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Short Legacy Effects of Growing Season Nitrogen Addition and Reduced Precipitation alter Soil Respiration during Nongrowing Season

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Abstract: The short legacy effects of growing season nitrogen (N) addition and reduced precipitation on nongrowing season soil respiration (*R*s), autotrophic respiration (*R*a), and heterotrophic respiration (*R*h) are still unclear. Therefore, a field manipulative experiment to determine the responses of nongrowing season *R*s and its components to growing season N addition and reduced precipitation was conducted in a temperate forest. The results show that growing season N addition and reduced precipitation significantly increased nongrowing season *R*s by regulating the response of *R*a and *R*h. The combination of N addition and reduced precipitation also showed a much stronger effect on *R*s and its components, but the magnitude and direction largely depended on the snowpack thickness. The effects of growing season N addition and reduced precipitation on nongrowing season *R*s and its components were mediated by different sampling periods. N addition significantly decreased *R*s by decreasing *R*h in early winter and significantly increased *R*s by increasing *R*a in deep winter and late winter. All treatments decreased temperature sensitivity (Q_{10}) of *R*s and *R*h. Our findings contribute to a better understanding of how nongrowing season *R*s and its components will change under growing season N addition and reduced precipitation and could improve predictions of the future states of the soil C cycle in response to climate change.

Keywords: N addition; reduced precipitation; nongrowing season soil respiration; carbon cycle

1. Introduction

Soil respiration (*R*s) plays an important role in regulating the global carbon (C) cycle and consists of both autotrophic respiration (*R*a) from roots and their symbionts and heterotrophic respiration (*R*h) from free-living soil microbes [1,2]. As the combined metabolism of soil microorganisms and plant roots, *R*s is inevitably influenced by both soil abiotic and biotic factors (e.g., soil temperature, soil water availability, and soil nitrogen (N) availability) [3,4]. However, rapid ongoing climate change, including drought and nitrogen deposition, would change soil biotic and abiotic factors, which may significantly affect *R*s and its components [5]. Thus, understanding the responses of *R*s and its components to climate change factors is crucial for predicting terrestrial ecosystems soil C dynamics in the future.

Soil N availability and moisture have been identified as important factors affecting *R*s and its components [6]. Numerous studies have been carried out to investigate the responses of *R*s and its components to N addition and reduced precipitation [7,8]. Their results showed that the effect of N addition or reduced precipitation on *R*s and its components were variations and inconsistencies, depending on ecosystem types, experimental treatments, and environmental conditions, etc. [8–10].

Moreover, the mechanisms of N addition and reduced precipitation on *R*s components may differ because the main controllers of different components of *R*s are variable [11–13]. For example, N addition may alter root biomass and activity, leading to a change in the relative contribution of *R*a to *R*s [9,14]. Although previous studies have greatly improved our understanding of *R*s and its components response to N addition and reduced precipitation, most measurements are conducted during the growing season. However, the short legacy effects of N addition and reduced precipitation in the growing season on nongrowing season *R*s and its components are unclear.

Previous studies indicated that there is still a lot of nongrowing season *R*s under snowpack and can globally contribute 5% to 60% of the total annual carbon dioxide flux from soils during nongrowing season [15,16]. However, in many temperate ecosystems, climate changes have been and will continue to be more pronounced during the nongrowing season than during the growing season [17]. Thus, this understanding has increased interest in the short legacy effects of growing season N addition and reduced precipitation on nongrowing season *R*s and its components. N addition and reduced precipitation may affect nongrowing season soil respiration and its components in several ways. First, nongrowing season *R*s and its components can be regulated by changes in C supply under N addition and/ or reduced precipitation. It has been demonstrated that nongrowing season soil freezing can increase over nongrowing season fine root mortality, then a large proportion of these relatively labile C compounds enter the soil, providing a fresh C source to the soil heterotrophic microbial community [1,18]. N addition and reduced precipitation in the growing season may affect nongrowing season fine root mortality by altering the growing season root morphological structure, which may alter the *R*s and the contribution of *R*a or *R*h to total soil respiration during nongrowing season. Moreover, N addition and reduced precipitation in the growing season also affect litter quality [19,20], altering the amount of relatively labile C compounds that enter the nongrowing season soil, which can change nongrowing season *R*s and its components. Second, reduced precipitation can directly decrease *R*s and its components by decreasing nongrowing season soil free-water content. Nongrowing season *R*h under reduced precipitation may suffer from reduced solute availability due to a reduction of the amount of free water during nongrowing season [21]. Third, N addition can directly increase *R*s and *R*h by increasing nongrowing season soil N availability. Further, N addition also may affect soil water icing by increasing soil salt concentration, which may alter the nongrowing season *R*s and its compounds. However, the direction and magnitude of the legacy effect of growing season N addition and reduced precipitation on nongrowing season *R*s and its compounds is unclear. Thus, further investigation of the responses of nongrowing season *R*s and its compounds to growing season N addition and reduced precipitation is still a key to understanding the comprehensive effects of climate changes on soil C cycling.

To investigate the short legacy effects of N addition, reduced precipitation, and their combination on the nongrowing season *R*s and its compounds, a field manipulative experiment with simulated reduction of 30% natural through-fall and increased 5 g N m^{-2} yr^{-1} N deposition in growing seasons was conducted in a temperate forest ecosystem. The objective of this study was to quantify the direction and magnitude of the short legacy of growing season N addition, reduced precipitation, and their combination on nongrowing season *R*s and its compounds. We hypothesized that growing season N addition would increase nongrowing season *R*s by increasing *R*a due to a decrease in the nongrowing season fine root mortality, and growing season reduced precipitation would decrease nongrowing season *R*s by decreasing *R*h due to a decrease in the amount of free water during nongrowing season. Because interannual variability in snowpack thickness may result in a discrepancy in the soil microenvironment, we further hypothesized that responses of nongrowing season *R*s and its components to N addition, reduced precipitation, and their combination would vary with interannual fluctuations in snowpack thickness.

