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Effects of elevated CO2 concentration and increased temperature on leaf quality responses of rare and endangered plants

Heon-Mo Jeong[1], Hae-Ran Kim[2], Seungbum Hong[1] and Young-Han You[3*]

Abstract

Background: In the study, the effects of elevated CO_2 and temperature on the nitrogen content, carbon content, and C:N ratio of seven rare and endangered species (*Quercus gilva*, *Hibiscus hambo*, *Paliurus ramosissimus*, *Cicuta virosa*, *Bupleurum latissimum*, *Viola raddeana*, and *Iris dichotoma*) were examined under control (ambient CO_2 + ambient temperature) and treatment (elevated CO_2 + elevated temperature) for 3 years (May 2008 and June 2011).

Results: Elevated CO_2 concentration and temperature result in a decline in leaf nitrogen content for three woody species in May 2009 and June 2011, while four herb species showed different responses to each other. The nitrogen content of *B. latissimum* and *I. dichotoma* decreased under treatment in either 2009 and 2011. The leaf nitrogen content of *C. virosa* and *V. raddeana* was not significantly affected by elevated CO_2 and temperature in 2009, but that of *C. virosa* increased and that *V. raddeana* decreased under the treatment in 2011. In 2009, it was found that there was no difference in carbon content in the leaves of the six species except for that of *P. ramosissimus*. On the other hand, while there was no difference in carbon content in the leaves of *Q. gilva* in the control and treatment in 2011, carbon content in the leaves of the remaining six species increased due to the rise of CO_2 concentration and temperature. The C:N ratio in the leaf of *C. virosa* grown in the treatment was lower in both 2009 and 2011 than that in the control. The C:N ratio in the leaf of *V. raddeana* decreased by 16.4% from the previous year, but increased by 28.9% in 2011. For the other five species, C:N ratios increased both in 2009 and 2011. In 2009 and 2011, chlorophyll contents in the leaves of *Q. gilva* and *H. hamabo* were higher in the treatment than those in the control. In the case of *P. ramosissimus*, the ratio was higher in the treatment than that in the control in 2009, but in 2011, the result was the opposite. Among four herb species, the chlorophyll contents in the leaves of *C. virosa*, *V. raddeana*, and *I. dichotoma* did not show any difference between gradients in 2009, but decreased due to the rise of CO_2 concentration and temperature in 2011. Leaf nitrogen and carbon contents, C:N ratio, and chlorophyll contents in the leaves of seven rare and endangered species of plant were found to be influenced by the rise and duration of CO_2 concentration and temperature, species, and interaction among those factors.

Conclusions: The findings above seem to show that long-term rise of CO_2 concentration, and temperature causes changes in physiological responses of rare and endangered species of plant and the responses may be species-specific. In particular, woody species seem to be more sensitive to the rise of CO_2 concentration and temperature than herb species.

Keywords: Global climate change, Endemic plants, Evergreen bread-leaved, *Quercus*, Photosynthesis, Leaf nitrogen

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Background

Climate change is likely to impact species both directly and indirectly. The same species may respond differently in different places, due to variations in exposure to climate change or differences in key habitats or species interactions.

Climate change over the past 30 years has produced numerous shifts in the distributions and abundances of species (Parmesan and Yohe 2003) and has been implicated in one species-level extinction (Pounds et al. 1999).

The Intergovernmental Panel on Climate Change predicted that approximately 20–30% of the plant and animal species would be at risk of extinction with a moderate increase in temperature of 1.5~2.5 °C (IPCC 2007). Thomas et al. (2004) predicted that 15~37% of 1103 plant and animal species will be committed to extinction based on mid-range climate change scenarios for 2050. Broennimann et al. (2006) predicted that the endemic flora of Southern Africa on average decreases with 41% in current endemic species richness and with 39% on species distribution range by 2050.

The IUCN which is the most widely used global threatened species list reported that 44,837 species have been assessed as threatened by 2008 and the number of threatened species keeps increasing every year since 2000 (Vié et al. 2009). In case of plants, the IUCN Red List of Threatened Species includes 15,674 species of mosses, ferns, gymnosperms, and angiosperms out of an estimated total number of species across those groups of 307,674 (IUCN 2012).

In Korea, Wildlife Protection Act has been enacted for establishing an institutional basic framework by integrating scattered and duplicated regulations relevant to wildlife protection in the Natural Environment Conservation Act and the law on conservation and game of wildlife etc. in 2004.

Climate change has been recognized as a threat to biodiversity. Consequences of climate change for the rare and endemic species are predicted to by fairly severe (Malcolm et al. 2006). Restricted-range endemic species may be especially vulnerable under climate change than generalist species (Brown et al. 1997; Maschinski et al. 2006).

The United Nations Convention on Biological Diversity (CBD) agreement at the 1992 UN Conference on Environment and Development is one of the most widely ratified treaties in the world. Since 2002, 193 parties to the CBD have committed themselves to substantially reducing rates of biodiversity loss by 2010. Thus, The UN has declared 2010 the "International Year of Biodiversity" with a key goal of increasing awareness of the importance of biodiversity for human well-being (Rands et al. 2010).

The government of Korea also is working hard to promote various events and establish master plans to conservation, management, and utilization of biological resources etc. in commemoration of the International Year of Biodiversity. However, the priority should be to carry out fundamental studies to predict responses of plants and animals under future climate change situation. In the world, the effects of elevated CO_2 concentration and increasing temperature on growth, development, and function of a large number of plant species have been investigated more than two decades (Long et al. 2004).

Many researchers have reported the changes in the nutrient contents of plant parts, especially nitrogen contents (Cotrufo et al. 1998). Nitrogen is the most important element that not only is the biggest amount of elements that plants absorb from the soil, but also contributes to the plant growth and development more than other nutritional elements (Ingestad 1981; Crawford and Glass 1998), and may plant a role in the response of plant to CO_2 (Yang et al. 2006).

The changes in plant nitrogen can have important ecosystem implications such as the net primary productivity (LeBauer and Treseder 2008), physiological processes (Curtis 1996; Wang et al. 2012), plant tissue quality (Knops et al. 2007), decomposition rate (Cotrufo et al. 1994), and herbivore population dynamics (Whittaker 1999); thus, a studies on the role of nitrogen in controlling plant response is necessary.

