Doctoral Thesis

Effects of climate warming on soil respiration and microbial community in warm temperate evergreen broad-leaved forests of Japan

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March 2013

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CHAPTER 1
General Introduction

1-1 Climate warming and the terrestrial ecosystem carbon cycle

Human activities, such as fossil-fuel burning, have resulted in an increase in the atmospheric CO_2 concentration from 280 ppm in pre-industrial times to 380 ppm at present and potentially to 700 ppm by the end of the 21st century (IPCC 2007). As a consequence of the increasing CO_2 concentration, global mean temperature increased by about 0.6 °C during the 20th century and is expected to increase by another 1.1–6.4 °C by the end of the 21st century (Houghton 1996; IPCC 2007).

Rapid increases in CO_2 and temperature affect almost all aspects of terrestrial carbon processes. Terrestrial ecosystems are intimately connected to atmospheric CO_2 levels through photosynthetic fixation of CO_2, sequestration of carbon into biomass and soil, and subsequent release of CO_2 through respiration and decomposition of organic matter (Fig. 1-1). Terrestrial ecosystems contain almost three times the amount of carbon

found in the atmosphere, with forests comprising by far the largest fraction (Schlesinger 1997). Forest ecosystems account for about 46% of the carbon stored in terrestrial ecosystems (IPCC 2001). Because soils contain more than two-thirds of the carbon in forest ecosystems (IPCC 2001), understanding the effect of climate warming on terrestrial ecosystems requires knowledge of how forest soil processes respond to soil warming.

1-2 Soil respiration and soil microbial community

Soil respiration is the sum of the autotrophic component produced by roots and the heterotrophic component originating from soil microbes that decompose organic materials (Fig. 1-1) (Bowden et al. 1993; Boone et al. 1998; Epron et al. 1999). Studies have suggested that these two components are differently influenced by climatic conditions (Boone et al. 1998; Epron et al. 2001; Dilustro et al. 2005) and respond differently to elevated atmospheric CO_2 or soil warming (Rustad et al. 2001; Pendall et al. 2004). Thus, separate estimates of these components are required for analyzing and modeling soil respiration and its response to climate change (Hanson et al. 2000).

In addition to the direct effects of climate change on soil respiration, soil warming–induced changes in the composition and biomass of soil

microbial communities have the potential to cause changes in soil carbon flow and are therefore likely to affect the global carbon cycle (Schimel and Gulledge 1998; Bardgett et al. 2008). Both field and chamber experiments have suggested that elevated temperature affects the biomass and composition of the soil microbial community (Allison and Treseder 2008; Frey et al. 2008; Schindlbacher et al. 2011).

1-3 Soil carbon cycling in warm temperate evergreen broad-leaved forests

One of the difficulties in assessing the impact of future climate change on forest ecosystem carbon cycles is that the response of soil respiration to climate change may vary widely depending on tree species. For example, George et al. (2003) reported that the annual fine-root respiration rate of *Pinus taeda* decreased by 17% and that of *Liquidambar styraciflua* increased by 86%, although both tree species were grown at similar CO_2 concentrations. The root respiration rates of *P. taeda* and *Pinus ponderosa* were similar at ambient CO_2 levels but differed significantly with elevation of CO_2 (Griffin et al. 1997).

The biomass and composition of soil microbial communities may also respond differently to elevated temperature, depending on the tree species.

Schindlbacher et al. (2011) found no change in microbial biomass and composition in a temperate, deciduous, broad-leaved forest, whereas other studies reported a reduction in fungal biomass and a shift in the microbial community in a mixed deciduous forest (Frey et al. 2008) and increases in bacterial and fungal biomass in a conifer forest (Allison and Treseder 2008).

The evergreen broad-leaved forest is a unique plant formation almost exclusively confined to the east coasts and adjacent islands of Asia (Kira 1978). This type of forest is the main natural vegetation type in the warm temperate zone of Japan (Suzuki 1982). Although the current area of evergreen broad-leaved forest is not extensive because of conversion to other land uses, the potential distribution covers the western half of the Japanese Archipelago. Evergreen broad-leaved forest has a much higher carbon cycling rate than forests in cool climates, such as cool temperate and boreal forests (Nakane et al. 1997). Ito et al. (2010), who examined the spatial pattern of soil respiration throughout the Japanese Archipelago using five carbon cycle models, estimated that evergreen broad-leaved forest had the highest soil respiration rate among the biomes of Japan. However, few data are available on the potential impact of climate change on soil respiration and the soil microbial community in warm temperate broad-leaved forests.

1-4 Objective and outline of this thesis

The objective of this research was to examine the effects of climate warming on the soil respiration and microbial community in evergreen broad-leaved forests in the warm-temperate zone of Japan.

In Chapter 2, I describe a multi-year open top chamber (OTC) experiment used to examine the potential effects of climate change (increased CO_2 level and temperature) on soil respiration (root respiration and heterotrophic respiration) in evergreen broad-leaved forests dominated by *Quercus glauca*, a common species in this forest type in western Japan. In Chapter 3, I discuss a field soil warming experiment conducted to examine the effect of increased soil temperature on the heterotrophic microbial community. In Chapter 4, I describe a pot culture experiment performed to examine the effects of elevated temperature on the rhizospheric soil microbial community associated with *Q. glauca* seedlings. In Chapter 5, I discuss my findings and conclusions.

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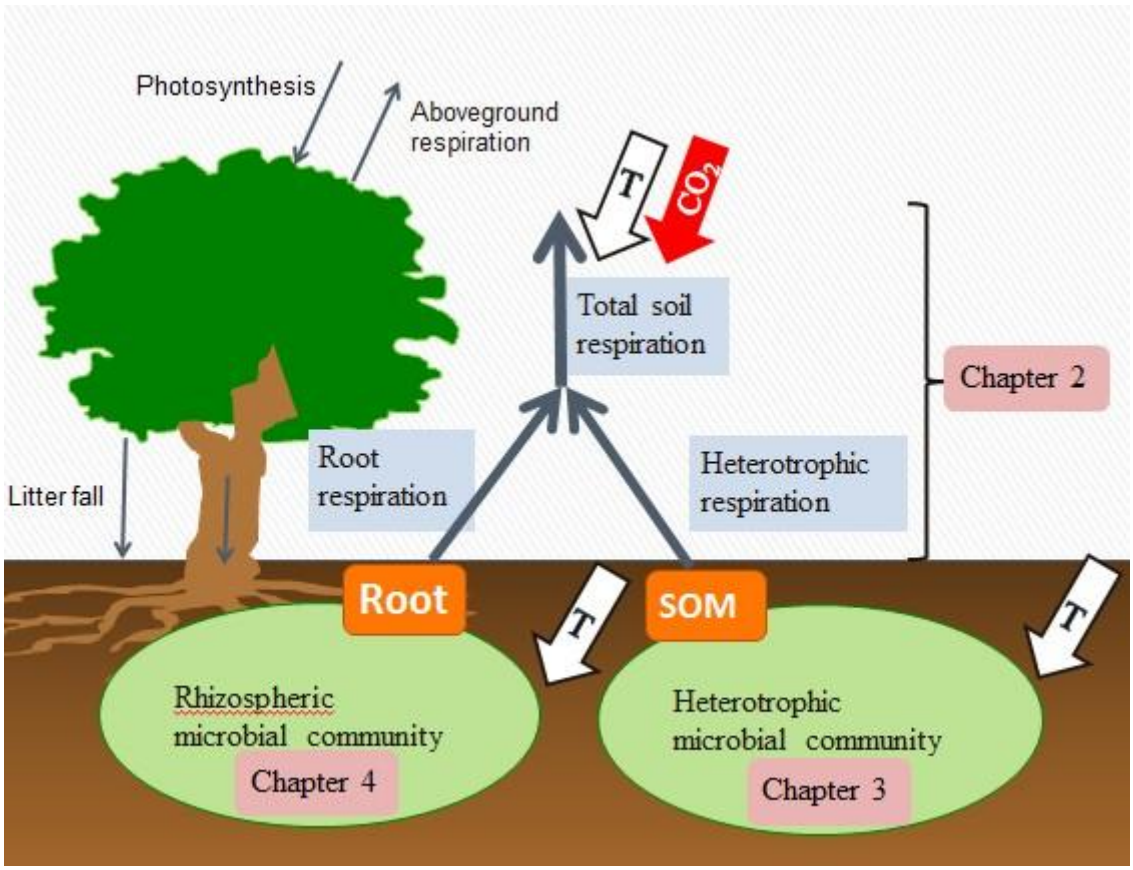

Fig. 1-1 Outline of the processes studied in this thesis. White arrows indicate the impact of elevated temperature (T), and red arrows indicate the impact of elevated CO_2.

CHAPTER 2
Impacts of elevated CO_2 and temperature on soil respiration in warm temperate evergreen *Quercus glauca* stands: an open-top chamber experiment

2-1 Introduction

To estimate the effects of climate change (elevated CO_2 and temperature) on soil respiration, experiments under near-natural soil and climate conditions are needed. Open-top chambers (OTCs) (Edwards and Norby 1999; Crookshanks et al.1998) are ideally suited to this purpose. However, previous studies have shown that the effects of elevated CO_2 and temperature on soil respiration vary with the duration of exposure (Rustad et al. 2001; Eliasson et al. 2005; Bernhardt et al. 2006), suggesting that multi-year experiments are important.

The aim of this study was to use a multi-year OTC experiment to examine the potential effects of climate change (increased CO_2 levels and temperatures) on soil respiration in evergreen broad-leaved forests dominated by *Quercus glauca*. This species, which tolerates drought and

nutrient-poor environments, occurs commonly in the evergreen broad-leaved forests of western Japan as a mid-succession species (Fujiwara 1981). Experimental stands of *Q. glauca* were grown in OTCs subjected to elevated temperatures and CO_2 concentrations. For 3 years, I examined the responses of total soil respiration and heterotrophic respiration in these stands to parameters indicative of climate change.

2-2 **Methods**

OTC experiment

This study was conducted as part of an OTC experiment to examine the effect of climate change on the evergreen broad-leaved forest ecosystem (Nakane et al. 2008). Six cuboidal OTCs (4 m long × 4 m wide × 5 m high) with open tops and made of transparent plastic sheets covering a metal frame were placed on the ground in the precision experimental field at the Biosphere Department's precision experimental at Hiroshima University in Higashi-Hiroshima, Japan (lat 34°24′N, long 132°44′E, 225 m a.s.l.) (Fig. 2-1a). The area has a warm temperate climate; the annual mean air temperature between 1991 and 2010 was 13.7 °C (Japan Meteorological Agency). Ambient air was drawn from outside into an air-conditioning system, enriched with CO_2, and introduced into the OTCs from a duct near

the bottom of the chamber. The air flowed out of the top of the chamber.

The CO_2 concentration 2 m above the ground in the inner part of each OTC was monitored with a CO_2 sensor (ZFP9GD11, Fuji-denki, Tokyo, Japan). Soil temperature at a depth of 5 cm in the center of each OTC was measured with a thermometer (Pt110-4-150D, Oyo Electric Co., Ltd, Kyoto, Japan). A soil moisture sensor (CIM-TRIME-EZ, Climater, Tokyo, Japan) placed in the middle of each chamber measured soil moisture at depths of 5 to 30 cm. A photon flux density sensor (IKS-27-30, Koito, Tokyo, Japan) was installed in the top of each chamber. All data were automatically recorded every 10 min by data loggers.

In October 2002, 36 seedlings of 3-year-old *Q. glauca* were planted directly into the ground within each OTC. They were thinned to eight individuals in 2004. Water supply systems were used to irrigate the plants weekly with sufficient water to maintain approximately similar soil moisture contents in all of the OTCs.

Experimental design

Each OTC was allocated to one of six combinations of temperature (two levels) and CO_2 (three levels) (Table 2-1). The treatments started in April 2003.

The CO_2 concentration in the OTCs varied considerably throughout the

experiment in both the ambient (A1, B1) and elevated (A2, A3, B2, B3) CO_2 treatments. However, the values relative to the ambient CO_2 concentration were close to those expected (Fig. 2-2); the average CO_2 concentrations for the 3 years of measurement were 413 ppm, 560 ppm, and 710 ppm in the ambient (A1, B1), mildly elevated (A2, B2), and highly elevated (A3, B3) treatments, respectively.