2. Materials and Methods

2.1. Study Site

The long-term field study sites are located in the Changbai Mountains Natural Reserve with an elevation of approximately 738 m height above sea level, in Jilin Province (42°24′ N, 128°06′ E), northeastern China. Climate type, soil properties, and plant species for the experimental area have been described in our previous publication [8,19,22,23]. The climate in the region belongs to a typical temperate-continental climate, with an average annual air temperature of 3.6 °C, and an average annual precipitation of 750 mm. The soil was developed from volcanic ash and is classified as Mollisols according to the American Soil Taxonomy [8,23]. Soil bulk density, total N contents, total carbon (C) contents, and total phosphorus (P) in the 0–20 cm soil layer were 0.57 g cm^{-3}, 7.17 g N kg^{-1}, 156.6 g C kg^{-1}, and 12.20 g P kg^{-1}, respectively. The plant community at the experimental region is a broadleaved Korean pine (*Pinus koraiensis*) mixed forest. The tree species are dominated by *Pinus koraiensis, Fraxinus mandshurica, Tilia amurensis,* and *Quercus mongolica*; the shrub species are dominated by *Euonymus alatus*, *Philadelphus schrenkii*, *Corylus mandshurica*, *Lonicera japonica*, and *Deutzia scabra*; and the herbaceous species are dominated by *Anemone raddeana, Cyperus microiria, A. cathayensis, Adonis vernalis, Funaria officinalis, Filipendula palmata,* and *Brachybotrys paridiformis*.

2.2. Experimental Design

To investigate the short legacy effects of N addition and reduced precipitation on nongrowing season *R*s and its components, a field manipulative experiment with two levels of N addition (control and 50 kg N hm^{-2} $year^{-1}$) and two levels of reduced precipitation (control and −30 of natural through-fall) was conducted in a temperate forest. The experiment was laid out in a split-plot design, with reduced precipitation manipulated at the plot level and N addition manipulated at the subplot level. Six 50 m × 50m plots were randomly established with three plots for ambient precipitation and the other three for reduced precipitation at May 2009 (three repetitions per treatment). An over 20 m wide buffer strip was set up between the plots. The reduced precipitation treatments were based on the precipitation record in the drought years of 1985, 1997, 1999, 2001, and 2003, which are close to 30% less than the long-term mean annual precipitation record in the study area in the last 30 years (Chinese Ecosystem Research Net). The rainout shelters were installed by using high light transmittance *c.* 95%, polycarbonate, V-shaped, translucent panels. The panels covered 30% of the plot area to intercept about 30% of through-fall in the growing seasons and were removed to allow snow to fall onto the forest floor. Each plot was divided into two 50 m × 25 m subplots with N addition and control subplots arranged randomly. Ammonium nitrate (NH_4NO_3) was applied as a form of N addition in each N addition subplot (50 kg N hm^{-2} $year^{-1}$). The N addition treatment was based on the N deposition record of 23 kg N hm^{-2} $year^{-1}$ for the study area [24], which was about double the rate of local N deposition. The fertilizer was weighed according to the N addition rate, mixed with 40 L of deionized water, and sprayed evenly onto the forest floor with a backpack sprayer. The additions were done monthly during the growing season (total six times from May to October). The control subplots received 40 L of deionized water without NH_4NO_3. All treatments started in May 2009 and continued to 2020.

2.3. Soil Respiration Measurement

To measure *R*s, three polyvinyl chloride collars (PVC, 20 cm in diameter, and 6 cm in height) were randomly and permanently established into the soil (with about 3 cm left above the ground) in each subplot in May 2015. To separate heterotrophic respiration (*R*h) from *R*s, a trenching method was used in the study. In each subplot, we randomly trenched three 1 m × 1 m area to 60 cm depth, approximately at the bottom of the root zone in study area. Trenches were lined using the micropore nylon meshes with pore sizes smaller than the diameter of a fine root. Then, each trench was refilled according to its original soil profiles. The PVC was permanently installed inside of each trench in May 2015. To minimize the effect of dead roots, the *R*h was determined over 1 year after trenching.

To investigate nongrowing season *R*s and its components, *R*s and *R*h were measured once a month from October to late May of 2016/2017, 2017/2018, and 2018/2019 by using the Li-8100Automated Soil Gas Flux System (Li-Cor Inc., USA). During the snow-covered period, all collars were checked and adjusted when a forecasted snowfall event was approaching to allow the snowpack to form or melt naturally in the collars during the nongrowing season. We added or removed one collar of the same size for each height adjustment and sealed the gaps between collars with water- and air-proof tape. Detailed information of adjusting soil collars in the nongrowing season has been described by Yan et al. (2019c) [25]. *R*s and *R*h were measured between 10:00 am and 12:00 am (Beijing time) and each measurement was repeated three times for each collar to produce a mean value. The soil temperature and soil volumetric water content at the 5 cm depth were determined simultaneously with *R*s or *R*h using soil moisture probes (Deltat Devices Ltd., Cambridge, England) and soil temperature probes (Omega Engineering Inc., Norwalk, CT, USA), respectively. The soil temperature and moisture probes were connected to LI-8100. The data of snow depth during the measurement periods was measured using a metric ruler. The difference between *R*s and *R*h was represented as the *R*a. Nongrowing season in this study was defined as October to May. Plants begin to turn green in later April and wilt in end of October in this area. Thus, the winter usually starts in November and ends in late April (base on phenology); October and May in the study area belong to the transitional months of the winter and growing seasons. Each winter (from November to April) was divided into three specified periods, the early winter (November and December), deep winter (January and February), and late winter (March and April) based on the snowpack thickness, soil temperature, and air temperature [26].

2.4. Statistical Analyses

A bivariate model was used to determine the relationship of *R*s or *R*h with soil temperature and moisture (Equation (1)).

$$R = ae^{bT}W^{c} \tag{1}$$

where R is soil respiration (*R*s or *R*h); T and W represent the soil temperature (°C) and soil volumetric moisture (%) at 5 cm depth, respectively; and a, b, and c are fitted model parameters.

The sensitivity of R to temperature was further calculated using the Q_{10} value (Equation (2)).

$$Q_{10} = e^{10b} \tag{2}$$

where b is the fitted parameter obtained from Equation (1).

The general linear mixed model (GLMM) for a split-plot with Fisher's multiple comparisons was used to analyze the effects of N addition, reduced precipitation, and their combined effect on *R*s, *R*h, Q_{10}, and the contribution of *R*h to *R*s. N addition, reduced precipitation, and their combined effects were considered fixed factors, and blocks as random effects were used to determine the effects of N addition, reduced precipitation, and their combined effect. To explore the relationship between *R*s and soil temperature, air temperature, and soil moisture, Pearson's correlation were performed. All statistical analyses were performed using the SPSS software version 17.0 (SPSS, IBM, Armonk, NY, USA).

