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Responses of Soil Respiration to the Interactive Effects of Warming and Drought in Alfalfa Grassland on the Loess Plateau

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Abstract: Elevated temperature and frequent drought events under global climate change may seriously affect soil respiration. However, the underlying mechanism of the effects of warming and drought on soil respiration is not fully understood in the context of the Loess Plateau. This study examined the response of soil respiration (Rs) to multiple factors, including warming (W), drought (P), and their interaction (WP), in the semi-arid grassland of the Loess Plateau in Northwest China. The research period was from May to November 2022, with an open-top heating box used for warming and a rain shelter used for drought. The results showed the following: (1) Rs ranged from 1.67 μmol m^{-2} s^{-1} to 4.77 μmol m^{-2} s^{-1}, with an average of 3.36 ± 0.07 μmol m^{-2} s^{-1}. The cumulative soil carbon flux ranged from 500.97 g C·m^{-2} to 566.97 g C·m^{-2}, and the average cumulative soil respiration was 535.28 ± 35.44 g C·m^{-2}. (2) Warming increased Rs by 5.04 ± 3.11%, but drought inhibited Rs by 3.40 ± 3.14%, and the interaction between warming and drought significantly reduced soil respiration by 11.27 ± 3.89%. (3) The content of particulate organic carbon (POC), dissolved organic carbon (DOC), soil organic carbon (SOC), and readily oxidized carbon (ROC) decreased with the increased soil depth. ROC after W and WP treatments was significantly higher than that of the control, and POC after P treatment was significantly higher than CK ($p < 0.05$). (4) The seasonal variation of soil respiration was positively correlated with soil temperature, soil water content, plant height, and leaf area index ($p < 0.05$), but the response rules differed during different regeneration periods. Soil water content; soil water content and leaf area index; and soil water content, soil temperature, and leaf area index were the factors that regulated the variation in soil respiration in the first, second, and third regeneration periods, respectively. These results clearly showed the limiting effect of drought stress on the coupling between temperature and soil respiration, especially in semi-arid regions. Collectively, the variations in soil respiration under warming, drought, and their interactions were further regulated by different biotic and abiotic factors. Considering future warming, when coupled with increased drought, our findings indicate the importance of considering the interactive effects of climate change on soil respiration and its components in arid and semi-arid regions over the next decade.

Keywords: warming; drought; soil respiration; water limited; semi-arid grassland

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1. Introduction

Global climate change, characterized by temperature elevation and altered rainfall patterns, poses a significant threat to the sustainable development of human society [1].

According to the report of the Intergovernmental Panel on Climate Change (IPCC), global climate change will continue to intensify, global temperatures will continue to rise, arid regions will expand, and extreme drought events caused by intense precipitation will increase in the future [2,3]. These changes have severe implications for the carbon cycle of terrestrial ecosystems. Soil respiration, as the second largest carbon flux between the atmosphere and terrestrial ecosystems, is an important regulator of the global carbon cycle and climate change [4]. Even slight changes in soil respiration can accelerate or decelerate the atmospheric carbon dioxide concentration, thus influencing the global carbon balance. Therefore, it is essential to study the increase in temperature and drought under climate change on soil carbon dynamics.

Soil respiration (Rs) mainly includes two components: autotrophic respiration (root respiration and rhizosphere microbial respiration) and heterotrophic respiration (soil microbial respiration and animal respiration) [5]. Rs is independently or synergistically regulated by several biotic and abiotic factors, including temperature, soil water content, substrate supply, aboveground biomass, enzymatic activity, and microbial community [6–10]. With the continuous intensification of the global climate, variations in temperature and moisture will have an interactive effect on Rs and its components to a certain extent, thus affecting the global carbon cycle [11,12]. Numerous simulation experiments conducted over the past two decades have demonstrated highly variable and complex responses of Rs to warming and drought. Studies have indicated that warming will lead to short-term increases, no effects, or negative effects on Rs in different grassland ecosystems [13,14]. Similarly, drought can significantly inhibit Rs, but it can also stimulate Rs [15–19]. The apparent inconsistency in these responses can be attributed to the interactions between biotic and abiotic factors [8–10]. For example, warming can increase microbial activity and soil organic carbon decomposition, releasing more carbon dioxide into the atmosphere [20]. Changes in precipitation can stimulate or inhibit plant photosynthesis and respiration by changing the soil environment and nutrient status [21,22]. In addition to the single factor effects, Rs is also influenced by the interaction of multiple global changes [23]. For instance, increased precipitation can compensate for the reduction in soil moisture caused by warming, while drought exacerbates soil moisture loss under warming [24]. However, to date, few experiments have investigated these two global change factors on carbon flux in the Loess Plateau ecosystem. Clearly, further understanding is needed, especially in relation to the interaction between warming and drought in arid and semi-arid regions.

