

Soil Respiration in Relation to Photosynthesis of *Quercus mongolica* Trees at Elevated CO₂

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Abstract

Knowledge of soil respiration and photosynthesis under elevated CO₂ is crucial for exactly understanding and predicting the carbon balance in forest ecosystems in a rapid CO₂-enriched world. *Quercus mongolica* Fischer ex Ledebour seedlings were planted in open-top chambers exposed to elevated CO₂ (EC = 500 μmol mol⁻¹) and ambient CO₂ (AC = 370 μmol mol⁻¹) from 2005 to 2008. Daily, seasonal and inter-annual variations in soil respiration and photosynthetic assimilation were measured during 2007 and 2008 growing seasons. EC significantly stimulated the daytime soil respiration by 24.5% (322.4 at EC vs. 259.0 mg CO₂ m⁻² hr⁻¹ at AC) in 2007 and 21.0% (281.2 at EC vs. 232.6 mg CO₂ m⁻² hr⁻¹ at AC) in 2008, and increased the daytime CO₂ assimilation by 28.8% (624.1 at EC vs. 484.6 mg CO₂ m⁻² hr⁻¹ at AC) across the two growing seasons. The temporal variation in soil respiration was positively correlated with the aboveground photosynthesis, soil temperature, and soil water content at both EC and AC. EC did not affect the temperature sensitivity of soil respiration. The increased daytime soil respiration at EC resulted mainly from the increased aboveground photosynthesis. The present study indicates that increases in CO₂ fixation of plants in a CO₂-rich world will rapidly return to the atmosphere by increased soil respiration.

Citation: Zhou Y, Li M-H, Cheng X-B, Wang C-G, Fan A-N, et al. (2010) Soil Respiration in Relation to Photosynthesis of *Quercus mongolica* Trees at Elevated CO₂. PLoS ONE 5(12): e15134. doi:10.1371/journal.pone.0015134

Editor: Andrew Hector, University of Zurich, Switzerland

Received: August 23, 2010; **Accepted:** October 25, 2010; **Published:** December 6, 2010

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Funding: This research was supported by the important research direction project of the Chinese Academy of Sciences (KZCX2-YW-JC404) and projects of key program of the National Natural Science Foundation of China (40930107). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The forest carbon balance is the net result of CO₂ fixation by aboveground photosynthesis and CO₂ release, notably the release from the belowground respiration of plant roots, rhizosphere, and soil organisms [1]. Almost 10% of the atmospheric CO₂ is released by soils each year [2], and this emission is more than the CO₂ released from fossil fuel combustion [3]. Soil respiration plays, therefore, a crucial role in the global carbon cycle and may be altered strongly by global environmental change [4–6].

Some studies found that elevated CO₂ had no effects on soil respiration [7,8], or, even suppressed soil respiration [9,10]. However, many studies using open-top chambers (OTCs) or Free-Air CO₂ Enrichment (FACE) experiments suggested that elevated CO₂ led to increased soil respiration rate [4,5,11–20]. For example, in a northern deciduous forest ecosystem, elevated CO₂ (534 μmol mol⁻¹) significantly stimulated soil respiration (+8–26%) compared to the controls over three growing seasons [19]. Soil respiration rate was significantly stimulated (+10–19%) by elevated CO₂ (580 μmol mol⁻¹) throughout 7 years of CO₂ enrichment in the Duke Forest FACE [18]. Data from four forest FACE experiments have shown that elevated CO₂ (544 μmol mol⁻¹) increased soil respiration by 16 to 39%, and the stimulation persisted for up to 6 years [5].

The rising concentration of atmospheric CO₂ commonly stimulated both photosynthesis and growth in forest trees [21–27], resulting in increased belowground carbon allocation [28,29]. Increased root biomass and root length stimulated root respiration [15,30,31]. After 6-years of CO₂ enrichment in a mid-successional lowland forest, root biomass and soil respiration increased by 50% and 30%, respectively [32].

There is growing evidence that soil respiration rate is closely correlated with aboveground photosynthesis [1,33–35]. A strong correlation between soil respiration and photosynthesis was observed in a mixed coniferous-deciduous temperate forest [36]. Sørensen et al. [6] reported that CO₂ respired by roots (rhizosphere) derived from the recently assimilated CO₂ accounted for 70% of the total soil respiration at a FACE facility. A large-scale tree-girdling experiment conducted in boreal forests showed that girdling reduced soil respiration by 54% relative to respiration on ungirdled control plots, indicating that current assimilation to roots is a key driver of soil respiration [1]. However, soil respiration does not respond to aboveground photosynthesis in complete synchrony. *In situ* radiocarbon labeling experiment in a black spruce forest revealed that the maximum ¹⁴C values in roots and rhizosphere respiration occurred 4 days after labeling [37]. Evidence from temperate and boreal forest ecosystems showed

that responses of root respiration to canopy photosynthesis lagged for a few hours to three weeks [33,38].