Air temperatures in the OTCs experienced wide seasonal fluctuations, but the values in the elevated temperature treatments (A1, A2, A3) were consistently higher than those in the ambient temperature treatments (B1, B2, B3) (Fig. 2-3a). The average air temperatures of the ambient and elevated treatments were 14.3 °C and 17.8 °C, respectively. The difference in soil temperature between the ambient and elevated temperature treatments was less clear, with the average temperatures for the 3 years being 15.4 °C and 16.8 ℃ , respectively (Fig. 2-3b).

Soil respiration measurements

Soil respiration rate was measured by using an open-flow gas exchange system (Lee et al. 2002) with an infrared gas analyzer (IRGA; LI-6252, Li-Cor, Lincoln, NE, USA) and a data logger (Model NR-1000; Keyence, Tokyo, Japan). The system comprised one reference line and four identical sample lines for each OTC. The reference line and sample lines were

pneumatically independent of each other. Each sample line was connected to one of four PVC soil respiration chambers (21 cm internal diameter and 15 cm high) that were placed in each OTC (Fig. 2-1b). The soil respiration chambers were composed of a lid and a body with one inlet and one outlet for air (Fig. 2-1b). In April 2003, the bodies of the respiration chambers were embedded in the ground in the OTCs. The lower 4 cm of the body was carefully inserted into the soil with minimal disturbance of the litter layer within the chamber. It was possible for spatial variations in the initial soil respiration rate between OTCs to prevent accurate assessment of the treatment effects. Therefore, to minimize the effects of any initial differences in soil respiration rate among OTCs, I chose the four positions of the chambers within each OTC in such a way that the averages of the soil respiration rates did not differ significantly between the OTCs. Soil respiration rates were measured with a portable infrared gas analyzer (LI-6400, Li-Cor).

Before the soil respiration measurements were begun, the lid of each chamber was placed on the body and the joint was sealed with PVC tape. Ambient air was introduced into the chambers at a flow rate of 1.4 L min^{-1} during summer (July to September) and 1.0 L min^{-1} during the other measurement periods (April to June, October to December), and the CO_2 concentration of the outflow air was measured with the IRGA. During CO_2

analysis, air flow to the IRGA was maintained at 0.5 L min^{-1} lower than the chamber flow-through rate; water vapor was removed by a drier before analysis. Soil respiration rates were measured once a month, from April to December, from 2003 to 2008. The rates in the three OTCs for each temperature treatment (ambient or elevated) were measured on the same date. One measurement cycle, in which the CO_2 concentrations of the five lines were measured in turn, was about 45 min. The cycle was repeated for 24 to 48 h every month for each treatment OTC. The mean value of the four lines (i.e. the data from the four chambers) was treated as the soil respiration rate at that time. Soil respiration measurements could not be taken from January to March, because the freezing temperatures prevented accurate measurement.

Total soil respiration is composed of root respiration and heterotrophic respiration (Hanson et al. 2000). Using the trenching method described by Lee et al. (2003), I also examined the effects of elevated CO_2 and elevated temperature on heterotrophic respiration rate. In this method, the roots existing in a given area are severed at the boundary of a small plot and the respiration rate of the trenched plot is regarded as the heterotrophic respiration rate. In April 2006, four trenched plots (each 50 × 50 cm) were established within each OTC (Fig. 2-1c) by making vertical cuts along the boundaries to 40 cm below the ground surface. Four root barriers

(corrugated fiberglass sheets) were inserted into the vertical cuts to prevent root regrowth into the plots. Soil respiration rates in the trenched plots were measured in the same manner as described above.

Data analysis

Differences in soil respiration rates between OTCs were analyzed with three-way ANOVA, with the treatments (CO_2 and temperature) and the month as main factors. To examine the effects of the trenching and root destruction over time, the differences in heterotrophic respiration between the 3 years were analyzed with one-way ANOVA.

The soil respiration rate (total soil respiration rate and heterotrophic respiration rate) (hourly mean value of the four lines) and the soil temperature data (hourly mean value) from each chamber in each year (April to December) were used to obtain the exponential regression equation:

$$R_T = \alpha e^{\beta T}, \quad (1)$$

where R_T is the soil respiration rate at soil temperature T (°C), and α and β are the regression coefficients.

The temperature dependence of soil respiration rate was indicated by

the Q_{10} value, which is the fractional change in rate with a 10 °C increase in temperature (Raich and Schlesinger 1992):

$$Q_{10} = e^{10\beta}, \quad (2)$$

where β is as determined from Eq. 1. The soil respiration rate at 15 °C (R_{15}) was used as the soil respiration potential:

$$R_{15} = \alpha e^{\beta 15}. \quad (3)$$

Two-way ANOVA was used to examine the effect of elevated CO_2 and temperature on soil respiration potential R_{15}, temperature dependence Q_{10}, and annual soil respiration rate.

Annual soil respiration rate, S_a, was calculated as the sum of the daily soil respiration rates S_d:

$$S_a = \sum S_d \quad (4)$$

and

$$S_d = 24 R_{15} Q_{10}^{((T-15)/10)}. \quad (5)$$

Daily soil respiration rate was calculated from the daily average soil temperature and Eq. 1. Because soil respiration measurements could not be

taken from January to March, the daily soil respiration rate during this period was estimated from the soil temperature by using Eq. 1.

2-3 Results

Soil respiration and Q_{10}

Total soil respiration rate in all chambers exhibited a clear seasonal pattern. Maximum respiration rates occurred during the summer, when soil temperatures were high; minimum respiration rates occurred during those early and late winter days when testing occurred, when soil temperatures were low (Fig. 2-4a). Elevated CO_2 and temperature had significant effects on total soil respiration rate (Table 2-2). In most cases, the highest soil respiration rate was observed in chamber A3, which had both elevated temperature and highly elevated CO_2 concentration. Conversely, the lowest soil respiration rate was in chamber B1 (ambient temperature and ambient CO_2) in most months (Fig. 2-4a). The differences between treatments were greater in summer than in winter.

Heterotrophic respiration displayed seasonal patterns and treatment effects similar to those of total soil respiration, although none of the treatment effects was significant (Fig. 2-4b, Table 2-2). The heterotrophic respiration rate in 2006 was significantly higher than those in the following

2 years ($P < 0.05$), but there was no significant difference in rates between 2007 and 2008.

Total soil respiration rate and heterotrophic respiration rate were significantly correlated with soil temperature in all treatments and were approximated by Eq. 1 (Fig. 2-5). Within each treatment, the R_{15} index of total soil respiration calculated from Eq. 1 was larger than that of heterotrophic respiration in each year (Table 2-3). The R_{15} values of total soil respiration and heterotrophic respiration were significantly increased ($P < 0.05$) by elevated CO_2 treatment, whereas no significant effect on R_{15} was detected for the elevated temperature treatments (Table 2-4).

The temperature coefficient Q_{10} of total soil respiration was within the range 1.7 to 2.9 and tended to be lower than that of heterotrophic respiration in the same treatment (Table 2-3). Neither elevated CO_2 nor elevated temperature affected the Q_{10} of total soil respiration, but the Q_{10} of heterotrophic respiration was significantly ($P < 0.01$) affected by elevated temperature (Table 2-4). No interaction between treatments (elevated CO_2 and elevated temperature) was observed for R_{15} and Q_{10} (Table 2-4).

Annual soil respiration

Annual soil respiration rate (Fig. 2-6) was estimated by Eqs. 4 and 5 using R_{15} and Q_{10} (Table 2-3) and the soil temperature data for the 3-year

experimental period. The effect of raised temperatures on annual total soil respiration rate (i.e. the difference between like-numbered A and B treatments) was not clear. However, the annual total soil respiration rate tended to increase with increasing CO_2 concentration in the order of B1<B2<B3 and A1<A2<A3 (Fig. 2-6). The effects of CO_2 concentration on annual heterotrophic respiration rate showed a similar trend. The increases in annual total soil respiration for the whole 3-year period relative to those under ambient conditions (B1) were 4%, 30%, 65%, 25% and 48% for A1, A2, A3, B2, and B3, respectively.

2-4 Discussion

The basic assumption of the trenching method is that the respiratory activity of the roots in the trenched area is completely suppressed because of the lack of an energy supply (Lee et al. 2003). However, previous studies have shown that this method tends to overestimate heterotrophic respiration rate, because roots severed from the main root stem can survive and maintain respiration for some months after severance (Hanson et al. 2000; Sulzman et al. 2005; Ngao et al. 2007). In addition, CO_2 derived from decomposing dead roots may increase the soil respiration rate in the trenched plot (Lee et al. 2003). In this study, heterotrophic respiration rates

were significantly higher in 2006 than in the following 2 years, and this result may have been due partly to the effect of trenching. In contrast, there was no significant difference between the heterotrophic respiration rates in 2007 and 2008. This is consistent with the results of previous studies indicating that the contribution of carbon emission through the decomposition of roots drops below 8% in the second year after trenching (Ohashi et al. 2000, Lee et al. 2003).

Elevated temperatures can affect soil respiration by changing soil respiration potential, increasing soil respiration rate, or both. A number of studies have reported that elevated temperature may increase soil respiration rate in experimental forest stands (Peterjohn et al. 1994; McHale et al. 1998; Edwards and Norby 1999). In this study, the difference in soil temperature between the ambient and elevated temperature treatments was relatively small (1.4 °C on average) compared with that in air temperature. However, a significant effect ($P < 0.05$) of temperature treatment on total soil respiration rates was detected, even though the effect of temperature on heterotrophic respiration rate was not significant ($P = 0.15$). The suggestion from this result is that root respiration rates were affected by the raised temperature. Although our research did not directly quantify the changes in belowground biomass and litter production in the rhizosphere, stimulation of extension, mortality, and

respiration in fine roots by elevated temperatures has been reported in other studies (Pregitzer et al. 2000; Wan et al. 2004).

Studies of the effects of elevated temperature on soil respiration potential are rare. Niinistö et al. (2004) observed that soil respiration rates at a given soil temperature in boreal forests were greater in elevated temperature treatments than in controls in the first year of their experiment. In this study, however, neither total soil respiration potential (R_{15}) nor heterotrophic respiration potential increased significantly under elevated temperatures.

Conversely, the elevated temperature treatments caused a significant change in the temperature sensitivity of heterotrophic respiration; the Q_{10} of heterotrophic respiration was significantly decreased by the elevated temperature. Bekku et al. (2003) reported a similar change in the Q_{10} of microbial respiration in a temperate soil exposed to experimental warming. Balser et al. (2006) suggested that soil temperature alters microbial community structure, with a concurrent change in temperature sensitivity.

These results indicate that elevated CO_2 increased total soil respiration rate significantly in the *Q. glauca* stands. This result is consistent with those of most elevated CO_2 concentration OTC experiments that have involved other forest tree species (Table 2-5). Because I did not detect a significant effect of elevated CO_2 on heterotrophic respiration rate, I

assume that the increase in total soil respiration rate was caused mainly by increased root respiration rate. Increases in root respiration rate under elevated CO_2 have been reported for a variety of tree species, although a few species have shown reductions in root respiration rates (Table 2-5).

In addition to the increases in total soil respiration rate, increases in total soil respiration potential and heterotrophic soil respiration potential were observed under elevated CO_2. Several mechanisms could explain these increases. First, elevated CO_2 concentrations promote fine root growth and biomass, which have a significant positive relationship with root respiration rate (Pregitzer et al. 2000). Second, root exudation and below-ground carbon distributions generally increase with elevated CO_2 concentration (Cheng 1999; Williams et al. 2000; Johnson et al. 1994; Rogers et al. 1994), and these can both stimulate heterotrophic respiration rate. Third, the additional litter produced under elevated CO_2 will eventually enter the soil organic matter and promote CO_2 efflux (Six et al. 1998).