Elevated CO_2 and temperature cause decline of plant tissue nitrogen concentration. In contrast, carbon content of plant organs increases, and thus the change in the relative proportion of carbon to nitrogen is considerably higher under increasing CO_2 concentration and temperature conditions (Fitter and Hay 2002).

The broad literature surveys have consistently found mean decreases in nitrogen of approximately 10–15% for plants grown at elevated CO_2 (Taub and Wang 2008). Cotrufo et al. (1998) determined decreases in nitrogen of 14% in aboveground and 9% in belowground. Also, elevated CO_2 causes reduction in nitrogen of 19 and 17% in woody and non-woody species respectively. Herbaceous species consistently had reduced leaf N-content under elevated CO_2, and hence smaller enhancements of photosynthesis, than woody species (Wertin et al. 2010).

According to Larsen et al. (2011), *Calluna vulgaris* and *Deschampsia flexuosa*, co-dominant species, grown in elevated CO_2 conditions had higher C:N ratio and lower N concentration than those growth in ambient ones. The leaves of *Q. gilva*, an endangered plant, had an increased C:N ratio due to their reduced nitrogen content grown under elevated CO_2 and temperature conditions (Kim and You 2010).

Such reduction in nitrogen concentration is that it results from N uptake, dilution by carbohydrate and plant secondary compounds, or changes in N allocation etc. (Taub and Wang 2008). Leaf nitrogen and carbon composition is affected through the accumulation of carbohydrates

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as a product of photosynthetic enhancement or relative tissue source–sink carbon demands (Körner et al. 1995).

Threatened plant species have less seed production and dispersal, lower rates of seedlings recruitment, and phenotypic plasticity in acclimating to a wider range of environmental conditions; thus, rare and endangered plants are particularly more vulnerable to changes in environment (Fischer et al. 1997).

Recently, researches are being carried out to obtain information about ecological responses of rare and endangered plants under elevated CO_2 and temperature (Kim and You 2010; Shin et al. 2012; Han et al. 2012), but unfortunately there is still a lack of studies on their biological and ecological responses under climate change situations (Aleric and Kirkman 2005). Moreover, there has been no study that tried to determine the effects of elevated CO_2 and temperature on changes in foliage biochemistry of various rare and endangered plants. Thus, the purpose of this study was to determine the changes in foliage biochemistry of

seven rare and endangered plant species by comparing leaf nitrogen, carbon, chlorophyll content, and C:N ratio under ambient and elevated CO_2 and temperature conditions.

Methods

Experimental design

This study was conducted in glass greenhouse (12 m length × 7.8 m width × 5 m height). The surface area of control and treatment is 46.8 m^2, respectively. The control was maintained at ambient CO_2 concentration, which averaged approximately 360 ± 9.2 ppm on a 24-h basis (Fig. 1). Treatment with elevated CO_2 concentration achieved by inputting a small quantity of pure CO_2 through two perforated plastic hoses so as to maintain the concentration at approximately 742.30 ± 16.92 ppm, twice that of the ambient (360.38 ± 9.19 ppm) concentration.

CO_2 concentration was monitored by a CO_2 sensor (TEL-7001, Onset computer, USA) installed at height of 1.5 m at 30-min intervals, and data was stored on data-
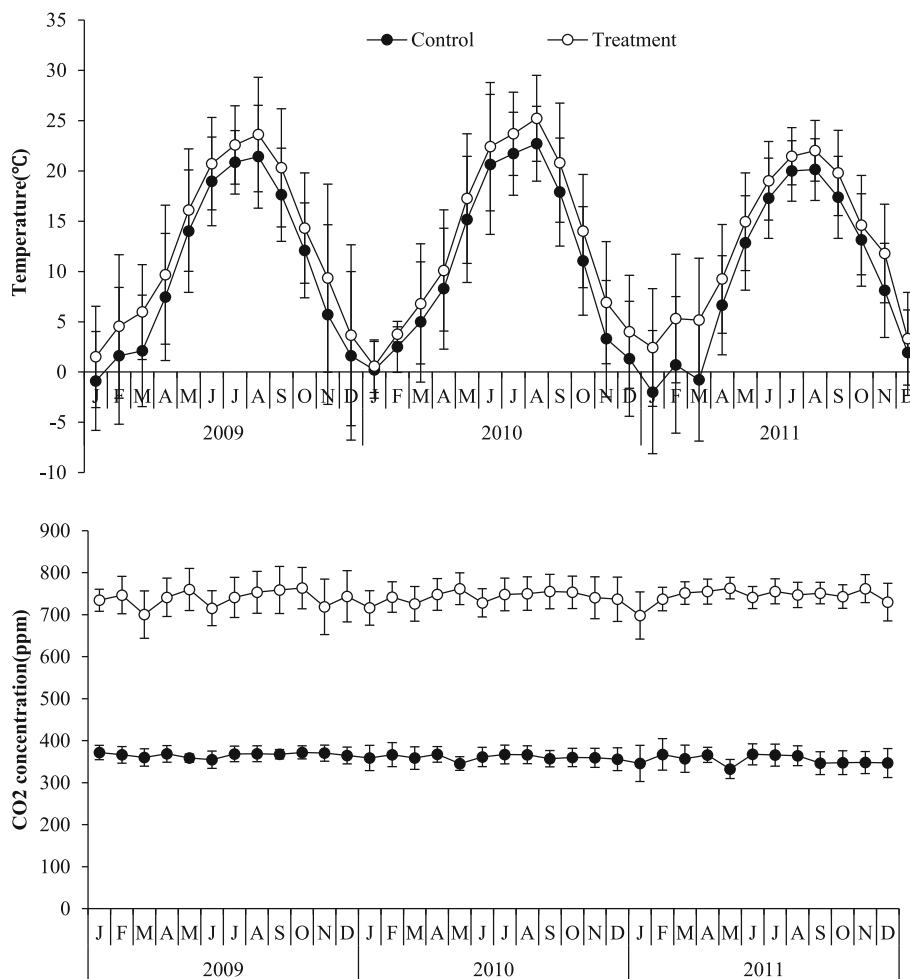
Fig. 1 Monthly mean air temperature and CO_2 concentration in control and treatment greenhouses throughout the experimental period from 2009 through 2011

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logger (HOBO U12, Japan) to evaluate the stability of the CO_2 concentration in the treatment. The CO_2 treatment lasted from May 2008 to June 2011. The mean temperature in the treatment was about 2.5 °C higher than the control. The air temperature was measured using a thermo recorder (TR-71 U, T&D Co., Japan) at the same height in the control and treatment greenhouses during the study period.