On the other hand, many environmental factors affect soil respiration [9]. Soil temperature has been recognized to be the most important environmental factor leading to seasonal and diurnal variations in soil respiration; and soil water content was considered to be the secondary variable affecting the temporal variation in soil respiration [39]. Soil respiration rate typically increases exponentially with increasing temperatures, and this relationship is often described using a Q_{10} (magnitude of increase in gas efflux over a 10°C change) [16,39–42]. The Q_{10} values varied greatly with vegetation types and environmental conditions [43]. King et al. [5] reported that Q_{10} values ranged from 1.2 to 4.8 in four FACE experiments with developing and established forests exposed to elevated CO₂ for 2–6 years. Unlike temperature, there is no common function used to model the relationship between soil respiration and soil moisture. In Siberian tundra systems, soil water significantly affected soil respiration in wet microsites but not in dry microsites [44]. However, the diel variation of soil respiration measured in dry valleys of Antarctica was explained by soil moisture variation [45].

As mentioned above, the responses of soil respiration to elevated CO₂ gained from the published data seem to vary with the variations of experimental facilities, duration of CO₂ exposure, plant species, soil property, etc. Hence, we investigated the soil surface respiration rate and plant photosynthesis of *Quercus mongolica* Fischer ex Ledebour plants exposed to elevated CO₂ in OTCs for three (2007) and four years (2008). The deciduous *Q. mongolica* widely distributed in northern China, Japan, Korea, Mongolia, and eastern Siberia, is a dominant tree species of natural forests in northeastern China. We hypothesize that elevated CO₂ in deciduous forest ecosystems stimulates soil respiration only during the peak growing season (hypothesis I). The rationale behind this hypothesis is that the photosynthesis of deciduous trees may be significantly affected by elevated CO₂ only when leaves are expanded but not yellowed. This means that a stimulation of soil respiration may not be detectable during either the early or the late growing season. Hence, a stimulation of soil respiration at elevated CO₂ in deciduous forests would be attributed mainly to the enhanced aboveground photosynthesis (hypothesis II). In addition, we also hypothesize that the seasonal variation of soil respiration is correlated with soil temperature, but not with soil moisture in the study area (hypothesis III), where the yearly precipitation reaches ~700 mm.

Results

Seasonal variation in soil respiration and photosynthesis

Soil respiration showed distinct temporal variation over time ($P < 0.001$ for both year and month time scale, Fig. 1A), with the highest values occurring in the peak growing season from June to August (Fig. 1A), when the soil was warm and the soil water content (SWC) was relatively high (Fig. 2). The lower values of accumulated daytime soil respiration occurred in May and October (Fig. 1A) when both soil temperature and soil moisture were low (Fig. 2). The accumulated daytime soil respiration ranged from 93.3 (EC) and 94.4 (AC) mg CO₂ m⁻² hr⁻¹ in October to 514.6 (EC) and 457.8 (AC) mg CO₂ m⁻² hr⁻¹ in August 2007. In 2008, the minimum and maximum accumulated daytime soil respiration were 103.9 mg CO₂ m⁻² hr⁻¹ in September and 481.3 mg CO₂ m⁻² hr⁻¹ in June at EC, 80.2 mg CO₂ m⁻² hr⁻¹ in September and 359.9 mg CO₂ m⁻² hr⁻¹ in August at AC (Fig. 1). No significant differences in soil respiration between EC