3. Results

3.1. Abiotic Factors

N additions had no significant effect on soil temperature or moisture ($p > 0.05$), while reduced precipitation showed significant effects on soil moisture ($p < 0.05$). Reduced precipitation decreased soil moisture significantly in early winter and before the snowmelt starts. Snowfall began on November of 2016/2017 and 2017/2018 but began on December of 2018/2019 (Figure 1). Trends in surface soil temperature were highly correlated with trends in air temperature and snowpack thickness (Figure 1). Soil temperature generally decreased with the declines in air temperature and increased with the increases in air temperature (Figure 1). The snowpack thickness tended to weaken the negative effect on soil temperature in all nongrowing seasons due to air temperature drop. The snowfall in the nongrowing season of 2018/2019 was significantly lower than that in the nongrowing season of 2016/2017 and 2017/2018 (Figure 1). The air temperature was lowest in the nongrowing season of 2017/2018 (Figure 1).

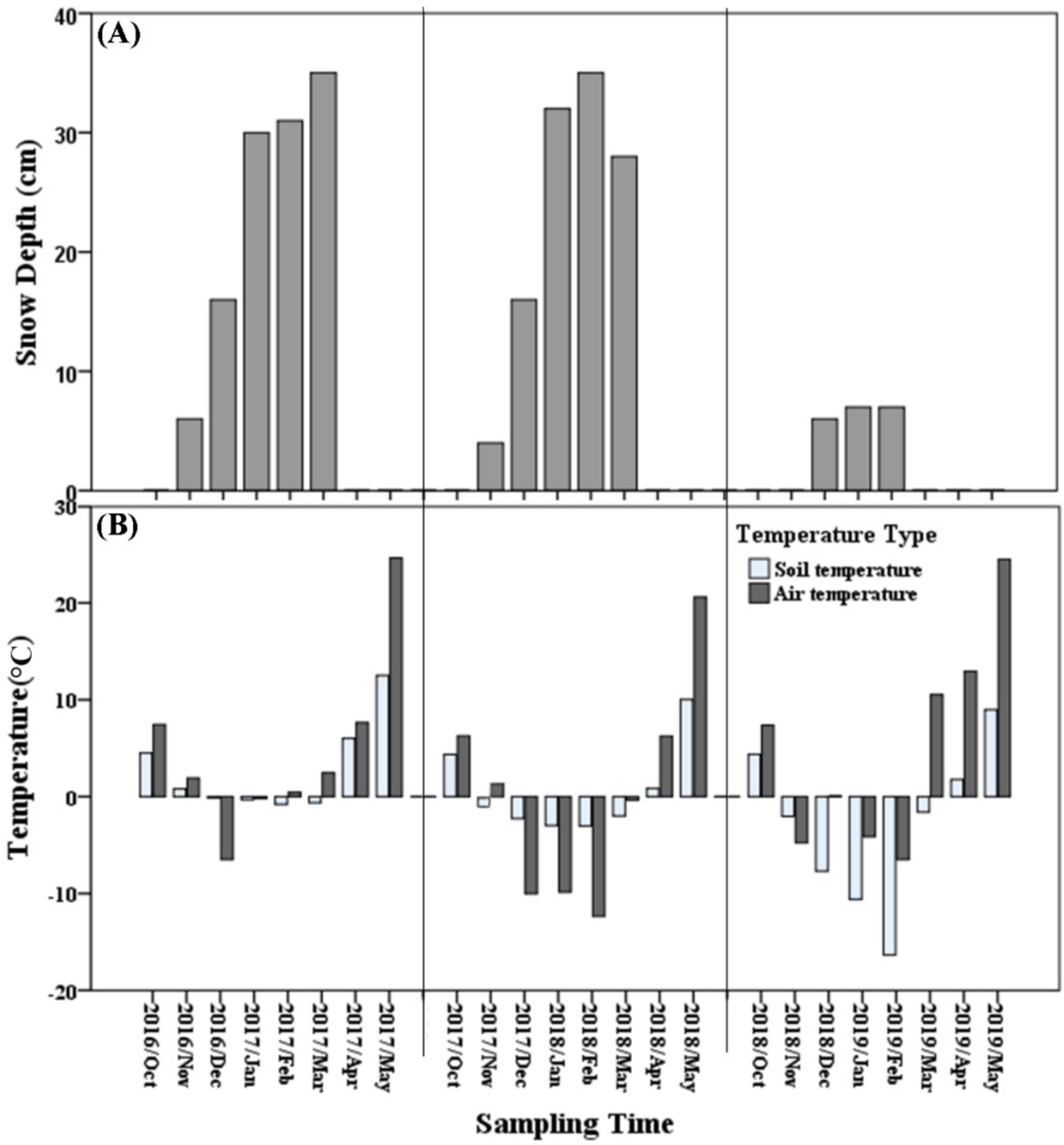

Figure 1. The (**B**) air temperature, soil temperature at 5 cm depth, and (**A**) snow depth during the measurement periods.

3.2. Soil Respiration and Its Components

The temporal dynamics of *Rs* and its components followed the seasonal pattern of soil temperature during the measurement periods, with obvious decreases after reduced temperature and snowfall events (Figure 2). In late winter, *Rs* and its components obviously increased after warming and started increasing from March onwards (Figure 2).

