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Responses of Soil, Heterotrophic, and Autotrophic Respiration to Experimental Open-Field Soil Warming in a Cool-Temperate Deciduous Forest

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ABSTRACT

How global warming will affect soil respiration (R_S) and its source components is poorly understood despite its importance for accurate prediction of global carbon (C) cycles. We examined the responses of R_S, heterotrophic respiration (R_H), autotrophic respiration (R_A), nitrogen (N) availability, and fine-root biomass to increased temperature in an open-field soil warming experiment. The experiment was conducted in a cool-temperate deciduous forest ecosystem in northern Japan. As this forest is subjected to strong temporal variation in temperature, on scales ranging from daily to seasonal, we also investigated the temporal variation in the effects of soil warming on R_S, R_H, and R_A. Soil temperature was continuously elevated by about 4.0°C from 2007 to 2014 using heating wires buried in the soil, and we measured soil respiratory processes in all four seasons from 2012 to 2014. Soil warming increased annual R_S by 32–45%, but the magnitude of the increase was different between the components: R_H and R_A were also stimulated, and increased by 39–41 and 17–18%, respectively. Soil N availability during the growing season and fine-root biomass were not remarkably affected by the warming treatment. We found that the warming effects varied seasonally. R_H increased significantly throughout the year, but the warming effect showed remarkable seasonal differences, with the maximum stimulation in the spring. This suggests that warmer spring temperature will produce a greater increase in CO_2 release than warmer summer temperatures. In addition, we found that soil warming reduced the temperature sensitivity (Q_{10}) of R_S. Although the Q_{10} of both R_H and R_A tended to be reduced, the decrease in the Q_{10} of R_S was caused mainly by a decrease in the response of R_A to warming. These long-term results indicate that a balance between the rapid and large re-

sponse of soil microbes and the acclimation of plant roots both play important roles in determining the response of R_S to soil warming, and must be carefully considered to predict the responses of soil C dynamics under future temperature conditions.

INTRODUCTION

The global terrestrial mean surface air temperature is expected to increase by 1.2–4.8°C compared with the mean value between 1986 and 2005 by the end of the 21st century (IPCC 2013). This warming is likely to greatly affect the carbon (C) cycles of terrestrial ecosystems by increasing soil respiration (R_S, which is defined as the CO_2 efflux from the soil surface; Bond-Lamberty and Thomson 2010). R_S is the largest natural source of CO_2 flux from terrestrial ecosystems into the atmosphere (Schlesinger and Andrews 2000). Therefore, accurate evaluation of R_S in various terrestrial ecosystems and prediction of future changes due to global warming represent urgent and challenging tasks for ecosystem research (Chen and others 2011). Numerous in situ observations of soil CO_2 efflux from around the world, and their application to modeling analyses and meta-analyses, have helped to reveal the spatial and temporal dynamics of soil C cycles (Bond-Lamberty and Thomson 2010; Wang and others 2014). However, we still face uncertainties in the spatial and temporal dynamics due to differences in site-specific factors, in the temperature sensitivities of plants and soil microbes, and in other ecological and biogeochemical factors that are responsible for determining soil C dynamics (Davidson and Janssens 2006). In addition, to accurately estimate future soil C dynamics under the influence of climate change, we must accumulate fundamental data on the responses of the soil C cycle and N dynamics under open-field conditions in the terrestrial ecosystem.

Open-field warming experiments have been conducted in various terrestrial biomes during the last two decades with the goal of clarifying the potential changes in ecophysiological traits and soil biogeochemical properties that will occur in response to the predicted future climate change (Rustad and others 2001; Dieleman and others 2012; Chung and others 2013). A recent meta-analysis (Lu and others 2013) of such experiments showed that the experimental warming stimulated C cycling in most of the biomes that have been studied. For example, the experimental warming increased both aboveground and belowground plant biomass and accelerated C fluxes such as R_S and the loss of litter mass. However, the results showed considerable variation in response to warming and that information on this response is insufficient for Asian forest ecosystems, where global warming is expected to have a remarkable influence on C sequestration (Houghton 2003; Pan and others 2011).

On the other hand, recent studies of soil C dynamics have highlighted the importance of the different sensitivities of autotrophic respiration (R_A) and heterotrophic respiration (R_H) to elevated temperature, since these components of R_S have different contributions to R_S, and differences in their sensitivity will have important consequences for future changes in R_S (for example, Gomez-Casanovas and others 2012; Wang and others 2014). R_S has two key components: R_A is derived from plant roots and associated rhizosphere symbionts, and depends on the C assimilated by plant photosynthesis, whereas R_H depends on the decomposition of soil organic matter and plant litter by soil microbes (Hanson and others 2000; Kuzyakov and Gavrichkova 2010). Therefore, the effects of elevated soil temperatures on the biotic and abiotic factors may cause different responses by R_A and R_H. For example, experimental warming in grasslands has shown that R_A and R_H responded differently to warming, with different response magnitudes or directions (Zhou and others 2007; Li and others 2013). However, these results were inconsistent with the findings in evergreen forests, where warming stimulated both R_H and R_A to similar degree and in the same direction (Schindlbacher and others 2009; Vogel and others 2014). Because experimental findings on the responses of the soil C cycle to warming and discrimination of the warming effects on R_H and R_A depend on the experimental conditions and durations, we currently lack an adequate understanding of these phenomena.

Several long-term warming studies (more than 6 years) showed different durations of the stimulatory effects of rising temperature on R_S (Melillo and others 2002, 2011; Zhou and others 2011). The increased R_S that results from a warming treatment may disappear over time, with R_S returning to pre-warming levels because of a hypothesized deple-

tion of labile C pools and thermal acclimation of microbial activity (Eliasson and others 2005; Bradford and others 2008) and of root activity (Jarvi and Burton 2013; Li and others 2013). However, these changes have not been thoroughly confirmed and the underlying mechanisms have not been fully clarified. In addition, our understanding of the thermal acclimation of respiratory processes in plants and soil microbes is inadequate because insufficient long-term warming experiments have been conducted; such experiments are necessary to provide the data required to support general conclusions about the acclimation processes. Therefore, studies on the temperature responses and thermal acclimation of R_H and R_A under long-term open-field warming would improve our understanding of how R_H and R_A contribute to the response of R_S to soil temperature increases.

Such open-field experiments must be conducted by considering the temporal changes in physical (for example, temperature and water availability) and ecological (for example, phenology) processes that govern the responses of C dynamics (Mo and others 2005). Whereas physical processes have been well studied, phenological processes have been considered less often in studies of R_H due to the difficulty in capturing the influence of aboveground processes on belowground responses (Kirschbaum 2013; Du and Fang 2014). However, the R_A component of R_S may be strongly linked to aboveground processes, because the variability of R_A appears to follow the seasonal changes in the supply of photosynthates to the roots in response to changes in leaf phenology (Curiel Yuste and others 2004; Savage and others 2013). In addition, the changes between seasons and years in the magnitude and direction of the warming effect on R_S may be explained by the combined effect of warming on R_H and R_A. For example, lower soil moisture caused by periods of summer drought may counteract the effect of warming on R_S (Schindlbacher and others 2012). On the other hand, R_H and R_A may respond less sensitively to increasing temperature during the summer than during spring and fall, when temperatures are lower than during the rest of the growing season and moisture levels are higher (Janssens and Pilegaard 2003). The seasonal dynamics of R_S at an experimental warming site have been described by Contosta and others (2011), but there have been no studies of the seasonality of warming effects on R_H and R_A in forest ecosystems. Clarifying this seasonality would reduce the uncertainty in assessment of the effect of warming on R_S, because the influence of global

climate change on ecosystem processes would also exhibit clear seasonality, particularly in temperate and cool-temperate regions (Orlowsky and Seneviratne 2012).