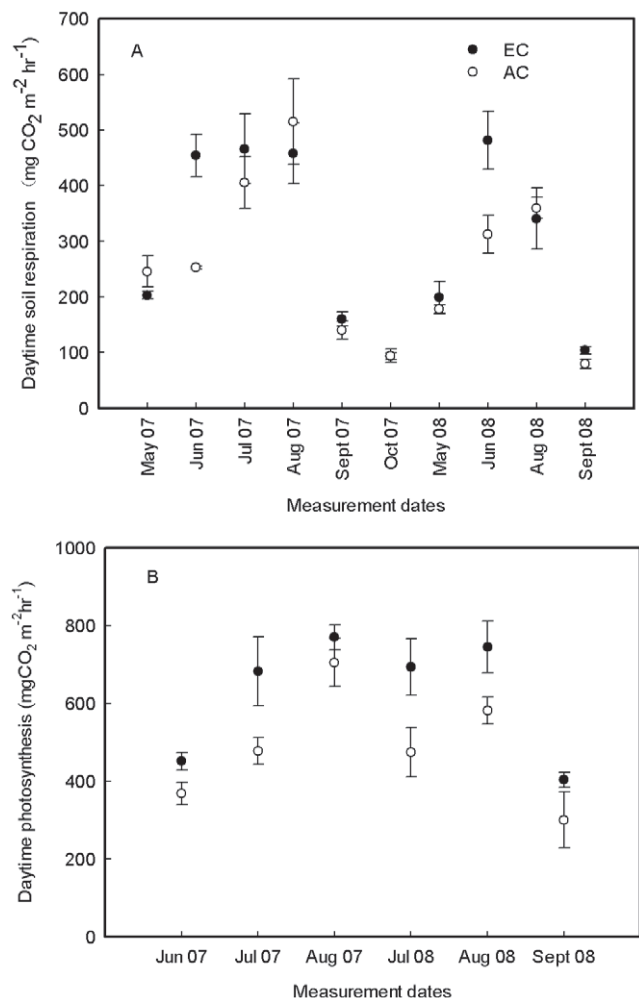


Figure 1. Seasonal and inter-annual variations of accumulated daytime soil respiration (A) and photosynthetic assimilation (B) under elevated CO₂ (EC) and ambient CO₂ (AC) during growing seasons in 2007 and 2008. Values are means $\pm$ SE ($n = 3$). doi:10.1371/journal.pone.0015134.g001

and AC were found during the early (May) and late growing season (October) (Fig. 1A; $P > 0.05$).

Within each CO₂ treatment, no significant inter-annual and seasonal variations in daytime photosynthesis of trees were found ($P > 0.05$; Fig. 1B). The average accumulated CO₂ assimilation was 624.1 mg CO₂ m⁻² hr⁻¹ at EC and 484.6 mg CO₂ m⁻² hr⁻¹ at AC across the two growing seasons.

Effects of elevated CO₂ on soil respiration and photosynthesis

Elevated CO₂ increased the overall accumulated daytime soil respiration significantly (Table 1), especially during the peak of the growing season (June to August) when photosynthetic rate was high and environmental factors, including temperature and moisture, were optimal (Fig. 1A, Fig. 2). The overall percentage increase caused by EC reached 24.5% (322.4 at EC vs. 259.0 mg CO₂ m⁻² hr⁻¹ at AC) for 2007 and 21.0% (281.2 at EC vs. 232.6 mg CO₂ m⁻² hr⁻¹ at AC) for 2008, with marked increase of 79.7% (June 2007), 54.1% (June 2008), and 29.6% (September 2008). The average accumulated daytime soil respiration were 306.0 mg CO₂ m⁻² hr⁻¹ at EC and 248.4 mg CO₂ m⁻² hr⁻¹ at AC over the two growing seasons (Table 1). However, during the

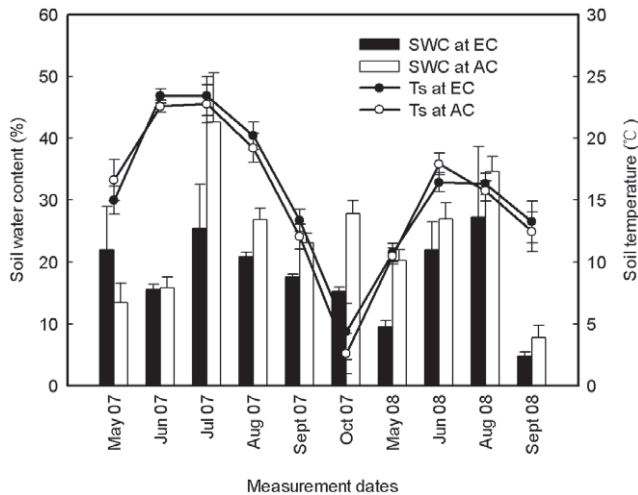


Figure 2. Monthly averaged soil water contents (SWC, $n=7$; mean ± 1 SE) and soil temperature at 5 cm depth (T_s , $n=21$; mean ± 1 SE) at elevated (EC) and ambient CO₂ (AC) during the experimental period.
doi:10.1371/journal.pone.0015134.g002

early and late growing season the soil respiration at EC did not significantly differ from that at AC ($P>0.05$, Fig. 1A).

