

Effect of Soil Moisture on the Response of Soil Respiration to Open-Field Experimental Warming and Precipitation Manipulation

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Abstract: Soil respiration (R_S , Soil CO₂ efflux) is the second largest carbon (C) flux in global terrestrial ecosystems, and thus, plays an important role in global and regional C cycling; moreover, it acts as a feedback mechanism between C cycling and global climate change. R_S is highly responsive to temperature and moisture, factors that are closely related to climate warming and changes in precipitation regimes. Here, we examined the direct and interactive effects of climate change drivers on R_S of *Pinus densiflora* Sieb. et Zucc. seedlings in a multifactor climate change experiment involving atmospheric temperature warming (+3 °C) and precipitation manipulations (−30% and +30%). Our results indicated that atmospheric temperature warming induced significant changes in R_S ($p < 0.05$), enhancing R_S by an average of 54.6% and 59.7% in the control and elevated precipitation plots, respectively, whereas atmospheric temperature warming reduced R_S by 19.4% in plots subjected to lower rates of precipitation. However, the warming effect on R_S was influenced by soil moisture. On the basis of these findings, we suggest that atmospheric temperature warming significantly influenced R_S , but the warming effect on R_S may be weakened by warming-induced soil drying in water-limited environments.

Keywords: soil respiration; climate change; warming effect; soil moisture

1. Introduction

Mean air temperatures and precipitation regimes across regional and global scales have been altered as a result of global climate change, and are expected to continue to change, engendering and exacerbating regional drought conditions, especially in mid-latitude regions [1–3]. These changes are likely to have significant impacts on soil respiration (R_S , soil CO₂ efflux), one of the largest fluxes in the global carbon (C) cycle [4]. As a critical process in the C cycle in terrestrial ecosystems, R_S plays an important role in regulating CO₂ flux from soil to the atmosphere [5]. Any potential change in R_S could, therefore, greatly affect the atmospheric CO₂ concentration, and subsequently affect climate change feedbacks [6]. Thus, a better understanding of the changes in R_S under present and future climate change would help guide projections of terrestrial C fluxes in the warming world.

Soil temperature and soil moisture, both of which are highly responsive to changes in air temperature and precipitation, are two of the primary abiotic drivers that regulate R_S [6,7]. As such, an increasing number of studies is focusing on the responses of R_S to climate warming (i.e., air and/or soil temperature warming) [6,8–11] and alterations in precipitation patterns [2,6,12,13]. These studies have revealed that not all ecosystems respond in a manner similar to these global climate change

drivers. For instance, Feng et al. [14], in a review of studies examining the effects of major global change drivers on R_S across China, noted that R_S response to these drivers differed among ecosystem types (e.g., forest, grassland, tundra). Similarly, Zhong et al. [10], in a review of studies focusing on how warming affects R_S on the Tibetan Plateau, found large variations among regions. Moreover, R_S response to global climate change drivers may also be dependent on the experimental treatment level (e.g., elevated temperature level, precipitation change) or experimental period [14].

R_S is a combined flux that consists of two biotic processes: autotrophic respiration, which originates from plant roots and the associated rhizosphere community, and heterotrophic respiration, which originates from soil microbes and fauna [6]. Because these two processes are sensitive to climate conditions, climate warming and transformations in precipitation patterns may also affect R_S indirectly by modifying autotrophic and heterotrophic respiration [12]. Nevertheless, to what degree the combination of global warming and changes in precipitation patterns will alter R_S , and the roles that other abiotic and biotic factors will play, remain unknown. Hence, in order to forecast global C cycling in the warming world, it is necessary to understand the impact of these changes on the regulation of R_S .