The Loess Plateau in China represents the largest loess accumulation area globally [25], and it is also an important component of China's three zones and four belts ecological barrier. Grassland is the most typical vegetation type in this region, accounting for approximately 42% of the total area. The carbon storage of the ecosystem is approximately 1.09–1.46 Pg C [26,27]. However, despite being China's big carbon (C) pool, the Loess Plateau suffers from severe soil erosion and degradation, making it one of the most critically affected regions worldwide [28]. Therefore, it plays a crucial role in the study of the global soil carbon cycle and climate change. Most of the Loess Plateau constitutes a semi-arid region with low precipitation, low soil water content, low vegetation coverage, and frequent extreme climatic events [29]. In order to restore the regional ecological environment, a large-scale project of converting farmland back to forests and grasslands has been implemented since 1999 [30]. Artificial grassland, the primary vegetation type resulting from the farmland conversion project on the Loess Plateau, possesses certain advantages in accelerating vegetation restoration and enhancing ecological stability [31]. However, because of the influence of a natural environment background, the recovery capacity is limited; the ecosystem in this area is extremely fragile, highly sensitive to changes in the external environment, and particularly vulnerable to global climate change [32]. Under the interactive effects of global changes, such as regional climate warming and frequent extreme droughts, soil carbon emissions from artificial grassland ecosystems on the Loess Plateau have attracted widespread attention. Understanding the trends and driving factors of Rs in artificial grassland ecosystems is of great significance for comprehending the regional carbon cycle and the conversion of ecosystem carbon sources and sinks on the Loess Plateau [30].

We aimed to investigate the response of Rs of artificial grassland in the Loess Plateau to global climate change, especially the response of climate warming and extreme drought events. We selected alfalfa, the dominant grass species in the Loess Plateau, to simulate field experiments of warming and extreme drought. The research period was from May to November 2022, with an open-top heating box used for warming and a rain shelter used for drought. Our objective was to address two questions: (1) How does soil C flux respond to warming, drought, and their interactions, whether independent or interacting? (2) What is the mechanism of regulating this response? We hypothesized that the (1) interaction between warming and drought significantly reduced soil respiration, and (2) the variation in soil respiration under warming, drought, and their interactions is regulated by different biotic and abiotic factors.

2. Materials and Methods

2.1. Study Area

This study was conducted at the National Field Scientific Observation and Research Station of Grassland Agroecosystem in Qingyang, Gansu Province. The experimental station is located in Shishe Township, Xifeng District, Qingyang City, Gansu Province (35°39′ N, 107°51′ E, altitude of 1297 m, Figure 1). The area is affected by a temperate semi-arid continental monsoon climate. The average precipitation for many years (1981–2020) is 537.5 mm, the average annual temperature is 9.4 °C, and the frost-free period is 150 days. In summer, the temperature is high and the rainfall is rainy. The rainfall in July increases, and the rainy season is mostly concentrated between September and October. The annual variation in rainfall is large. The regional soil type is dark loessial soil, and the pH value is between 8.0–8.5.

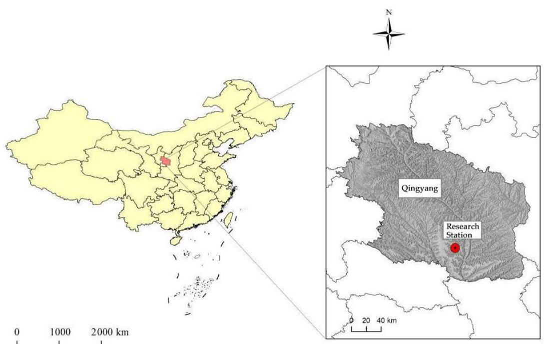

Figure 1. Location map of the research area.

2.2. Experimental Design

In order to simulate the process of Rs under warming and drought conditions, a completely randomized block design was used in this study. Two factors (warming and drought) and four treatments were set up: control (CK), warming (W), drought (−50% precipitation, P), and warming × drought (warming and −50% precipitation, WP). Each treatment was set up with four replicates, a total of 16 experimental plots, each plot area 4 m × 4 m (16 m^2), and 1 m spacing between treatment plots (Figure 2). It is convenient for regular manual monitoring to avoid the experimental error caused by the interference of the test to the adjacent cells. To control the experimental variables, the influencing variables, such as vegetation type and nutrient level in the sample plot, were controlled equally.

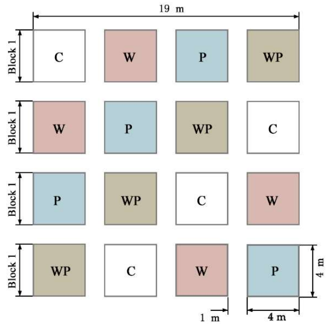

Figure 2. Layout of the experiment design.C, control treatment; W, warming treatment; P, drought treatment by reducing 50% precipitation; WP, combined warming and drought treatment.

In order to simulate the effect of warming on Rs caused by climate warming, an open top chamber (OTC) was set up in the experiment, which could provide warming to the enclosure throughout the entire year. The material was acrylic organic glass with a transmittance of more than 90%, which was a hexagonal truncated cone. After the warming treatment, the temperature increased by approximately 0.6 °C. According to the semi-arid climate characteristics and change trend in the Loess Plateau, we proposed achieving drought treatment by reducing the precipitation by 50%. The effect of a drought was achieved by installing a canopy to cover 50% of the area of the test area. The height of the canopy from the ground was 1.30–1.60 m. The 20 cm wide tile-shaped transparent groove plate was used to intercept the rainwater and flow it into the rainwater collection device through the PVC sink and catheter. At the same time, 60 cm deep PVC plates were buried around the sample, avoiding the influence of the lateral transport of soil moisture between small areas. In order to eliminate the difference between the treatments caused by the light transmittance of the PVC pipeline in the canopy, the canopy was also placed in the area without drought treatment, and the pipeline was inverted to achieve the effect of natural precipitation. After treatment, the soil moisture significantly decreased and the drought effect was significant.