Elevated CO₂ consistently increased daytime CO₂ assimilation during the measurement period (Fig. 1B, Table 1). On average, elevated CO₂ increased photosynthesis by 28.8% across the two growing seasons. The highest stimulation percentage of $>40\%$ occurred in July for both years. The average values of accumulated CO₂ assimilation were $624.1 \text{ mg CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$ at EC and $484.6 \text{ mg CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$ at AC over the two growing seasons ($P<0.01$; Table 1).

Responses of soil respiration to soil temperature and soil moisture

No difference in soil temperature at a depth of 5 cm (T_s) between CO₂ treatments was detected (Tables 1 and 2; Fig. 2). T_s exhibited pronounced seasonal fluctuation ($P<0.001$) and inter-annual variation ($P<0.001$) (Fig. 2). Patterns of changes in T_s were similar between the two CO₂ treatments (Fig. 2).

Table 1. Effects of CO₂ treatment on soil respiration rate, photosynthesis, soil temperature at 5 cm depth, soil water content and plant growth (plant height and basal diameter), tested by paired-samples T test.

	Elevated CO ₂	Ambient CO ₂
Soil respiration ($\text{mg CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$)	$306.0 \pm 52.2a$	$248.4 \pm 41.6b$
Photosynthesis ($\text{mg CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$)	$624.1 \pm 52.9a$	$484.6 \pm 56.1b$
Soil temperature ($^{\circ}\text{C}$)	$16.9 \pm 0.6a$	$16.6 \pm 0.6a$
Soil water content (% in quality)	$18.6 \pm 1.9b$	$23.9 \pm 1.7a$
Plant height (cm)	$106.4 \pm 1.2a$	$86.8 \pm 1.1b$
Basal diameter (cm)	$2.2 \pm 0.1a$	$1.9 \pm 0.1b$

Different letters between elevated and ambient CO₂ in each row indicated significant difference at $P<0.05$ level.

Means ($\pm$ SE) across two growing seasons were given.

doi:10.1371/journal.pone.0015134.t001

Soil respiration increased exponentially with T_s for both CO₂ treatments (Fig. 3A). T_s explained $>72\%$ of the variations in soil respiration (Table 2). No significant difference in Q_{10} values between the two CO₂ treatments was detected for both 2007 and 2008 ($P>0.05$; Table 2). Across the two growing seasons, the Q_{10} values were about 1.6 for both CO₂ treatments (Table 2).

Soil respiration increased with increasing SWC during the growing season for both CO₂ treatments, showing a positive correlation between soil respiration and SWC (Fig. 3B, Table 3). SWC at EC exhibited similar seasonal trend to AC and peaked in July and August (Fig. 2). In 2007, SWC ranged from 15.2% in October to 25.4% in July (19.4% on average) at EC and from 13.4% in May to 42.6% in July (24.9% on average) at AC (Table 2). In 2008, the mean SWC was 17.0% at EC with the lowest value of 4.8% in September and the highest value of 26.1% in July, whereas the mean SWC was 22.2% at AC with the lowest value of 7.7% in September and the highest value of 34.6% in August (Table 2). Elevated CO₂ significantly decreased SWC (Table 1) by 11.7% in 2007 and 20.6% in 2008.

Growth responses

The plant height and basal diameter at EC were significantly greater than those at AC across the two growing seasons (Fig. 4, Table 1). The plants stop growing after October until next May; therefore, the height and basal diameter in October 2007 were similar to those in May 2008 (Fig. 3). The plant height increased by 19.2 cm and 17.6 cm at EC and 17.6 cm and 19.6 cm at AC for 2007 and 2008, respectively. Similarly, the mean basal diameter increased by 0.4 cm at EC and 0.3 cm at AC for each year.

Relationships between soil respiration and biotic and abiotic factors

Daytime soil respiration was positively correlated with T_s ($P<0.01$), SWC ($P<0.01$), and photosynthetic assimilation ($P<0.05$) for both AC and EC treatment (Table 3). In addition, positive correlation between T_s and SWC was also found during the two growing seasons (Table 3).

Discussion

The seasonal pattern of soil respiration in our study showed that soil respiration was higher during the middle growing season just when the soil temperature, soil moisture and photosynthesis were also higher. These results are consistent with the findings of Niinistö et al. [16] and Pregitzer et al. [46], who found that the greatest soil respiration in forest ecosystems occurred during the peak growing season, and the lowest soil respiration was measured in spring and autumn when soil temperatures was relatively low and the canopy density was low.