Plant materials

Among the seven species of plant used in this research, the following six species—*Q. gilva*, *H. hamabo*, *C. virosa*, *B. latissimum*, *V. raddeana*, and *I. dichotoma*—are on the list of endangered species class II designated by the Ministry of Environment. The remaining one, *P. ramosissimus*, is what the Korea Forest Service designates as a rare species of plant and classifies as an endangered species (EN). *H. hamabo* and *P. ramosissimus* used in this research were 3-month-old seedlings supplied by the National Institute of Environmental Research in 2007. The seeds of other five species were sowed in 2007, and in April 2008, to make roots of the young plants grow, they were moved to the soil in greenhouse. We fertilized 0.5% of the sand weight and subsequently applied organic fertilizer, which contains an ammonium nitrogen content below 170 mg·L^{-1} and nitrate nitrogen at a concentration of 150–330 mg·L^{-1} (Monsanto Korea Inc., Seoul). All plants were watered twice or three times per week to prevent them from suffering from water stress.

Leaf C:N ratio, nitrogen and carbon contents

We used to experiment with 1-year-old collected leaves. The samples of leaves were dried for 2 days at 65 °C. After the leaves were dried, the samples were pulverized into fine powder with a blender (AKM-369 s; Eupa, Seoul, Korea). The nitrogen and carbon contents were determined using an automatic elemental analyzer (Flash EA 1112 series; Thermo Fisher Scientific, Rochester, NY, USA) at the Center for Research Facilities, Chungnam National University. The C:N ratio was calculated as the ratio of carbon content to nitrogen content. Chlorophyll content measurement on leaves of rice cultivars was made with hand-held chlorophyll content meter (Chlorophyll Content Meter-20, ADC Bioscientific), which measures the absorbance of a small portion of leaf using differential transmission at two wavelengths, 665 and 940 nm.

Statistical analysis

The determined effects of elevated CO_2 and temperature on the foliage chemistry parameters of seven rare and endangered species were confirmed via one-way ANOVA, and the statistical differences between the control and treatment groups were evaluated by Fisher's least significant difference test as post-hocs, with significance set at $p = 0.05$. Multivariate analysis of variance (MANOVA) was used to analyze the effects of elevated CO_2 concentration and temperature, term of exposure, and species and their interactions within each species. All statistical analyses were performed at a 0.05 level of confidence with STATISTICA 8 software (Statsoft, Inc., Tulsa, OK, USA).

Results and discussion

Leaf nitrogen concentration

Elevated CO_2 and temperature reduced the leaf nitrogen content of three woody species in 2009 and 2011 (Fig. 2). The leaf nitrogen content of *Q. gilva* grown under the control and treatment was 18.90 ± 0.49 mg g^{-1}, 12.47 ± 0.59 mg g^{-1} in 2009, and was 18.04 ± 0.73 mg g^{-1}, 11.21 ± 0.27 mg g^{-1} in 2012, respectively. For *H. hamabo*, the nitrogen content was found to be increased by 23.0% in 2009, and 12.93% in 2011, when compared to those grown in control. The leaf nitrogen content of *P. ramosissimus* grown under the control and treatment was 31.44 ± 1.55 mg g^{-1}, 24.21 ± 0.18 mg g^{-1} in 2009, and was 28.78 ± 0.83 mg g^{-1}, 25.06 ± 1.11 mg g^{-1} in 2012, respectively.
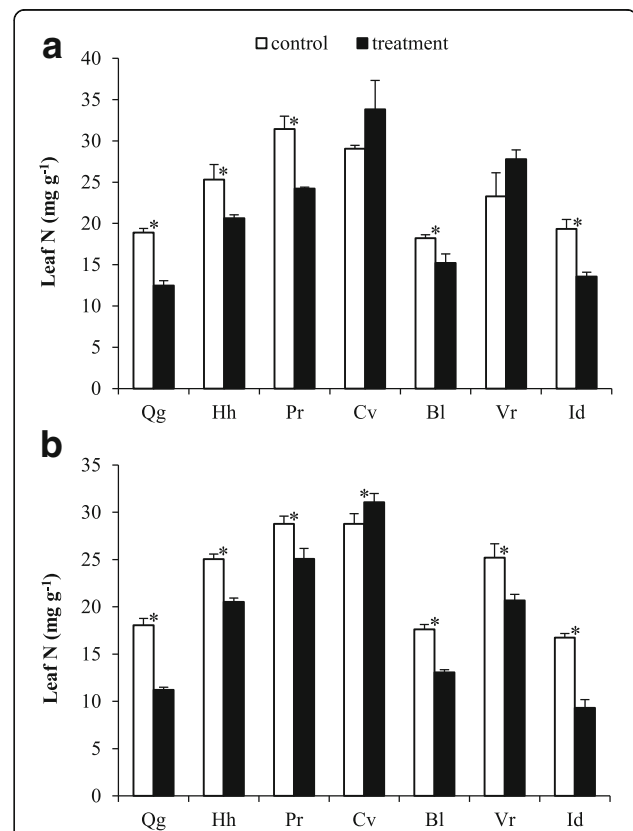
Fig. 2 Leaf nitrogen content of *Q. gilva* (Qg), *H. hamabo* (Hh), *P. ramosissimus* (Pr) *C. virosa* (Cv), *B. latissimum* (Bl), *V. raddeana* (Vr), and *I. dichotoma* (Id) grown under the control and treatment in 2009 (**a**) and 2011(**b**). Asterisk signs on the bars indicate significant differences between control and treatment within each species (Fisher's least significant difference, *P* < 0.05)

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Unlike woody species, four non-woody species seem to have different responses to elevated CO_2 and temperature (Fig. 2). The leaf nitrogen content of *C. virosa* was not significantly affected by elevated CO_2 and temperature in 2009, but increased by 7.92% compared with the control in 2011. The percent of decrement in nitrogen content due to elevated CO_2 and temperature for *B. latissimum* reached 16.52 and 25.86% in 2009 and 2011, respectively. The leaf nitrogen concentration of *V. raddeana* was not significantly affected by elevated CO_2 and temperature in 2009, but decreased by 17.97% compared with the control in 2011. The leaf nitrogen concentration of *I. dichotoma* grown under the control and treatment was 19.34 ± 1.13 mg g^{-1}, 13.57 ± 0.53 mg g^{-1} in 2009, and was 16.73 ± 0.44 mg g^{-1}, 9.32 ± 0.87 mg g^{-1} in 2012, respectively.