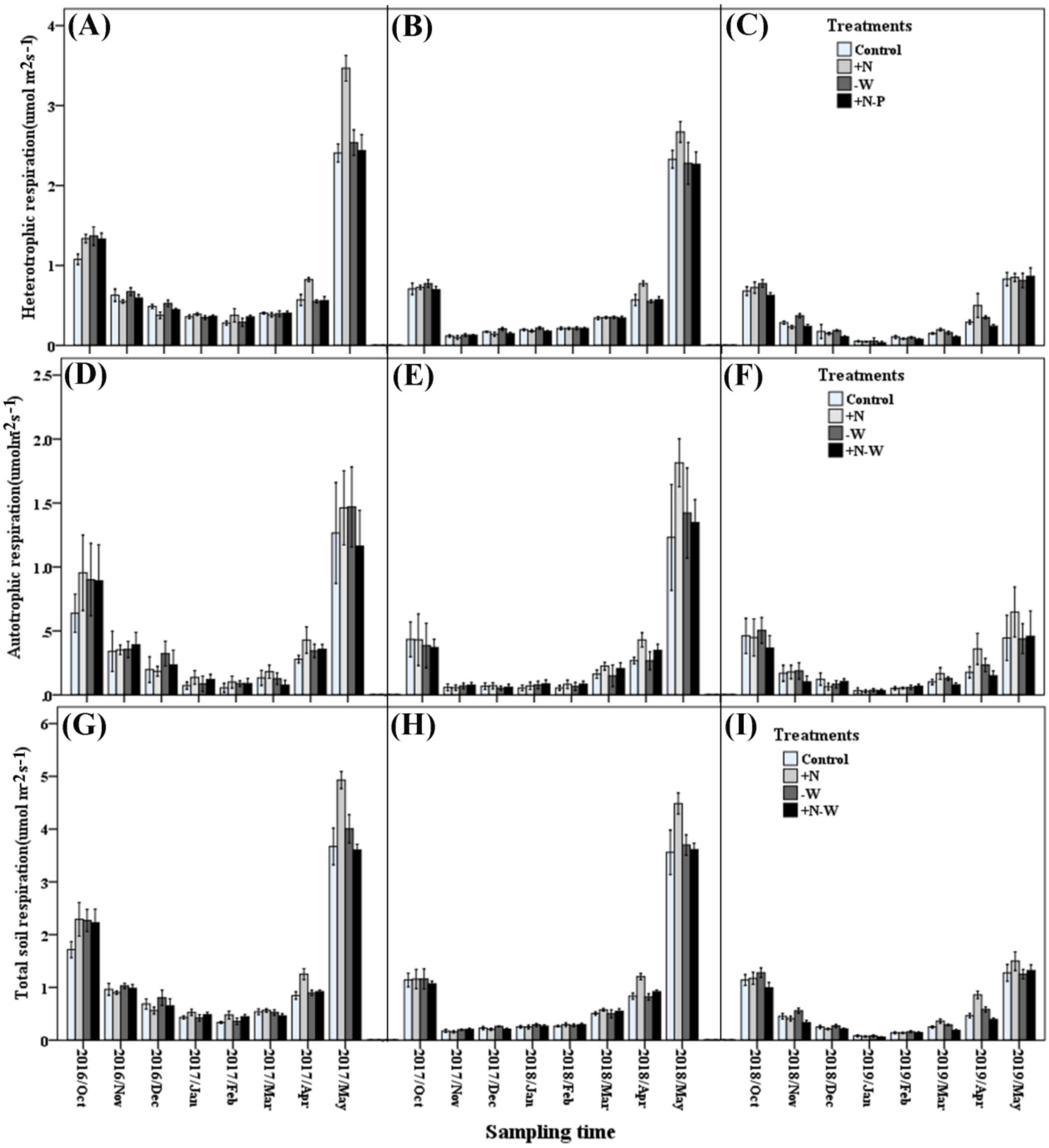

Figure 2. Temporal changes in heterotrophic respiration (**A**–**C**), autotrophic respiration (**D**–**F**), and total soil respiration (**G**–**I**), among the different treatments during the measurement periods in the 2016/2017, 2017/2018, and 2018/2019. The error bars are standard errors. +N represents the N addition treatments; -W represents the precipitation reduction treatments; and +N-W represents the combined effect of N addition and precipitation reduction.

The *R*s, *R*h, and *R*a had a significant positive response to N addition and reduced precipitation in the whole nongrowing season, although the responses of the *R*s, *R*h, and *R*a to N addition and reduced precipitation were not completely consistent in different periods of the nongrowing season (Table 1 and Figures 3 and 4). N addition increased the nongrowing season mean *R*h by 6%, 10%, and 20%, while it increased the *R*a by 35%, 44%, and 41%, resulting in a significant increase in *R*s by 13%, 19%, and 26% in 2016/2017, 2017/2018, and 2018/2019, respectively (Figure 3). These findings indicated that the effects of the N addition on *R*a were greater than on *R*h in the nongrowing season. Reduced precipitation increased the nongrowing season mean *R*h by 3%, 5%, and 21%, while it increased the *R*a by 30%, 8%, and 13%, resulting in a significant increase in *R*s by 7%, 5%, and 20% in 2016/2017, 2017/2018, and 2018/2019, respectively (Figure 3). These results suggest that the snowpack thickness tended to strengthen the positive effect on *R*a and tended to weaken the positive effect on *R*h due to reduced precipitation in nongrowing season. The combined effect of N addition and reduced precipitation increased the nongrowing season *R*s, *R*h, and *R*a in 2016/2017 but decreased the nongrowing season *R*s, *R*h, and *R*a in 2018/2019 (Figure 3), suggesting that the snowpack thickness altered the response direction of *R*s, *R*h, and *R*a to the combined effect. Moreover, the different periods of nongrowing season also regulated the effects of N addition and reduced precipitation on the nongrowing season *R*s, *R*h, and *R*a (Figure 4). In early winter, the interesting result was that N addition decreased the *R*h significantly, resulting in a significant decrease in *R*s (Figure 4).

Table 1. Effects of different treatments and sampling time on nongrowing season soil respiration and its components.

Treatment or Sampling Times	*p* Value			
	df	*R*s	*R*a	*R*h
+N	1	<0.001	0.008	<0.001
-W	1	<0.001	0.201	<0.001
Sampling times	23	<0.001	<0.001	<0.001
+N*-W	1	<0.001	<0.001	<0.001
+N*sampling times	23	<0.001	0.067	<0.001
-W*sampling times	23	<0.001	0.848	<0.001
+N*-W*sampling times	23	<0.001	0.002	<0.001

* represents the interaction.