2.3. Measurement Protocols

Among the Rs measurement plots, a PVC collar (20 cm in diameter and 10 cm in height) was inserted into the soil to a depth of 5 cm at the center of each plot for measuring the soil CO_2 efflux. Since May 2022, the CO_2 efflux was measured using a LI-6800 (LI-COR, Lincoln, NE, USA). Rs was measured twice a month in the growing season and once a month in the non-growing-season during the study period. All of the soil respiration measurements were carried out between 09:00 and 11:00 a.m. (local time). Each treatment took roughly 1–2 min.

2.4. Soil Temperature and Moisture Measurements

The Ts and VWC at the 10 cm depth were measured simultaneously with the soil respiration rates using the 8150–203 soil temperature probe and GS1 soil moisture sensor (LI-COR, Lincoln, NE, USA), respectively. Meteorological data (Ta, PAR and precipitation) were recorded every half-hour using a PC200W automatic meteorological station (Campbell Scientific, Logan, UT, USA) placed within 50 m of the experimental field.

2.5. Aboveground Biomass and Soil Carbon Fractions

The alfalfa plant height and leaf area index were measured simultaneously using soil respiration. The plant height was measured by taking 10 plants that were randomly selected by tape, and the average values were taken. LAI was measured using LAI2000 (LI-COR, Lincoln, NE, USA). The alfalfa was cut on 29 June and 26 August when the late bud to flowering stage was 10%. Therefore, the beginning of the growing season to 29 June was defined as regeneration period 1, 29 June to 26 August was regeneration period 2, and 26 August to the end of the growing season was regeneration period 3. Moreover, 0–10 cm, 10–20 cm, 20–30 cm, and 30–50 cm soil layers were collected for the soil carbon component determination in September 2022. The soil organic carbon (SOC) and particulate organic carbon (POC) were determined using the potassium dichromate volumetric method and external heating method. Soil microbial biomass carbon (MBC) was extracted by chloroform fumigation using K_2SO_4, readily oxidized carbon (ROC) using the $KMnO_4$ oxidation method, and dissolved organic carbon (DOC) was determined by ultraviolet spectrophotometry.

2.6. Data Analysis

Repeated-measure ANOVA tests were used to test the effects of warming and drought on Rs and soil carbon fractions. Significant differences were evaluated at the $p < 0.05$ level. Duncan's method was used to compare the differences between treatments. Exponential and linear fittings were used to evaluate the relationships between soil respiration and abiotic and biotic factors (soil temperature and moisture, H and LAI). We used $(WP - P)/P \times 100\%$ and $(W - C)/C \times 100\%$ to represent the effect of drought on soil warming (relative variation, %). The drought effects with and without soil warming were $(WP - W)/W \times 100\%$ and $(P - C)/C \times 100\%$, respectively [33].

A statistic equation was used to calculate the cumulative repiration:

$$X = \text{Rs} \times 3600 \times 24 \times 12 \times 10^{-6} \quad (1)$$

$$CO_2 - C(g{\cdot}m^{-2}) = \sum_{i=1}^{n} Xi \quad (2)$$

where X is daily soil respiration, Rs is measured soil respiration (μmol m^{-2} s^{-1}), 12 is the molar mass of $CO_2 - C$(g mol^{-1}), 3600 and 24 are conversion coefficients of time, i is the first measurement of soil respiration rate, and n is the monitoring number.

The relationships between soil respiration and abiotic and biotic factors (soil temperature, soil moisture, and AGB) were evaluated using exponential and quadratic fitting. All of the statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Origin (version 21.0) was used for plotting.

3. Results

3.1. Changes in Environmental and Biomass Factors

During the experimental period, air temperature (Ta) and rainfall exhibited significant seasonal patterns (Figure 3). Ta gradually increased from winter to summer, followed by a decline after reaching the peak daily maximum temperature of 26.6 °C in August, with an average annual temperature of 10.0 °C, which was about 0.4 °C higher than the long-term average (9.7 °C, from 1970 to 2021). During the study period, the seasonal variation of precipitation was large; the period from June to October received the most annual precipitation (~82.2%), with winter and early spring mostly without precipitation. Abnormal lower precipitation in August led to summer drought. The annual cumulative precipitation was 432.9 mm, which was approximately 20.4% lower than the long-term average (544.0 mm, from 1970 to 2021).

The overall seasonal pattern of soil temperature (Ts) was not significant due to unusual temperature fluctuations caused by abnormal temperature rise in spring and concentrated precipitation in June and late July (Figure 4A). Except for a slight increase in September, the temperature gradually decreased after August. Ts showed no significant difference between treatments ($p > 0.05$, Figure 4A). The soil volumetric water content (VWC) exhibited three peaks on 28 June, 24 July, and 5 September, with the highest point occurring on 24 July (Figure 4B). Under drought treatment, VWC was significantly lower than in the control and warming treatments ($p < 0.05$). The seasonal patterns in alfalfa height and leaf area index (LAI) were similar throughout the experimental period, with both significantly decreasing after being mowed twice. Lodging occurred when the alfalfa reached a certain height, leading to a divergence between LAI and alfalfa height on 21 August and 18 October (Figure 4C,D). Although there was no significant difference between treatments ($p > 0.05$), the growth of alfalfa under warming was higher than that under other treatments in the same period.