Seven rare and endangered plants showed species-specific responses on leaf nitrogen concentration under the elevated CO_2 concentration and temperature conditions (Table 1). The leaf nitrogen concentrations of three woody species grown under the treatment were lower than those grown under the control in 2009 and 2011. Among the three woody plants, leaf nitrogen concentration of *Q. gilva*

was decreased by 34.02% in 2009 and 37.83% in 2011 under the elevated CO_2 and temperature conditions (Fig. 2).

McGuire et al. (1995) found mean reduction in leaf nitrogen of 21% for woody plants grown at elevated CO_2. In *Eucalyptus saligna*, evergreen trees, foliar nitrogen contents were approximately 28.5% lower in plants grown under the elevated CO_2 and temperature than those grown under the ambient CO_2 and temperature (Murray et al. 2013). Compared to results in other studies of various woody species, *Q. gilva* showed the highest percentage reduction in leaf nitrogen under elevated CO_2 concentration and high temperature situations.

In herbaceous species, the leaf nitrogen concentration was found to increase, decrease or remain the same under elevated CO_2 and temperature. The leaf nitrogen concentration of *C. virosa* and *V. raddeana* was not significantly affected by elevated CO_2 and temperature in 2009, while both species showed different responses in 2011. *B. latissimum* and *I. dichotoma* both showed decreased leaf nitrogen concentration under elevated CO_2 concentration and temperature in 2009 and 2011.

Table 1 Multivariate analysis of variance (MANOVA) for effects of elevated CO_2 + temperature and term of CO_2 exposure and their interactions on parameters of seven rare and endangered plant species in Korea

Species	Factor	Nitrogen		Carbon		C:N ratio		Chlorophyll	
		F	P	F	P	F	P	F	P
Q. gilva	CO_2 + T (C)	443.714	**< 0.000**	5.075	0.054	572.304	**< 0.000**	596.564	**< 0.000**
	Term(T)	11.391	**< 0.010**	9.253	**< 0.016**	30.533	**< 0.001**	3.384	0.084
	C x T	0.391	0.549	1.913	0.204	9.979	**< 0.013**	1.151	0.299
H. hamabo	CO_2 + T (C)	63.690	**< 0.000**	38.787	**< 0.000**	112.189	**< 0.000**	149.995	**< 0.000**
	Term(T)	0.112	0.747	3.610	0.094	0.592	0.464	10.161	0.006
	C x T	0.015	0.906	4.268	0.073	0.242	0.636	15.324	**< 0.001**
P. ramosissimus	CO_2 + T (C)	82.444	**< 0.000**	24.945	**< 0.001**	105.848	**< 0.000**	0.002	0.967
	Term(T)	2.254	0.172	133.247	**< 0.000**	4.097	0.078	0.395	0.538
	C x T	8.448	**< 0.020**	0.789	0.400	8.085	**< 0.022**	34.940	**< 0.000**
C. virosa	CO_2 + T (C)	10.244	**< 0.013**	46.843	**< 0.000**	8.842	**< 0.018**	106.980	**< 0.000**
	Term(T)	1.901	0.205	89.992	**< 0.000**	4.981	0.056	7.251	**< 0.016**
	C x T	1.270	0.292	16.764	**< 0.003**	1.864	0.209	15.215	**< 0.001**
B. latissimum	CO_2 + T (C)	102.856	**< 0.000**	10.530	**< 0.012**	139.643	**< 0.000**	24.231	**< 0.000**
	Term(T)	13.350	**< 0.006**	10.004	**< 0.013**	29.131	**< 0.001**	27.580	**< 0.000**
	C x T	4.302	0.072	11.399	**< 0.010**	17.432	**< 0.003**	11.333	**< 0.004**
V. raddeana	CO_2 + T (C)	0.000	0.998	43.719	**< 0.000**	1.703	0.228	19.348	**< 0.000**
	Term(T)	6.717	**< 0.032**	323.299	**< 0.000**	19.841	**< 0.002**	0.290	0.598
	C x T	20.296	**< 0.002**	25.002	**< 0.001**	23.863	**< 0.001**	8.252	**< 0.011**
I. dichotoma	CO_2 + T (C)	207.594	**< 0.000**	405.465	**< 0.000**	119.363	**< 0.000**	3.196	0.093
	Term(T)	55.856	**< 0.000**	297.333	**< 0.000**	47.078	**< 0.000**	0.600	0.450
	C x T	3.252	0.109	285.869	**< 0.000**	21.323	**< 0.002**	2.219	0.156

The bold data in the table means significantly different among the environmental treatment and interaction within each species ($p < 0.05$)

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Table 2 Multivariate analysis of variance (MANOVA) for effects of elevated CO_2 + Temperature, species and term of CO_2 exposure and their interactions on photosynthesis parameters of seven rare and endangered plant species in Korea

Factor	Nitrogen		Carbon		C:N ratio		Chlorophyll	
	F	P	F	P	F	P	F	P
CO_2 + T (C)	165.241	**< 0.000**	170.497	**< 0.000**	518.301	**< 0.000**	12.571	**< 0.001**
Term (T)	36.434	**< 0.000**	305.477	**< 0.000**	123.077	**< 0.000**	25.461	**< 0.000**
Species (S)	338.648	**< 0.003**	408.496	**< 0.000**	409.411	**< 0.000**	492.478	**< 0.000**
C x T	9.795	**< 0.000**	71.039	**< 0.000**	55.399	**< 0.000**	61.905	**< 0.000**
C x S	29.841	**< 0.000**	4.035	**< 0.002**	78.397	**< 0.000**	130.498	**< 0.000**
T x S	2.482	**< 0.034**	31.495	**< 0.000**	20.267	**< 0.000**	10.115	**< 0.000**
C x T x S	7.531	**< 0.000**	5.061	**< 0.000**	11.934	**< 0.000**	2.224	**< 0.046**

The bold data in the table means significantly different among the environmental treatment and interaction within each species ($p < 0.05$)

There was a significant effect of short- and long-term exposure to elevated CO_2 concentration and temperature on leaf nitrogen concentration (Table 2).