Previous studies found that the increase magnitude of soil respiration caused by elevated CO₂ varied greatly with the tree species or vegetation cover [5,12,19]. The present study with deciduous *Q. mongolica* under elevated CO₂ showed a monthly increase in soil respiration by 23.0%. Similarly in deciduous forests, elevated CO₂ has been found to stimulate soil respiration by 39% in stands of paper birch and trembling aspen [15], and 8–26% in aspen forests [19]. In evergreen forests, elevated CO₂ stimulated the soil respiration by 24% in loblolly pine (*Pinus taeda* L.) plantation [18], 50–57% in ponderosa pine [11,12], 23–37% in 20-year-old Scots pines (*Pinus sylvestris* L.) [16], and 20% in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings [47].

Consistent with our hypothesis I (see Introduction), the present study found that elevated CO₂ significantly stimulated soil

Table 2. Temperature response functions of soil respiration ($\mu\text{mol m}^{-2}\text{s}^{-1}$) to soil temperature ($^{\circ}\text{C}$); soil temperature and soil water content (% in quality) during growing seasons in 2007 and 2008.

	Fitted equation	R ²	Q ₁₀	T _s (Min)	T _s (Max)	T _s (Mean)	SWC (Min)	SWC (Max)	SWC (Mean)
2007									
Elevated CO ₂	SR = 0.7096 exp (0.0822 T _s)	0.7776	1.62 ± 0.03a	4.4 ± 2.3	23.4 ± 0.6	16.6 ± 2.9a	15.2 ± 0.7	25.4 ± 7.1	19.4 ± 1.7a
Ambient CO ₂	SR = 0.8243 exp (0.0641 T _s)	0.7225	1.56 ± 0.08a	2.6 ± 1.6	22.8 ± 1.6	16.0 ± 3.1a	13.4 ± 3.2	42.6 ± 7.9	24.9 ± 4.3b
2008									
Elevated CO ₂	SR = 0.3707 exp (0.1438 T _s)	0.7390	1.54 ± 0.16a	10.8 ± 0.7	16.5 ± 0.8	14.2 ± 1.4a	4.8 ± 0.7	26.1 ± 4.8	17.9 ± 4.5a
Ambient CO ₂	SR = 0.4506 exp (0.1250 T _s)	0.7736	1.57 ± 0.05a	10.5 ± 0.6	17.9 ± 0.9	14.2 ± 1.7a	7.7 ± 2.1	34.6 ± 2.4	22.2 ± 9.9b

SR soil respiration rate, T_s soil temperature, SWC soil water content.

Different letters between elevated and ambient CO₂ within the same year and the same category indicated significant difference at $P < 0.05$ level ($n = 3$).

doi:10.1371/journal.pone.0015134.t002

respiration from June to August (Fig. 1A, Table 1) and this stimulation was significantly correlated with enhanced photosynthesis (Fig. 1, Table 3). Increased plant height and basal diameter under elevated CO₂ implied higher root biomass. Thus, the increase in respiring roots and photosynthate availability at

elevated CO₂ may result in pronounced increase in soil respiration compared to ambient CO₂. Increased root biomass and production at elevated CO₂ have already been recognized to be the most common reason resulting in increased soil respiration [12,16,19,48–51]. Previous studies indicated that there was a close relationship between aboveground photosynthesis and soil respiration since root respiration consumes recently fixed photosynthates from foliages [1,13,46,52–54]. Andrews and Schlesinger [53] attributed the increase in forest soil respiration to increased root and rhizosphere respiration under elevated CO₂. Pregitzer et al. [19] reported that the recently fixed carbon by photosynthesis accounted for 60–80% of soil respiration during the peak growing season. Although there are still uncertainties regarding the relative contribution of roots or rhizosphere to the total soil respiration in the present study, higher soil respiration associated with higher photosynthesis under elevated CO₂ may imply a greater carbon input to roots/rhizosphere. Thus, consistent with our hypothesis II (see Introduction), the increase in soil respiration at elevated CO₂ is mainly attributed to increased aboveground photosynthesis.

Soil respiration varied significantly with year and month ($P < 0.05$), which may be a combined result of temporal variation in soil temperature, aboveground photosynthesis, and root growth rate. King et al. [5] found that the inter-annual variation of soil respiration in four FACE systems was determined by changes in soil temperature influencing plant photosynthesis and root growth. Regardless of CO₂ treatment, soil respiration increased exponen-