The aim of this study was to examine R_S response to changes in temperature and precipitation by exposing *Pinus densiflora* Sieb. et Zucc. seedlings to various temperatures, precipitation amounts, and combinations of the two. Based on previous studies that indicated that warming increased the root collar diameter, above- and below-ground biomass of *P. densiflora* seedlings, and soil microbial activity [9,15–17], we first hypothesized that warming would enhance R_S by increasing both autotrophic and heterotrophic respiration. Given that shifts in precipitation can undoubtedly change soil conditions (i.e., temperature and moisture), which will in turn change the activities of root and soil microbial activity [18], we then hypothesized that elevated precipitation would also enhance R_S and that reduced precipitation would reduce R_S . Moreover, a combination of warming and precipitation manipulation may aggravate the changes in soil conditions and the responses of *P. densiflora* seedlings and soil microbes. Thus, we also hypothesized that the response of R_S to warming would vary under different precipitation manipulations.

2. Materials and Methods

2.1. Experimental Design

The experiment was conducted on the grounds of Korea University, located in Seoul, South Korea (37°35'36" N, 127°1'31" E). We chose *P. densiflora* seedlings for this experiment because this species is one of the representative temperate coniferous trees in South Korea [8]. Mean air temperature and annual precipitation were 13.6 °C and 792.1 mm, respectively, in 2015, and 12.5 °C and 1450.5 mm, respectively, from 1981 to 2010 (Korea Meteorological Administration, 2016). In April 2013, a total of 18 experimental plots (1.5 m × 1.5 m with a 50 cm buffer between the plots) containing 45 2-year-old *P. densiflora* seedlings were established in the study site. The soil at this site is classified as loamy sand (80% sand, 14% clay, 6% silt) [19].

The experimental treatment system was established in April 2013 and consisted of six different treatments with three replicates: two levels of atmospheric warming (control (C) and +3 °C (W)) were crossed with three levels of precipitation (control (P0), −30% (P−), and +30% (P+)). The six treatments consisted of (1) atmospheric temperature control and precipitation control (C*P0), or the “ambient” treatment; (2) atmospheric warming and precipitation control (W*P0); (3) atmospheric temperature control and reduced 30% precipitation (C*P−); (4) atmospheric warming and reduced 30% precipitation (W*P−); (5) atmospheric temperature control and elevated 30% precipitation (C*P+); and (6) atmospheric warming and elevated 30% precipitation (W*P+). An infrared heater (FTE-1000; Mor Electric Heating Instrument Inc., Grand Rapids, MI, USA) was used to elevate the air temperatures in the warming plots. The infrared heaters were set at a height of 60 cm above the *P. densiflora* seedling canopy in warmed plots; dummy heaters (without warming lamps) were set at the same height in

non-warmed plots. A transparent panel was used to reduce natural precipitation in the decreased precipitation plots, and an automatic pump and drip-irrigation system was used to elevate precipitation in the plots with higher precipitation levels. These changes in air temperature and precipitation were designed to simulate climate change conditions expected in Korea over the next 50 years, based on RCP 8.5 climate-change scenarios.

2.2. Field Measurements

Soil respiration was measured in the morning between 9:00 and 12:00 on 19 June, 19 August, and 20 October, 2015 using a closed-chamber system with a portable diffusion-type, non-dispersive infrared (NDIR) CO₂ sensor (GMP343, Vaisala CARBOCAP, Helsinki, Finland) and a polyacrylics chamber (10 cm in diameter, 12 cm in height). R_S measurements and calculations were based on the methodology described by Noh et al. [9]. Briefly, CO₂ concentrations in the chamber were recorded every 5 s for 300 s using a handheld controller and logger (MI-70, Vaisala CARBOCAP, Helsinki, Finland) coupled with the NDIR CO₂ sensor; the first 30 s of data after the placement of the chamber were excluded from subsequent analyses. R_S was calculated using the equation (Equation (1)):

$$R_S = \frac{dCO_2}{dt} \times \frac{PV}{ART}, \quad (1)$$

where P is the atmospheric pressure, V is the volume of the headspace gas within the chamber, A is the soil surface area enclosed by the chamber, R is the gas constant, and T is the air temperature (K).