In three woody plants, mean percentage of changes in leaf nitrogen concentration was − 25.2 and − 22.9% after short- and long-term of exposure to elevated CO_2 concentration and temperature, respectively. The declination rate of leaf nitrogen concentration of herbaceous species was 10 times as high in 2011 as in 2009. Thus, leaf nitrogen concentration was declined significantly more long-term exposure to elevated CO_2 concentration and temperature for herbaceous (− 19.8%) compared with woody (− 2.4%) species.

According to Wertin et al. (2010), the leaf N content of herbaceous species responded more strongly than woody species to elevated CO_2 concentration. However, Cotrufo et al. (1998) found a mean decrease of 19% in woody and 17% in non-woody-leaf nitrogen in response to growth in elevated CO_2 regimes. The inconsistency of the results of this present study with those in previous studies may be due to differences in sensitivity to environmental changes (Kleijn et al. 2008). In general, rare and endangered species are more responsive to environmental variables than widespread species (Gaston and Kunin 1997).

Leaf carbon concentration

The leaf carbon content of all species except for *P. ramosissimus* was not significantly affected by elevated CO_2 and temperature in 2009 (Fig. 3). The carbon content of *P. ramosissimus* was 435.17 ± 1.62 mg g^{-1} in the control and 439.67 ± 1.74 mg g^{-1} in the treatment. However, in 2012, the leaf carbon content of all species except for *Q. gilva* was significantly increased by elevated CO_2 concentration and temperature (Fig. 3).

The leaf carbon content of *H. hamabo* and *P. ramosissimus* grown under the treatment has increased from 416.01 ± 3.03, 444.67 ± 1.55 to 432.68 ± 1.20, 447.81 ± 1.19 in comparison to the control, respectively. The highest increases came in leaf carbon content of *I. dichotoma*, up 6.3%, to
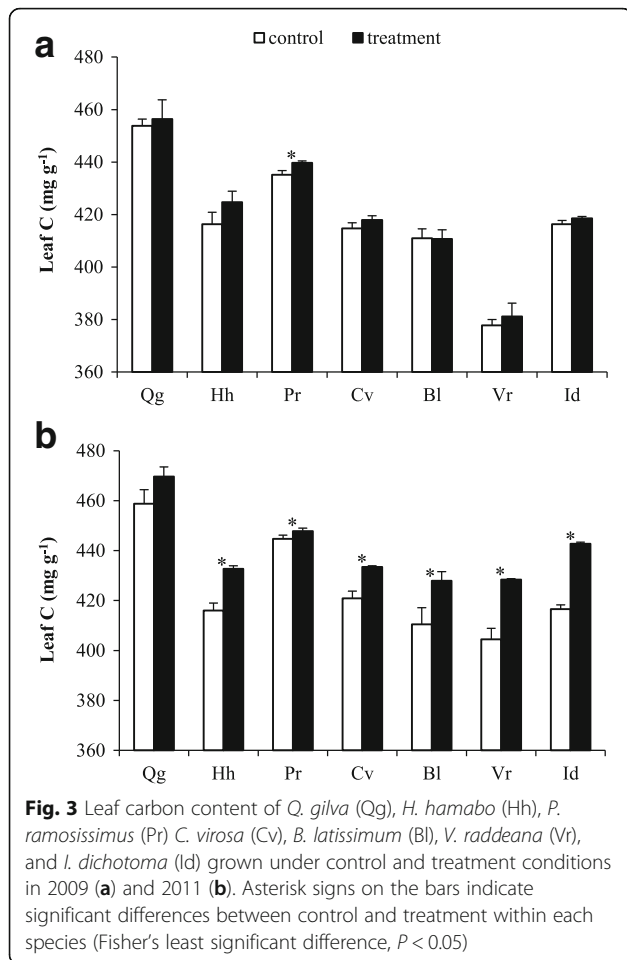
442.77 ± 0.60 mg g^{-1} from 416.55 ± 1.72 mg g^{-1} among four non-woody species. The leaf carbon contents were stimulated significantly more under elevated CO_2 and temperature for herbaceous species (4.86%) compared with woody species (2.36%).

The increases in leaf carbon content by elevated CO_2 concentration and temperature is generally positively related to the accumulation of carbohydrates as a product of photosynthetic enhancement (Tjoelker et al. 1999). In 2009, there were no significant effects of elevated CO_2 and temperature on leaf carbon concentration of all species except for *P. ramosissimus*. However, elevated CO_2 and temperature increased leaf carbon concentration for six of the seven species studied in 2011. The sole exception was *Q. gilva*, where resulted in a no significant affects in leaf carbon. The leaf carbon concentration of woody plants grown in the treatment was increased by 1.21% in 2009 and 2.36% in 2011. In the treatment, four herbaceous plants showed a slight increment in leaf carbon concentration in 2009 whereas those species had relatively higher increases in 2011.