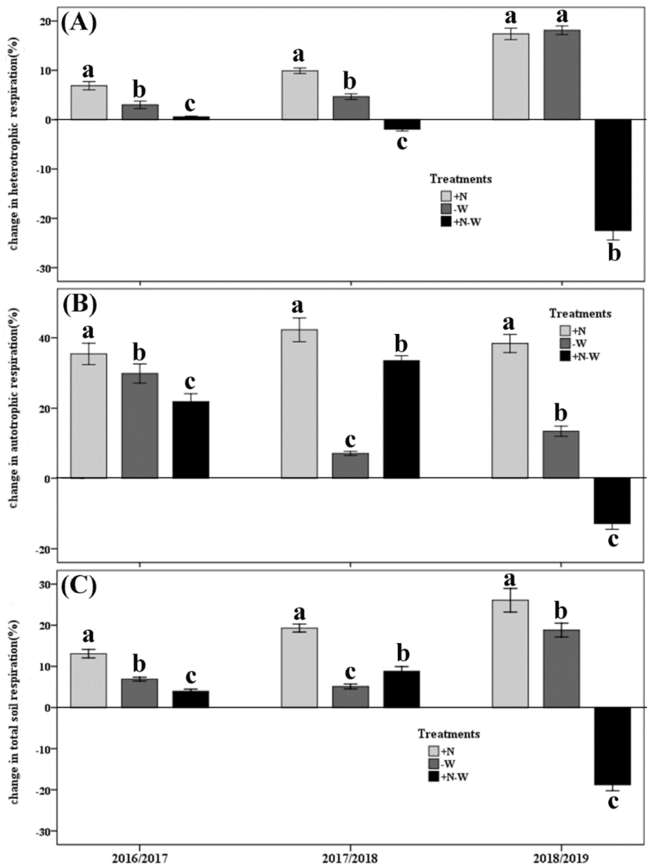

Figure 3. The changes of different treatments versus control treatment in the nongrowing season measurement periods. Significant differences among the different treatments are indicated by different letters. The error bars are standard errors. +N represents the N addition treatments; -W represents the precipitation reduction treatments; and +N-W represents the combined effect of N addition and precipitation reduction.

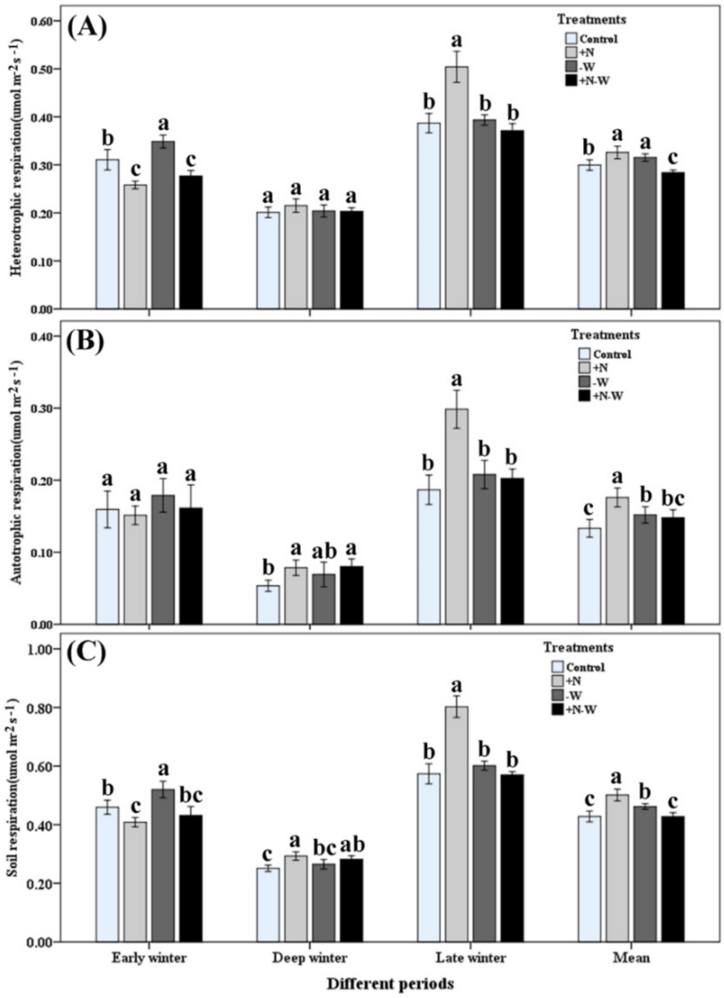

Figure 4. The effects of different treatments on total soil respiration (**C**), heterotrophic respiration (**A**), autotrophic respiration (**B**) and their mean value during the different periods in the nongrowing season of 2016/2017, 2017/2018, and 2018/2019. Significant differences among the different treatments are indicated by different letters. The error bars are standard errors. +N represents the N addition treatments; -W represents the precipitation reduction treatments; and +N-W represents the combined effect of N addition and precipitation reduction.

The contributions of the *R*h to *R*s had also a clear seasonal pattern (Figure 5). The contributions of the *R*h to *R*s began to increase in the nongrowing season but then declined after March due to the increase of root respiration (Figure 5). In general, the contribution of the *R*h to *R*s was higher (55%–90%) than that of the *R*a in all treatments in nongrowing season. N addition significantly decreased the contribution of the *R*h to *R*s in all nongrowing season, whereas reduced precipitation only significantly decreased the contribution of the *R*h to the *R*s in the 2018/2019 nongrowing season but did not show a significant effect in 2016/2017 and 2017/2018 nongrowing season, suggesting that the effect of reduced precipitation on the contributions of *R*h to *R*s may be regulated by soil temperature and snowpack thickness in different nongrowing seasons. The combined effect of N addition and reduced precipitation also greatly decreased the contribution of the *R*h to the *R*s in 2017/2018 and 2018/2019 nongrowing season but did not show a significant effect in 2016/2017 nongrowing season. In summary, all treatments significantly decreased the contributions of *R*h to *R*s in nongrowing season (Figure 5).