In the present study, we investigated the effects of soil warming on R_A, R_H, and R_S by combining open-field soil warming treatments with trenching and non-trenching treatments in a Japanese cool-temperate deciduous broadleaf forest, which is an important representative vegetation type in the humid temperate zone of Monsoon Asia. The open-field warming treatment has been conducted since 2007. In this experiment, we examined the soil N availability, fine-root biomass, and C and N contents in the soil and roots to provide insights into the causes of the changes in R_A and R_H caused by soil warming. R_A is influenced by the changes in root biomass and in the root N concentration, which affect growth and physiological factors such as cell protein contents and nutrient assimilation (Ryan and others 1996). In addition, R_H is influenced by the change in soil C and N concentrations because R_H depends on the quality and quantity of the soil organic matter that is available to microbes (Eliasson and others 2005). We hypothesized that (1) soil warming would increase annual R_S, but that thermal acclimation of R_H and R_A would occur in responses to relatively long-term warming, leading to thermal acclimation of R_S, and (2) the impacts of warming on R_S would vary seasonally as a result of seasonal changes in R_H and R_A, which may respond to changes in the phenology of plants and in soil microbes. Based on data from our open-field warming experiment, we discuss possible consequences of the temperature responses of the ecophysiological and biogeochemical components of soil CO_2 efflux and of C and N dynamics.

MATERIALS AND METHODS

Study Sites

Our study was conducted at the Tomakomai experimental forest (TOEF, 42°40′N, 141°36′E, 80 m a.s.l.) of Hokkaido University. TOEF is located in a flat 2715-ha area with a cool-temperate climate in northern Japan. Mean annual air temperature and precipitation were 6.3°C and 1051 mm, respectively (1999–2008). The snow-free period is usually from mid-April to December, and the snow depth during the rest of year averages 0.7 m. The study site is a deciduous broadleaf forest dominated by more than 300-year-old *Quercus crispula* Blume with a canopy height of 18–20 m. Co-dominant tree species are *Ostrya japonica* Blume, *Acer mono*

Maxim., *Cercidiphyllum japonicum* Sieb. et Zucc., and *Tilia japonica* Simonkai. The forest floor is covered with a perennial evergreen dwarf bamboo (*Sasamorpha borealis*). The soil type is a Volcanogenous Regosol with a depth of 0.5 m. Additional details of the site are given by Nakamura and others (2014). The site is contributing data to the following networks: JaLTER (Japan Long-term Ecological Research Network), JapanFlux (part of the AsiaFlux Network), and J-BON (Japan's Biodiversity Observation Network).

Experimental Warming and Environmental Parameters

Three control plots and three warming plots (each 5 m × 5 m), with one oak tree in the center of each plot, were established in the spring of 2007. Electric heating wires were inserted horizontally into the soil in each warming plot at a depth of 5–10 cm, at horizontal intervals of 20 cm. The soil in the control plots was also cut using a flat-bladed shovel to create a disturbance similar to those in the warmed plots. The warming systems were designed to automatically remain 5°C warmer than the control plots by using thermocouples and a temperature controller (E5CS5-U, Omron Inc., Tokyo, Japan). The temperature difference was set to be the same as that used in the soil warming studies at the Harvard Forest (for example, Peterjohn and others 1994), and this differential falls within the range of the projected increase in global mean surface air temperature that will occur by the late 21st century (IPCC 2013). The thermocouples were inserted at a depth of 5 cm in all subplots. The soil warming started in May 2007 and operated throughout the year, except for a temporary period without warming during the winter of 2014.

The volumetric soil moisture content at a depth of 0–10 cm was measured using 5TM soil moisture sensors (Decagon Devices, Pullman, Washington, USA) in each subplot ($n = 6$). The data were recorded at 30-min intervals using Em50 dataloggers (Decagon Device). All sensors in the heated plots were placed halfway between adjacent heating wires. Air temperatures and precipitation were measured by weather stations adjacent to the site (www.jma.go.jp).

Trenching Treatment

To separate R_S to R_A and R_H, root trenching was applied because it provides a reasonable estimate of annual respiration rates in forest ecosystems when methodological side-effects such as disturbances in the plant-soil system are accounted for (Subke and others 2006; Díaz-Pinés and others 2010). A trench subplot (50 cm × 50 cm) was established inside each of the control and warmed plots at the study site in late October 2012. The trenched subplots were at least 3 m from the nearest tree trunk. Roots in the trenched subplots were cut to a depth between 45 and 50 cm below the ground surface, which represented the approximate depth of the solid bedrock. No roots were removed from the trenched plots, except for some subterranean roots of dwarf bamboo (>0.5 cm in diameter) and their attached secondary fine roots near the soil surface (0–5 cm), which were removed with minimum disturbance to the soil structure. To inhibit root ingrowth, pieces of 0.3-cm-thick polycarbonate board were inserted to reach the bottom of each vertical cut in the trench, and their joins were sealed with additional strips of the board. Any new plants were removed manually from the trenched subplots. The trenched subplots in the warmed plots were also equipped with heating wires, following the same design as in the non-trenched parts of the plot. Soil temperature and moisture sensors were installed in all trenched subplots. To minimize the transient response caused by decomposition of dead roots, the trenching treatment was conducted 6 months before we began measuring the R_H from the trenched subplots (Lee and others 2003). Then, R_A is calculated as the difference between R_S and R_H.

Fine-Root Biomass and Total Soil C and N

Soil N availability was evaluated using the ion exchange resin bag method (Binkley and Matson 1983). Three resin bags with 14 ml of the anion and 14 ml of the cation (Sybron IONAC ASB-1P OH^- and C-251 H^+, Sybron International, Milwaukee, WI, USA) were placed at 5 cm below the surface mineral soil in each plot during the growing season (139 days) in 2013. The ammonium and nitrate that became attached to the resin were extracted in 100 ml of 2 M KCl solution, and the inorganic N concentrations were determined using a Lachat flow-injection auto analyzer (QuikChem AE, Lachat instrument, USA). In June, we randomly collected three soil samples from the surface mineral horizon (0–5 cm) using a soil core sampler (5 cm in diameter) at each of control and warmed plots. All roots were removed from the fresh soil samples and divided into coarse (≥2 mm in diameter) and fine roots (<2 mm in diameter), and were then oven-dried at 75°C to a constant weight.

Subsamples of the remaining mineral soil were passed through a 2-mm sieve and oven-dried at 105°C to a constant weight, and the dry weight was used to calculate the bulk density of each 5-cm sample. They were ground to a particle size smaller than 0.25 mm, and used to determine the concentration of C and N using an NC analyzer (Sumigraph NC-22F, Shimadzu, Kyoto, Japan). Total soil C contents were calculated by multiplying the C concentration in the sample and the soil bulk density at each soil depth.