After long-term exposure to elevated CO_2 concentration and temperature, the leaf carbon concentration was increased by 2.21% for woody plants and 6.52% for herbaceous plants. The changes in carbon content of non-woody species were three times higher than woody species. It means that herbaceous plants were more responsive than woody plants to elevated CO_2 and temperature.
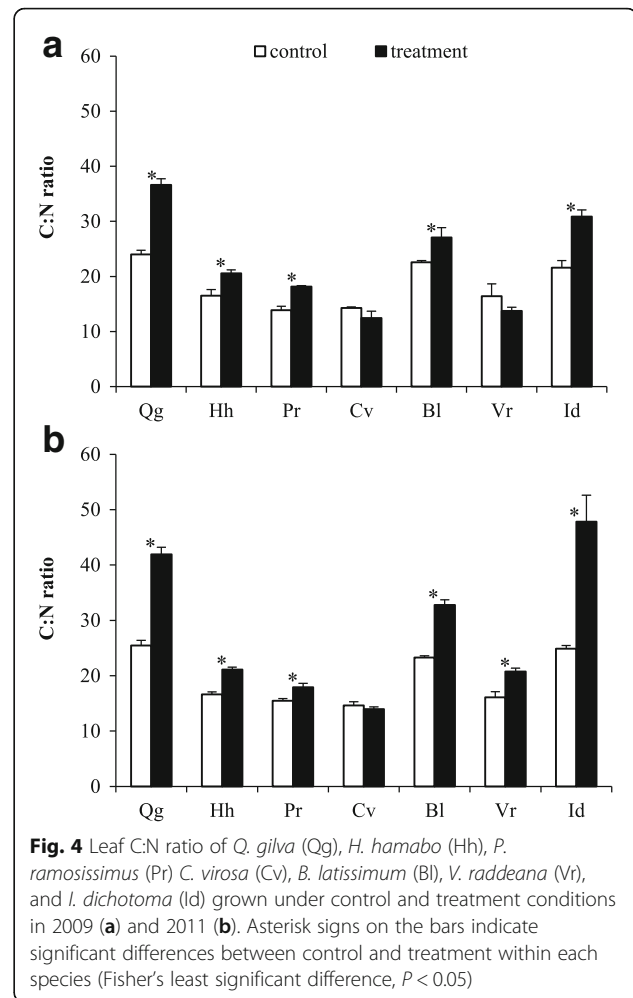
Leaf C:N ratio

Elevated CO_2 concentration and temperature stimulated the leaf C:N ratio of three woody species in 2009 and 2011 (Fig. 4). The leaf C:N ratio of *Q. gilva* grown under the control and treatment was 24.02 ± 0.76, 36.62 ± 1.14 in 2009, and was 25.45 ± 0.92 mg g^{-1}, 41.90 ± 1.31 mg g^{-1} in 2012, respectively. The leaf C:N ratio of *H. hamabo* grown in the treatment increased up to 24.72% from 16.50 ± 1.10 to 20.58 ± 0.59 in 2009 and up to 26.94% from 16.62 ± 0.43 to 21.09 ± 0.46 in 2011 in comparison to those grown in the control. The leaf C:N ratio of *P. ramosissimus*

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Fig. 3 Leaf carbon content of *Q. gilva* (Qg), *H. hamabo* (Hh), *P. ramosissimus* (Pr) *C. virosa* (Cv), *B. latissimum* (Bl), *V. raddeana* (Vr), and *I. dichotoma* (Id) grown under control and treatment conditions in 2009 (**a**) and 2011 (**b**). Asterisk signs on the bars indicate significant differences between control and treatment within each species (Fisher's least significant difference, $P < 0.05$)

Fig. 4 Leaf C:N ratio of *Q. gilva* (Qg), *H. hamabo* (Hh), *P. ramosissimus* (Pr) *C. virosa* (Cv), *B. latissimum* (Bl), *V. raddeana* (Vr), and *I. dichotoma* (Id) grown under control and treatment conditions in 2009 (**a**) and 2011 (**b**). Asterisk signs on the bars indicate significant differences between control and treatment within each species (Fisher's least significant difference, $P < 0.05$)

grown under the control and treatment was 13.87 ± 0.74, 18.16 ± 0.16 in 2009, and was 15.46 ± 0.40 mg g^{-1}, 17.89 ± 0.74 mg g^{-1} in 2012, respectively.

However, for *C. virosa*, the leaf C:N ratios were not significantly affected by elevated CO_2 and temperature in 2009 and 2011. The leaf C:N ratio of *B. latissimum* grown in the treatment increased up to 20.04% from 22.57 ± 0.33 to 27.09 ± 1.77 in 2009 and up to 40.63% from 23.29 ± 0.30 to 32.76 ± 0.93 in 2011 in comparison to those grown in the control. The C:N ratio of *V. raddeana* was not significantly affected by elevated CO_2 and temperature in 2009, but increased by 28.87% compared with the control in 2011. Like *B. latissimum*, the leaf C:N ratio of *I. dichotoma* grown in the treatment increased up to 43.16% from 21.57 ± 1.33 to 30.88 ± 1.17 in 2009 and up to 92.20% from 24.89 ± 0.56 to 47.83 ± 4.76 in 2011 in comparison to those grown in the control.

Under high CO_2 concentration and temperature, plant tissue nitrogen content decreased whereas carbon content increased. Both of these together resulted in an increase of C:N ratio (Leakey et al. 2009). The changes in plant C:N ratio affect litter quality, rate of decomposition, insect herbivore as well as the balance vegetative and reproductive

growth (Fageria and Baligar 2005). Bernier et al. (1981) found that a low C:N ratio might delay or reduce reproductive development.

Three woody plants grown in the treatment have a higher leaf C:N ratio than those grown in the ambient in 2009 and 2011. Among the three species for *Q. gilva* leaf C:N ratio increased by 52.49% in 2009 and 64.60% in 2011 under the elevate CO_2 and temperature conditions. Murray et al. (2013) reported an average increase in C:N ratio of 33.3% for *Eucalyptus tereticornis*. In four herbaceous plants, the leaf C:N ratio of *B. latissimum* and *I. dichotoma* was higher in treatment than in control in 2009 and 2011 whereas *C. virosa* did not exhibit increased leaf C:N ratio when growing in elevated CO_2 and temperature condition. In the treatment, *V. raddeana* showed no change in leaf C:N ratio in 2009 but the ratio of carbon to nitrogen for those species increased by 28.87% in 2012.

However, herbaceous plants showed the marked difference between the short-term and the long-term responses of leaf C:N ratio to elevated CO_2 and temperature (Fig. 6). The leaf C:N ratio of three woody species increased by

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36.06% after short-term exposure to elevated CO_2 and temperature. In contrast to the short-term response, long-term exposure of three woody plants to elevated CO_2, and temperature caused a slightly increase in leaf C:N ratio (8.44%). However, herbaceous plants showed the opposite response to length of CO2 and temperature exposure. The rate of increment in leaf C:N ratio of four non-woody species after long-term exposure (34.76%) to elevated CO_2 and temperature was higher than after short-term exposure (8.05%).