Annual total soil respiration rate ($P < 0.001$), heterotrophic respiration rate ($P < 0.01$), and root respiration rate ($P < 0.001$) were all significantly affected by elevated CO_2. Under ambient temperatures, annual total soil respiration rate increased by 24% when CO_2 was elevated ×1.4 and 48% when CO_2 was elevated ×1.8 (compared with ambient CO_2 treatment).

Under the same CO_2 concentrations, annual total soil respiration rates were only a little higher under elevated temperature than under ambient temperature (Fig. 2-6). Previous studies indicated that soil CO_2 efflux is generally sensitive to temperature. In this study, elevated temperature caused an increase (4-23%) in annual soil respiration which is smaller than those in other forest types (Table 2-6). For the combination of elevated CO_2 and elevated temperature, the responses were 30% (A2) and 65% (A3) higher, respectively, than under the ambient (B1). These increases were comparable to the results of an OTC study (CO_2 at 700 ppm) in south China yielding about a 29% increase in CO_2 efflux on average (Deng et al. 2010). Mean soil CO_2 efflux also increased by 35% to 59% under combined treatment (elevated CO_2 of 700 μmol mol^{-1} and elevated temperature with an average annual increase of 5 °C) in a 20-year-old plantation of Scots pine (*Pinus sylvestris*) in the boreal zone of Finland (Niinistö et al. 2004).

Annual total soil respiration rates under ambient conditions (B1) from 2006 to 2008 were estimated to be 640 to 960 g C m^{-2} $year^{-1}$. These values are somewhat smaller than those reported for evergreen broad-leaved climax forests of *Quercus sessilifolia* and *Castanopsis cuspidata* in Nara, Japan (990 to 1290 g C m^{-2} $year^{-1}$; Nakane 1975). This may be due partly to the fact that our experimental stand was relatively young (7 to 10 years

old) during the experimental period.

Using the average of five models, Ito et al. (2010) estimated the annual total soil respiration rate of an evergreen broad-leaved forest in Japan to be 12.1 Mg C ha^{-1} $year^{-1}$. Applying the 25% to 65% increase in soil respiration that we found in response to elevated CO_2 to the total area of evergreen broad-leaved forests of Japan (14.1×10^3 km^2; Ito et al. 2010) would cause the CO_2 efflux from the soil to increase by 3 to 7.9 Mg C ha^{-1} $year^{-1}$ (in total, 4 to 11×10^6 Mg $year^{-1}$) under elevated CO_2 concentrations of the order examined in this study.

The results indicate that future climate change (especially CO_2 elevation) may significantly impact soil respiration in warm temperate evergreen broad-leaved forests dominated by *Q. glauca*.

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Table 2-1. The open-top chamber treatments

Treatment	Air temperature	CO_2 concentration
A1	ambient + 3 ℃	ambient
A2	ambient + 3 ℃	ambient × 1.4
A3	ambient + 3 ℃	ambient × 1.8
B1	ambient	ambient
B2	ambient	ambient × 1.4
B3	ambient	ambient × 1.8

Table 2-2. Results of three-way ANOVAs for the effect of elevated temperature and elevated CO_2 on total soil respiration rate and heterotrophic respiration rate.

Treatment effect	Total soil respiration rate		Heterotrophic respiration rate	
	F	*P*	*F*	*P*
Temperature	4036	*	2.061	ns
CO_2	13386	***	1.699	ns
Month	47455	***	0.598	ns
Temperature × CO_2	115	ns	0.384	ns
Temperature × month	270	ns	1.397	ns
CO_2 × month	787	ns	0.131	ns
Temperature × CO_2 × month	323	ns	0.292	ns

* $P < 0.05$; *** $P < 0.001$; ns not significant

Table 2-3. Total soil and heterotrophic respiration potential R_{15} (soil respiration at 15 ℃) and Q_{10} of soil respiration for 2006–2008 in each open-top chamber.

	R_{15} (mg C m^{-2} h^{-1})			Q_{10}		
Treatment	2006	2007	2008	2006	2007	2008
Total soil respiration						
A1	67.0	68.7	75.4	2.43	2.25	1.77
A2	89.1	96.9	93.1	2.23	1.99	1.70
A3	74.0	128.6	95.2	2.90	2.04	2.15
B1	91.0	66.2	60.4	2.01	2.46	2.47
B2	97.1	88.3	89.3	2.59	2.50	2.01
B3	107.5	103.5	91.5	2.43	2.67	2.39
Heterotrophic respiration						
A1	52.1	62.1	54.9	2.62	1.63	1.86
A2	56.2	51.2	52.7	2.83	2.36	2.70
A3	60.9	104.2	59.7	3.16	1.73	2.70
B1	42.0	38.7	36.6	3.67	3.22	3.10
B2	83.0	59.5	58.7	2.70	2.67	2.68
B3	67.6	73.2	53.3	3.48	2.63	3.46

Table 2-4. Results of ANOVAs for the effect of elevated temperature and elevated CO_2 on soil respiration at 15 °C and Q_{10} of total soil respiration and heterotrophic respiration.

	Total soil respiration		Heterotrophic respiration	
Treatment effect	R_{15}	Q_{10}	R_{15}	Q_{10}
CO_2	*	ns	*	ns
Temperature	ns	ns	ns	**
CO_2 × temperature	ns	ns	ns	ns

* $P < 0.05$; ** $P < 0.01$; ns not significant

Table 2-5. Effects of elevated CO_2 on soil respiration rate in experimental forest stands in open-top chamber experiments.

Species	CO_2 (ppm)	Exposure period (months)	Soil respiration rate			Reference
			Total	Root	Microbial	
Conifers						
Pinus ponderosa	525	26	+		ns	Johnson et al. 1994
Pinus ponderosa	700	26	+		ns	Johnson et al. 1994
Pinus sylvestris	700	20		-		Crookshanks et al. 1998
Pinus sylvestris	+350	6	+	+		Janssens et al.1998
Pinus sylvestris	+200	12	+			Pajari 1995
Deciduous broad-leaved trees						
Fraxinus excelsior	700	20		-		Crookshanks et al. 1998
Quercus petraea	700	20		-		Crookshanks et al. 1998
Acer saccharum	700	36	+	+	ns	Edwards and Norby 1999
Acer rubrum	700	36	+	+	ns	Edwards and Norby 1999
Mixed forest (evergreen broad-leaved trees and conifers)						
Castanopsis hystrix	700	30	+			Deng et al. 2010
Syzygium hancei	700	30	+			Deng et al. 2010
Pinus massoniana	700	30	+			Deng et al. 2010
Schima superba	700	30	+			Deng et al. 2010
Acmena acuminatissima	700	30	+			Deng et al. 2010
Ormosia pinnata	700	30	+			Deng et al. 2010
Evergreen broad-leaved trees						
Quercus glauca	560	72	+	+	ns	**Present study**
Quercus glauca	710	72	+	+	ns	**Present study**

+ increased; – decreased; ns not significant

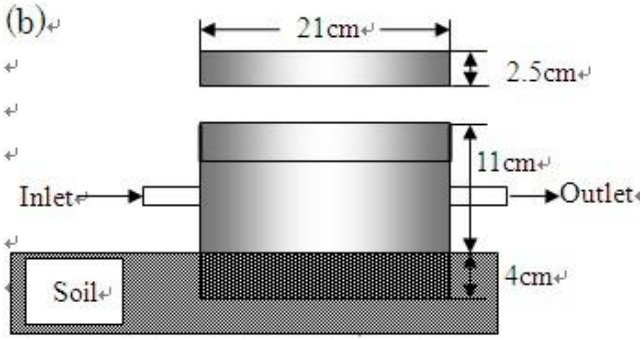
Table 2-6. Effects of elevated temperature on soil respiration rate in experimental forest stands.

Species	Elevated temperature (℃)	Exposure period (years)	Soil respiration rate			Reference
			Heterotrophic	Root	Total	
Conifer forest						
Pinus sylvestris	5	4			+27-34%	Niinistö et al. 2004
Picea mariana	0.4-0.9	3			+20%	Bergner et al. 2004
Deciduous broad-leaved forest						
Acer rubrum	4	3	+12%	-		Edwards and Norby 1999
Mixed forest (deciduous broad-leaved trees and conifers)						
Picea abies	4	2	+39-45%		+45-47%	Schindlbacher et al. 2009
Abies alba	4	2	+39-45%		+45-47%	Schindlbacher et al. 2009
Fagus syltica	4	2	+39-45%		+45-47%	Schindlbacher et al. 2009
Evergreen broad-leaved forest						
Quercus gluaca	3	3			+4-23%	**Present study**

+ increased; – decreased

(a)

(b)



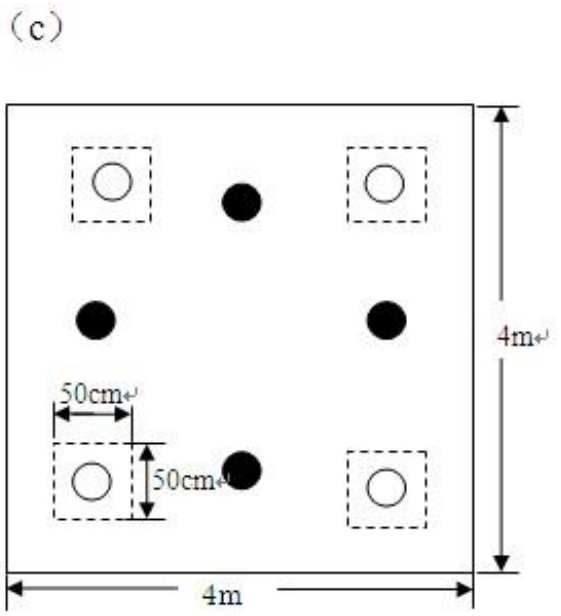


Fig.2-1 Experimental equipment and layout. (**a)** One of the open-top chambers (OTCs). **(b)** Diagram of a respiration chamber used for the measurement of soil respiration (21 cm internal diameter and 15 cm high). (**c)** Layout of the control and trench plots within the OTCs. Each plot contained four total soil respiration chambers (filled circles) and four heterotrophic respiration chambers (empty circles). Dashed boxes show trench plots (each 50 × 50 cm).

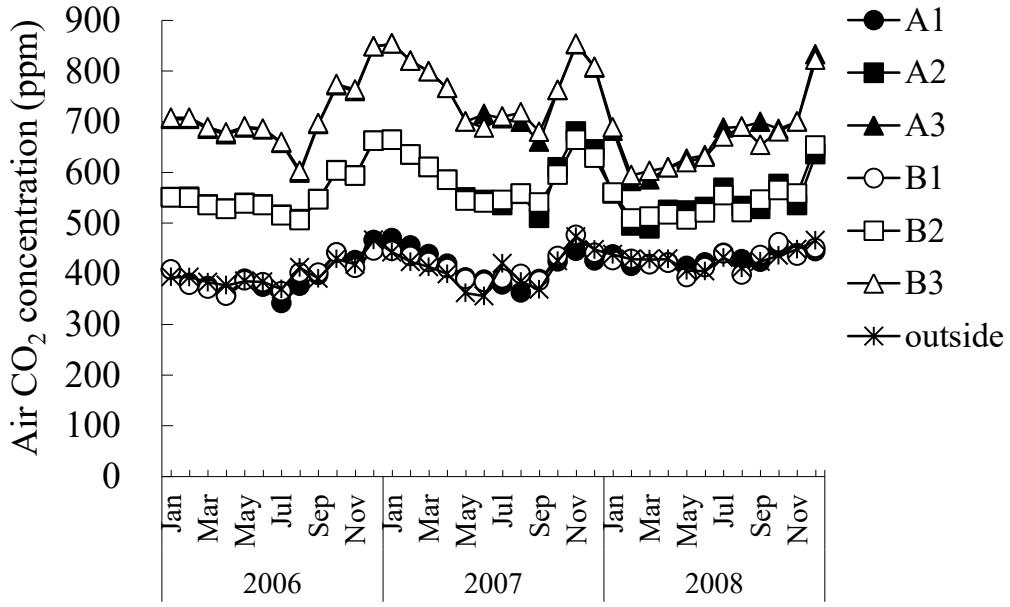
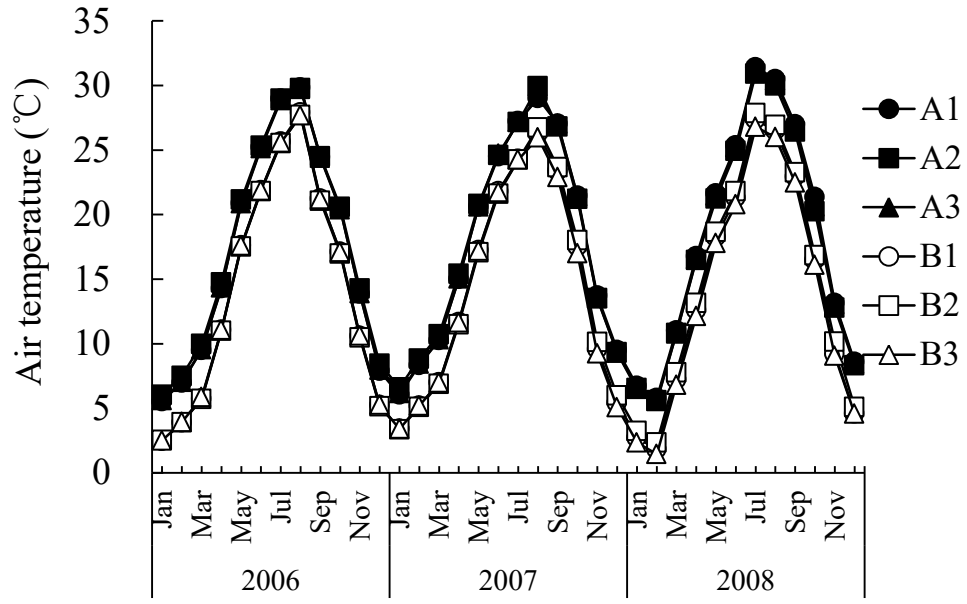