Soil CO_2 Flux Measurements

Soil CO_2 fluxes (μmol CO_2 m^{-2} s^{-1}) were manually measured using a closed-chamber system (20 cm in diameter, 7 cm in height) equipped with a diffusion-type non-dispersive infrared gas analyzer (GMP343, Vaisala, Helsinki, Finland). The chamber size was sufficiently small that it did not require a fan inside the chamber to ensure the mixing of gases. Three soil collars (20 cm in diameter, 5 cm in height) for each of the control and warmed plots and one soil collar for each of the trenched subplots in both control and warmed plots were inserted into the soil to a depth of 3 cm to ensure an airtight seal. A total of 24 collars were installed at locations with similar conditions in terms of the understory vegetation density, and not close to the tree's root collar or stumps. During the flux measurements, chamber lids with round rubber seals were attached to the fixed collars. CO_2 concentrations were recorded every 5 s for 300 s using dataloggers (MI-70, Vaisala). Respiration rates were calculated from a linear regression of the increase in the CO_2 concentration after the first 60 s (Bekku and others 1995). Simultaneously with these measurements, soil temperature and moisture at depths of 5 cm were measured at three to four points near all soil collars using a portable thermometer (CT-419WP, CUSTOM, Tokyo, Japan) and a time-domain reflectometry sensor (CS620, HydroSense, Campbell Scientific, Logan, UT, USA). Soil temperature and moisture data were used in determining the relationship between soil temperature and moisture and respiration rates. Soil moisture values measured at multiple points using the CS620 sensor ($n = 54$) were also used to correct the overestimation of moisture content by the 5TM sensor (mentioned above).

To investigate the impact of soil warming on R_S, R_H, and R_A, we measured the soil CO_2 flux three times a day at 2-month intervals, from mid-October 2012 to early November 2014. The seasonally representative measurement dates were decided based on four leaf development stages (dormancy,

leaf expansion, maturity, and senescence) that were determined based on the leaf area index (LAI) at the study site (Figure 1A). We also defined the four different stages to represent winter, spring, summer, and fall seasons based on threshold values of LAI. LAI of the forest canopy was estimated by the incident photosynthetically active photon flux density (PPFD) above the forest canopy and in the understory from 2012 to 2013. PPFDs were measured continuously at 5-min intervals using quantum sensors (IKS-27, Koito Industry Co. Ltd, Japan) and summed to provide daily values. LAI was calculated using the following equation (cf. Muraoka and others 2013):

$$LAI = -\frac{1}{0.42}\left(\ln\frac{PPFD_{understory}}{PPFD_{abovecanopy}} - \ln(0.4)\right), \quad (1)$$

where 0.42 is the constant that represents attenuation of the photosynthetically active radiation into the canopy (T. Hiura, unpublished data), and 0.4 is the ratio of PPFD in understory to PPFD above canopy measured before leaf expansion.

The respiration rates were measured in the morning, between 07:00 and 10:00 (hereafter, the "08:00" measurement time), between 11:30 and 13:30 (hereafter, "12:00"), and between 15:00 and 17:00 (hereafter, "16:00"). These categories cover the most important periods of diurnal variation in both soil temperature and respiration rates.

Data Analysis

We calculated R_A as the difference between R_S measured in the non-trenched subplots and R_H measured in the corresponding trenched subplots. To accurately calculate R_A from R_S and R_H, it is necessary to use the same soil temperature (T) due to the high sensitivity of these processes to temperature. Any small differences in soil temperature between the non-trenched and trenched subplots were corrected using the following equation:

$$R_H{'} = f(T_{nontrench}) + \text{bias}\,[R_H - f(T_{trench})], \quad (2)$$

where $R_H{'}$ represents the adjusted value of the measured R_H, and $f(T)$ represents the temperature dependence of R_H in the subplots. In addition, to accurately calculate the warming effect, small differences in the temperature increase between the control and warmed plots were corrected using the following equation:

$$R_W{'} = f(T_C + \Delta T) + \text{bias}\,[R_W - f(T_W)], \quad (3)$$

where $R_W{'}$ represents the adjusted value of the measured mean respiration rate in the warmed
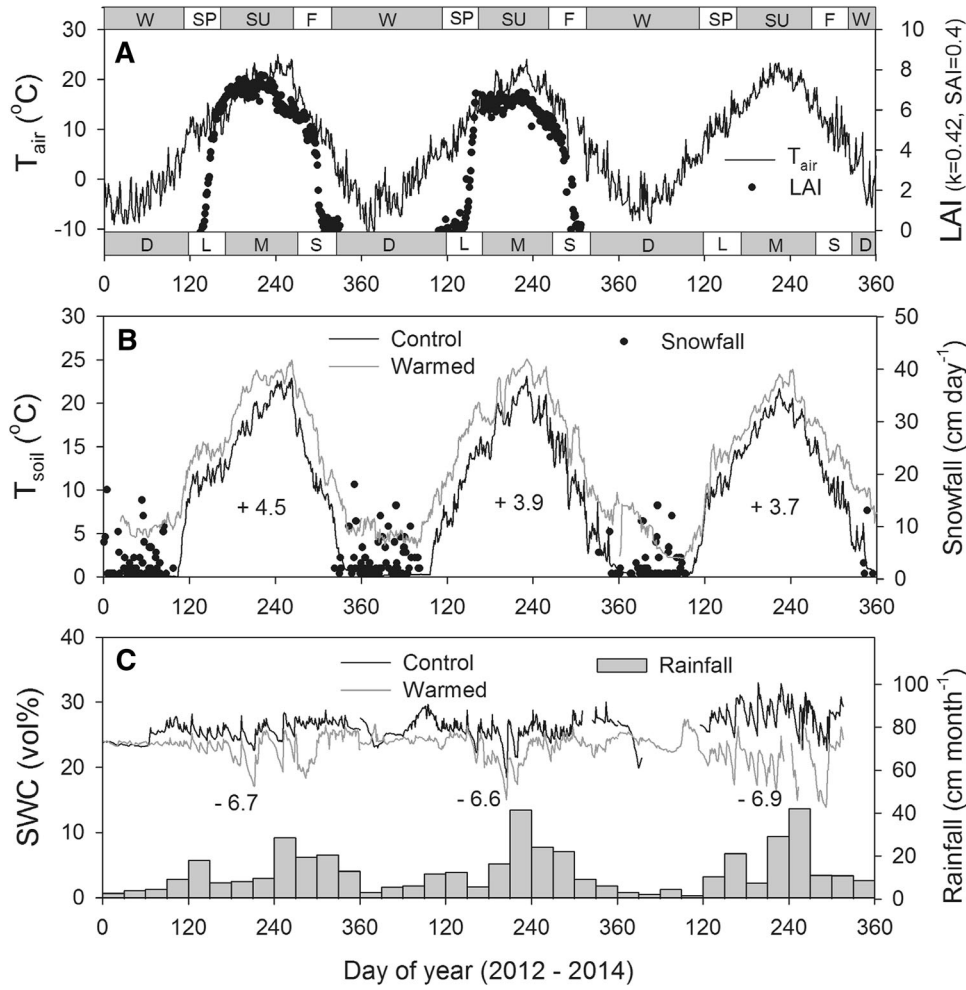
Figure 1. Seasonal variations in **A** the daily mean air temperature (T_{air}) and leaf area index (LAI calculated using the equation presented in the "Method" section), **B** soil temperature (T_{soil}) at a depth of 5 cm and snowfall, and **C** volumetric soil water content (SWC) at a depth of 5 cm and rainfall. *W* winter, *SP* spring, *SU* summer, *F* fall, *D* dormancy, *L* leaf expansion, *M* maturity, *S* senescence. The *numbers* in **B** and **C** represent the mean differences in soil temperature and soil water content, respectively, between the control and warmed plots (i.e., warmed value–control value).

subplots (R_W), $f(T)$ represents the temperature dependence of R_W in the subplots, and ΔT is mean value of the temperature increase during the respiration measurements. To describe the temperature dependence of the respiration rates, we used the following exponential function:

$$R = R_0 \exp^{bT}, \qquad (4)$$

where R is the CO_2 efflux (µmol CO_2 m^{-2} s^{-1}), R_0 is the respiration rate at a reference temperature of 0°C, b is a regression coefficient related to temperature sensitivity ($Q_{10} = e^{10b}$), and T is the soil temperature (°C) at a depth of 5 cm. This exponential function can be rewritten as $R = R_{10}Q_{10}^{(T-10)/10}$; here, R_{10} is the respiration rate at a reference temperature of 10°C (Mo and others 2005). Q_{10} was used to analyze the temperature sensitivities of R_S, R_H, and R_A per plot.

