (i.e., bulk density, VWC, AWC, TN, and AHN) in the 0–10 cm soil layer had significant effects on the Re and Rs under different precipitation treatments.
DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

XL carried out the measurement of Rs and Re, collected soil samples, assisted in the determination of soil physical properties, acquired, collected, and analyzed the experimental data, and wrote manuscripts. As the applicant of this natural science fund project, YY obtained financial and instrument support, designed the site layout of the precipitation control experiment, assembled the ST and humidity monitor, guided the installation of polyvinyl chloride soil collars, guided and assisted the collection of soil and plant samples, discussed data analysis, and revised the manuscript. LF helped complete the layout of the experimental site. All the authors made important contributions to this study.

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