Air temperature was measured using infrared temperature sensors (SI-111, Campbell Scientific, Logan, UT, USA), soil temperature was measured at a depth of 5 cm using temperature sensors (107-L34, Campbell Scientific, Logan, UT, USA), and soil moisture was measured at a depth of 10 cm using reflectometer probes (CS616, Campbell Scientific, Logan, UT, USA) ($n = 18$). Air temperature, soil temperature, and soil moisture were logged every 30 min, and the data were recorded using a data logger (CR3000, Campbell Scientific, Logan, UT, USA).

2.3. Statistical Analysis

Repeated measures ANOVA was used to test the effects of warming, precipitation manipulation, and their interaction on soil temperature, moisture, and R_S . We used the relative R_S between the warming plots and non-warmed plots to assess the effect of warming on R_S under different precipitation regimes. Fisher's least significant difference (LSD) test was used to analyze differences in air temperature, soil temperature, soil moisture, and R_S among the treatments. In addition, covariance and linear regression analyses were used to assess the relationships between R_S and soil moisture. All statistical analyses were performed with SAS v.9.3 (SAS Systems, Cary, NC, USA), and significance was set at $p \leq 0.05$. All associated data were available in Tables A1 and A2.

3. Results

3.1. Soil Temperature and Moisture

During the study period, only atmospheric warming and sampling month had significant effects on soil temperature ($p < 0.05$ and $p < 0.01$; Table 1). Warming increased air temperatures around the canopy surface by 3.09 °C, 2.34 °C, and 2.89 °C on average in the control, reduced, and elevated precipitation plots, respectively (Table 2), but the differences in soil temperature induced by atmospheric warming were lower than the differences in air temperature among the precipitation treatments. Warming increased soil temperatures by 0.63 °C, 0.28 °C, and 0.44 °C on average in the control, reduced, and elevated precipitation plots, respectively (Table 2). Although only warming and month had significant effects on soil moisture (all $p < 0.01$; Table 1), on average, soil moisture varied significantly among the treatments (Table 2). Compared with the ambient treatment (C*P0), all other treatments reduced soil moisture by 0.44 Vol% to 1.94 Vol% (Table 2). In addition, warming had a

drying effect on soil moisture, reducing it by an average of 1.87 Vol%, 1.13 Vol%, and 0.85 Vol% in the control, reduced, and elevated precipitation plots, respectively (Table 2).

Table 1. Repeated-measure ANOVAs for soil temperature (ST), soil moisture (SM), and soil respiration (R_S) in response to warming, precipitation manipulation, and their interaction.

Effect	df	ST			SM			R_S	
		F	p		F	p		F	p
Warming (W)	1	6.1900	0.0285	*	12.060	0.0046	**	6.4500	0.0259
Precipitation (P)	2	1.5400	0.2535	ns	0.7300	0.5019	ns	1.8800	0.1948
Month (M)	2	2924.7	<0.0001	**	89.360	<0.0001	**	22.620	<0.0001
W × P	2	0.3100	0.7397	ns	0.6700	0.5293	ns	5.4300	0.0209
W × M	2	2.3800	0.1138	ns	0.3000	0.7451	ns	0.0500	0.9553
P × M	4	1.2200	0.3304	ns	1.7300	0.1763	ns	2.3900	0.0786
W × P × M	4	0.3300	0.8565	ns	0.0800	0.9869	ns	0.1000	0.9831

× = interaction effect; ns = not significant ($p > 0.05$); * = significant ($p < 0.05$); ** = significant ($p < 0.01$).

Table 2. Mean air temperature (AT), soil temperature (ST), soil moisture (SM), and soil respiration (R_S) under different treatments, presented as mean ± standard error.