To assess the warming effects on measured CO_2 fluxes, the relative warming effect sizes were described as percentages using the following equation:

Warming effect (%) $= ([R_W/R_C] - 1) \times 100$, (5)

where R_W and R_C are the mean respiration rates in the warmed and control subplots, respectively.

Annual respiration rates were estimated by applying the fitted Q_{10} functions to the daily mean soil temperatures using the following equation:

$$R_C = \Sigma f_{control}(Q_{10c}, T_{control}) \text{ and } R_W = \Sigma f_{warmed}(Q_{10w}, T_{warmed}), \qquad (6)$$

where R_C and R_W are the estimated CO_2 fluxes in the control plot and warmed plots, respectively, and Q_{10c} and Q_{10w} are Q_{10} functions derived from the control and warmed plots, respectively. To estimate the annual respiration rates, including the respiration rate during the winter, we used data collected from April to November. The mean contribution of R_H to R_S was determined as the ratio of the estimated annual R_H to the estimated annual R_S. To correct for differences caused by different temperature increases among the years (that is, the actual increases rather than the target 5°C in-
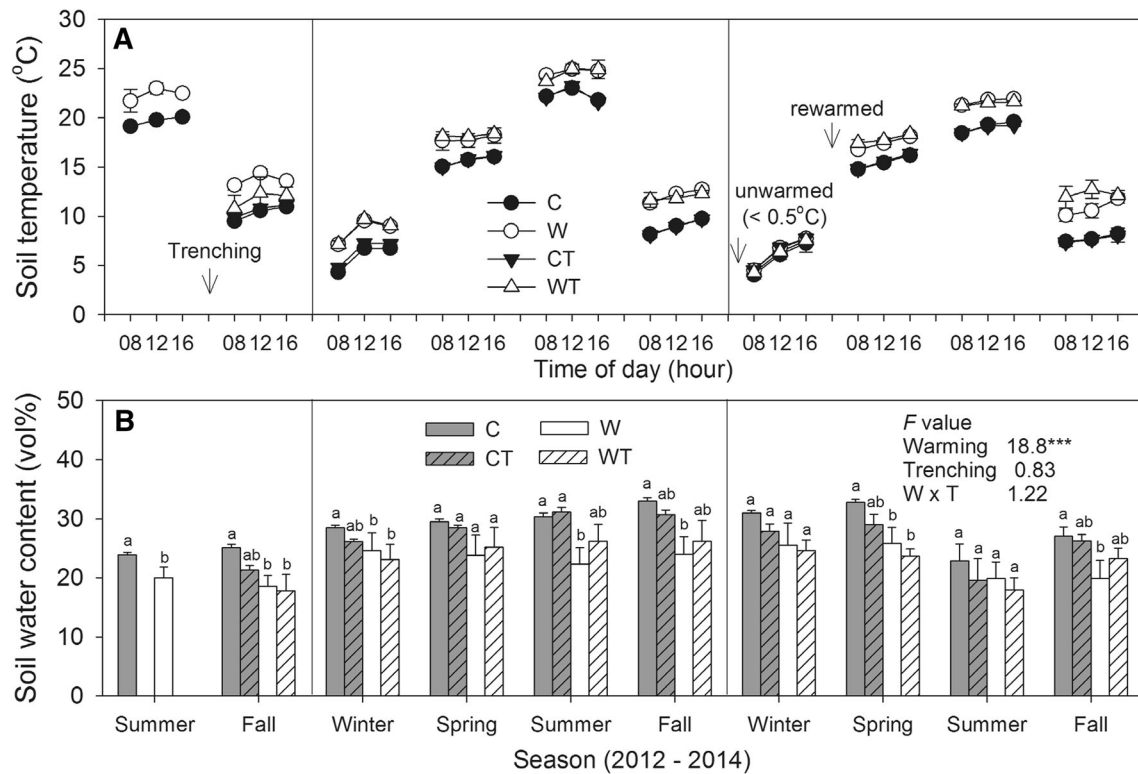
Figure 2. Temporal variations in **A** soil temperature and **B** soil water content in the untrenched and trenched subplots of the control and warmed plots. Times: 08:00, between 07:00 and 10:00; 12:00, between 11:30 and 13:30; 16:00, between 15:00 and 17:00. Plot types: C, untrenched control; CT, trenched control; W, untrenched warmed; WT, trenched warmed. *Bars* represent the mean ± one standard error (*n* = 3). For a given season, *bars labeled with differences letters* differ significantly among the treatments (*P* < 0.05).

crease), we normalized the warming effect to provide a value per 1°C increase by dividing the estimate by ΔT, which is the annual mean value of the increased temperature in each year.

To evaluate the effect of thermal acclimation on the prediction of future soil CO_2 fluxes, we used both Q_{10c} and Q_{10w} to simulate the annual respiration rates for a 4°C temperature increase, which was the mean value of the temperature increase compared with the temperature in the control plots during the 3 years of the study (Figure 1B).

Three-way analysis of variance (ANOVA) was used to examine the effects of soil warming, measurement time (08:00, 12:00, and 16:00), and season, and their interactions on soil temperature, soil moisture, and the CO_2 fluxes (R_S, R_H, R_A, and R_H/R_S). Two-way ANOVA was used to examine the effects of season and time of day (and their interaction) on the warming effect size on R_S, R_H, and R_A and to examine the effects of warming and trenching on soil temperature and soil moisture. Tukey's studentized range test was used to detect significant differences in soil temperature and soil

moisture among plot types, and Student's *t* test was used to detect significant differences in all C and N compounds, in temperature sensitivity (Q_{10}), and in other variables between the control and warmed plots. The effects were considered to be significantly different if $P < 0.05$. The statistical analyses were performed using version 9.4 of the SAS software (SAS Institute 2013).

RESULTS

Experimental Conditions

The soil warming treatments increased the annual mean soil temperatures at a depth of 5 cm by 4.5°C in 2012, 3.9°C in 2013, and 3.7°C in 2014 (Figure 1B). The soil temperature fluctuated in response to the seasonal pattern of air temperature ($r^2 = 0.93$, $P < 0.001$). During the period of the respiration observations (except for the winter in 2014), the soil temperatures around the soil collars in the warmed plots were higher than those in the control plots by average of 3.0°C in 2012, 3.0°C in 2013, and 2.1°C in 2014 (Figure 2A).