Fig. 2-2 Monthly average air CO_2 concentrations in each open-top chamber and outside during the period January 2006 to December 2008.

(a)



(b)

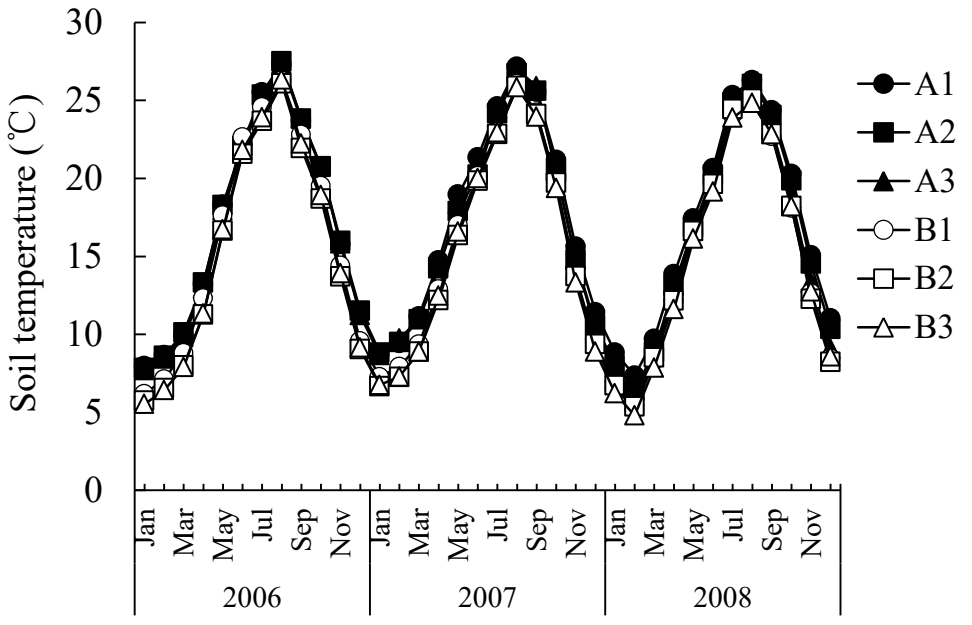
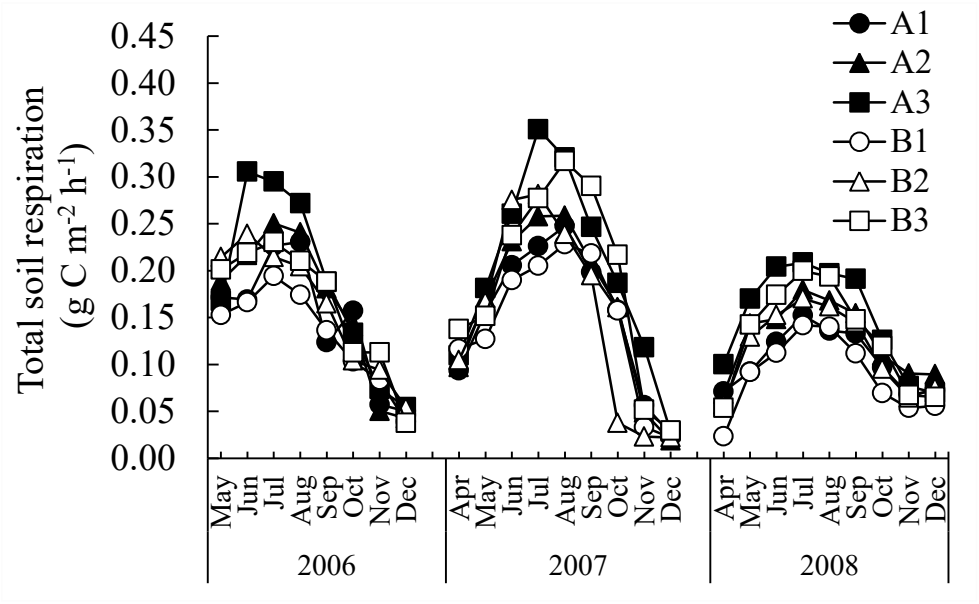


Fig. 2-3 Monthly average values of (**a)** air temperature in each open-top chamber (OTC) and (**b)** soil temperature at a depth of 5 cm in each OTC during the period January 2006 to December 2008.

(a)



(b)

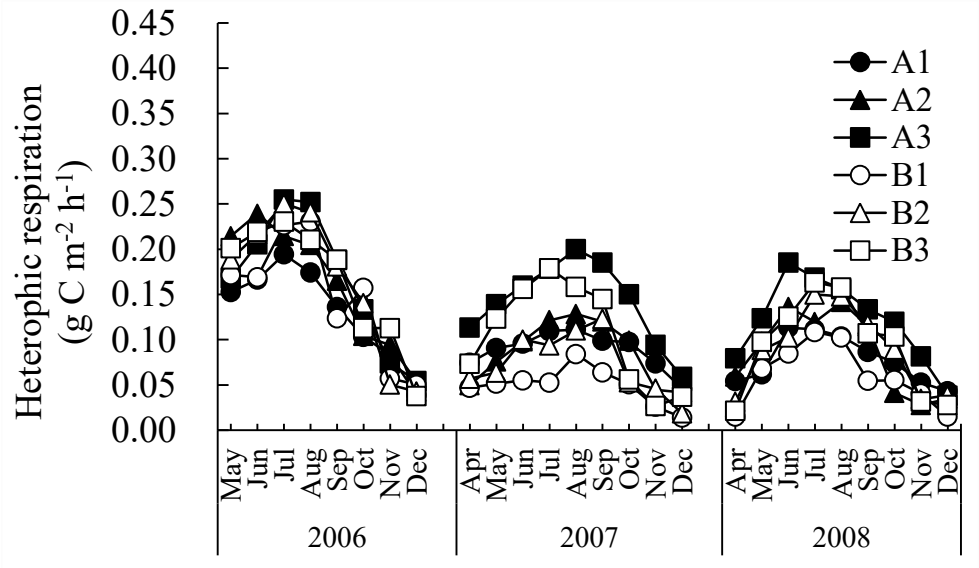


Fig. 2-4 Monthly measurements of hourly rates (mean value of four lines from each open-top chamber) of (**a)** total soil respiration and (**b)** heterotrophic respiration. Rates are shown as hourly means of 48-h measurements.

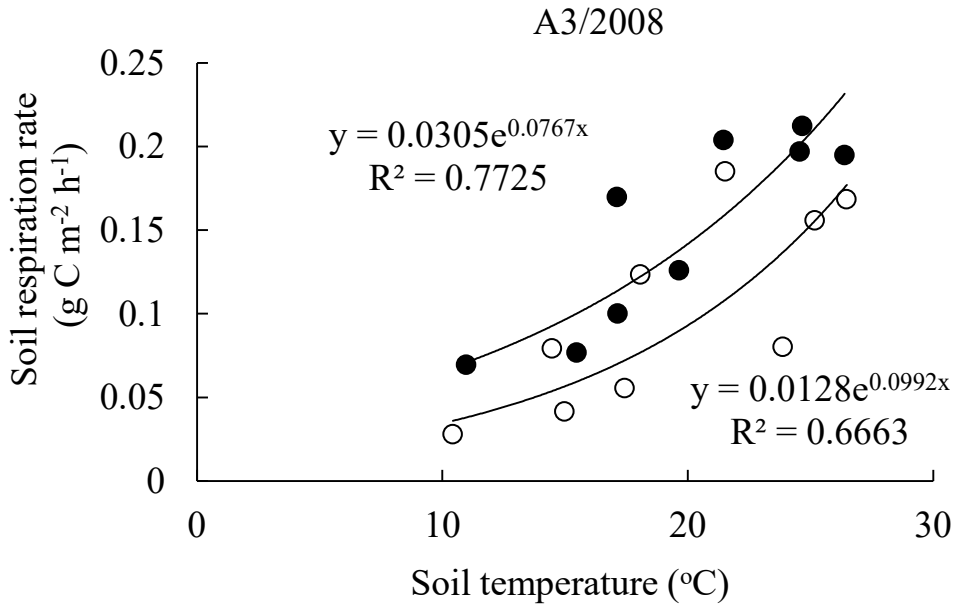


Fig. 2-5 Relationship between monthly average of soil respiration and soil temperature in chamber A3 (ambient CO_2 concentration × 1.8, ambient air temperature +3 °C) in 2008. Solid circles, total soil respiration; open circles, heterotrophic respiration.

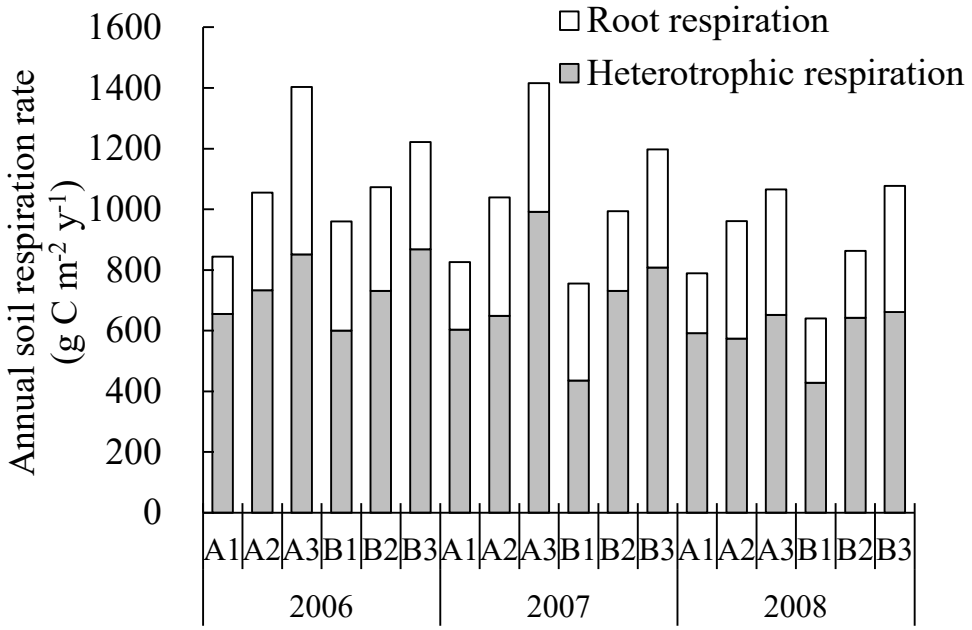


Fig. 2-6 Mean annual carbon effluxes from total soil respiration and from heterotrophic and root respiration.