Variables	C*P0	W*P0	C*P−	W*P−	C*P+	W*P+
AT (°C)	21.02 ± 3.22 a	24.11 ± 3.37 a	21.30 ± 3.34 a	23.64 ± 3.23 a	21.00 ± 3.24 a	23.90 ± 3.34 a
ST (°C)	21.80 ± 2.82 a	22.43 ± 2.90 a	22.36 ± 2.86 a	22.65 ± 2.91 a	22.08 ± 2.80 a	22.52 ± 2.79 a
SM (Vol %)	7.34 ± 0.56 a	5.47 ± 0.50 b	6.53 ± 0.43 ab	5.40 ± 0.39 b	6.90 ± 0.39 a	6.04 ± 0.36 ab
R_S ($\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	2.02 ± 0.46 a	3.12 ± 0.49 ab	2.76 ± 0.41 ab	2.23 ± 0.38 a	2.36 ± 0.48 ab	3.76 ± 0.55 b

W = warming; P = precipitation manipulation; C*P0 = atmospheric temperature control and precipitation control; W*P0 = atmospheric warming and precipitation control; C*P− = atmospheric temperature control and reduced precipitation; W*P− = atmospheric warming and reduced precipitation; C*P+ = atmospheric temperature control and elevated precipitation; and W*P+ = atmospheric warming and elevated precipitation. Values followed by a different letter are significantly different to each other ($p < 0.05$).

3.2. Treatment Effects on R_S

Warming and month also significantly affected R_S ($p < 0.05$ and $p < 0.01$, respectively), as did the interactive effect of warming and precipitation manipulation ($p < 0.05$; Table 1). Compared with the ambient treatment, all other treatments significantly enhanced R_S by 16.9% to 86.6% (Table 2). R_S exhibited similar temporal variations under all treatments over the course of the study period, increasing from June to August and subsequently decreasing thereafter (Figure 1). However, the relative R_S between warmed plots and non-warmed plots under different precipitation levels exhibited the opposite temporal variation, with a smaller R_S in August than in June and October (Figure 2a). Moreover, R_S in June and October differed significantly among the three precipitation treatments. Warming had a positive effect on R_S in the control and elevated precipitation plots, but a negative effect on R_S in reduced precipitation plots, since the relative R_S in the latter plots was negative (Figure 2b). Specifically, warming enhanced R_S by an average of 54.6% and 59.7% in the control and elevated precipitation plots, respectively, whereas warming reduced R_S by 19.4% in reduced precipitation plots.

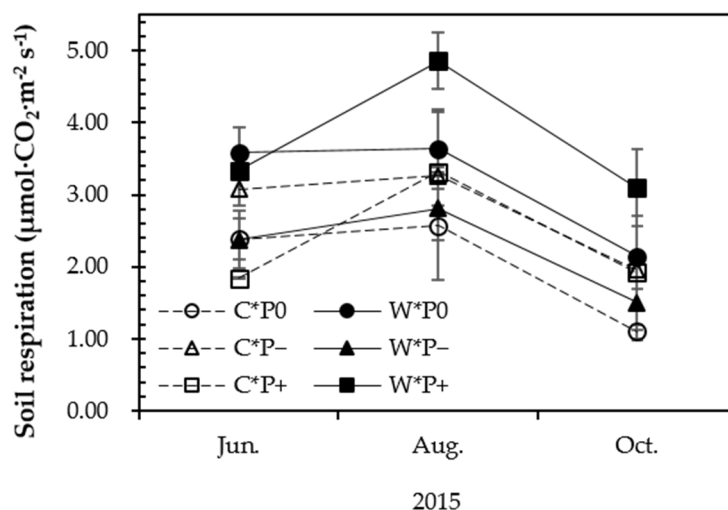


Figure 1. Variations in soil respiration among treatments. Vertical bars indicate the standard error. Treatments: C*P0 = atmospheric temperature control and precipitation control; W*P0 = atmospheric warming and precipitation control; C*P- = atmospheric temperature control and reduced precipitation; W*P- = atmospheric warming and reduced precipitation; C*P+ = atmospheric temperature control and elevated precipitation; and W*P+ = atmospheric warming and elevated precipitation.