Soil warming decreased the annual mean soil moisture content by 6.7 vol% in 2012, 6.6 vol% in 2013, and 6.9 vol% in 2014, and the differences were significant ($P < 0.01$) (Table 2; Figure 1C, 2B). The mean volumetric moisture content at a depth of 0–10 cm in the non-trenched control plots ranged from 13.9 to 33.8 vol%, with an annual average of 27.1–29.6 vol%. The soil moisture fluctuated in response to the seasonal pattern of precipitation (Figure 1C). There was no significant difference in soil temperature between the non-trenched and trenched subplots ($P > 0.05$, Figure 2A). The trenching technique has potential problems associated with the changes in soil moisture that results from a lack of in-plot transpiration in the trenched plots (Bond-Lamberty and others 2011; Noh and others 2012). However, in our study, it appears that it was not necessary to account for changes in soil moisture conditions between the non-trenched and trenched subplots, since there was no difference in soil moisture compared with the non-trenched parts of the plot in both the control treatment and the warming treatment ($P > 0.05$, Figure 2B). Soil water contents at well-drained and cool humid regions such as our study site, where rainfall is frequent, may therefore show little influence of trenching.

Table 1 summarizes the results in the control and warmed plots in terms of the main soil and root parameters. None of the differences in N availability and in fine-root biomass were statistically significant between the plots. Soil warming marginally decreased the total soil N concentration ($P = 0.051$), from 6.6 mg g^{-1} in the control plot to 5.0 mg g^{-1} in the warmed plot. Soil warming did not significantly affect the root C and N concentrations, but marginally decreased the root C:N ratio ($P = 0.064$), from 37.6 in the control plot to 30.9 in the warmed plot (Table 1). The total C contents in the soil from 0 to 15 cm in depth were 42.1 and 36.0 Mg C ha^{-1} in the control and warmed plots, respectively, but the difference was not significant.

Responses of R_S, R_H, and R_A to Warming

The seasonal and diurnal variations in R_S, R_H, and R_A and results of statistical analysis are shown in Figure 3 and Table 2, respectively. In all respiration parameters, the seasonal variations were clear and significant (Table 2, $P < 0.0001$). On the other hand, we did not find a significant diurnal change in respirations (Table 2, $P > 0.05$). Significantly, higher R_S values were observed in the warmed plots than in the control plot for every season except for the summer season (Figure 3A, $P < 0.05$). Significant higher R_H values were observed in the warmed plots for every season than in the control plots, but R_A was significantly higher in the warmed plots at only a single time of day in spring seasons (Figure 3B, C).

The contribution of R_H to R_S was significantly increased by soil warming (Table 2, $P = 0.0004$). Mean R_H/R_S was 70.6% in the control plots and 73.7% in the warming plots. Figure 4 shows the seasonal patterns of the soil warming effect size on soil respiration rates. The warming effect (%) on R_S and R_H was significantly affected by the season ($P < 0.05$), but not by the time of day. Then, using a second-order polynomial function, we found a weak ($r < 0.53$) but statistically significant regression between season (day of year) and the warming effect on R_S and R_H ($P < 0.01$). Seasonal variations in the warming effect were clear, with lower effects in summer than in other seasons. The warming effect on R_A was often negative (that is,

Table 1. Soil N Availability, Fine-Root Biomass, and C and N Concentration in the Fine Roots and Soil of the Topsoil Layer (0–5 cm)

	Control	Warmed	P value
N availability (NH$_4^+$ + NO$_3^-$ ppm bag^{-1})	23.4 (5.1)	24.7 (2.9)	0.639
Fine-root biomass (<2 mm, g m^{-2})	96.9 (19.3)	99.2 (19.4)	0.941
Live fine-root C concentration (mg g^{-1})	479.1 (7.0)	460.2 (8.9)	0.058
Live fine-root N concentration (mg g^{-1})	13.1 (0.4)	15.4 (1.8)	0.269
Root C:N ratio	37.6 (1.58)	30.9 (34.9)	0.064
Total soil C concentration (mg g^{-1})	92.0 (13.8)	72.9 (7.4)	0.165
Total soil N concentration (mg g^{-1})	6.6 (0.9)	5.0 (0.5)	0.051
Soil C:N ratio	13.9 (0.1)	14.3 (0.3)	0.153
Total soil C content (Mg C ha^{-1})[a]	42.1 (7.9)	36.0 (1.7)	0.448

Reported values are means ± one standard error (n = 3).
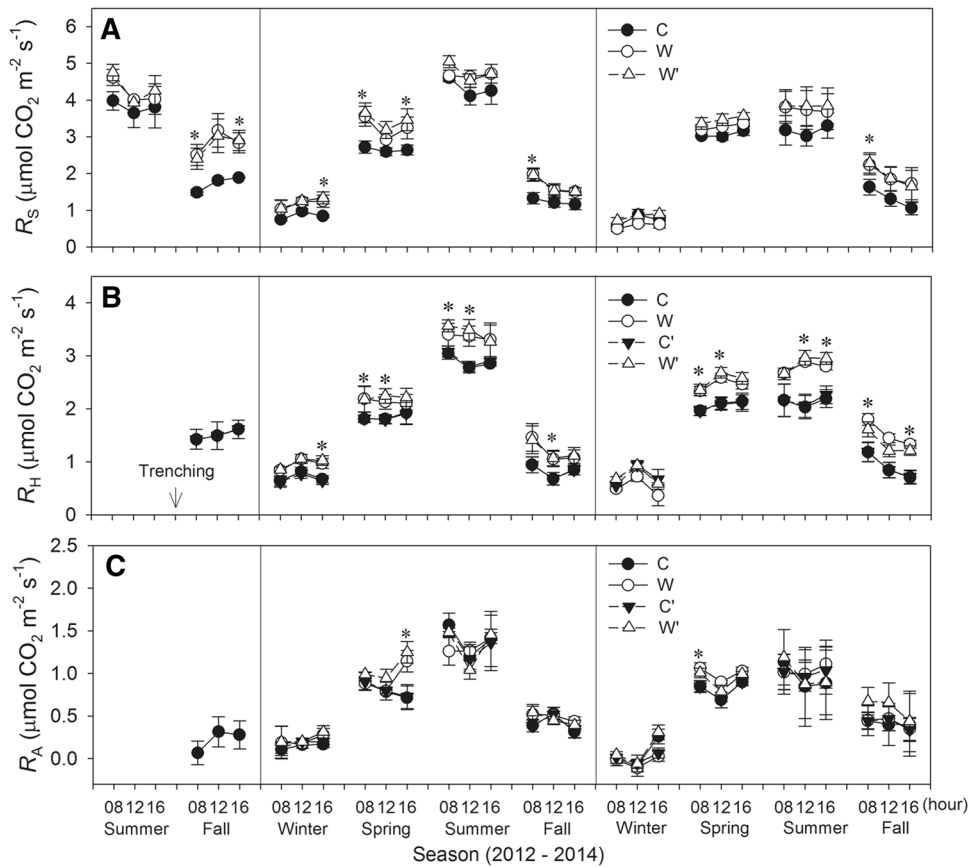*[a]0–15 cm soil depth.*

Figure 3. Temporal variations of **A** R_S, **B** R_H, and **C** R_A in the control and warmed plots. C' and W' are the temperature-corrected values (see the "Methods" section for details). *Bars* represent mean ± one standard error ($n = 3$). *Values labeled with an asterisk* differ significantly between the control and warmed plots ($P < 0.05$).