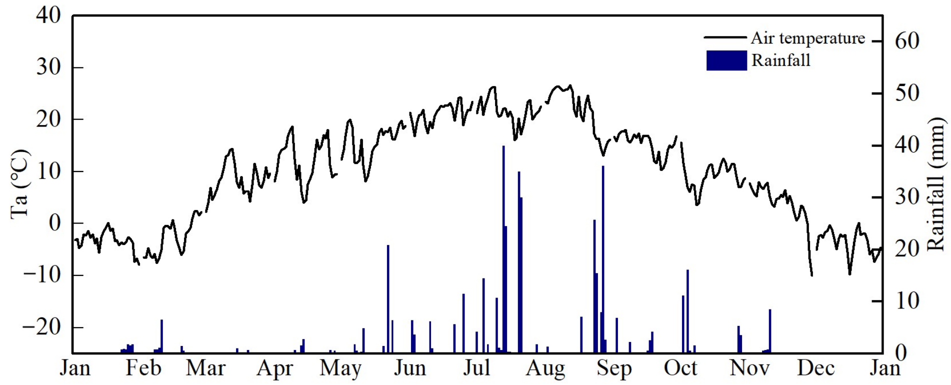

Figure 3. Seasonal variation of air temperature and rainfall in 2022.

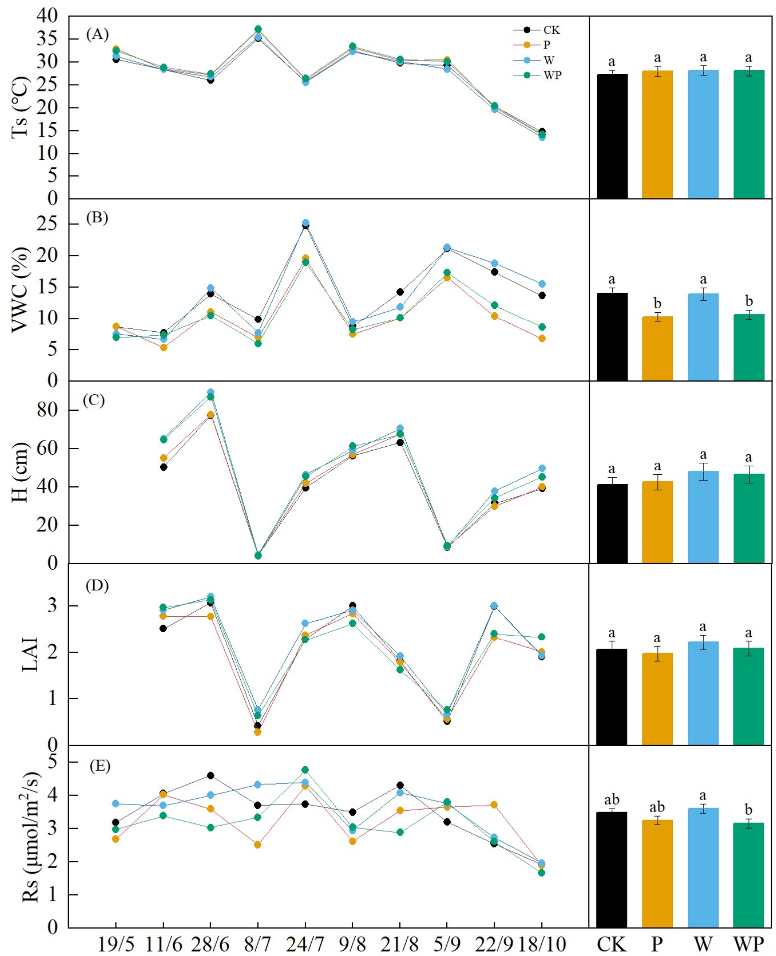

Figure 4. The seasonal variations and differences among treatments in soil temperature (Ts) (**A**), soil volumetric water content (VWC) (**B**), alfalfa height (H) (**C**), leaf area index (LAI) (**D**), and soil respiration(Rs) (**E**). CK, control treatment; P, −50% precipitation treatment; W, warming treatment; WP, combined warming and −50% precipitation. Different letters represent significant difference at $p < 0.05$.

3.2. Variation in Soil Respiration

Soil respiration (Rs) exhibited distinct seasonal changes, following a single–peak trend. It gradually increased at the beginning of the growing season and decreased after August, with two exceptions on 8 July and 9 August when a declining trend was observed (Figure 4E). Overall, there was a significant difference in Rs between W and WP throughout the growing season ($p < 0.05$). Warming generally stimulated Rs, while plots subjected to drought treatment displayed lower soil carbon emissions compared with the control.

Soil warming substantially stimulated Rs by 5.04% under ambient precipitation (W–C), but suppressed it when drought occurred (WP–P, Figure 5). Drought decreased Rs by 3.40% under ambient temperatures (P–C), and decreased it by 11.27% under warming conditions (WP-W, Figure 5).