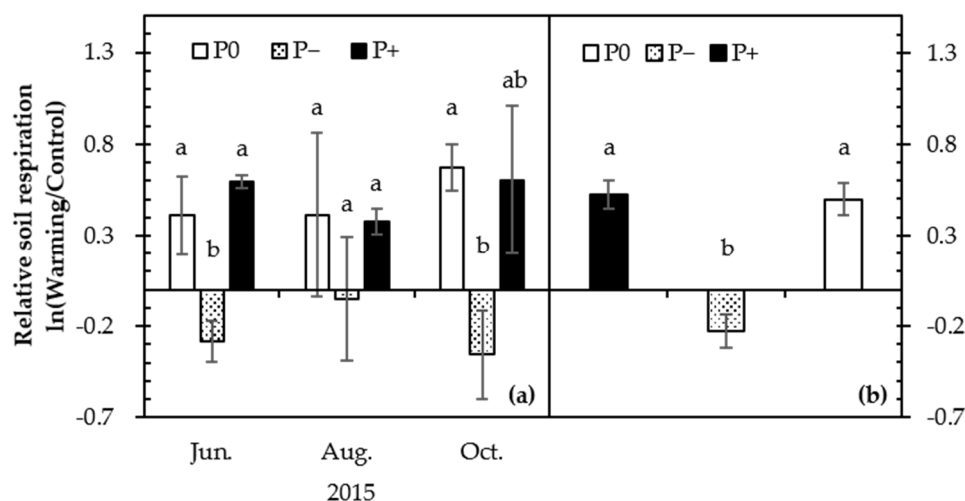


Figure 2. (a) Variations in relative soil respiration between the warmed plots and non-warmed plots subjected to three precipitation manipulations; (b) mean relative soil respiration between the warmed plots and non-warmed plots under different precipitation manipulations. Vertical bars indicate the standard error. Different lower-case letters represent significant differences ($p < 0.05$). Treatments: P0 = precipitation control; P- = reduced precipitation; P+ = elevated precipitation.

3.3. Correlations Between R_S and Soil Moisture

There was a positive correlation between R_S and soil temperature ($r = 0.52$, $p < 0.01$) and moisture ($r = 0.33$, $p < 0.05$) across all plots. Specifically, there were significant positive correlations between R_S and soil moisture in both warmed plots ($r = 0.72$, $p < 0.01$) and non-warmed plots ($r = 0.60$, $p < 0.01$; Figure 3a). The dependency of R_S on soil moisture in warmed plots was higher than that in non-warmed plots. The relative R_S was positively correlated with the relative soil moisture between warmed plots and non-warmed plots ($r = 0.42$, $p < 0.05$; Figure 3b).