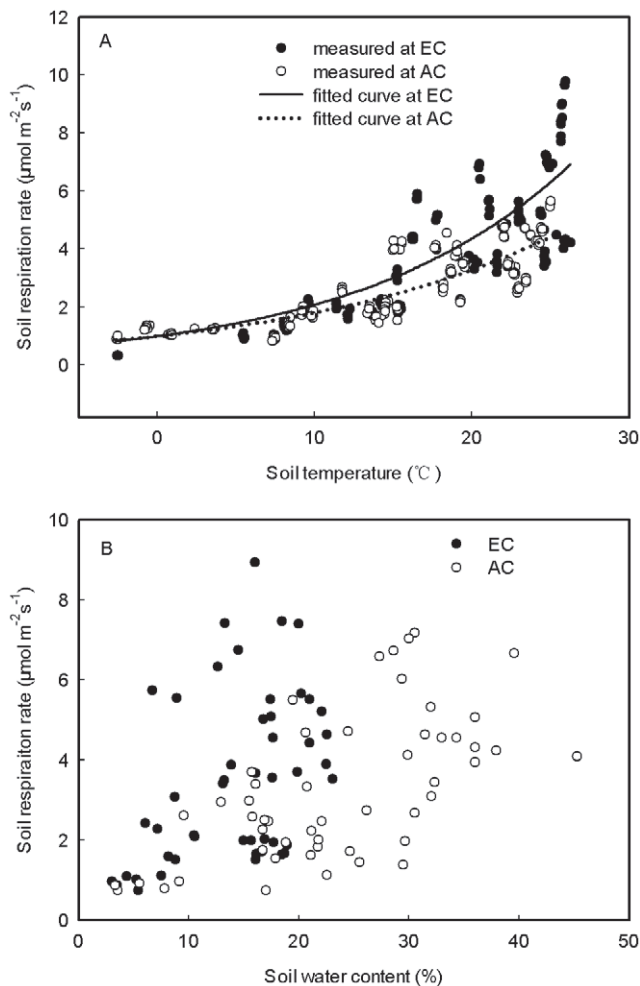


Figure 3. Soil respiration in relation to soil temperature at 5 cm depth (A) and soil water content (B) at elevated (EC) and ambient CO₂ (AC) during growing seasons. Trend lines were generated for each CO₂ treatment using exponential regression.
doi:10.1371/journal.pone.0015134.g003

Table 3. The Pearson's correlation coefficient among soil respiration, photosynthesis, plant height, basal diameter, soil temperature at 5 cm depth and soil water content at elevated and ambient CO₂ during growing seasons in 2007 and 2008.

	SR	P _N	H	D	T _s	SWC
SR	1.000					
P _N	0.226*	1.000				
H	−0.067	0.153	1.000			
D	−0.229	0.246	0.956**	1.000		
T _s	0.678**	0.024	−0.245	−0.341	1.000	
SWC	0.436**	0.169	−0.075	−0.107	0.239*	1.000

SR soil respiration, P_N photosynthesis, H plant height, D basal diameter, T_s soil temperature at 5 cm depth, SWC soil water content

*indicates correlation is significant at 0.05 level and

**at 0.01 level (2-tailed).

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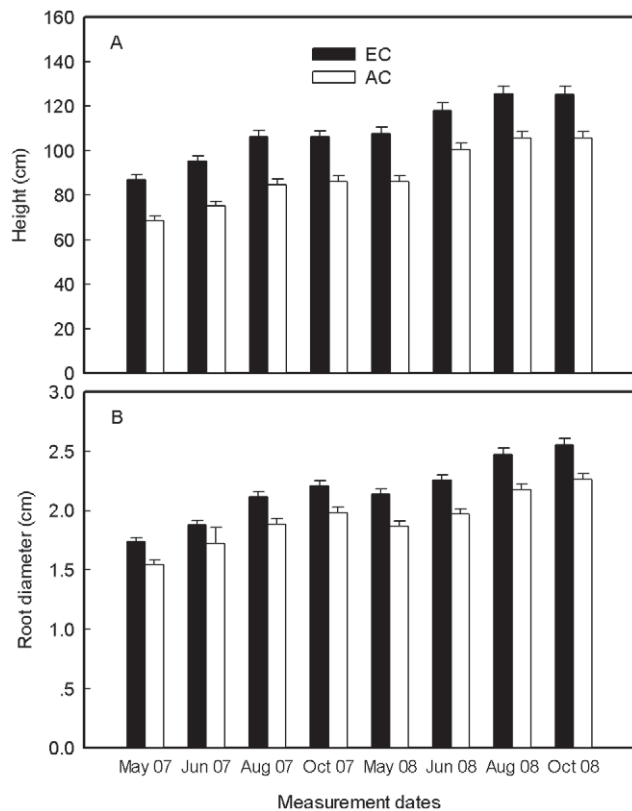


Figure 4. Growth (mean $\pm$ 1 SE) of plant height (A) and basal diameter (B) under elevated and ambient CO₂ during growing seasons in 2007 and 2008.

doi:10.1371/journal.pone.0015134.g004

tially with soil temperature [29,55,56]. Variations in soil temperature alone accounted for over 70% of the variation in soil respiration at both elevated and ambient CO₂ found in our study (Table 2). This finding is comparable to past work which found that soil temperature explained 70% of the variation in soil respiration in a *Pinus cembra* forest [57], 72% of the variation in a spruce forest [58], and 80% of the variation in a mixed temperate hardwood forest [59].