Elevated CO_2 and temperature generally increases foliar C:N ratio by promoting the accumulation of carbohydrates and diluting foliar nitrogen content (Taub and Wang 2008). Leaf nitrogen content was lower but carbon content was higher in the leaves under elevated CO_2. Consequently, the change in the relative proportion of carbon to nitrogen was considerably higher in the leaves under elevated CO_2. Murray et al. (2013) found that elevated CO_2 concentration reduced leaf nitrogen content but increased starch content, sugar, and total nonstructural carbohydrate. Thus, C:N ratio of *E. tereticornis* increased by 43.91%.

In the present study, changes in leaf C:N ratio of non-woody species were more responsive than woody species after long-term exposure to CO_2 concentration and temperature, although herbaceous plants showed no significant difference between control and treatment after short-term exposure to elevated CO_2 and temperature.

Leaf C:N ratio was relatively more response to elevated CO_2 and temperature in *B. latissimum* and *I. dichotoma* with the thick, broad leaves than in *C. virosa* and *V. raddeana* with the thin, narrow leaves.

Fig. 5 Chlorophyll content concentration of *Q. gilva* (Qg), *H. hamabo* (Hh), *P. ramosissimus* (Pr) *C. virosa* (Cv), *B. latissimum* (Bl), *V. raddeana* (Vr), and *I. dichotoma* (Id) grown under control and treatment conditions in 2009 (**a**) and 2011 (**b**). Asterisk signs on the bars indicate significant differences between control and treatment within each species (Fisher's least significant difference, $P < 0.05$)

Leaf chlorophyll content

The leaf chlorophyll content of three woody species grown under the treatment was higher than those grown under the control in 2009 (Fig. 5). The chlorophyll content of foliage for *Q. gilva* was 37.66 ± 0.67 $\mu mol g^{-1}$ s^{-1} in the control and 47.64 ± 0.62 $\mu mol g^{-1}$ s^{-1} in the treatment, and that of *H. hamabo* was 24.06 ± 0.60 $\mu mol g^{-1}$ s^{-1} in the control and $29.84 \pm 0.65 \mu mol g^{-1}$ s^{-1} in the treatment in 2009. In *P. ramosissimus*, leaf chlorophyll content was increased by 12.22% under the elevated CO_2 concentration and temperature conditions. Among four herbaceous species, the leaf chlorophyll content of *C. virosa* was 21.46 ± 1.49 $\mu mol g^{-1}$ s^{-1} in the control and 18.90 ± 0.71 $\mu mol g^{-1}$ s^{-1} in the treatment, but the others were not significantly affected by elevated CO_2 and temperature in 2009.

The leaf chlorophyll content of *Q. gilva* and *H. hamabo* increased by 23.56 and 12.25% under the elevated CO_2 concentration and temperature conditions in 2011, respectively. However, for *P. ramosissimus*, the chlorophyll content in leaves reduced by 11.16%. The leaf chlorophyll content of four herbaceous species was reduced by
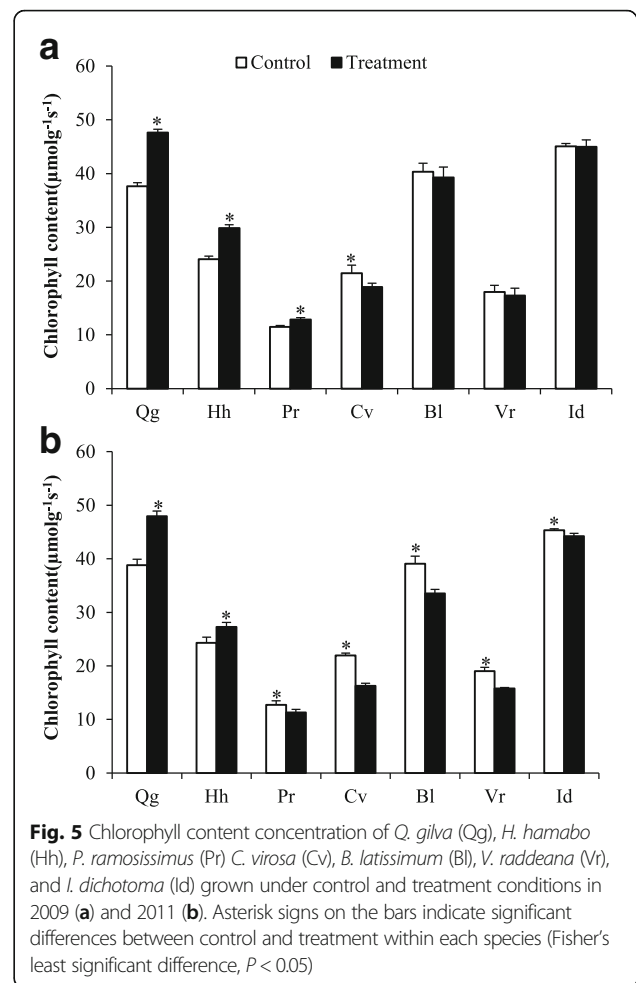
elevated CO_2 and temperature. The chlorophyll content in leaves of *C. virosa and B. latissimum* grown in the treatment increased up to 25.80 and 14.18%, compared to those grown in the control, respectively. The leaf chlorophyll content increases due to elevated CO_2 and temperature for *V. raddeana* and *I. dichotoma* reached 17.03 and 2.43%, respectively.

Chlorophyll is the most abundant biological pigments and light harvesting component. The loss of chlorophyll is a highly visible indicator of such events as disease, industrial pollution, and temperature extremes (Hendry and Grime 1993).

Elevated CO_2 concentration and temperature negatively affect plant growth and photosynthetic capacity by reducing leaf chlorophyll content. In general, declines in leaf chlorophyll have been reported in plants in response to elevated CO_2 concentration and temperature (Lewis et al. 1999). Chlorophyll content decreased with the accumulation of non-structural carbohydrate under elevated CO_2 concentration and temperature. Li et al. (2013) found that starch particles accumulate in the chloroplasts of the dwarf

bamboo growth under elevated CO_2 concentration. Non-structural carbohydrate accumulation is generally thought to physically distort the chloroplast (DeLucia et al. 1985) and possibly hinder CO_2 diffusion within the chloroplast (Makino 1994).

