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 (Rs), autotrophic respiration (Ra), and heterotrophic respiration (Rh) are still unclear. Therefore, a field manipulative experiment to determine the responses of nongrowing season Rs 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 Rs by regulating the response of Ra and Rh. The combination of N addition and reduced precipitation also showed a much stronger effect on Rs 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 Rs and its components were mediated by different sampling periods. N addition significantly decreased Rs by decreasing Rh in early winter and significantly increased Rs by increasing Ra in deep winter and late winter. All treatments decreased temperature sensitivity ($Q_{10}$) of Rs and Rh. Our findings contribute to a better understanding of how nongrowing season Rs 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 (Rs) plays an important role in regulating the global carbon (C) cycle and consists of both autotrophic respiration (Ra) from roots and their symbionts and heterotrophic respiration (Rh) from free-living soil microbes [1,2]. As the combined metabolism of soil microorganisms and plant roots, Rs 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 Rs and its components [5]. Thus, understanding the responses of Rs 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 Rs and its components [6]. Numerous studies have been carried out to investigate the responses of Rs and its components to N addition and reduced precipitation [7,8]. Their results showed that the effect of N addition or reduced precipitation on Rs 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 Rs components may differ because the main controllers of different components of Rs are variable [11–13]. For example, N addition may alter root biomass and activity, leading to a change in the relative contribution of Ra to Rs [9,14]. Although previous studies have greatly improved our understanding of Rs 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 Rs and its components are unclear.

Previous studies indicated that there is still a lot of nongrowing season Rs 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 Rs and its components. N addition and reduced precipitation may affect nongrowing season soil respiration and its components in several ways. First, nongrowing season Rs 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 Rs and the contribution of Ra or Rh 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 Rs and its components. Second, reduced precipitation can directly decrease Rs and its components by decreasing nongrowing season soil free-water content. Nongrowing season Rh 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 Rs and Rh 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 Rs and its compounds. However, the direction and magnitude of the legacy effect of growing season N addition and reduced precipitation on nongrowing season Rs and its compounds is unclear. Thus, further investigation of the responses of nongrowing season Rs 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 Rs 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 Rs and its compounds. We hypothesized that growing season N addition would increase nongrowing season Rs by increasing Ra due to a decrease in the nongrowing season fine root mortality, and growing season reduced precipitation would decrease nongrowing season Rs by decreasing Rh 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 Rs 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, Fumaria 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 Rs 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 × 50 m 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$_4$NO$_3$) 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$_4$NO$_3$. All treatments started in May 2009 and continued to 2020.

2.3. Soil Respiration Measurement

To measure Rs, 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 (Rh) from Rs, 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 Rh 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-8100 Automated 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)).

\[
R = ae^{bT}W^c
\]

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)).

\[
Q_{10} = e^{10b}
\]

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 $R_s$ 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, $R_s$ 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 Rs, Rh, and Ra had a significant positive response to N addition and reduced precipitation in the whole nongrowing season, although the responses of the Rs, Rh, and Ra 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 Rh by 6%, 10%, and 20%, while it increased the Ra by 35%, 44%, and 41%, resulting in a significant increase in Rs 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 Ra were greater than on Rh in the nongrowing season. Reduced precipitation increased the nongrowing season mean Rh by 3%, 5%, and 21%, while it increased the Ra by 30%, 8%, and 13%, resulting in a significant increase in Rs 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 Ra and tended to weaken the positive effect on Rh due to reduced...
precipitation in nongrowing season. The combined effect of N addition and reduced precipitation increased the nongrowing season Rs, Rh, and Ra in 2016/2017 but decreased the nongrowing season Rs, Rh, and Ra in 2018/2019 (Figure 3), suggesting that the snowpack thickness altered the response direction of Rs, Rh, and Ra 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 Rs, Rh, and Ra (Figure 4). In early winter, the interesting result was that N addition decreased the Rh significantly, resulting in a significant decrease in Rs (Figure 4).

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

| Treatment or Sampling Times | df | Rs  | Ra  | Rh  |
|----------------------------|----|-----|-----|-----|
| +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*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.
The contributions of the Rh to Rs had also a clear seasonal pattern (Figure 5). The contributions of the Rh to Rs 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 Rh to Rs was higher (55%–90%) than that of the Ra in all treatments in nongrowing season. N addition significantly decreased the contribution of the Rh to Rs in all nongrowing season, whereas reduced precipitation only significantly decreased the contribution of the Rh to the Rs 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 Rh to Rs 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 Rh to the Rs 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 Rh to Rs 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 Rh, Ra, and Rs 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 Rs and Rh, respectively. The soil temperature and moisture explained more variations in nongrowing season Rs and Rh in the control treatments than those in other treatments. In this study, the fitted Q_{10} values of the Rs ranged from 3.70 to 4.07, and the Q_{10} value of Rh ranged from 3.87 to 4.55 (Table 3). The Q_{10} values of the Rs and Rh were also higher in control treatment than that in other treatments. N addition and the combined effect significantly decreased the Q_{10} value of Rs, 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 Rh. Those results indicated that N addition and reduced precipitation could reduce the sensitivity of nongrowing season Rs and Rh to soil moisture and temperature.
4. Discussion

and change in microbial communities. Our previous results in the study site also confirmed that N
nongrowing season N mineralization [16], which could be attributed to an increase in microbial activity
resulting in an increase of R activity of nongrowing season microorganisms and alter the microbial community composition,
nongrowing season this study.