Table 2. Statistical Significance (P values) of the Three-Way ANOVA for the Effects of Soil Warming (S), Time of Day (T), Season (S), and Their Interactions on the Variables

Factor	DF	T_S	SWC	R_S	R_H	R_A	R_H/R_S
Warming (W)	1	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.1076	**0.0004**
Time of day (T)	2	**<0.0001**	0.9565	0.5017	0.7223	0.1447	0.9661
Season (S)	3	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.9470
W × T	2	0.8329	0.9119	0.9788	0.8892	0.4147	0.9597
W × S	3	0.2559	0.5539	0.7109	0.3651	0.6294	0.7056
T × S	6	0.2664	0.9997	0.6771	0.3204	0.4070	0.9934
W × T × S	6	0.9976	0.9998	0.9996	0.9117	0.9207	0.9997

Significant differences are highlighted in bold ($P < 0.05$).
DF degree of freedom.

warming decreased R_A), depending on the time of day, and this was especially clear in the winter and summer, but the effects of season and time of day were not statistically significant.

Temperature Sensitivities of R_S, R_H, and R_A

Table 3 shows the Q_{10} values and other variables derived from the relationship between soil temperature and respirations from 2012 to 2014. R_S,

R_H, and R_A were all strongly significantly correlated with temperature ($r^2 > 0.71$), except for R_A in 2014, which had a weak but significant correlation ($r^2 = 0.37$), whereas the soil water content showed no significant relationship with R_S, R_H, or R_A (Figure S1). Soil warming decreased the temperature sensitivities (Q_{10} values) of R_S, R_H, and R_A, and the difference in R_S was significant in 2012 and 2013 ($P < 0.05$), but only marginally significant in 2014 ($P < 0.10$) and for the 3-year period as a whole
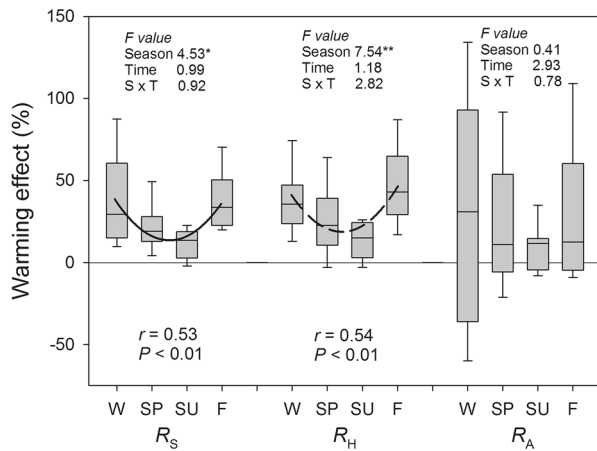
Figure 4. Seasonal variations in the soil warming effect (%) on R_S, R_H, and R_A. Results are reported for the two-way ANOVA with season (S) and time of day (T). *Values labeled with an asterisk* are statistically significant (*$P < 0.05$; **$P < 0.01$). *Box plots* present the median, 95% confidence interval, and minimum and maximum values. The *lines* represent statistically significant quadratic equations for the relationship between the season (day of year) and the warming effect. *W* winter, *SP* spring, *SU* summer, *F* fall.

(Table 3). Figure 5A shows the estimated R_S, R_H, and R_A calculated from the model coefficients in the control and warmed plots. Figure 5B shows the seasonal variation of the increases in soil C loss as a result of R_H and R_A caused by the warming treatment. The increase in soil C loss caused by warming resulted from increases in R_H and R_A: for R_H, the increase was greatest (0.58 g C m^{-2} d^{-1}) in the summer (between the 180th and 270th of day of the year), when soil temperatures were highest (22.5°C), whereas for R_A, the increase was greatest (0.12 g C m^{-2} d^{-1}) in the spring and fall, when soil temperatures were between 12 and 14°C in the control plot.

Annual R_S, R_H, and R_A

Figure 6 shows the annual total R_S, R_H, and R_A in the control and warmed plots from 2012 to 2014. In 2012, soil warming (+4.5°C) increased the annual R_S by 45%. In 2013, soil warming (+3.9°C) increased the estimated annual R_S by 34%, R_H by 41%, and R_A by 18%. In 2014, soil warming (+3.7°C) increased the estimated annual R_S by 32%, R_H by 39%, and R_A by 17%.

Because these percentages resulted from different magnitudes of warming, we normalized the effect of warming on R_S, R_H, and R_A per 1°C warming to allow a comparison based on the same temperature increase. The normalized magnitude

of the effect of warming on R_S, R_H, and R_A was an increase of 9.0, 10.4, and 4.6%, respectively, in 2013, and of 9.1, 10.3, and 4.4% in 2014 (Figure 6).

To examine the influence of thermal acclimation on estimation of annual R_S, R_H, and R_A for 4°C of warming, we used the Q_{10} values derived from the control and warmed plots shown in Table 3. The annual respiration rates estimated based on Q_{10} in the control plots were overestimated by 0.68 Mg C ha^{-1} (8.3%) for R_S, by 0.14 Mg C ha^{-1} (2.3%) for R_H, and by 0.61 Mg C ha^{-1} (28.0%) for R_A compared with the values estimated based on Q_{10} in the warmed plots (Figure 7).

DISCUSSION

The most pronounced changes in the belowground C pools and fluxes caused by soil warming were attributed to the stimulation of both R_S and R_H. However, R_A responded differently, and we will discuss the different responses of R_H and R_A to elevated temperature with reference to the warming effects on other variables, the temporal variability, changes in the temperature sensitivities of the components of soil respiration, and their implications for forest C cycles under future climate warming.

Soil N Availability and Fine-Root Biomass

We expected that soil N availability would be decreased by soil warming, which in turn would induce an increase in root biomass in the warmed plots because of the substantial reduction of solute N pools by preventing the soil from freezing during winter at this warming site (Ueda and others 2013). In general, greater microbial activity at warmer temperatures increases net N mineralization (Bonan and Van Cleve 1992; Rustad and others 2001), and hence sufficient N availability may lead to a decrease in root biomass because fewer roots are required to take up sufficient N (Zhou and others 2011). On the other hand, the warming-induced increase in plant productivity can directly enhance both root biomass and root activity (Yin and others 2013). However, this study shows that soil warming did not increase N availability during the growing season and that the soil warming plot had a marginally lower total soil N concentration ($P = 0.051$; Table 1). At our warming site, long-term soil warming significantly decreased mean soil moisture, by a 3-year mean of 6.7%. The decreases in soil moisture and in N pools may offset the effect

Table 3. Temperature Sensitivity (Q_{10}) and Variables Derived from Relationships Between Soil Temperatures and Respiration Rates ($R = R_0 \times \exp^{bT}$)

	R_0		R_{10}		r^2		Q_{10}		P
	Control	Warmed	Control	Warmed	Control	Warmed	Control	Warmed	
2012									
R_S	0.532 (0.104)	0.883 (0.079)	1.491 (0.036)	1.834 (0.160)	0.93 (0.02)	0.79 (0.03)	2.87 (0.23)	2.08 (0.01)	**0.028**
2013									
R_S	0.483 (0.013)	0.588 (0.085)	2.346 (0.062)	2.155 (0.129)	0.96 (0.01)	0.94 (0.01)	2.79 (0.04)	2.41 (0.14)	**0.048**
R_H	0.369 (0.060)	0.469 (0.033)	1.579 (0.102)	1.605 (0.189)	0.94 (0.01)	0.90 (0.02)	2.70 (0.21)	2.27 (0.05)	0.125
R_A	0.096 (0.011)	0.115 (0.011)	0.646 (0.021)	0.551 (0.010)	0.79 (0.07)	0.79 (0.05)	3.63 (0.34)	2.87 (0.14)	0.108
2014									
R_S	0.602 (0.064)	0.884 (0.073)	1.530 (0.133)	1.714 (0.208)	0.83 (0.01)	0.81 (0.07)	2.56 (0.08)	2.08 (0.18)	0.069
R_H	0.476 (0.097)	0.565 (0.091)	1.098 (0.141)	1.209 (0.108)	0.82 (0.03)	0.89 (0.02)	2.39 (0.20)	2.19 (0.17)	0.494
R_A	0.146 (0.043)	0.265 (0.013)	0.430 (0.088)	0.471 (0.184)	0.71 (0.04)	0.37 (0.16)	3.22 (0.46)	2.07 (0.49)	0.163
2012–2014									
R_S	0.571 (0.024)	0.749 (0.107)	1.481 (0.074)	1.636 (0.153)	0.90 (0.01)	0.83 (0.02)	2.59 (0.04)	2.22 (0.14)	0.058

R_0 basal respiration rate, b coefficient that represents the Q_{10}. R_{10} respiration rate at a reference temperature of 10°C. Reported values are means ± one standard error (n = 3). Significant differences are highlighted in bold (P < 0.05).