The responses of woody and non-woody species to elevated CO_2 and temperature did differ significantly in the leaf chlorophyll. On average, the leaf chlorophyll content of three woody plants increased by 20.91% in 2009 and 8.22% in 2011 under the treatment. However, mean leaf chlorophyll content of herbaceous plants grown in the treatment decreased by 4.63% in 2009 and 14.86% in 2011 (Fig. 5). The same result was found in rice; chlorophyll content was decreased in the leaves of the seedlings grown at elevated CO_2 concentration (Nakano et al. 1997).

As a result of the effect of short and long term exposure to elevated CO_2 concentration and temperature on leaf chlorophyll content of seven rare and endangered plants, unlike woody plants, chlorophyll content of four herbaceous plants decreased considerably after long-term exposure to elevated CO_2 concentration and temperature (Fig. 6). The reduction in leaf chlorophyll content affects plant photosynthetic capacity, growth, and development directly and indirectly. Thus, elevated CO_2 concentration and temperature will have a more negative effect on herbaceous plants rather than woody plants.

The leaf chlorophyll content of three woody species was increased by elevated CO_2 concentration and temperature. However, the longer period of exposure to elevated CO_2 concentration and temperature, the more increment in

chlorophyll content reduces. In *P. ramosissimus*, chlorophyll content of plants grown in treatment was lower than plants grown in control after long-term exposure to elevated CO_2 concentration and temperature. Because woody species respond differently according to term of exposure to elevated CO_2 concentration and temperature, it is necessary to conduct long-term experiments on the effects of elevated CO_2 concentration and temperature on woody plants responses.

In conclusion, leaf chemical components of seven rare and endangered plants were significantly affected by elevated CO_2 concentration and temperature as well as term of exposure, species, and their interactions (Table 2). Especially, woody and herbaceous plants showed different responses to term of exposure to elevated CO_2 concentration and temperature. The present study has also shown that changes in foliage biochemistry of woody and non-woody plants were expressed differently during short- and long-term growth at high CO_2 concentration and temperature.

Three woody plants were significantly affected by the increase in CO_2 concentration and temperature for a short period of time while, as time passes, these species exhibited acclimation to long-term CO_2 enrichment and increased temperature. In the herbaceous plants, however, short-term exposure to elevated CO_2 concentration and temperature did not influence biochemical responses related to leaves, but the variation in the physiological response is higher than the woody plants with 3~4 times when being exposed in the long-term period.
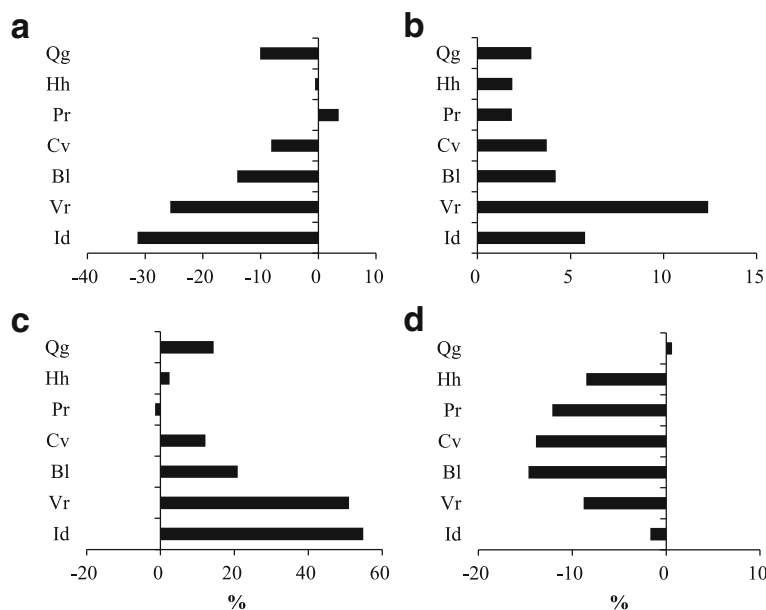
Fig. 6 Percentage variation of leaf nitrogen (**a**), carbon content (**b**), C:N ratio (**c**), chlorophyll content (**d**) of *Q. gilva* (Qg), *H. hamabo* (Hh), *P. ramosissimus* (Pr) *C. virosa* (Cv), *B. latissimum* (Bl), *V. raddeana* (Vr), and *I. dichotoma* (Id). The change in percent for each parameter is calculated as the change in percent for measured values in 2011 compared to measured values in 2009, under the elevated CO_2 and temperature

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These results suggest that elevated CO_2 concentration and temperature have a significant effect on woody plants in a short time, but those species adapt to environmental changes in the long term. However, there is no change in herbaceous plant's initial response to elevated CO_2 concentration and temperature, while long-term exposure to these changed circumstances may have a significant effect on non-woody plants.

Conclusions

As a result of comparative study on leaf chemical responses of rare and endangered plant species grown under the ambient and elevated CO_2 and temperature in 2009 and 2011, leaf biochemical contents of seven plant species were affected by elevated CO_2 and temperature, period of exposure, species specific, and their interactions.

The changes in environmental factors primarily affect plant physiological characteristics, and then this has an effect on the plant growth and development. Thus, it is important to determine the effect of rapid environmental changes on plant physiological responses under expected climatic and atmospheric change quickly and accurately.

In the present study, seven rare and endangered plants were more responsive than common species to elevated CO_2 concentration and temperature conditions, and then these changes in physiological responses of the plants will affect negatively plant growth and development. Also, there were differences between groups of species, with woody species responding short-term exposure and non-woody species responding long-term exposure to elevated CO_2 and temperature. Thus, it is necessary to evaluate the long-term impact of elevated CO_2 and temperature on various types of plants.

Abbreviations
ANOVA: Analysis of variance; MANOVA: Multivariate analysis of variance

Availability of data and materials
Not applicable.

Authors' contributions
All authors conducted a survey together during the study period. JHM wrote the manuscript. YYH participated in the design of the study and examined the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
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