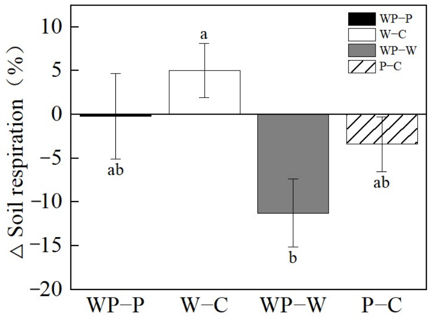

Figure 5. Relative changes (%) in soil respiration (Rs), induced by drought without soil warming (P–C) and with soil warming (WP–W), and by soil warming without throughfall reduction (W–C) and with throughfall reduction (WP–P) in 2022. Different letters represent significant difference at $p < 0.05$.

Over the experimental period, the average cumulative soil respiration was 535.28 g C·m^{-2}. Warming enhanced cumulative soil respiration, while P and WP limited cumulative soil respiration (Figure 6). Cumulative soil respiration was 7.48% and 13.17% higher in the W treatment compared with P and WP, respectively (Figure 6). Throughout the entire growing season, cumulative soil respiration was significantly lower in WP compared with W ($p < 0.05$), while no significant difference was observed between CK and P ($p > 0.05$).

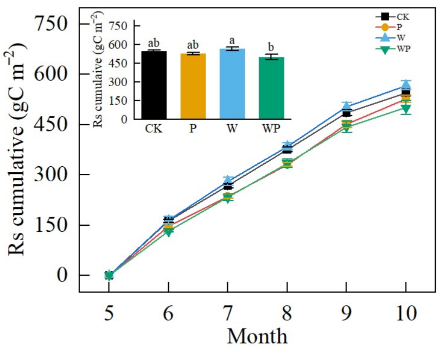

Figure 6. Cumulative soil CO_2 efflux (Rs) from May to October 2022 under the four treatments. Different letters represent significant difference at $p < 0.05$.

3.3. Variation in Soil Carbon Fractions

The changes in particulate organic carbon (POC), dissolved organic carbon (DOC), soil organic carbon (SOC), and readily oxidizable carbon (ROC) in different soil depths in 2022 were analyzed (Figure 7). In general, the soil carbon content decreased with the change in soil depth. W, P, and WP increased POC, with P significantly higher than CK ($p < 0.05$, Figure 7E). No significant differences were observed in DOC between treatments in terms of the depth and total content ($p > 0.05$), although DOC was higher under drought stress compared with the control (Figure 7B,F). SOC did not vary significantly, except for WP, which exhibited significant differences compared with W and P at the 20–30 cm depth range ($p < 0.05$). SOC remained relatively stable across different depths (Figure 7C,G). WP had the highest content of ROC, followed by W (Figure 7D,H), and WP and W were significantly higher than that of the CK and P treatments ($p < 0.05$).

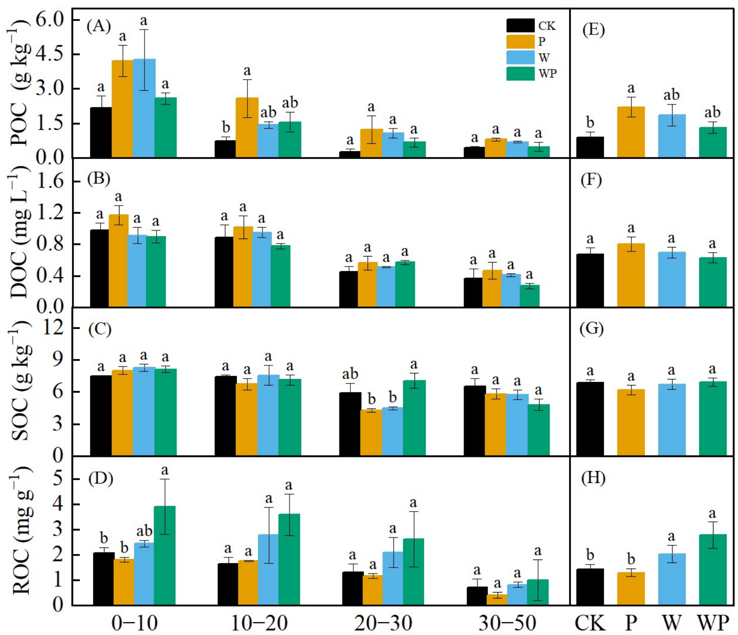

Figure 7. Layered content of particulate organic carbon (POC) (**A**), dissolved organic carbon (DOC) (**B**), soil organic carbon (SOC) (**C**), and readily oxidized carbon (ROC) (**D**). Differences in POC (**E**), DOC (**F**), SOC (**G**), ROC (**H**) among treatments. Different letters represent significant difference at $p < 0.05$.

While there were no significant differences in microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) among treatments ($p > 0.05$), W and WP decreased MBC compared with CK, P increased MBC but had no significant effect on MBN, and the WP treatment moderately decreased MBN (Figure 8A). The soil microbial biomass carbon-to-nitrogen ratio (MBC/MBN) did not vary significantly among the different treatments ($p > 0.05$), but the MBC/MBN ratios under P and WP were higher than that of CK, while W showed a lower ratio compared with CK (Figure 8B).