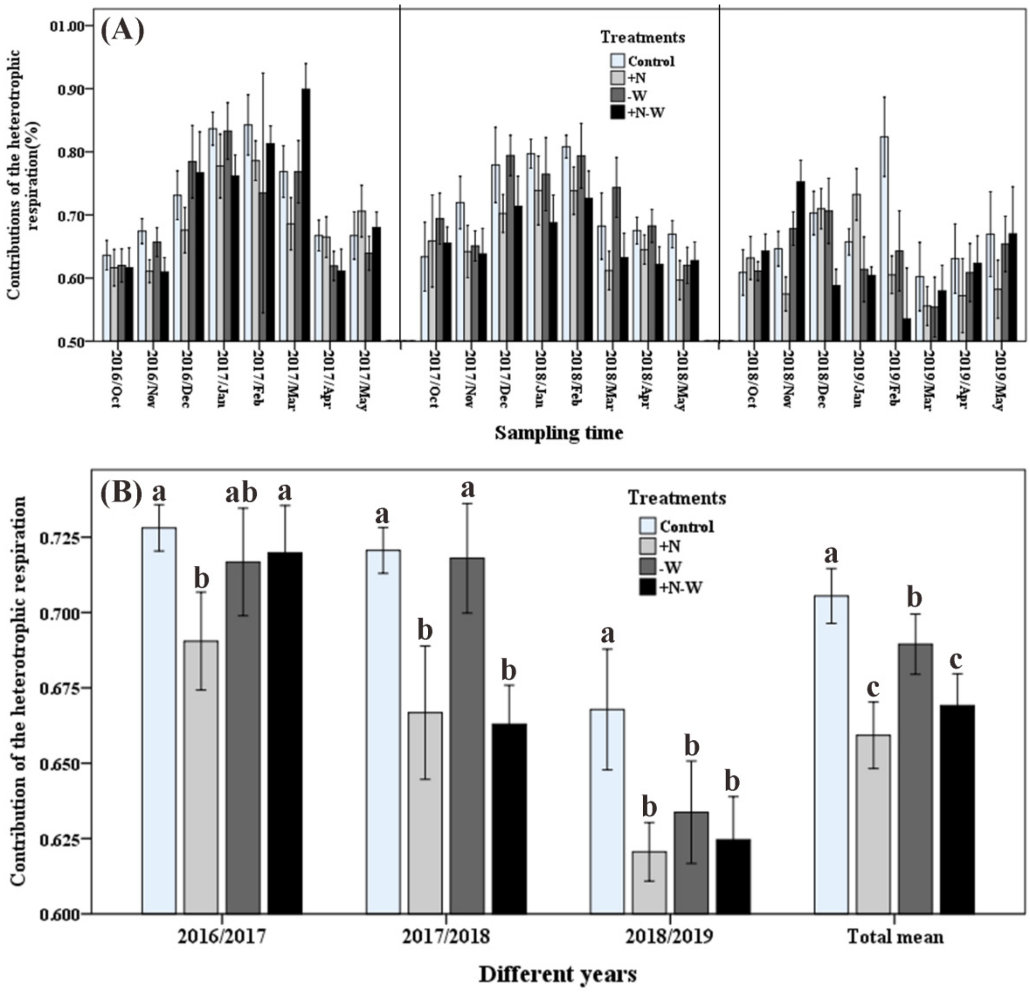

Figure 5. The contribution of heterotrophic respiration to total soil respiration in the each measurement periods (**A**) and each nongrowing season (**B**) in the 2016/2017, 2017/2018, and 2018/2019. Significant differences among the different treatments are indicated by different letters. The error bars are standard errors. +N represents the N addition treatments; -W represents the precipitation reduction treatments; and +N-W represents the combined effect of N addition and precipitation reduction.

3.3. Relationships of Soil Respiration with Environmental Factors

The *R*h, *R*a, and *R*s under all treatments were significantly positively correlated with soil temperature, soil moisture, and air temperature (Table 2). In the study, the soil moisture and temperature explained 64%–80% and 48%–75% of the variations in nongrowing season *R*s and *R*h, respectively. The soil temperature and moisture explained more variations in nongrowing season *R*s and *R*h in the control treatments than those in other treatments. In this study, the fitted Q_{10} values of the *R*s ranged from 3.70 to 4.07, and the Q_{10} value of *R*h ranged from 3.87 to 4.55 (Table 3). The Q_{10} values of the *R*s and *R*h were also higher in control treatment than that in other treatments. N addition and the combined effect significantly decreased the Q_{10} value of *R*s, while reduced precipitation did not show a significant effect (Table 3). However, compared with the control, all treatments significantly decreased the Q_{10} values of *R*h. Those results indicated that N addition and reduced precipitation could reduce the sensitivity of nongrowing season *R*s and *R*h to soil moisture and temperature.

Table 2. The correlation coefficients between nongrowing season soil respiration and soil temperature at 5 cm depth, air temperature, and soil moisture.

Treatments	Soil Temperature		Air Temperature		Soil Moisture	
	R^2	p Value	R^2	p Value	R^2	p Value
Control	0.701	<0.001	0.625	<0.001	0.542	<0.001
+N	0.731	<0.001	0.659	<0.001	0.612	<0.001
-W	0.774	<0.001	0.569	<0.001	0.647	<0.001
+N-W	0.561	<0.001	0.551	<0.001	0.605	<0.001

Table 3. Parameter estimates of models for nongrowing season *R*s and *R*h as a function of soil temperature (T) and moisture (M) in the 0–5 cm soil from May 2016 to October 2017 in different treatments (mean ± SE).

Models	a	b	c	R^2	p	Q_{10}
$Rs = ae^{bt}M^c$						
Control	2.35 ± 0.09 ***	0.14 ± 0.01 **	0.70 ± 0.03 ***	0.80	<0.001	4.07 ± 0.10a
+N	1.97 ± 0.12 ***	0.13 ± 0.01 *	0.66 ± 0.04 ***	0.76	<0.001	3.71 ± 0.21b
-W	2.12 ± 0.14 **	0.13 ± 0.00 **	0.68 ± 0.08 ***	0.78	<0.001	3.94 ± 0.19ab
+N-W	1.88 ± 0.10 *	0.13 ± 0.01 **	0.58 ± 0.04	0.64	<0.001	3.70 ± 0.12b
$Rh = ae^{bt}M^c$						
Control	1.78 ± 0.14 ***	0.15 ± 0.01*	0.56 ± 0.06 ***	0.75	<0.001	4.55 ± 0.09a
+N	1.72 ± 0.09 ***	0.14 ± 0.02	0.45 ± 0.08 ***	0.62	<0.001	4.12 ± 0.28b
-W	1.69 ± 0.18 ***	0.14 ± 0.01 *	0.52 ± 0.10 ***	0.59	<0.001	4.15 ± 0.14b
+N-W	1.63 ± 0.21 ***	0.14 ± 0.01 *	0.45 ± 0.04 ***	0.48	<0.001	3.87± 0.19b

The Q10 value is obtained from b (Q10 = e10b). a, b, and c are regression coefficients. -P represents the precipitation reduction treatments; +N represents the N addition treatments; and +N-P represents the N addition and the precipitation reduction treatments. Different letters in the same year among treatments indicate significant differences ($p < 0.05$). Data are mean ± SE. ANOVA: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

There was higher soil CO_2 flux in transitional months of the nongrowing season and growing seasons (May or October) compared with in the nongrowing season (from November to April) in this study, which was similar to a previous study [27]. High soil CO_2 flux in October may be due to high litter input and decomposition from the recently finished growing season; while there was high soil CO_2 flux after soil thaw (in May), which may be due to the increased activity of soil microbial and plant roots and more soil C available to decompose [27,28]. Low soil CO_2 flux throughout the nongrowing season was attributed to decreasing fine root and microbial activity, declining substrate availability, and lower soil moisture and temperature [29,30]. Generally, both phenology and microclimate drive the seasonal patterns of soil CO_2 flux in nongrowing seasons (including nongrowing season and transitional months).