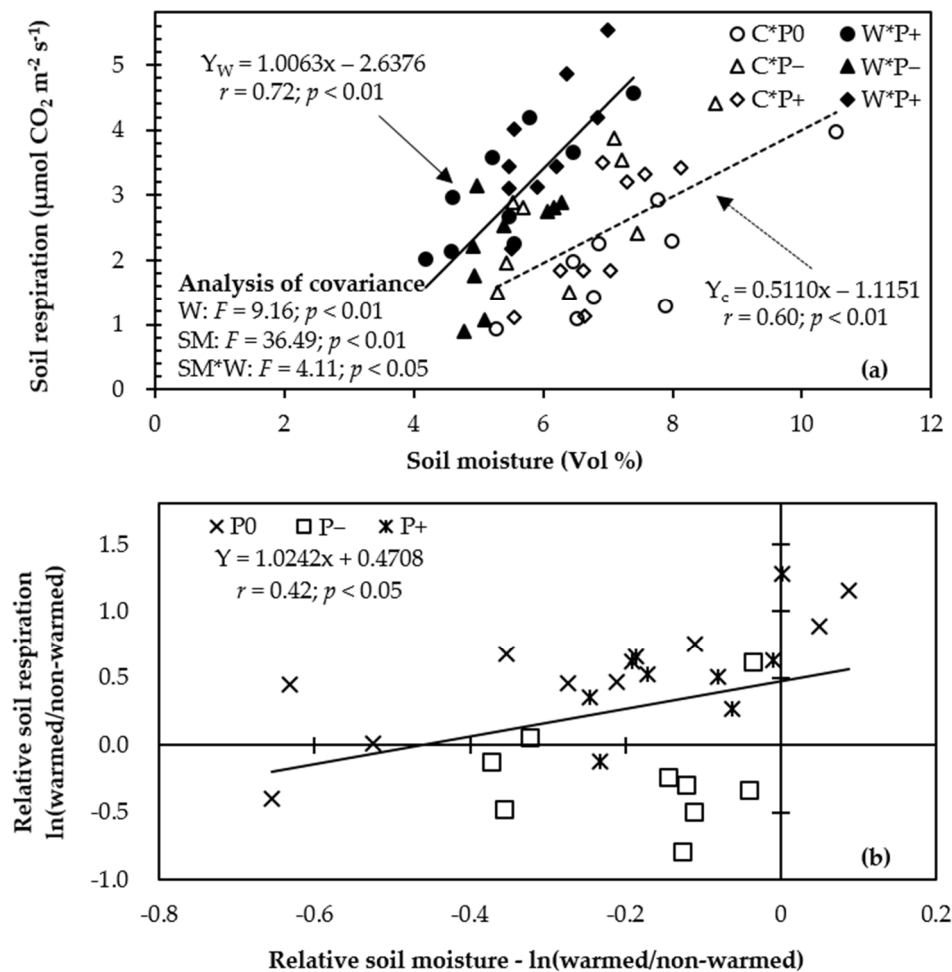


Figure 3. (a) Soil respiration plotted against soil moisture in warmed plots (YW) and non-warmed plots (YC); (b) relative soil respiration between warmed and non-warmed plots plotted against relative soil moisture between the warmed plots and non-warmed plots. Treatments: P0 = precipitation control; P- = reduced precipitation; P+ = elevated precipitation; C*P0 = atmospheric temperature control and precipitation control; W*P0 = atmospheric warming and precipitation control; C*P- = atmospheric temperature control and reduced precipitation; W*P- = atmospheric warming and reduced precipitation; C*P+ = atmospheric temperature control and elevated precipitation; and W*P+ = atmospheric warming and elevated precipitation.

4. Discussion

4.1. Effects of Treatments on R_S

Generally, we found that warming and the interaction of warming and precipitation manipulation had significant effects on R_S , whereas precipitation manipulation alone had no effect on R_S (Table 1). A previous experiment conducted at the same site in 2015 reported that precipitation manipulation alone had no effect on above- and below-ground seedling biomass [15,16]. Given that plant growth has a strong influence on R_S via root respiration, the lack of plant biomass response to precipitation manipulation could explain that the precipitation manipulation did not significantly affect R_S in our study, a result that was consistent with the findings of Wei et al. [18].

Furthermore, the significant positive effects of warming and the interaction of warming and precipitation manipulation on R_S were likely due to the treatment effects on the seedlings, which could directly contribute to R_S , and the treatment effects on soil conditions (i.e., temperature and moisture), which could result in site-specific soil conditions and indirectly affect the R_S . In this study, soil

temperature remained largely unaffected by treatment, but the soil dried out, likely due to increased seedling transpiration rates, especially in warmed and reduced precipitation plots. According to previous studies in the same study site, warming not only increased the root collar diameter and above- and below-ground biomass of seedlings [15,16], but also altered the soil microbial activity and community [17]. Thus, the warming effect on R_S might be primarily driven by the seedling and soil microbial responses to the treatments. Hence, combining the results of the present study and other studies, we suggest that the shifts in R_S caused by the treatment-induced modifications to seedling growth and soil conditions might involve a complex of mechanisms that interact to determine root responses to variable soil environments, changes in the soil microbial community, and allocation of assimilated C in the plant–soil–microbe system [5,6,11].

Soil at the study site was a loamy sand [19], and thus, soil moisture at the study site was naturally low. As such, it is possible that soil moisture played a stronger role than did soil temperature in determining R_S over the course of the study period. We found that although warming generally enhanced R_S in the control and elevated precipitation plots, warming reduced R_S in the reduced precipitation plots, despite soil temperatures being increased by warming (Table 2). The drier conditions caused by the combination of warming and reduced precipitation most likely led to this decline in R_S . This finding was consistent with those of other studies showing that soil drying induced by warming can offset the effects of increasing temperature in a water-limited environment [5,6,14].