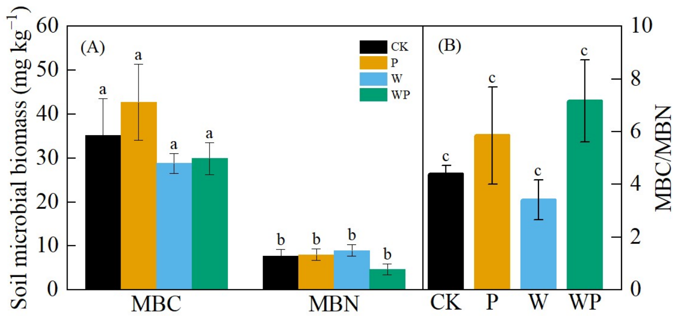

Figure 8. Soil microbial biomass carbon (MBC) and soil microbial biomass carbon nitrogen (MBN) (**A**) and carbon nitrogen ratio value (**B**). Different letters represent significant difference at $p < 0.05$.

3.4. Relationships between Soil Respiration and Environmental Factors

Based on the observed values across all of the treatments, Rs exhibited an exponential increase with Ts ($R^2 = 0.36$, $p < 0.05$, Figure 9A). Rs also showed a linear positive correlation with VWC, H, and LAI ($p < 0.05$, Figure 9B–D).

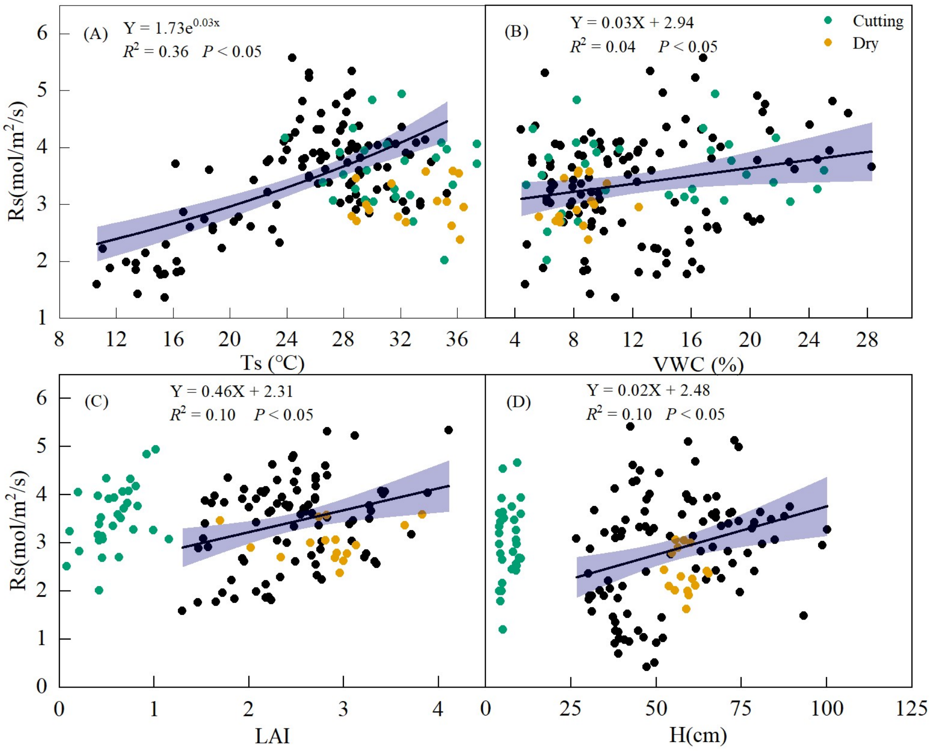

Figure 9. Relationships between soil respiration (Rs) and soil temperature (Ts) (**A**), soil volumetric water content (VWC) (**B**), leaf area index (LAI) (**C**), and alfalfa height (H) (**D**) during the whole growing season. The yellow and green points represent the values of the dry period and the mowing period, respectively. Black dots represent values during normal periods.

The relationship between soil respiration (Rs) and Ts, VWC, and LAI was analyzed using linear models at different regeneration stages (Figure 10). There was a significant negative correlation between Rs and Ts, except during regeneration period 3 ($p < 0.05$). Rs exhibited a linear increase with increasing VWC ($p < 0.05$, Figure 10B,E,H). Rs also showed a linear increase with increasing LAI during regeneration periods 2 and 3 ($p < 0.05$, Figure 8F,I), but no correlation was found during regeneration period 1 (Figure 10C).

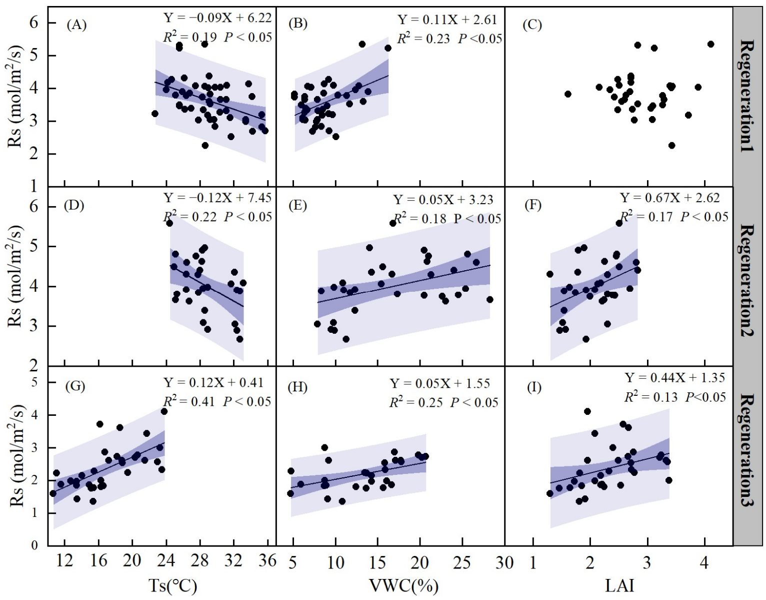

Figure 10. Relationships between soil respiration (Rs) and soil temperature (Ts) in regeneration period 1 (**A**), regeneration period 2 (**D**), regeneration period 3 (**G**). Relationships between soil respiration