CHAPTER 3
Effects of experimental warming on the soil heterotrophic microbial community in a warm temperate evergreen broad-leaved forest: a three-year field experiment

3-1 Introduction

The results of chapter 2 showed that elevated temperature caused a significant change in the temperature sensitivity (Q_{10}) of heterotrophic respiration though the rates were not significantly affected. One of the possible explanations of this phenomenon is that soil warming alters the quality or quantity of soil microorganisms, with a concurrent change in temperature sensitivity. It has been suggested that temperature sensitivity of heterotrophic respiration varies with changes in microbal biomass (Conant et al. 2008a) and in microbial community composition (Balser et al. 2006) induced by soil warming. However, there is little available information relevant to the potential impact of climate warming on soil microbial communities in warm temperate evergreen broad-leaved forests.

In this chapter, a field experiment was conducted to examine the effect

of soil warming on the soil heterotrophic microbial community in a natural warm temperate evergreen broad-leaved forest. Infrared heaters were used to increase the soil temperature of experimental plots in a forest stand for three years and used phospholipid fatty acid (PLFA) analysis to examine changes in the heterotrophic microbial community.

3-2 Materials and methods

The experiment, which is part of a project to examine the response of forest soil carbon cycle to experimental warming (Liang et al. 2008), was conducted in a secondary forest with an area of approximately 1.6 km^2 located in the central part of the city of Higashi-Hiroshima in western Japan (34°41′N, 132°72′E; 335 m a.s.l). The forest is dominated by broad-leaved evergreen trees such as *Quercus glauca, Symplocos kuroki*, and *Ilex pedunculosa*, the tree density and canopy height being 1450 stems ha^{-1} and about 10 m, respectively. The soils are classified as coarse-textured, residual, immature soils with a parent rock of granite (Japan National Land Survey Division, Land and Water Bureau).

Ten plots on the forest floor within a circular area with a diameter of 40 m were randomly chosen. In order to prevent root regrowth into the plots, all the plots were treated by the trenching method described by Lee

et al. (2003).Ten trench plots were established (each 1 × 1 m) by making vertical cuts along the boundaries to a depth of 40 cm below the ground surface. Four root barriers (corrugated fiberglass sheets) were inserted into the vertical cuts. The large chambers (0.9 m × 0.9 m × 0.5 m, L × W × H) (Fig. 3-1) described by Liang et al. (2003) were inserted into the trench plots. Each chamber was closed for 4 mins by turns for measuring soil CO_2 efflux. The chambers opened and closed automatically so that inputs of rainfall and litter would occur naturally. The plots were divided into two groups, five of which, the warming plots, were warmed continuously with infrared heaters beginning in October 2007. The other five served as controls. In each warming plot a single 90 cm × 15 cm infrared heater was suspended 2 m above the ground. The soil temperature and moisture content of each plot were measured continuously at soil depths of 5 and 15 cm. Data were stored on data-loggers at 10-minute intervals by the automatical chamber system (Fig. 3-2).

Soil samples for PLFA analysis were collected from the Ah horizon (0–5 cm) of the warming and control plots in October 2010. Three cores (5 cm diameter × 5 cm deep) were taken from each plot. We mixed the three cores from each plot to obtain one composite sample. The samples were returned to the laboratory, freeze-dried, sieved (<2 mm) to remove gravel and dead roots, and stored at –80 °C until PLFA analysis.

Lipids were extracted by using a Bligh and Dyer (1959) extraction, as modified by White et al. (1979) and Frostegård et al. (1993a). Briefly, an aliquot of 1–2 g (dry weight) of soil was extracted with a chloroform-methanol-citrate buffer mixture (1:2:0.8). The lipids were separated into neutral lipids, glycolipids, and phospholipids on a silicic acid column (Sep-Pak™ plus silica; Waters Corp., Milford, MA, USA) (Arao et al. 2001). The phospholipids were esterified with a HCl-Methanol Reagent (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) (Stoffel et al. 1959). The resulting fatty acid methyl esters were separated with a gas chromatograph (GC-2014; Shimadzu Corp., Kyoto, Japan) equipped with a capillary column (30 m DB-5 ms, Phenyl-Methyl/Silicone; J&W Scientific Inc., Folsom, CA, USA). Helium was used as a carrier gas. Peak areas were quantified by adding methyl nonadecanoate fatty acid (19:0) as an internal standard. The fatty acid nomenclature described by Frostegård et al. (1993a) was used. The total content of PLFAs (TotPLFAs) was used as a metric of total microbial biomass (Frostegård et al. 1993a). The fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy17:0, 18:1ω7c, and cy19:0 were chosen to represent bacterial PLFAs (BactPLFAs) (Frostegård et al. 1993a; Federle 1986). The amount of 18:2ω6,9 was used as a metric of fungal biomass (FungPLFA) (Federle 1986; Frostegård and Bå th 1996).

PLFAs are classifiable into several categories according to their

molecular structure (Yoshitake et al. 2006). In this study PLFAs were classified into five categories (straight chain saturated fatty acids, branched chain fatty acids, unsaturated fatty acids, cyclopropyl fatty acids (cy17:0, cy19:0), and 18:2ω6,9) and summed their mole percents. Cyclopropyl fatty acids and branched chain fatty acids are known to be characteristic of gram-negative bacteria and gram-positive bacteria, respectively (Federle 1986). Previous studies have shown that this classification is useful for detection of shifts in microbial community structure (Yoshitake et al. 2006).

Student's *t*-test was used to test for significant differences in soil properties and PLFA content between the warming and control plots. All statistical analyses were carried out with SPSS software (v. 19.0; SPSS Inc., Chicago, IL). I considered differences to be significant if the type I error rate (p) was 5% or less.

3-3 Results and discussion

Warming consistently increased soil temperatures by about 3 °C throughout the three-year experimental period. The ranges of daily average soil temperature were 4.1–22.6 °C and 7.1–25.8 °C in the control and warming plots, respectively. The corresponding ranges of soil

moisture content were 20.5–37.6% and 20–36.5%, respectively. The soil moisture content of the warming and control plots did not differ significantly (Student's *t*-test, $p > 0.05$ Table 3-1) during the three-year experimental period.

The TotPLFA content, a metric of soil microbial biomass, was not significantly affected by the three years of experimental soil warming (Student's *t*-test, $p > 0.05$, Table 3-2). A similar insensitivity of microbial biomass to soil warming has been reported in several previous studies (Schindlbacher et al. 2011; Zhang et al.2005; Zogg et al. 1997), although Petersen and Klug (1994) reported some change in soil microbial biomass in response to soil warming.

Bacterial and fungal biomass responed differently to soil warming: a significant increase in BactPLFA content occurred in the warming plots (Student's *t*-test, $p < 0.05$, Table 3-2), whereas FungPLFA content was not significantly affected by the three years of soil warming. As a result, the ratio of fungal to bacterial biomass decreased by 35% (Table 3-2), an indication of a significant shift in the composition of the microbial community.

Differences in the relative abundance of cyclopropyl fatty acids, which are characteristic of gram-negative bacteria (Harwood and Russell 1984), provided further evidence of a temperature-induced shift in community

composition (Student's t-test, $p < 0.05$, Table 3-2). In addition, some new fatty acids (15:0, 17:0, 17:1ω7, i18:0, 20:4ω6, and 20:5ω3) were detected in the warming plots, whereas the fatty acid 16:1 became undetectable (Fig. 3-3), a further indication of changes in the composition of the microbial community.

Results of previous soil-warming field studies of microbial communities in forest ecosystems have been rather inconsistent though bacterial biomass was generally unaffected by elevated temperatures (Table 3-3). Schindlbacher et al. (2011) reported that a 4 ℃ increase in soil temperature during the snow-free season had no influence on the microbial community composition in a temperate, mountain forest soil. In contrast, Frey et al. (2008) reported a significant reduction in total microbial biomass and a shift in microbial community composition in a mixed deciduous forest after 12 years of soil warming of 5 ℃ above the ambient temperature. In a study of an Alaskan boreal forest, Allison and Treseder (2008) reported that bacterial and fungal abundance declined by more than 50% in a closed-top greenhouse treatment. However, because their warming treatment caused drying of the soil, the observed change in microbial biomass may have been partly due to the change in moisture content. In this study the soil moisture content did not differ significantly between treatments, and the observed shift in the microbial community

can therefore be attributed solely to the effect of warming. Considering the relatively short duration (3 years) of this study and the lower warming temperature (+3 ℃) compared to some previous work (Schindlbacher et al. 2011; Frey et al. 2008), I conclude that the soil bacterial community in this warm-temperate, evergreen broad-leaved forest was remarkably sensitive to warming effects.

It has been suggested that bacterial community composition influnces heterotrophic respiration and can potentially influence soil carbon storage (Cleveland et al. 2007). Although studies have yet to be carried out of the impact of climate change on members of the soil microbial community that are symbiotic with plants, these results suggest that changes in the heterotrophic micorobial community are among the mechanisms thorough which climatic warming affects the carbon cycle of warm temperate evergreen broad-leaved forest.

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Table 3-1. The soil temperature (ST) and the soil water content (SWC) in the control and warming treatment.

	ST(-5cm, ℃)	SWC(%)
Control	13.6	22.6
Warming	16.1	22.0
P	***	0.26

*** $p<0.001$

Table 3-2. Fatty acid content (nmol g^{-1}) and the proportion of fatty acids (mole percent) in the microbial communities from the control and warming plots after three years. Mean and standard deviation (in parentheses, $n = 5$) are given.

	Warming (± SD)	Control (± SD)	*p*
Fatty acid content (nmol g^{-1})			
TotalPLFA	376.2 (86.6)	378.7 (84.6)	n.s.
BactPLFA	115.4 (17.7)	90.0 (7.0)	*
FungalPLFA	21.6 (8.9)	25.7 (7.9)	n.s.
F/B ratio	0.20 (0.06)	0.30 (0.07)	*
Fatty acid Proportions (mole percent)			
Straight chain saturated fatty acids	25.3% (5%)	23.9% (2%)	n.s.
Branched chain fatty acids	28.4% (6%)	26.1% (2%)	n.s.
Cyclopropyl fatty acids	12% (6%)	5.5% (2%)	*
Unsaturated fatty acids	37.7% (3%)	40% (6%)	n.s.
18:2ω6,9	5.7% (2%)	6.8% (1%)	n.s.

Significant differences between the warming and control estimated by Student's *t*-test.

n.s., not significant; *, $p < 0.05$

Table 3-3. Effects of elevated temperature on soil microbial communities in experimental forest stands.