4.1. Effects of N Addition on Nongrowing Season Soil Respiration and Its Components

It has been demonstrated that the response of different soil respiration components (*R*h and *R*a) to N addition determines the direction of soil respiration (*R*s) response [8,11]. Over the entire nongrowing season, N addition significantly enhanced the *R*h and *R*a, resulting in the positive effects on *R*s in this study.

Based on the previous studies, several mechanisms may explain the positive response of nongrowing season *R*h, *R*a, and *R*s to N addition. First, N addition may directly increase the activity of nongrowing season microorganisms and alter the microbial community composition, resulting in an increase of *R*h. A previous study showed that N addition generally accelerated nongrowing season N mineralization [16], which could be attributed to an increase in microbial activity and change in microbial communities. Our previous results in the study site also confirmed that N addition affected soil microbial activity and community composition in the nongrowing season [26], which may increase litter decomposition, soil N mineralization, and *R*s. Second, the positive response of nongrowing season *R*h, *R*a, and *R*s to N addition can be explained by alleviating the limitation of nongrowing season soil C availability. Nongrowing season *R*s is always C limited and can respond rapidly to increased labile C availability [18,31]. On the one hand, N addition may enhance growing season plant growth and organic C inputs (e.g., increase litter biomass) to the soil by alleviating soil N limitation [3], which may increase the soil C availability in nongrowing season, leading to increased *R*h and *R*s. On the other hand, the increase of nongrowing season labile C availability under N addition could be attributed largely to the increase in the fine biomass and exudation that stimulated nongrowing season *R*a and *R*h. Although we did not determine the nongrowing season fine root biomass in this study, our previous study found that fine root biomass responded positively to N addition during growing seasons in the experimental site [32]. Thus, we speculated that an increase in nongrowing season *R*a under N addition might be related to the increase of fine root biomass and exudation. Moreover, the enhancement of fine root biomass during the growing season may increase the number of fine root deaths, increasing these relatively labile C compounds entering the soil, and then stimulating the *R*h, and enhancing the *R*s. Third, N addition may decrease the possibility of nongrowing season fine root mortality and then increase nongrowing season soil *R*a by altering growing season fine root morphology. The rate of fine root mortality was reduced by an increase in fine root diameter [6,33]. In the study site, fine root diameter during growing seasons significantly increased with N addition [23,32], which may increase the anti-interference ability of fine roots and decrease the possibility of nongrowing season fine root mortality, thus resulting in increased nongrowing season soil *R*a.

However, the different periods of nongrowing season regulated the responses of nongrowing season *R*h, *R*a, and *R*s to N addition. In early winter, the interesting result was that N addition significantly decreased the *R*h, resulting in a significant decrease in *R*s. This result is consistent with a previous study [10]. The negative response of *R*h to N addition in early winter may be explained by microbial growth strategies. In the growing season, the temperate forest is usually limited by the soil N availability [34]. N addition alleviates the restriction of soil nitrogen availability on soil microorganisms and may alter the microbial community from k strategy to r strategy [35]. The increase of soil N availability makes the soil environment more superior, which is conducive to the growth of microorganisms, but the anti-interference ability of microorganisms is relatively reduced. When nongrowing season comes, the microorganism of r strategy was unable to resist the change of microenvironment (reduced temperature and the cycles of soil thawing and freezing), which could cause a lot of microbial death, resulting in the decrease of *R*h in the early winter. With the end of thawing and freezing in early winter, the soil surface was frozen, and the availability of soil substrate played a key role in regulating the *R*h and *R*s. Thus, the nutrients released by the death of microorganisms during the early winter and increased soil C availability by N addition would promote the *R*h and *R*s in the deep winter and late winter. However, in this study, we did not directly measure the dynamics of fine roots and microorganisms in the nongrowing season, and the mechanism of the legacy effect of growing season N addition on nongrowing season respiration may need to be paid more attention to in future studies.

4.2. Effects of Reduced Precipitation on Nongrowing Season Soil Respiration and Its Components

The effect of reduced precipitation on nongrowing season *R*h, *R*a, and *R*s was similar to that of N addition, and nongrowing season *R*h, *R*a, and *R*s also had a significantly positive response to reduced precipitation in this study. Our previous studies found that reduced precipitation also significantly increased *R*h, *R*a, and *R*s during the growing seasons [8], indicating that the reduced precipitation treatment in the study site not only did not lead to serious soil water shortage but also was beneficial to improving the activity of plant roots and microorganisms. Thus, in this study, the slightly reduced precipitation in the growing season can stimulate nongrowing season *R*h, *R*a, and *R*s through several processes correlated with changes in the soil properties and biological physiology. First, microbial biomass and activity in the nongrowing season were regulated by soil water concentration. With the decrease of soil temperature in the nongrowing season, soil water will form ice crystals that destroy the structure of the microbial cell membrane [36]. The more soil moisture, the more ice crystals formed in the soil and the greater the damage to microorganisms. Therefore, the reduced precipitation may reduce microbial mortality and increase *R*h and *R*s. Furthermore, relatively low water content could increase soil structural disruption by freezing in nongrowing season [37]. Additionally, soil structural disruption would increase organic matter concentrations of macro and microaggregate fractions [37], which may stimulate microbial activity, thereby enhancing soil *R*h and *R*s. Moreover, reduced precipitation also increased fine root biomass in this study [32], which could lead to an increase in soil labile C availability during nongrowing season. In nongrowing season, the increase of root biomass can not only directly increase *R*a but also enhance *R*h by root exudates and root deaths, resulting in an increase of total *R*s. In general, the legacy effect of reduced precipitation in the growing season may accelerate the nongrowing season soil C cycle.