(Rs) and soil volumetric water content (VWC) in regeneration period 1 (**B**), regeneration period 2 (**E**), regeneration period 3 (**H**). Relationships between soil respiration (Rs) and leaf area index (LAI) in regeneration period 1 (**C**), regeneration period 2 (**F**), regeneration period 3 (**I**). Regeneration period 1 is from May to June. Regeneration period 2 is from July to August. Regeneration period 3 is from September to October. The blue regions are the confidence band and prediction band, respectively.

4. Discussion

4.1. Variation Characteristics of Soil Respiration

Seasonal dynamics of Rs are widespread in different ecosystems; however, the range in numerical variation may vary depending on the ecosystem type. In our study, Rs were ranged from 1.67 µmol m^{-2} s^{-1} to 4.77 µmol m^{-2} s^{-1} during the growing season, with an average of 3.36 µmol m^{-2} s^{-1}, which was within the range of global grassland soil respiration (0.13 µmol m^{-2} s^{-1}~7.27 µmol m^{-2} s^{-1}) [34]. However, the values obtained in our study were higher than the annual average soil respiration reported in grassland ecosystems (2.05 µmol m^{-2} s^{-1}) and farmland ecosystems on the Loess Plateau (1.7 µmol m^{-2} s^{-1}) [35,36]. This discrepancy could be attributed to the high density of artificial grassland. It is known that root biomass has a significant impact on Rs [13,37]. Therefore, developed dense roots can promote autotrophic respiration and increase Rs. A large amount of aboveground biomass not only meets the substrate supply required for Rs, but also serves as the main source of litter formation, which has a positive effect on the soil organic matter content and the formation of buffering interfaces to regulate the surface microenvironment, thereby stimulating Rs. The study of Moyes et al. (2013) [38] and Mottee et al. (2018) [39] supported these findings. Another factor that may lead to a higher average Rs is a high soil organic carbon content, which is different from long-term cultivated farmland.

4.2. Effect of Warming on Soil Respiration

The response of Rs to warming and its driving factors have been widely studied in various ecosystems. Studies found that warming stimulated Rs in the boreal, temperate, arid, and Mediterranean [14,40–43]. Our results show that warming relatively increased Rs by 5.04%, which was similar to previous studies. Under the warming treatment, the plant height exceeded that of CK, and the increased aboveground biomass contributed to the input of organic carbon from plants to the soil. This increase in organic carbon input, as reflected by the higher content of POC and ROC (Figure 7), suggests that elevated temperatures could accelerate the migration rate of substrate particulate organic carbon and readily oxidizable carbon in the soil [44], thereby promoting soil mineralization and root development to increase the root biomass [13,45]. Additionally, warming led to a slight reduction in the carbon–nitrogen ratio of soil microbial biomass (Figure 8), indicating less nitrogen limitation in the soil. This reduction in nitrogen limitation was manifested by the promotion of underground carbohydrate distribution and increased growth of aboveground and underground plant parts [46]. All of the above are beneficial for increasing the contribution rate of autotrophic respiration to Rs and stimulating Rs. The study by Lu et al. [47] also found that warming significantly enhanced the plant carbon pool in both aboveground and underground parts in a grassland experiment. However, compared with other studies, the increase in Rs under W was not statistically significant ($p > 0.05$, Figure 4E). This lack of significance could be attributed to the fact that warming did not significantly increase Ts ($p > 0.05$; Figure 4A), which was due to high vegetation coverage. In our study station, the density and height of vegetation reduced the duration of solar radiation received by the ground, leading to reduced energy absorption and limited temperature rise.

4.3. Effect of Drought on Soil Respiration

In the context of future climate change, extreme drought and rainfall events are expected to occur more frequently, and their impact on ecosystem processes is often greater than the effects of warming and nitrogen deposition [48,49]. Most studies have shown that drought reduces Rs [46,50]. However, the response of Rs to drought varies across different ecosystems. Studies have shown that drought stress can reduce Rs in mesic and xeric ecosystems [15,16], while stimulating it in aquatic ecosystems [17–19]. In our experiment, drought relatively reduced Rs by 3.4%. This was consistent with previous studies on the semi-arid grassland ecosystem in Inner Mongolia and the Mediterranean ecosystem [14,49,51–53]. In arid and semi-arid ecosystems, water availability is the primary factor influencing the productivity and carbon source for Rs. Therefore, the lack of water causes many microorganisms to enter a dormant state, and restrict root and microbial growth, impede substrate and enzyme diffusion in the soil, limit soil biological activities, and consequently reduce autotrophic and heterotrophic respiration [54,55].