Species	Elevated temperature (℃)	Exposure period (years)	Biomass			Reference
			Total	Bacteria	Fungi	
Conifer forest						
Picea mariana	Ts 0.5			-	-	Allison and Treseder 2008
Picea mariana	0.4–0.9	3		ns	ns	Bergner et al. 2004
spruce-fir	5		-			Arnold et al.1999
Deciduous broad-leaved forest						
Acer saccharum	5,15,25	16 weeks	-			Zogg et al. 1997
Quercus velutina	5	12	-		-	Frey et al. 2008
Acer rubrum	5	12	-		-	Frey et al. 2008
Betula papyrifera	5	12	-		-	Frey et al. 2008
Acer pensylvanicum	5	12	-		-	Frey et al. 2008
Mixed forest (deciduous broad-leaved trees and conifers)						
Picea abies	4	6	ns	ns	ns	Schindlbacher et al. 2011
Fagus sylvatica	4	6	ns	ns	ns	Schindlbacher et al. 2011
Abies alba	4	6	ns	ns	ns	Schindlbacher et al. 2011
Picea abies	4	4		ns		Kuffner et al. 2012
Fagus sylvatica	4	4		ns		Kuffner et al. 2012
Abies alba	4	4		ns		Kuffner et al. 2012
Evergreen broad-leaved forest						
Quercus gluaca	3	3	ns	+	ns	**Present study**

+ increased; – decreased; ns not significant

Fig. 3-1 The automatical open top chamber

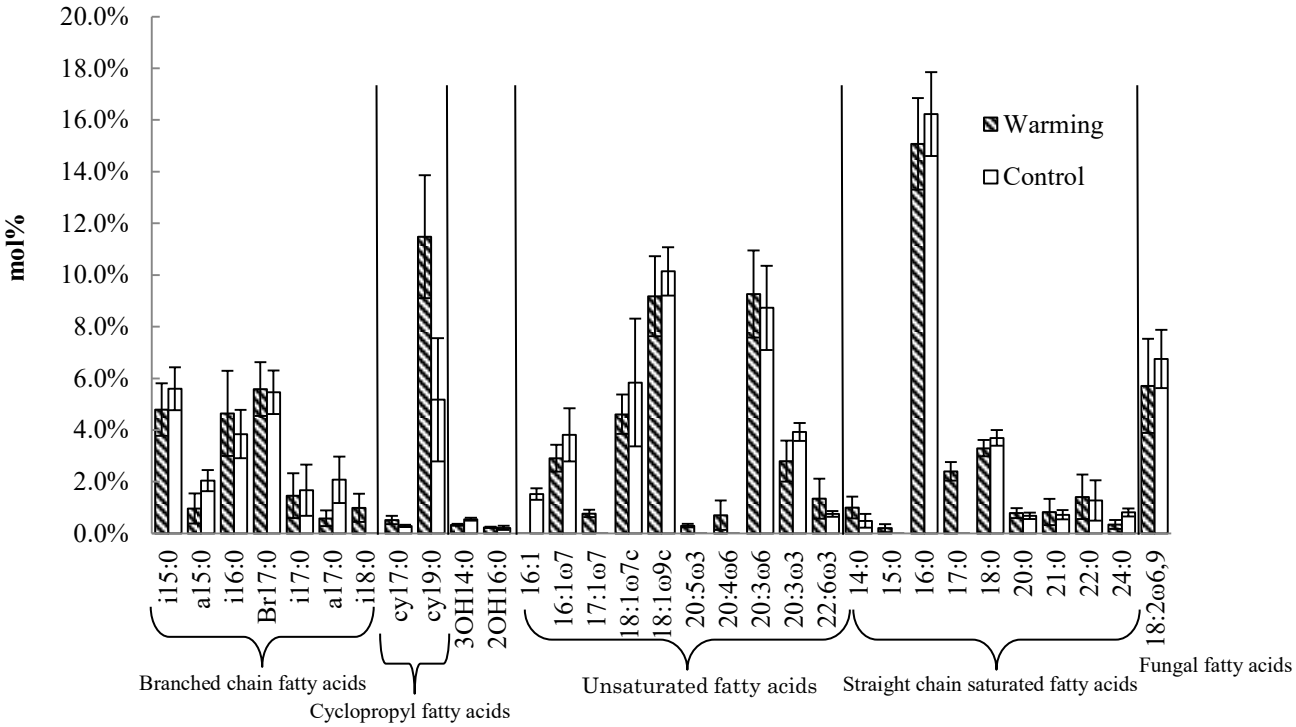

Fig. 3-2 Changes in the mole fraction of selected phospholipid fatty acids (PLFAs) extracted from the soils of the control and warming plots. Error bars are one standard error of the mean of five plots. W: warming; C: control.

CHAPTER 4
Effects of elevated temperature on the rhizospheric soil microbial community associated with *Quercus glauca* seedlings

4-1 Introduction

The rhizosphere is the narrow zone of soil directly adjacent to plant roots (Linderman 1988). The rhizospheric microbial community includes bacteria and fungi that are directly influenced by root exudates (Bertin et al. 2003), as well as mycorrhizal fungi that colonize the host plant's roots, and this community plays an important role in the soil carbon cycle (Jones et al. 2004). Previous studies indicated that the biomass and composition of rhizospheric microbial communities differed significantly from those of bulk soil microbial communities (Kent and Triplett 2002; Shi et al. 2011).

As described in Chapter 3, a field experiment showed that the heterotrophic microbial community in the bulk soil was significantly affected by experimental warming. However, because the soil microbial community inhabiting the rhizosphere and that in bulk soil respond differently to warming (Kandeler et al. 1998), it is necessary to examine the

effects of elevated temperature on the rhizospheric microbial community as well.

In this chapter, I describe a pot culture experiment conducted to examine the effect of warming on the rhizospheric microbial community associated with *Quercus glauca* seedlings.

4-2 Materials and methods

Seeds of *Q. glauca* were collected from the Higashi-Hiroshima campus of Hiroshima University in December 2011. All the seeds were put in polyethylene bags and stored in a refrigerator at 4 ℃ until the incubation experiment. Just before the experiment began, the surface of the seeds was sterilized by soaking them in 70% ethanol for 1 min and then in sodium hypochlorite solution (active Cl approximately 5%) for 1 min, and then rinsing them five times with sterile water. They were allowed to germinate on wet filter paper in plastic dishes under sterile conditions.

A nylon bag (45-μm mesh; 2 cm in diameter and 4 cm deep) was used to separate the rhizospheric soil from the bulk soil. Two germinated seeds were planted in each mesh bag and then put into a pot (4 cm in diameter and 8 cm deep) (Fig. 4-1). The soil used in this study was weathered granite soil collected from the central part of Ube city, Yamaguchi Prefecture,

southwestern Japan. The soil was sieved to remove large particles of rock (>5 mm). Approximately 60 g of soil was put in each pot. All pots were maintained under the same conditions at room temperature (20 °C) for 1 month.

Three incubators (LH-200RDS; Nihon Ikakikai Co., Osaka, Japan) were used to control the temperature. Before the incubation experiment, the inside of the incubators was sterilized with 70% ethanol. Twenty replicate pots were put in each of the three incubators, which were maintained at 15, 18, and 21 °C, respectively (Fig. 4-2). The 15 ℃ condition, which is near the ambient annual mean air temperature of Higashi-Hiroshima, served as the control. Fluorescent lamps were used to provide a photosynthetic photon flux density (400–700 nm) of approximately 300 μmol m^{-2} s^{-1}, with a 12-h light photoperiod. Sufficient water was used to irrigate all the plants every 2 days.

The plants were harvested in June 2012, at 3 months after planting. Pots with dead plants were excluded from the analysis. Stems were cut at the soil surface, and oven-dried weight (70 ℃ for 2 days) of leaves, stems, and roots were measured. Soil samples were collected for Phospholipid fatty acid (PLFA) analysis. Soil in the mesh bags was collected as the rhizospheric soil and that outside of the bags as the bulk soil. The samples were returned to the laboratory, freeze-dried, sieved (<2 mm) to remove

gravel and dead roots, and stored at –80 °C until PLFA analysis. PLFAs in the soil samples were analyzed by using the method described in Chapter 3.

One-way ANOVA was used to test the significance of differences in plant dry weights and PLFA contents among the treatments. All statistical analyses were carried out with SPSS software (v. 19.0; SPSS Inc., Chicago, IL, USA). Differences were considered to be significant at $P < 0.05$.

4-3 Results

At 3 months after planting, no significant effects of warming on total aboveground biomass or belowground biomass were observed (one-way ANOVA, $P>0.05$; Fig. 4-3).

In all the bulk soil samples, neither TotPLFA contents nor FungPLFA contents were significantly affected by the warming (one-way ANOVA, $P>0.5$; Fig. 4-4). BactPLFA was below the detectable limit.

In all the rhizospheric soil samples, the TotPLFA content, an estimate of microbial community biomass, did not change significantly among treatments (one-way ANOVA, $P>0.05$; Fig. 4-5a). However, the FungPLFA contents of rhizospheric soil samples in the warming treatments (18 and 21 ℃) were significantly higher than that in the control (one-way ANOVA,

$P<0.05$; Fig. 4-5b). As in the bulk soil, BactPLFA was below the detectable limit.

4-4 Discussion

In this study, I used a weathered granite soil with a low organic matter content to detect changes in the microbial community in the rhizosphere. Therefore, it is likely that soil PLFA levels in this experiment were lower than those in natural forest soils. In fact, the TotPLFA contents in this experiment were much lower than those measured in forest soil (as described in Chapter 3). The low BactPLFA (below the detectable limit) can also be explained by the low organic matter content.

The TotPLFA content and FungPLFA content in the bulk soil did not differ significantly among the temperature treatments. This is in accord with the result of the field experiment (Chapter 3), which showed that the fungal biomass was unaffected by the elevated temperature (+3 °C). In rhizospheric soil, however, FungPLFA was increased by the elevated temperatures, although no significant change in TotPLFA was detected. These results suggest that microbial communities in bulk soil and those in rhizospheric soil respond differently to temperature.

Because *Q. glauca* is an ectomycorrhizal plant (Kinoshita et al. 2007), the FungPLFA detected in this study is likely to include the biomass of mycorrhizal fungi. According to previous studies, the responses of mycorrhizal fungi to warming are variable, ranging from no response (Heinemeyer et al. 2004) to significant increases in biomass (Rygiewicz et al. 2000; Staddon et al. 2002; Urcelay et al. 2003; Fitter et al. 2004). Mycorrhizal fungi play an important role in the forest carbon cycle (Hobbie 2006; Kinoshita et al. 2007). The increase in FungPLFA in the rhizospheric soil suggests that an increase in mycorrhizal fungi is among the mechanisms by which climate warming affects the carbon cycle of evergreen broad-leaved forest in the warm temperate zone.

Although there were some limitations to this study, including the short experimental period (3 months) and low organic matter content of the weathered granite soil, this study shows that the rhizospheric fungal community is sensitive to elevated temperature.

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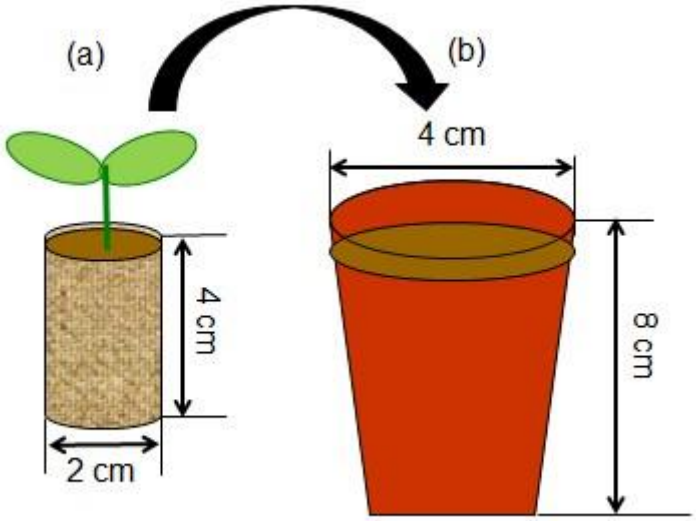


Fig. 4-1 The nylon bag (45-μm mesh) and pot used in the experiment.

Fig. 4-2 Pots with plants in the incubators.