The responses of nongrowing season *R*h, *R*a, and *R*s to reduced precipitation treatment were also not completely consistent in different periods of the nongrowing season. During the early winter, reduced precipitation significantly increased *R*s, which was attributed to the increase of *R*h. This result can be explained by the first mechanism that the decrease in soil moisture can reduce the number of ice crystals and decrease microbial death [36]. In the deep winter, we found no significant response of *R*h, *R*a, and *R*s to reduced precipitation, but a small positive effect. During the deep winter, only a small amount of free water may remain on the surface of soil particles. The *R*h was strongly dependent on water availability in frozen soil [38]. Reduced precipitation could also have a negative effect on the *R*h and *R*s by reducing free water availability during deep winter. Thus, reduced precipitation might have no significant effects on nongrowing season *R*h and *R*s because the positive effect from decreasing microbial mortality was partly offset by the negative effect on decreasing free water availability. Although the reduced precipitation had a similar effect on soil respiration in deep winter and late winter, the mechanism might be different. In the late winter, the snow began to melt, and the soil was subjected to freeze-thaw cycle. Due to the melting of a large amount of snow, it is possible to reset the water content of the two water treatments (reduced precipitation and control) to the same level. In addition, the effect of the freeze-thaw cycle on *R*h, *R*a, and *R*s may be stronger than that of reduced precipitation treatment, so precipitation reduction did not significantly affect *R*h, *R*a, and *R*s during the late winter.

4.3. Combined Effects of N Addition and Reduced Precipitation on Nongrowing Season Soil Respiration and Its Components

The previous study suggested that soil water was needed to transport substrates, thus reduced precipitation would inevitably affect the response of *R*h, *R*a, and *R*s to N addition [39]. In this study, the individual effect of N addition was greater than the combined effect of N addition and reduced precipitation, indicating that the positive effects of N addition on nongrowing season *R*h, *R*a, and *R*s could be weakened or offset by reduced precipitation in the 2016/2017 and 2017/2018 nongrowing season. This result was consistent with a previous study [8]. The effect of reduced precipitation on the response of nongrowing season *R*h, *R*a, and *R*s to N addition might be attributed to soil "freezing drought". During the nongrowing season, freezing of soil water leads to the sharp decline of soil free-water and the formation of "freezing drought". Under the "freezing drought" condition, the weak effect of reduced precipitation on soil free-water would greatly limit the diffusion of soil substrate, which could reduce the promotion of N addition on nongrowing season *R*h, *R*a, and *R*s. Moreover, the effect of reduced precipitation on the response of nongrowing season *R*h, *R*a, and *R*s to N addition could also be attributed to a large increase in N accumulation due to decreases in soil free-water, creating a general "salt effect" of the N addition [40], thereby decreasing nongrowing season *R*h, *R*a, and *R*s. However, in nongrowing season extreme conditions (thin or no snowpack), there is a deeper layer of soil frozen and less free water. In this case, reduced precipitation might change the direction of the effect of N addition on nongrowing season *R*h, *R*a, and *R*s by aggravating the "salt effect" caused by N addition, which might support the results of the 2018/2019 nongrowing season in this study. Thus, with the decrease of snow cover, the soil free-water decreases significantly, and the effect of N addition on nongrowing season *R*h, *R*a, and *R*s may change from a positive effect to a negative effect. Understanding the combined effects of N addition and reduced precipitation on nongrowing season *R*s and its components will improve our ability to predict the response of nongrowing season soil C cycle to climate change.

4.4. Adjustment of Soil Respiration and Its Components by N Addition and Reduced Precipitation

During the measurement periods, the contributions of *R*h to *R*s showed clear seasonal patterns, with the highest value in the deep winter (January and February). It has been proved that the tolerance of soil microorganisms to low temperature is much greater than that of plant roots [41]. Although the dynamics of fine root activity and microbial activity were not measured in the study, we speculated that different tolerances of plant roots and microorganisms to low temperature might be the main reasons for the seasonal changes in the contributions of *R*h to *R*s. Furthermore, N addition, reduced precipitation, and their combined effect significantly decreased the contributions of *R*h to *R*s in the nongrowing season, which is similar to previous studies [8,42]. This indicated that the increase in the *R*a might be more intense than the increase in *R*h under different treatments. Because the trenching method also affects the contributions of *R*h to *R*s [43], it may be necessary to combine multiple measurement methods to accurately evaluate the contribution of *R*h to *R*s under climate changes in the future.

In this study, we also found nongrowing season *R*s and its components exhibited significant positive relationships with soil temperature and moisture, which was consistent with previous studies suggesting that soil temperature and moisture generally play an important role in controlling soil respiration processes [11,44,45]. The temperature sensitivity of *R*s (Q_{10}) reflects the sensitivity of the soil C cycle to climate [46]. The Q_{10} of *R*s and *R*h was higher in the nongrowing season (varied from 3.70 to 4.07 and from 3.87 to 4.55, respectively) than that in the growing season (varied from 1.83 to 2.23 and from 2.02 to 2.47, respectively) in this study site [8]. Seasonal variation in the root biomass and production, the microbial community composition, and the liquid water availability may lead to the high Q_{10} of *R*s and *R*h during the nongrowing season [44]. However, N addition, reduced precipitation, and their combined effect had a negative effect on the Q_{10} of nongrowing season *R*s and *R*h, which was consistent with previous studies [11,43]. The changing pattern of Q_{10} in the nongrowing season under different treatments is different from that in the growing season [8], which may be attributed to the change of soil microenvironment and metabolism of plant roots and soil microbes caused by soil freezing in the nongrowing season. Moreover, a previous study found that the Q_{10} was influenced by both soil C quantity and C complexity [31]. Eberwein et al. (2015) [31] proved the C quality-Q_{10} hypothesis that is decreased in Q_{10}-value with labile C addition. Thus, the negative response of Q_{10} of nongrowing season *R*s and *R*h to the N addition and reduced precipitation may be due to the increase of soil labile C input through the increase of microbial mortality, root mortality, and root exudates in the nongrowing season.

5. Conclusions

Our results suggested that growing season N addition and reduced precipitation had increasing effects on the nongrowing season *R*h, *R*a, and *R*s, but the effect highly depended on the sampling periods and thickness of snowpack. The reduced precipitation during the growing season decreased the positive effect of N addition on the nongrowing season *R*h, *R*a, and *R*s in the 2016/2017 and 2017/2018 year with thick snowpack, while it changed the direction of N addition effect in the 2018/2019 year with thin snowpack. Moreover, decreased Q_{10} of the nongrowing season *R*h and *R*s under the N addition and reduced precipitation was observed in this study, suggesting that the increasing N deposition and drought may decrease nongrowing season soil C losses in the scenario of global warming during the nongrowing season in the future. Our results indicate that the legacy effect of growing season N addition and reduced precipitation on nongrowing season *R*s and its components should be considered in assessing the effects of global climate changes on soil C cycle in terrestrial ecosystems.

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