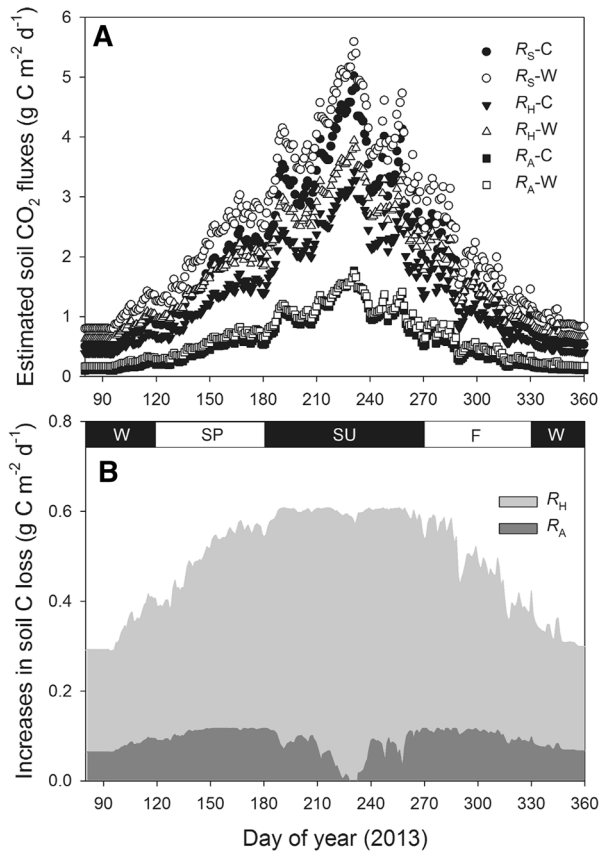**Figure 5.** **A** Estimated R_S, R_H, and R_A in the control and warmed plots and **B** increases in the soil C loss as a result of changes in R_H and R_A caused by soil warming. *W* winter, *SP* spring, *SU* summer, *F* fall.

of increasing temperature on N availability by decreasing microbial activity and the resulting N mineralization (Wang and others 2006; Allison and Treseder 2008).

As a result, soil warming did not significantly alter fine-root biomass or the root C and N concentrations, and the root N concentration in our study site was consistent with the values (11.5–11.7) in a recent study in a hardwood forest dominated by *Acer saccharum* Marsh. (Jarvi and Burton 2013). However, soil warming decreased the root C:N ratio (by 18%), and the decrease was marginally significant (*P* = 0.064; Table 1), as has been found in a study in a mixed hardwood forest dominated by *Quercus rubra* and *Acer rubrum* (Zhou and others 2011). The decreased C:N ratio suggests increases in root mortality and decomposition rates (Withington and others 2006). In a companion experiment based on a minirhizotron technique at this study site, we found that root production and mortality were higher in the warmed plots than in the control plots, and there was higher seasonal
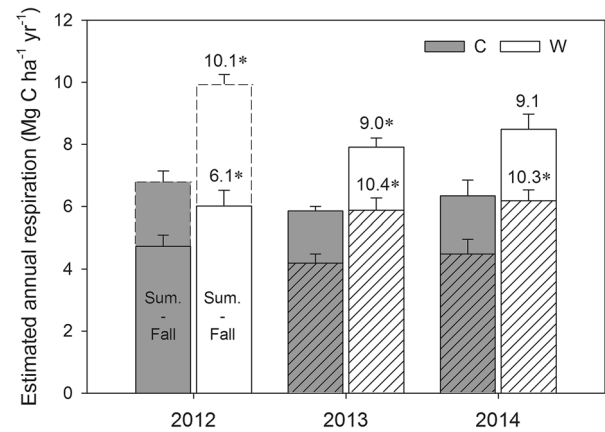

Figure 6. Estimated annual R_S (total bar length), R_A (*top part of the bars*), and R_H (*cross-hatched part of the bar*) from 2012 to 2014. The *dashed line* in 2012 indicates the estimated annual R_S including the period (winter and spring) when no R_S measurements were available. Annual R_S and R_H were estimated using the model coefficients and daily mean soil temperature in the control and warmed plots for each year. The *values above the bars* denote the normalized warming effect of the respiration rates per 1°C temperature increase. *Bars* represent the mean ± one standard error (*n* = 3). *Numbers labeled with an asterisk* differ significantly between the control and warming treatments (*P* < 0.05). Treatments: *C* control, *W* warmed. Season: *Sum* summer.
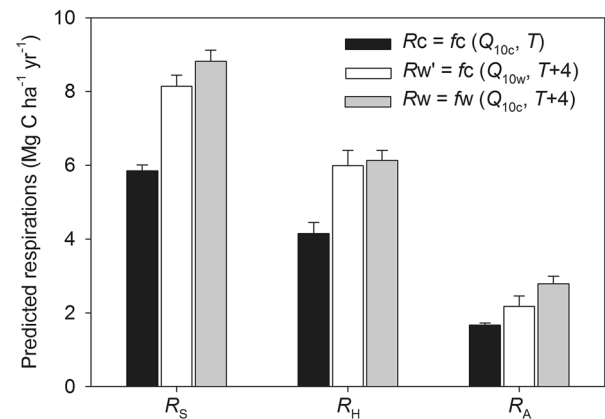

Figure 7. Annual R_S, R_H, and R_A predicted at a 4°C higher temperature using the daily mean soil temperature in the control plot and the two different model coefficients in the control and warmed plots. Q_{10c} and Q_{10w} were derived from the control and warmed plots, respectively.

variability in the warmed plots (K. Fukuzawa and others, unpublished data). The much lower total soil C concentration in the long-term warmed plots suggests that the most labile C (contributed by root mortality) may have contributed to prolonging the

warming effects on R_H; however, the difference was not statistically significant ($P = 0.165$). Our on-going study of root turnover rates, labile and stable C pools will provide insights to understand long-term warming effects on the soil C pool as a result of the balance between plant C inputs and soil C losses in the warmed plots.

Responses of R_S and R_H to Warming

A recent meta-analysis (Wang and others 2014) showed that R_S tends to be increased under experimental warming conditions. In our mature temperate forest, the increases in R_S caused by soil warming during 3 years of measurements were within the range of 24–76% increases reported in other soil warming experiments in a range of forest ecosystems (for example, Peterjohn and others 1994; Rustad and Fernandez 1998; Melillo and others 2002; Bronson and others 2008; Schindlbacher and others 2009; Hagedorn and others 2010). The 32% to 45% increases of R_S caused by soil warming at our study site were less than the 47% increase of R_S (at +4°C) in a coniferous forest in a cool humid climate (Schindlbacher and others 2009), but higher than the 28% increase during the first 6 years in a temperate deciduous forest (at +5°C) (Melillo and others 2002).