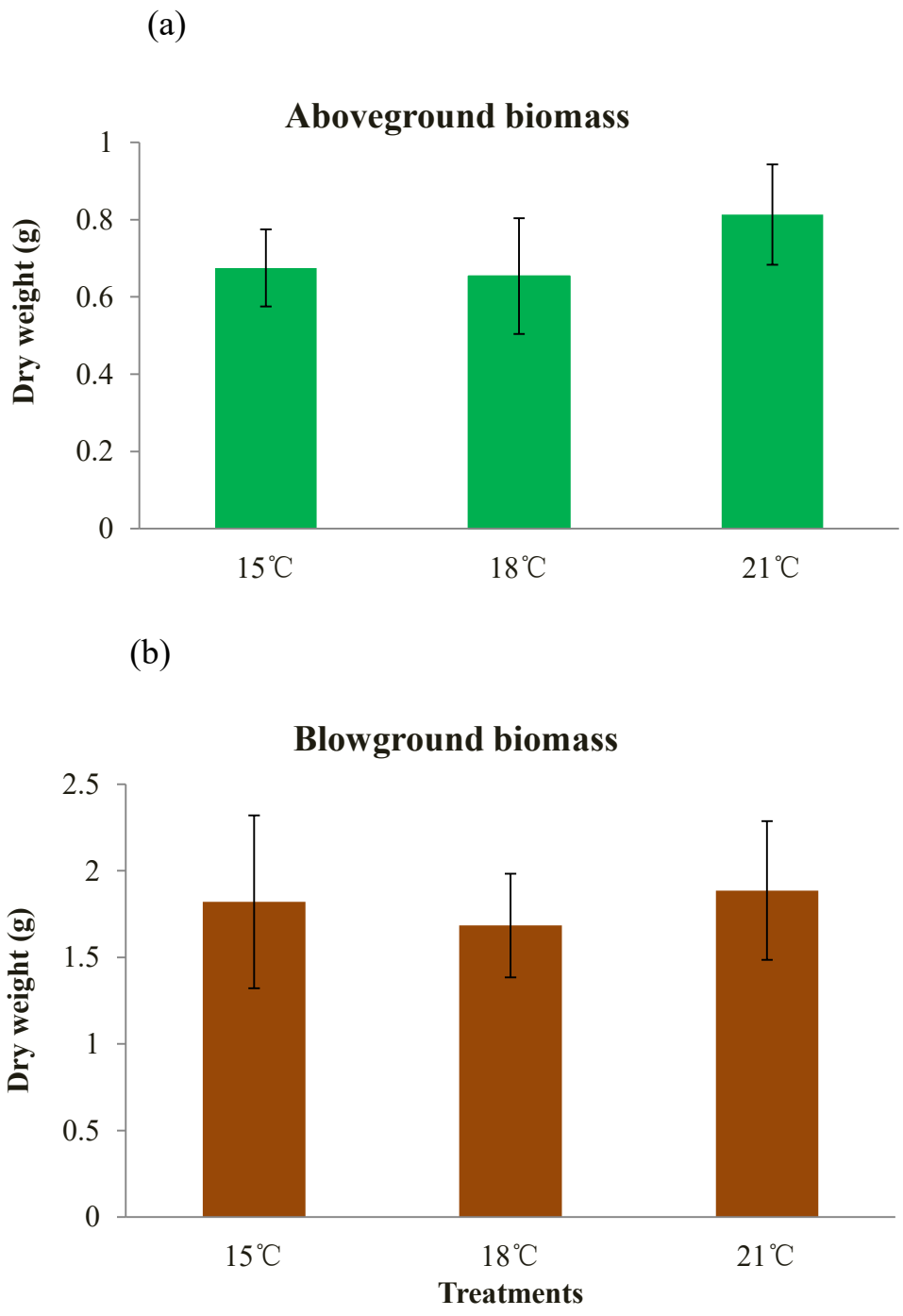
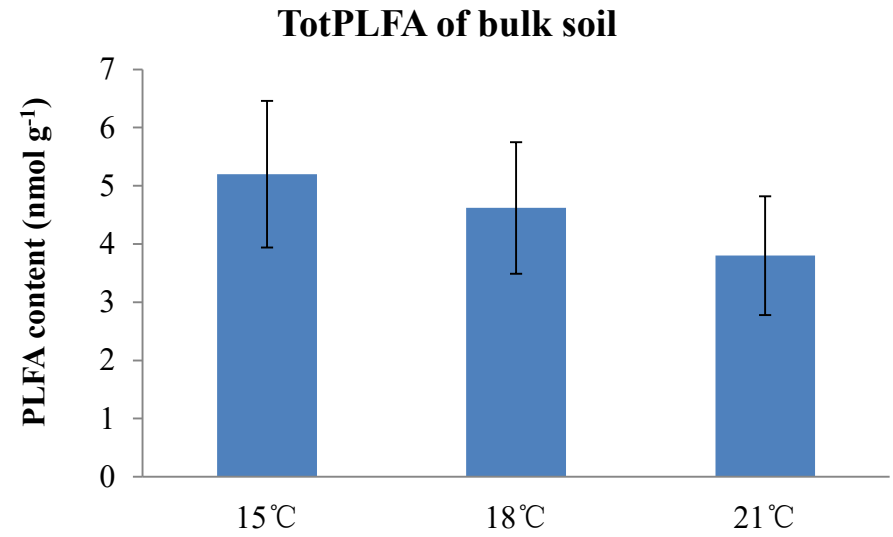


Fig. 4-3 The total dry weight of the **(a)** aboveground biomass and **(b)** belowground biomass in the three temperature treatments. Vertical bars represent ± SD (n = 7–13). No significant differences were observed among temperature treatments (one-way ANOVA, P>0.05).

(a)



(b)

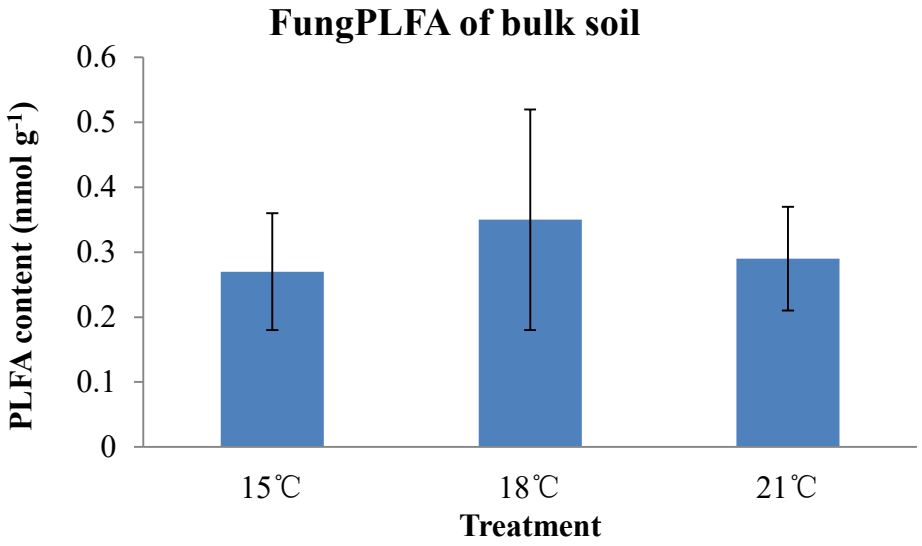


Fig. 4-4 The **(a)** total PLFA contents and **(b)** fungal PLFA contents in bulk soil in the three temperature treatments. Vertical bars represent ± SD (n = 7–13). Neither TotPLFA nor FungPLFA was significantly affected by the temperature treatments (one-way ANOVA, P>0.05).

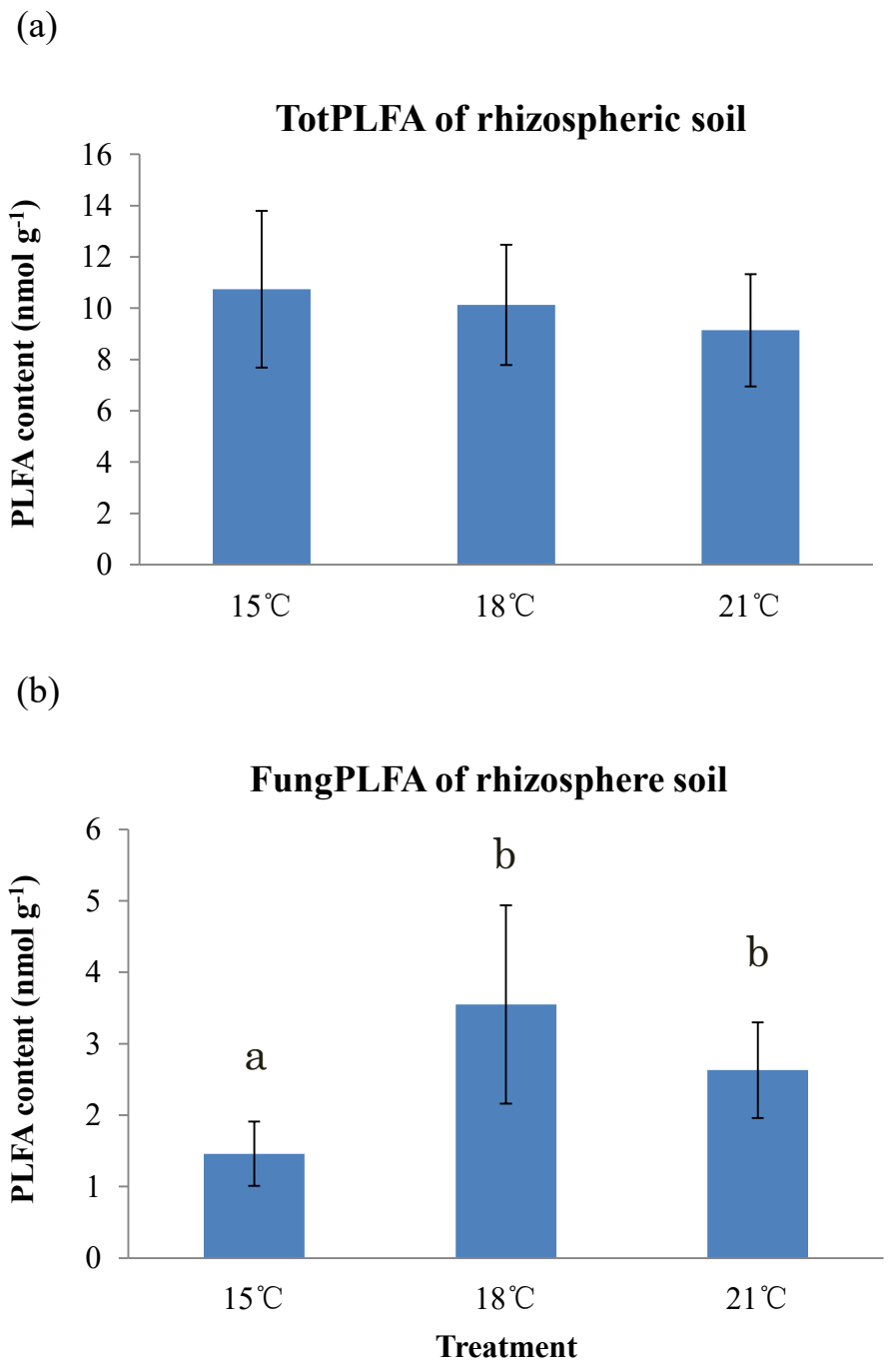


Fig. 4-5 The **(a)** total PLFA contents and **(b)** fungal PLFA contents in rhizospheric soil in the three temperature treatments. Vertical bars represent ± SD (n = 7–13). The TotPLFA contents showed no significant difference, whereas FungPLFA contents did differ significantly across treatments

(one-way ANOVA, $P<0.05$). Bars with the same letter are not significantly different (Tukey-Kramer test, $P>0.05$).

CHAPTER 5
General discussion and conclusions

Previous studies indicated that soil respiration is generally sensitive to temperature (Luo et al. 2001; Rustad et al. 2001; Niinistö et al. 2004). When natural ecosystems were exposed to experimental warming, the soil respiration rate generally increased (Hobbie 1996; Edwards and Norby 1999; Melillo et al. 2002; Bergner et al. 2004; Niinistö et al. 2004; Schindlbacher et al. 2009). In this study, elevated temperature caused an increase in annual soil respiration of 4–23% in the open-top chamber experiment (Chapter 2). However, the impact of elevated temperature on soil respiration was small compared with that of elevated CO_2. In addition, neither elevated CO_2 nor elevated temperature had a significant effect on the heterotrophic respiration rate. These findings indicate that stimulation of the root respiration rate by elevated CO_2 and temperature is among the important mechanisms by which climate change affects the ecosystem carbon cycle of evergreen broad-leaved forests in the warm temperate zone.