season, N addition significantly enhanced the R N addition determines the direction of soil respiration (Rs) response [8,11]. Generally, both phenology and microclimate drive
season was attributed to decreasing fine root and microbial activity, declining substrate availability, and lower soil moisture and temperature [29,30]. Low soil CO
litter input and decomposition from the recently finished growing season; while there was high soil
CO flux in October may be due to high 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 CO2 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 CO2 flux in October may be due to high litter input and decomposition from the recently finished growing season; while there was high soil CO2 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 CO2 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 CO2 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 (Rh and Ra) to N addition determines the direction of soil respiration (Rs) response [8,11]. Over the entire nongrowing season, N addition significantly enhanced the Rh and Ra, resulting in the positive effects on Rs in this study.

Based on the previous studies, several mechanisms may explain the positive response of nongrowing season Rh, Ra, and Rs 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 Rh. 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

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 |
|------------|------------------|----------------|--------------|
|            | R²     | p Value | R²     | p Value | R²     | 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 Rs and Rh 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 | Rs = aeMT | Rh = aeMT |
|--------|-----------|-----------|
| a      | b         | c         | R²     | p       | Q10     |
| Control | 2.35 ± 0.09 | 0.14 ± 0.01 | 0.70 ± 0.03 | 0.80 | <0.001 | 4.07 ± 0.10 |
| +N     | 1.97 ± 0.12 | 0.13 ± 0.01 | 0.66 ± 0.04 | 0.76 | <0.001 | 3.71 ± 0.21 |
| -W     | 2.12 ± 0.14 | 0.13 ± 0.00 | 0.68 ± 0.08 | 0.78 | <0.001 | 3.94 ± 0.19 |
| +N-W   | 1.88 ± 0.10 | 0.13 ± 0.01 | 0.58 ± 0.04 | 0.64 | <0.001 | 3.70 ± 0.12 |

The Q10 value is obtained from b (Q10 = e^bt). 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.
addition affected soil microbial activity and community composition in the nongrowing season [26], which may increase litter decomposition, soil N mineralization, and Rs. Second, the positive response of nongrowing season Rh, Ra, and Rs to N addition can be explained by alleviating the limitation of nongrowing season soil C availability. Nongrowing season Rs 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 Rh and Rs. 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 Ra and Rh. 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 Ra 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 Rh, and enhancing the Rs. Third, N addition may decrease the possibility of nongrowing season fine root mortality and then increase nongrowing season soil Ra 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 Ra.

However, the different periods of nongrowing season regulated the responses of nongrowing season Rh, Ra, and Rs to N addition. In early winter, the interesting result was that N addition significantly decreased the Rh, resulting in a significant decrease in Rs. This result is consistent with a previous study [10]. The negative response of Rh 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 Rh 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 Rh and Rs. Thus, the nutrients released by the death of microorganisms during the early winter and increased soil C availability by N addition would promote the Rh and Rs 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 Rh, Ra, and Rs was similar to that of N addition, and nongrowing season Rh, Ra, and Rs also had a significantly positive response to reduced precipitation in this study. Our previous studies found that reduced precipitation also significantly increased Rh, Ra, and Rs 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 Rh, Ra, and Rs 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 Rh and Rs. 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 Rh and Rs. 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 Ra but also enhance Rh by root exudates and root deaths, resulting in an increase of total Rs. 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 Rh, Ra, and Rs to reduced precipitation treatment were also not completely consistent in different periods of the nongrowing season. During the early winter, reduced precipitation significantly increased Rs, which was attributed to the increase of Rh. 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 Rh, Ra, and Rs 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 Rh was strongly dependent on water availability in frozen soil [38]. Reduced precipitation could also have a negative effect on the Rh and Rs by reducing free water availability during deep winter. Thus, reduced precipitation might have no significant effects on nongrowing season Rh and Rs 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 Rh, Ra, and Rs may be stronger than that of reduced precipitation treatment, so precipitation reduction did not significantly affect Rh, Ra, and Rs 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 Rh, Ra, and Rs 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 Rh, Ra, and Rs 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 Rh, Ra, and Rs 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 Rh, Ra, and Rs. Moreover, the effect of reduced precipitation on the response of nongrowing season Rh, Ra, and Rs 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 Rh, Ra, and Rs. 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 Rh, Ra, and Rs 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 Rh, Ra, and Rs may change from a positive effect to a negative effect. Understanding the combined effects of N addition and reduced precipitation on nongrowing season Rs 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 Rh to Rs 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 Rh to Rs. Furthermore, N addition, reduced precipitation, and their combined effect significantly decreased the contributions of Rh to Rs in the nongrowing season, which is similar to previous studies [8,42]. This indicated that the increase in the Ra might be more intense than the increase in Rh under different treatments. Because the trenching method also affects the contributions of Rh to Rs [43], it may be necessary to combine multiple measurement methods to accurately evaluate the contribution of Rh to Rs under climate changes in the future.

In this study, we also found nongrowing season Rs 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 Rs (Q_{10}) reflects the sensitivity of the soil C cycle to climate [46]. The Q_{10} of Rs and Rh 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 Rs and Rh 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 Rs and Rh, 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 Rs and Rh 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 Rh, Ra, and Rs, 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 Rh, Ra, and Rs 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 Rh and Rs 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 Rs 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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