Elevated CO₂ did not change the temperature sensitivity of soil respiration since no significant difference in Q_{10} values between elevated and ambient CO₂ was found (Table 2). Similarly, King et al. [5] also found that the temperature sensitivity of soil respiration appeared to be unaffected by elevated CO₂. However, soil respiration rates were found to be more sensitive to changes in soil temperature at elevated CO₂ than at ambient CO₂ [9]. For example, the Q_{10} values were 2.4 in ambient CO₂ versus 2.8 in elevated CO₂ in a ponderosa pine forest [9], 1.5 to 3.2 at ambient CO₂ and 1.2 to 4.8 at elevated CO₂ in a *Populus* plantation [5], 1.9 to 3.3 in a mixed temperate forest [60]. The Q_{10} values of $\sim$ 1.6 for both CO₂ treatments found in the present study (Table 2) are similar to the values of 1.4 to 1.8 found in a ponderosa pine plantation [61]. The temperature sensitivity of soil respiration has been recognized to be positive with substrate supply [9]. Elevated CO₂ increased the carbon allocation to roots, providing additional substrates for root respiration [62,63], and further leading to an increased temperature sensitivity of root respiration and soil respiration. On the other hand, decreased soil moisture resulted from elevated CO₂ can result in a decrease in activity and biomass of soil organisms [64], consequently causing reduction in soil

microbial respiration and soil respiration. In the present study, no apparent CO₂ effects on Q_{10} were found (Table 2). This result may be resulted from an interaction between elevated CO₂ and soil moisture since elevated CO₂ increased the aboveground photosynthesis and root respiration (Fig. 1), but decreased the soil moisture (Fig. 2).

The contribution of soil water content to soil respiration was found to depend on vegetation cover and soil properties [39,44,45]. The mathematical relationship between soil respiration and soil moisture is relatively complex if a correlation between them exists [65,66]. In six forest plantations located at Rwanda in African, soil respiration is mainly related to soil water content which explained 36–77% of the temporal variation in soil respiration [67]. The quadratic relationship between soil respiration and soil water content in a tallgrass prairie accounted for 26% of the variation in soil respiration [65]. Soil moisture is also considered as an important factor affecting soil respiration in our study because a significantly positive correlation between soil water content and soil respiration has been detected (Table 3). This finding is consistent with the results gained in a semiarid grassland ecosystem [68], but did not support our hypothesis III (see Introduction). Elevated CO₂ decreased soil water content in the present study (Table 1, Fig. 2), probably due to greater water loss caused by greater leaf area. Since the precipitation in the research area mainly occurs during the peak growing season (from June to August) when temperature is also relatively higher, soil water content is then positively correlated with soil temperature in our study (Table 3).

Conclusion

An enhanced photosynthetic assimilation leading to increased plant growth at elevated CO₂ implies greater root respiration consuming the recently fixed carbon. Increased photosynthetic assimilation and aboveground growth may create dense canopy to fix more carbon but also stimulate belowground respiration. Hence, the present study indicates that increase in CO₂ fixation of plants in a CO₂-rich world will rapidly return to the atmosphere by increased soil respiration.

Materials and Methods

Research area and experiment design

The experiment was conducted at the Research Station of Changbai Mountain Forest Ecosystem (42°24' N, 128°05' E, 738 m a.s.l.), Jilin Province, northeastern China. The annual mean air temperature is 3.6°C and annual mean precipitation is 695 mm [69]. The maximum air temperature and over 60% precipitation occurred in June to August. Ten hexagon OTCs (4.0 m in both height and diameter) with clear glass were established nearby the research station. Uniform local forest soil with a total organic carbon of 9.0% was used in OTCs [70]. Three-year-old plants, with similar plant size in height and basal diameter, of *Q. mongolica* from a nearby plantation were planted at a spacing of 0.5×0.5 m in OTCs in spring 2005.