On the other hand, there was only a small difference (about 1% point) in the normalized "warming effect" during our 3 years research (Figure 6). The stimulatory effect of soil warming on R_S may be declined during long-term warming because of depletion of the labile C pools (Bradford and others 2008). However, in our study, R_S was still greatly stimulated (by 32% compared with the control), even after 8 years of warming (5 years before the study period and 3 years during the study), and this reflected the increased R_H. In addition, soil warming did not significantly alter the temperature sensitivity of R_H (Table 3). Therefore, in our temperate forest, we would expect long-lasting warming effects on both R_H and R_S because of the constant supply of predominantly non-stabilized and readily decomposable C pools via plant litter (cf. Reichstein and others 2005). This is supported by the fact that increased root litter (discussed earlier) produced under soil warming represents decreased root longevity based on the C:N ratio. Recent warming manipulation experiments combined with trenching treatments showed that short-term soil warming continuously increased R_H during a 3-year experimental period in a grassland (Li and others 2013) and during a 2-year experimental period in a coniferous forest

(Schindlbacher and others 2009). On the other hand, in our long-term warmed soils, as the total soil C content was lower than that in the control (-14%, $P = 0.448$), the soil warming site may have had a much lower level of soil labile C; however, the difference was not significant, so more research will be required to test this hypothesis by separately quantifying levels of labile and stable soil C (Xu and others 2012). This predicted decrease in labile C pools during long-term warming would probably result from the increase in R_H, which would consume the labile C as a respiratory substrate.

The contribution of R_H to R_S was 71 and 74% in the control and warmed plots, respectively, which falls within the range reported for temperate deciduous forests (29–101%; Subke and others 2006). In this study, we used trenching to distinguish between R_H and R_A. Because of the organic matter derived from residual dead roots, trenching plots would typically result in a transient increase (by 8% after 1 year) of R_S (Lee and others 2003). Considering the transient effect of these residual dead roots, the contribution of R_H to R_S in this study may have been overestimated to some extent. On the other hand, R_H in the trenched subplot is not equivalent to R_H in the non-trenched plots due to exclusion of the "rhizosphere priming effect", in which root exudates can stimulate R_H (Kuzyakov and Gavrichkova 2010). In this case, the trenching technique would lead to underestimation of the warming effect on R_H because the decreased labile C pools would decrease the magnitude of the warming effect on R_H, leading to overestimation of R_A. The net effect of the underestimate and overestimate is not clear, and should be clarified in future research.

Responses of R_A to Warming

Soil warming increased the annual R_A by 17% to 18% during 2013 and 2014 at our long-term warmed site. Our study provided new field evidence that R_A still responded to warming even after 8 years of warming. In a long-term warming experiment by Melillo and others (2011) in a mixed deciduous forest, 7 years of warming decreased R_A due to a decline in fine-root biomass. The warming effect on R_A is determined by a combination of the changes in root biomass and the R_A per unit root biomass. As we did not find any changes in root biomass, this suggests that R_A per unit root biomass increased in the warmed plot.

On the other hand, soil warming decreased the temperature sensitivity of R_A compared with the control. The temperature sensitivity of R_A was

lower in the warmed plots (Q_{10} = 2.07–2.87) than that in the control plots (3.22–3.63), but the difference was not statistically significant. This apparent decrease in Q_{10} may have resulted from a warming-induced moisture deficit (Jarvi and Burton 2013). Decrease in soil moisture, especially during the warm summer, may cause a decreased response of R_A to temperature, since low water availability causes stomatal closure and reductions in photosynthetic C assimilation.

We hypothesized that R_A and R_H would respond differently to warming. The magnitude of increment in R_A caused by warming was smaller than that for R_H, which supports our hypothesis. A previous study in a temperate evergreen forest revealed that short-term soil warming stimulated both R_H and R_A to the same degree (Schindlbacher and others 2009). However, in other research, the change in R_A was distinct from the warming-induced change in R_H because R_A is also influenced by changes in both above- and below-ground productivity (Zhou and others 2011; Li and others 2013). If long-term warming decreases root activity, including the production of fine roots and the root tissue N concentration, then the effect of warming (a decrease) would be stronger for R_A than R_H.

Seasonal Variation of the Warming Effect

As we expected, the responses of R_S and R_H to warming varied seasonally. The seasonality of the effect on R_S was smallest in the summer (Figures 3, 4), which is consistent with a previous result in a mature hardwood forest (Contosta and others 2011). Our results indicate that the warming effect on R_S is largely dependent on the different seasonal patterns of the warming effect on R_H and R_A. The increases in R_S during the winter and summer were contributed mainly by the stimulation of R_H by warming (Figure 5B). The most pronounced increase in R_A was observed during the spring, when leaf expansion occurred (Figure 3C). It is also known that soil warming at our study site increased the photosynthetic activity of forest-floor plants and changed leaf phenology during the spring (Ishioka and others 2013). These warming-induced changes in leaf phenology would cause the pronounced response of R_A to warming, suggesting potential linkage of C transportation between photosynthetic production and root respiration. Considering the different temporal variations of the warming effects on R_H and R_A, our results suggest that a warmer or extended spring might have larger effects on annual R_H and R_A and hence R_A than a warmer or extended summer. However, the seasonal variation in the responses of R_H and R_A to warming remains uncertain because we did not consider the seasonal variations in the availability of labile substrates that were removed by the root trenching (Kirschbaum 2013).

Temperature Sensitivities in Forest C Cycles Under Climate Warming

In our long-term warming experiment, we quantified the effects of soil warming on R_S, R_H, and R_A. We then demonstrated a difference in the temperature response curve of R_H and R_A indexed by Q_{10}, and between control and warmed plots. We found that the effect of warming on annual R_H was stronger than that on annual R_A due to the lack of a decrease in temperature sensitivity (Q_{10}) of R_H with increasing temperature. R_H contributed more than R_A to prolonging the effect of warming on R_S. This result supports a recent meta-analysis, which found that R_H and R_A responded differently to rising temperature (Wang and others 2014). As a result, our long-term warming slightly increased the contribution of R_H to R_S due to the lower responses of R_A. If the increase in plant C uptake caused by warmed-induced changes in photosynthetic capacity does not compensate for the increased loss of soil C that results from R_H, then the C budget would change to show decreased C sequestration in this forest (Norby and Luo 2004; Melillo and others 2011).

We also found a decrease in Q_{10} of R_A caused by warming, and this resulted in acclimation of R_S to warmer temperature. Thermal acclimation of R_S has been discussed mainly in relation to temperature-induced weakening of the positive correlation between warming temperatures and increased C sequestration (for example, Luo and others 2001; Kirschbaum, 2004) The increased loss of C due to increased R_S would be lessened by reducing plant photosynthetic or respiratory activity or capacity as the plants acclimated to warming (Atkin and Tjoelker 2003). As has been shown in our simple model estimation (Figure 7), R_S would be overestimated remarkably if we applied temperature response (Q_{10}) obtained under current environmental condition (that is, control plots in this study) to the simulated future condition with 4°C higher temperature (Figure 6) (Curiel Yuste and others 2010). To more accurately predict the changes of R_S subjected to climate changes, our results suggest that models should account for when and how much R_H and R_A will acclimate to changing temperatures.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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