4.2. Soil Moisture and Warming Effect on R_S

Our results indicated that there might be a warming effect/soil moisture threshold on R_S , which suggested that the warming effect on R_S would be influenced by soil moisture within this water-limited environment. Several lines of evidence support this conclusion. First, it was observed that R_S generally increased with increasing soil moisture in both warmed plots and non-warmed plots; however, covariance analysis revealed a significant interaction between warming and soil moisture ($p < 0.05$). Moreover, warming caused a shift in the R_S -moisture response curve (Figure 3a). These patterns might be the result of complex interactions among the mechanisms involved in the warming effect and soil moisture. Second, we also observed that warming had a positive effect on R_S in the control and elevated precipitation plots but a negative effect on R_S in reduced precipitation plots (Table 2 and Figure 2b). Third, warming effects on R_S were positively correlated with soil moisture among all precipitation treatments (Figure 3b).

Both autotrophic and heterotrophic respiration were correlated with soil moisture. In previous studies at the same site, it was reported that warming enhanced root biomass, as well as soil microbial biomass and activity [15–17]. Hence, we concluded that warming enhanced R_S by increasing both autotrophic and heterotrophic respiration under sufficient soil available water [7,20,21]. Soil moisture can greatly influence the diffusion of soluble nutrients, and consequently reduce available nutrients for soil microbes and uptake by roots [22,23]. In water-limited environments, increasing competition for nutrients between roots and soil microbes induced by water stress might therefore offset, at least somewhat, the positive effect of warming on microbial biomass and activity [24]. On the other hand, when soil moisture is limited, microbes and plant roots have to invest more energy to produce protective secondary compounds, which would hamper their growth and the amount of C allocated to respiration [25]. Therefore, in water-limited environments, the negative warming effect of R_S might be attributed to reductions in heterotrophic and autotrophic respiration associated with drought induced by warming.

5. Conclusions

The results of our study indicated that the direct and/or interactive effects of changes in air temperature and precipitation regimes would likely alter R_S . Both R_S and the warming effect on R_S , reflected by the relative R_S between warmed and non-warmed plots, varied within treatments, suggesting that treatment-induced changes in R_S and the warming effect on R_S were directly

and/or indirectly related to soil conditions (temperature and moisture) and the growth of seedlings. Most notably, soil moisture appears to be a key factor controlling C fluxes from soil to atmosphere, and warming-induced soil drying may weaken or offset the atmospheric warming effect on R_S in water-limited environments. Our study demonstrated the preliminary response of R_S to the effects of warming and altered precipitation regimes on *P. densiflora* seedlings. Given that *P. densiflora* is a common temperate coniferous tree species and is widely distributed throughout South Korea [26], future monitoring will provide important parameters for predicting the response of forest belowground C turnover to a warmer climate.

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Author Contributions: Guanlin Li, Seongjun Kim, Seung Hyun Han and Hanna Chang were responsible for the collection of data from the study site; Guanlin Li and Seongjun Kim analyzed the data; Yowhan Son conceived and designed the experiments; Guanlin Li wrote the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest. The funding sponsors had no role in the design of this study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix

Table A1. Mean air temperature (AT), soil temperature (ST), soil moisture (SM), and soil respiration (R_S) measured in June, August and October, 2015 under different treatments, presented as mean \pm standard error.