However, elevated temperature caused a significant change in the temperature sensitivity (Q_{10}) of heterotrophic respiration (Chapter 2). In addition, there was a significant increase in BactPLFA content in the

warming plots, and the ratio of fungal to bacterial biomass decreased after 3 years of experimental warming, an indication of a significant shift in the microbial community structure in the evergreen broad-leaved forest (Chapter 3). Previous studies indicated that bacterial biomass was generally unaffected by elevated temperature (Table 3-3). The results of this study (Chapter 3) indicated that the heterotrophic bacterial community in Japanese evergreen broad-leaved forest is more sensitive to elevated temperature than those in other forest types.

The response of fungal biomass to soil warming varied among forest types (Table 3-3). For example, Frey et al. (2008) reported that warming of soils led to a decrease in the fungal community in a deciduous broad-leaved forest ecosystem. In contrast, Schindlbacher et al. (2011) found that fungal biomass was unaffected by soil warming (+4°C) for 6 years in a temperate mountain forest with deciduous broad-leaved trees and conifers. In the present study (Chapter 3), no significant change in fungal biomass in the heterotrophic microbial community was detected after 3 years of experimental warming. In the field experiment, the influence of roots and the associated microbial community was excluded by using the trenching method. Therefore, the impacts of elevated temperature on soil microbial communities might have been underestimated. In fact, the pot culture experiment (Chapter 4) showed that rhizospheric fungal biomass was

sensitive to elevated temperature conditions.

Although studies on the effect of elevated temperature in rhizospheric microbial communities are rare, several studies examined the effect of elevated CO_2 on rhizospheric microbial communities. They reported that the fungal biomass in the rhizosphere was sensitive to elevated CO_2 conditions. Thus, the findings of the present and previous studies suggest that the rhizospheric fungal biomass would be affected by future climate change (elevated CO_2 and temperature). Because the soil microbial community has a profound influence on soil carbon flow (Chen et al. 2005; Hawkes et al. 2005), these findings clarify the mechanisms by which climate change might affect the carbon cycle in evergreen broad-leaved forests in the warm temperate zone (Fig. 5-1).

Although many questions remain, including the possible increase in productivity that could result from elevated CO_2 and temperature, the results of my research suggest that future climate change may significantly affect soil respiration and the soil microbial community in evergreen broad-leaved forests in the warm temperate zone.

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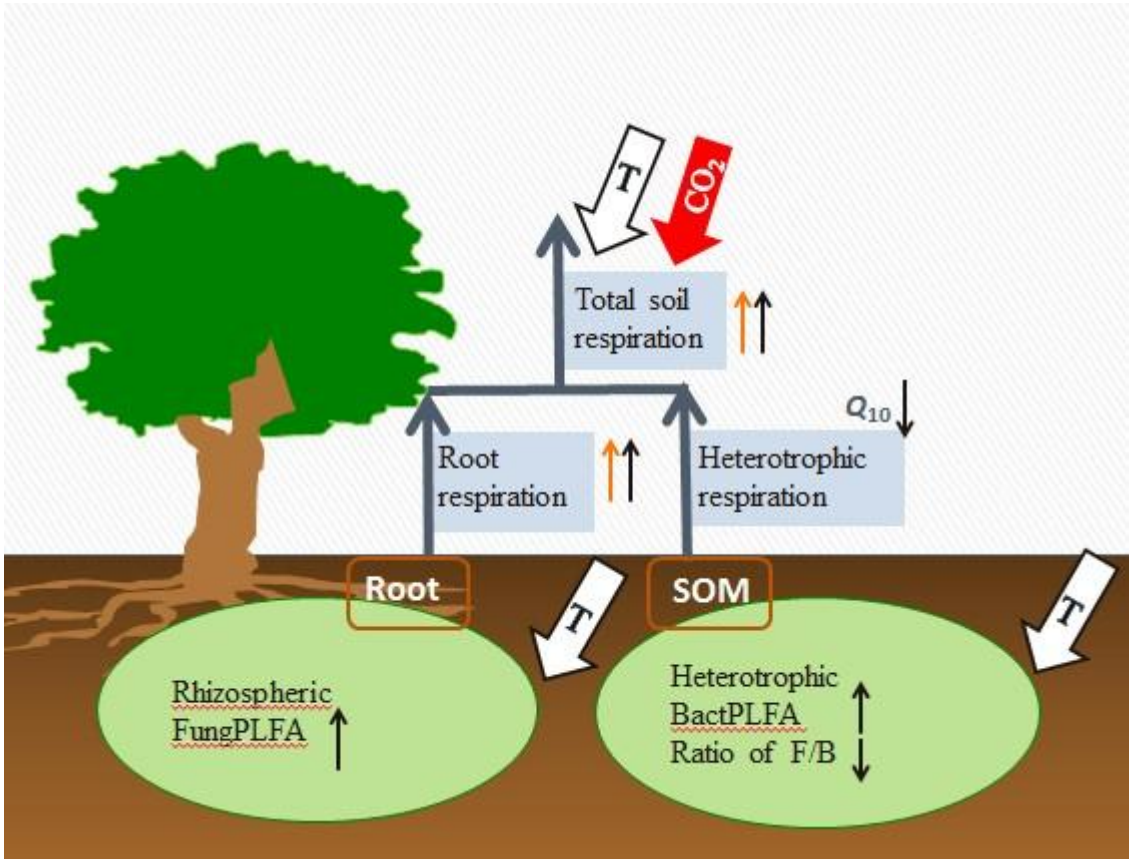

Fig. 5-1 Proposed mechanisms for the effects of climate change on soil carbon flow in evergreen broad-leaved forests in the warm temperate zone. White arrows indicate the impact of elevated temperature (T), and red arrows indicate the impact of elevated CO_2. Black arrows indicate the responses to elevated temperature (T), and orange arrows indicate the responses to elevated CO_2.

Acknowledgments

There are many people that need to be acknowledged for their help over the past 5 years. First and foremost, I would like to thank my advisor, Professor Takayuki Nakatsubo, for his continual support and advice throughout my doctor course. I would like to thank Professor Kaneyuki Nakane, my advisor at master course in Hiroshima University, for giving me encouragement and support. I would also like to acknowledge my committee members, Professor Hiroshi Sakugawa, Professor Naoki Sakurai and Dr. Motomu Toda, for their thoughtful contributions to my research.

I also grateful to Dr. Akiko Sasaki of Hiroshima University and Dr. Shinpei Yoshitake of river basin research center in Gifu University, for useful advice and suggestions in PLFA analysis. I wish to thank Dr. Nanshen Liang of the National Institute for Environmental Studies for helpful comments and Dr. Bahman Jabbarian Amiri of Hiroshima University for help with the field experiment. Dr. Asami Ogura of Hiroshima University deserves special thanks for the encouragement.

I also thank the members of the Nakatsubo and Nakane Laboratory, Hiroshima University, for providing assistance in the field work.

Last, I would like to acknowledge my friends and family who supported me through this process along the way.

Abstract

CHAPTER 1: General introduction

In Chapter 1, I introduce the relationship between soil carbon flow and climate warming. I review previous studies, which suggested that the responses of soil respiration and soil microbial communities to future climate change may vary widely across forest types.

Evergreen broad-leaved forest is the main natural vegetation type in the warm temperate zone of Japan. The potential land area of this forest type covers the western half of the Japanese Archipelago. Evergreen broad-leaved forest has a much higher carbon cycling rate than forests in cool climates, such as cool-temperate and boreal forests. However, few data are available on the potential impact of climate change on evergreen broad-leaved forests.

The objective of this study is to elucidate the effect of climate warming on the soil respiration and microbial community (heterotrophic and rhizospheric) in the evergreen broad-leaved forest of the warm temperate zone of Japan.

CHAPTER 2: Impacts of elevated CO_2 and temperature on soil respiration in warm-temperate evergreen *Quercus glauca* stands: an open-top chamber experiment

In this chapter, I describe an open-top chamber experiment that was conducted for 3 years to examine the effects of elevated CO_2 and temperature on soil respiration in experimental stands of *Quercus glauca*, an evergreen tree species common in the warm-temperate zone of Japan. Seedlings of *Q. glauca* were planted in open-top chambers and treated with ambient and elevated (ambient × 1.4, ambient × 1.8) CO_2 concentrations and ambient and elevated (+3 ℃) air temperatures.

Elevated CO_2 significantly increased the total soil respiration rate ($P < 0.001$) and the soil respiration rate at 15 °C (R_{15}) ($P < 0.05$) but had no significant effect on the temperature-response coefficient Q_{10}. Although temperature significantly affected the total soil respiration rate ($P < 0.05$), neither the R_{15} nor the Q_{10} of total soil respiration was affected significantly by the air temperature increase. However, the Q_{10} of heterotrophic respiration was ($P < 0.01$) significantly affected by elevated temperature. The annual soil respiration rate, estimated from R_{15}, Q_{10}, and soil temperature data, tended to increase with elevated CO_2 concentration. These results suggest that the soil respiration rate in Japanese broad-leaved

forests dominated by *Q. glauca* is sensitive to elevated CO_2 and is likely to increase under future climatic conditions.

CHAPTER 3: Effects of experimental warming on the soil heterotrophic microbial community in a warm temperate evergreen broad-leaved forest: a three-year field experiment

The experiments described in Chapter 2 revealed that elevated temperature caused a significant change in the temperature sensitivity (Q_{10}) of heterotrophic respiration, which might have been caused by a change in the structure of the microbial community.

To elucidate the effect of climate warming on the soil heterotrophic microbial community in evergreen broad-leaved forests in the warm temperate zone, a soil warming experiment was conducted in a natural secondary forest in Higashi-Hiroshima in western Japan. Ten trench plots (1 m × 1 m) with barriers to prevent root regrowth were established in the forest. The plots were divided into warming and control treatments. Infrared heaters were used to increase the soil temperature of the warming plots by about 3 °C for 3 years. Phospholipid fatty acid (PLFA) analysis was used to examine the composition of the soil heterotrophic microbial community. There were no significant differences in the total content of PLFAs (TotPLFAs) and fungal PLFAs (FungPLFAs) between the warming

and control plots. However, warming caused an increase in bacterial PLFAs (BactPLFAs), resulting in a lower ratio of FungPLFAs to BactPLFAs (F/B ratio) in the warming plots. In addition, PLFAs characteristic of Gram-negative bacteria increased in the warming plots. These results indicate that the soil heterotrophic microbial community in this evergreen broad-leaved forest is sensitive to climate warming.

CHAPTER 4: Effects of experimental warming on the rhizospheric soil microbial community associated with *Quercus glauca* seedlings

The rhizosphere is generally defined as the narrow zone of soil directly adjacent to, and affected by, plant roots. Rhizodeposition is an important source of carbon for microbes in the rhizosphere. In order to elucidate the effects of climate warming on the rhizospheric soil microbial community of *Q. glauca*, I conducted an incubation warming experiment in the laboratory. Three incubators were used to create different temperature conditions (15, 18, and 21 ℃), and 40 *Q. glauca* seedlings growing in mesh bags (to separate rhizospheric soil from the bulk soil) were placed in each incubator. After 3 months, PLFA analysis was conducted to examine changes in the rhizospheric microbial community.

There were no significant differences in TotPLFAs in rhizospheric soil

between the warming and control treatments. However, the FungPLFA contents in the warming treatments (18 and 21 ℃) were significantly higher than that in the control ($P < 0.05$). These results indicate that the soil rhizospheric microbial community associated with *Q. glauca* seedlings is sensitive to climate warming.

CHAPTER 5: General discussion and conclusions

As described in Chapter 2, total soil respiration was significantly increased by elevated CO_2 and temperature. Although the effect on the heterotrophic respiration rate was not significant, the increased root respiration rate due to elevated CO_2 and temperature is an important mechanism by which climate change will affect the ecosystem carbon cycle of evergreen broad-leaved forests in the warm temperate zone. In addition, the temperature sensitivity of heterotrophic respiration was changed by elevated temperature, which may have been caused by alteration of the microbial community structure. The experiments described in chapters 3 and 4 suggest that predicted climate warming will alter the soil microbial community in the bulk soil and the rhizosphere of this type of forest. Although many questions remain, including the possible increase in productivity that could result from elevated CO_2 and temperature, these results and those of previous studies indicate that the soil respiration and

microbial community in evergreen broad-leaved forests of the warm temperate zone may be significantly affected by climate change.