CO₂ fumigation treatments (AC = ambient CO₂ concentration of 370 $\mu\text{mol mol}^{-1}\text{CO}_2$ for 4 chambers, and EC = elevated CO₂ concentration of 500 $\mu\text{mol mol}^{-1}\text{CO}_2$ for the other 6 chambers) began in 2005. Elevated CO₂ was supplied in daytime during each growing season from the beginning of May to the end of October. Elevated CO₂ has been supplied to the chambers by pipes connected to industrial CO₂ tanks outside the chambers. The concentrated CO₂ was pumped into the chambers from a height of 1.6 m and was diffused. The CO₂ concentration (500 $\pm$ 100 $\mu\text{mol mol}^{-1}\text{CO}_2$) was recorded every 10 min by CO₂

sensors (SenseAir, Sweden) installed in the center of each chamber and automatically adjusted to the target concentration by controlling the input amount of concentrated CO₂. All chambers accepted natural rainfall during the experimental period.

Soil respiration measurements

The soil respiration presented in this study is defined as an integrating respiration of all components including root respiration, microbial and soil fauna respiration, and chemical process emission, but excluding the respiration of litter. Soil surface respiration was measured from 6:00 am to 6:00 pm at a two-hours' interval from May to October in 2007 and repeated in 2008, using a LI-6400 portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA) in closed circuit with a LI-6400-09 soil respiration chamber (SRC). The measurements were carried out according to the methods described in detail by Tingey et al. [9]. Soil respiration collars, constructed from PVC pipe, were randomly distributed in the center and on the edge of each OTC. We did not find any significant difference in soil respiration measured on the edge of OTCs compared to that measured in the chamber center. Collars remained in the same measurement locations throughout the measurement. The collars were inserted approximately 2 cm into the soil depth, and matched well with the SRC to avoid possible leakage. All litter and herbs were carefully removed during the experiment period. To ensure parallel measurements and to diminish the effects of environmental variations of temperature and humidity on parameters measured, we selected two points (measurement locations) at each chamber, and three chambers per treatment only.

The T_s was recorded with soil respiration rate concurrently by a portable temperature probe attached to the analyzer which was inserted into the soil adjacent to the SRC. The 0–5 cm soil layer under each SRC was cored after each measurement. The soil was weighed before and after dried at 100°C for 48h. The SWC was expressed as a percentage of water mass to dry soil mass. The soil respiration rate per treatment for each measurement time was based on the average of the data from the two locations in three OTCs.

The soil respiration was measured only during the daytime from 6 am to 6 pm corresponding to the photosynthesis measurements.

Photosynthesis and growth measurements

The daily course of photosynthesis was also measured concurrently during the daytime from 6 am to 6 pm at a two-hours' interval on clear days during the growing seasons in 2007 and repeated in 2008. Photosynthetic measurements were conducted using the same system of LI-6400 (LI-COR, Lincoln, NE, USA). All measurements were made *in situ* on fully sunlit

leaves in trees at respective growth CO₂ concentrations (AC, EC). Plant height and basal diameter of all individuals in each OTC were measured during early, peak and late growing season each year.

Q₁₀ of soil respiration

Scatter plots were used to determine the relationship between soil respiration and T_s for 2007 and 2008, respectively, using the data gained from a complete growing season (May - October) in each year. The scatter plots were fitted by an exponential relationship between measured data of soil respiration rate (y) and T_s (x): $y = ae^{bx}$, where a and b are coefficients. The Q₁₀ values were then calculated using $Q_{10} = e^{10b}$.

Estimating accumulated daytime soil respiration and CO₂ assimilation

Accumulated daytime soil respiration was obtained by integrating values measured during the daytime for each treatment. Integrated values were calculated by determining the area under each segment of two consecutive measurement points and then summing the segments for a total daytime soil respiration. For a detailed method description, see Vose et al. [12]. The same method was also applied for calculating the daytime CO₂ assimilation, using the daily course of net photosynthetic rate.

Statistical analyses

Statistical analyses were performed with SPSS 13.0 software program (SPSS Inc, 2004). The normality test was carried out using P-P test on datasets prior to statistical analyses to verify a normal distribution. We used paired-samples T test to test the differences in soil respiration, photosynthesis, soil temperature at 5 cm depth, soil water content, and plant growth in height and basal diameter between EC and AC within each measurement date. One-way ANOVA was used to compare the difference in Q₁₀ values between EC and AC. The Pearson correlation was used to detect the correlation among soil respiration, photosynthesis, tree growth and environmental factors.

Acknowledgments

We thank Guozheng Song for maintaining the experimental instruments at the Changbai Mountain Research Station and Junqiang Zheng for the suggestion in the statistical analysis.

Author Contributions

Conceived and designed the experiments: YZ SH. Performed the experiments: YZ X-BC C-GW A-NF L-XS X-XW. Analyzed the data: YZ M-HL. Wrote the paper: YZ M-HL.

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