Month	Treatment	AT	ST	SM	R_S
		(°C)	(°C)	(Vol %)	($\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\text{ s}^{-1}$)
June	C*P0	23.22 \pm 0.24	23.91 \pm 0.33	7.03 \pm 0.39	2.39 \pm 0.28
	W*P0	26.16 \pm 0.56	25.09 \pm 0.39	5.20 \pm 0.34	3.58 \pm 0.35
	C*P−	23.49 \pm 0.19	24.72 \pm 0.12	6.14 \pm 0.54	3.08 \pm 0.23
	W*P−	26.45 \pm 0.41	25.18 \pm 0.36	4.94 \pm 0.02	2.38 \pm 0.41
	C*P+	23.17 \pm 0.11	24.03 \pm 0.29	6.64 \pm 0.22	1.84 \pm 0.00
	W*P+	26.30 \pm 0.01	24.72 \pm 0.21	5.87 \pm 0.21	3.34 \pm 0.11
August	C*P0	25.16 \pm 0.20	25.26 \pm 0.10	8.43 \pm 1.10	2.57 \pm 0.75
	W*P0	28.65 \pm 1.54	25.58 \pm 0.22	6.45 \pm 0.56	3.63 \pm 0.55
	C*P−	25.67 \pm 0.00	25.69 \pm 0.14	7.39 \pm 0.67	3.26 \pm 0.89
	W*P−	27.27 \pm 1.04	25.93 \pm 0.19	6.18 \pm 0.06	2.81 \pm 0.04
	C*P+	25.21 \pm 0.07	25.65 \pm 0.12	7.67 \pm 0.25	3.32 \pm 0.06
	W*P+	28.08 \pm 0.46	25.87 \pm 0.05	6.73 \pm 0.19	4.86 \pm 0.39
October	C*P0	14.68 \pm 0.05	16.22 \pm 0.42	6.56 \pm 0.75	1.10 \pm 0.10
	W*P0	17.53 \pm 1.32	16.63 \pm 0.16	4.78 \pm 0.41	2.14 \pm 0.07
	C*P−	14.74 \pm 0.28	16.68 \pm 0.26	6.06 \pm 0.70	1.95 \pm 0.26
	W*P−	17.20 \pm 0.00	16.84 \pm 0.59	5.09 \pm 0.18	1.50 \pm 0.52
	C*P+	14.63 \pm 0.27	16.56 \pm 0.29	6.37 \pm 0.42	1.92 \pm 0.79
	W*P+	17.30 \pm 0.58	16.97 \pm 0.35	5.52 \pm 0.02	3.10 \pm 0.53

C*P0 = atmospheric temperature control and precipitation control; W*P0 = atmospheric warming and precipitation control; C*P− = atmospheric temperature control and reduced precipitation; W*P− = atmospheric warming and reduced precipitation; C*P+ = atmospheric temperature control and elevated precipitation; and W*P+ = atmospheric warming and elevated precipitation.

Table A2. The code of statistical analysis performed with SAS v.9.3 (SAS Systems, Cary, NC, USA).

Repeated measures ANOVA	<pre> data RMANOVA; do P = 'n', 'd', 'T'; do T = 'c', 'w'; do s = 1 to 3; input M1–M3; output; end; end; end; cards; proc glm; class P T; model M1–M3 = P T P*T/nouni; repeated t 3 (6 8 10) contrast (1)/summary print; run; </pre>	<pre> /P = precipitation manipulation; n = precipitation control; d = reduced precipitation; i = elevated precipitation/ /T = warming; c = atmospheric temperature control; w = atmospheric warming/ /month/ </pre>
Fisher's least significant difference (LSD) test	<pre> data LSD; do a = 1 to 6; do i = 1 to 3; input x @@; output; end; end; cards; proc anova; class a; model x = a; means a/LSD; run; </pre>	<pre> /a = Treatment/ </pre>
Analysis of Linear regression	<pre> data regression; input ST SM RS @@; cards; proc corr pearson; var ST SM RS; run; </pre>	<pre> /ST = soil temperature; SM = soil moisture; RS = CO₂ efflux/ </pre>
Analysis of covariance	<pre> data covariance; input T \$ RS ST SM @@; cards; proc sort; by T; run; proc glm; class T; model RS = T SM; run; </pre>	<pre> /T = warming; ST = soil temperature; SM = soil moisture; RS = CO₂ efflux/ </pre>

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