4.4. Interactive Effect of Warming and Drought on Soil Respiration

In most ecosystems (forest, farmland, grassland, wetland, and desert), most climatic factors have a positive effect on Rs, but the average respiration rate of single-factor treatments is lower than that of double-factor treatments [46]. Nonetheless, in our experiment, Rs decreased by 11.27% under WP, aligning with the findings of Zhou et al. (2016) [46] who integrated the effects of 150 global climate factors on Rs. When warming and drought scenarios coexist, water-limited soils generally exhibit reduced sensitivity to temperature changes, with water becoming a limiting factor, particularly in arid and semi-arid regions [56]. Rs becomes more responsive to changes in water availability [57]. Drought stress disrupts the temperature–soil respiration coupling by impeding the diffusion of soluble carbon substrates and extracellular enzymes, thus limiting microbial activity [58]. This will limit heterotrophic respiration. On the other hand, under drought conditions, soil warming further intensifies soil water stress, which may exceed the threshold that is suitable for the metabolism of plant roots, resulting in a decrease in C allocation to belowground [42]. This will reduce autotrophic respiration. Additionally, soil water deficit significantly reduces the temperature sensitivity of Rs [59–61]. In cases where a small degree of warming does not substantially impact the soil moisture's physical and chemical properties, the dominant influence of soil moisture overwhelms the effect of temperature, as observed in several field experiments [49,62,63]. Overall, in future climate change scenarios, not only are changes in the magnitude of climate variables expected, but so as changes in their interannual and interannual variability [1]. The net carbon dioxide emissions from soil to the atmosphere will ultimately depend on the specific balance between warm and drought [64].

4.5. Responses of Soil Respiration to Environmental and Biotic Factors

Numerous studies have emphasized the crucial role of temperature in regulating Rs, with changes in Ts generally accounting for most of the seasonal variation in Rs [65,66]. In our study, a significant exponential relationship between Ts and Rs was observed throughout the growing season ($p < 0.05$), indicating that temperature was the main regulating factor of Rs dynamics. However, positive effects of Ts and Rs were observed during periods of sufficient VWC (Figure 10G), and negative effects were observed during periods of higher temperature (Figure 10A,D). The main reason was that high Ts resulted in increased evapotranspiration and reduced VWC. The negative impact of water limitation offset the positive effect of high temperature and vigorous growth, leading to different responses of Rs to temperature during different periods of regeneration.

In arid and semi-arid ecosystems, VWC is considered one of the most important factors affecting Rs [54,67,68]. Our research results in the semi-arid grassland ecosystem of the Loess Plateau support this conclusion, indicating a positive correlation between VWC and Rs (Figure 9B). The results of different regeneration periods also reinforce this conclusion (Figure 10B,E,H). Similar conclusions were reached in Inner Mongolia grasslands by Dong et al. [69]. Water directly impacts root and microbial processes, thereby influencing soil CO_2 emissions [70,71]. Water limitation in grassland growth can lead to reduced litter input, diminished supply of photosynthate and other residues to roots [72,73], and decreased autotrophic respiration and Rs. Moreover, soil water limitation can impede the diffusion of organic solutes near microorganisms or reduce the microbial population size, resulting in reduced heterotrophic respiration and Rs [55,74,75].

Aboveground and belowground biomass are major biological factors influencing the Rs, as the material source for Rs is derived from photosynthesis, with aboveground biomass representing the primary product of photosynthesis [76]. In our study, Rs significantly increased with the increase in LAI and plant height ($p < 0.05$, Figure 7C,D). There was also a linear positive correlation between Rs and aboveground biomass at regeneration periods 2 and 3 ($p < 0.05$, Figure 10F,I). This is mainly because the increase in aboveground biomass enhanced the photosynthetic capacity, leading to a greater distribution of photosynthetic products to the root system and subsequent increases in belowground biomass. Root respiration is a component of Rs, and the quantity of root biomass directly determines the strength of Rs [77]. Additionally, the input of aboveground litter is crucial for carbon accumulation in the soil. Aboveground biomass facilitates litter accumulation, which promotes microbial growth, decomposition rates, and the synthesis and secretion of extracellular enzymes, all contributing to soil carbon mineralization and subsequent carbon emissions [78]. This conclusion is consistent with findings in the semi-arid grasslands of Hungary, the arid grasslands of Inner Mongolia, and the semi-arid grasslands of the Loess Plateau described in this study [79,80].

5. Conclusions

The conducted operational experiments provide valuable insights into the response of soil respiration to warming and drought. Our findings indicate that warming enhances soil respiration, while drought inhibits it. The interaction between warming and drought results in a significant reduction in soil respiration, emphasizing the limiting effect of drought stress on the temperature-soil respiration coupling, particularly in semi-arid regions. Furthermore, our results highlight the significance of soil temperature, soil water content, plant height, and leaf area index in regulating soil respiration. The exponential model successfully explains the relationship between soil temperature and respiration, while soil respiration demonstrates a linear positive correlation with soil water content, plant height, and leaf area index. These findings hold great importance for vegetation restoration efforts in degraded land within the context of global climate change. The research will provide scientific support for optimizing the carbon sequestration/emission reduction management plan of artificial grassland ecosystems in the Loess Plateau under the interaction of global change in the future. However, the duration of the experiment may be crucial for evaluating the response of the C process to environmental changes, as the effect of global change drivers on soil respiration will significantly change over time. Therefore, in future field experiments, the temporal variation in soil respiration and its components should be considered to prolong our understanding of the feedback of terrestrial carbon cycling on global change.

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