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A new viewpoint to understand the response of leaf dark respiration to elevated CO_2 concentration

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Abstract

By investigating the R_D–C_a (dark respiration rate–atmospheric CO_2 concentration) and P_N (net photosynthetic rate)–C_a curves of bamboo (*Fargesia denudata*) and poplar (*Populus cathayanna*), we found that: (*1*) the minimal R_D was close to ambient CO_2 concentration, and the elevated or decreased atmospheric CO_2 concentration enhanced the R_D of both species; (*2*) the response curves of R_D–C_a were simulated well by quadratic function. This phenomenon might be an inherent property of leaf R_D of *F. denudata* and *P. cathayanna*. If this was true, it implies that effect of CO_2 on R_D could be interpreted with the relationship of R_D–C_a curves and the quadratic function.

Additional key words: controlled environment chambers; *Fargesia denudata*; net photosynthetic rate; *Populus cathayanna*; species differences.

Introduction

Dark respiration rate (R_D) is an important part of carbon sequestration for individual plants, communities, and even ecosystems. With the research progress in plant growth and photosynthesis, responses of R_D to elevated carbon dioxide concentration (EC) have been studied more often. For instance, leaf R_D increased in response to EC in some studies (Potvin and Strain 1985, Nijs *et al.* 1988, Baker *et al.* 1992, Thomas and Griffin 1994), while it decreased in other ones (Spencer and Bowes 1986, Wullschleger and Norby 1992, Baxter *et al.* 1995, Bunce and Ziska 1996). Other authors even found no effect of EC (den Hertog *et al.* 1993). Mechanisms about the effects of EC were still at a speculative stage due to the diversity of the soundings. The proposed mechanisms concerning reduced R_D were usually related to changes in intercellular CO_2 concentration (Raghavendra and Vani 1989, Shaish *et al.* 1989), enzymatic activity (Monning 1983, Kerbel *et al.* 1988), or dark CO_2 fixation rate (Hammel *et al.* 1979, Gale 1982). Those concerning stimulated R_D are related to increase in content of non-structural saccharides (Azcón-Bieto and Osmond 1983, Farrar 1985, Norby *et al.* 1986), growth rate (Thomas and Griffin 1994), and the enhancement of other pathways of respiration under EC (Lambers 1985). Because of the existence of various effects, the impact of future EC on R_D cannot be convincingly predicted and further research is still needed (Amthor 1991).

Most experiments on CO_2 elevation have measured R_D at ambient CO_2 concentration (C_a) and EC only. We determined the P_N–C_a (net photosynthetic rate to C_a) curves of bamboo (*F. denudata*) and poplar (*P. cathayanna*) leaves under eight CO_2 concentrations and photosynthetically active radiation (PAR*)*, respectively. Then, the R_D–C_a curves were fitted by a quadratic function. The aim was to provide a new viewpoint to interpret effects of EC on R_D.

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Abbreviations: C_a – atmospheric (ambient) CO_2 concentration; CEC – controlled environment chamber; EC – elevated CO_2 concentration; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; PPFD – photosynthetic photon flux density; R_D – dark respiration rate.

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Materials and methods

Experiment site and plants: The plants were obtained from field plots of a CEC experiment that is exploring the response of sub-alpine plant community to EC at the Maoxian Ecological Station, Chinese Academy of Sciences (31°41′07″N, 103°53′58″E, 1 800 m a.s.l.). The average CO_2 concentration over one growth season was around 360 µmol mol^{-1} and the long-term means of annual precipitation and air temperature were 800 mm and 12 °C. The CEC experiment system consisted of eight independent, self-controlled, and enclosed-top chambers. The system enables assessing the medium to long-term effects and adaptations of global climate change (for example, EC, elevated temperature, and their interaction) on plant processes at the community level. In 2006, the CO_2 concentration in the EC-chambers was within 700–800 µmol mol^{-1} for 91.3 % of the whole exposure time. The CEC system provides a wide variety of climatic conditions that are similar to natural regimes under high-frigid conditions in south-western China. Natural plant communities (40×40×40 cm) were selected, dug out, filled into woody boxes, and transferred into growth chambers on 20 November, 2005. These communities were exposed to C_a and EC for 24 h every day from March 1st, 2006. We chose poplar (*Populus cathayanna*) and bamboo (*Fargesia denudata*) in the communities for measuring leaf P_N and R_D.

Field measurements: Four leaves, the third or the fourth one to the apex of the main stem of bamboos or poplars exposed to C_a, were chosen and the P_N and R_D were measured with the *LI-6400* Portable Photosynthesis System (*LI-COR*, Lincoln, NE, USA). PAR was supplied by the *LI-6400* photosynthetic system with a red-blue, cold light resource and was set at 1 500, 1 200, 800, 300, 100, 50, 20, and 0 µmol m^{-2} s^{-1}, respectively. At each PAR, the measurements of P_N and R_D started at 400 µmol mol^{-1} of CO_2. Once the steady state was reached, the CO_2 concentration was gradually lowered in the sequence of 400, 200, 100, 50, 0, and then increased stepwise to 400, 800, 1 200, and 1 500 µmol mol^{-1}. Leaves were kept in leaf chamber for at least 30 min to acclimate to each pair of PAR and CO_2 concentration. CO_2 was injected into the open circulating gas-stream of the photosynthesis system using an auto-controlled CO_2 injector. Data from these sequential measurements of P_N and R_D were taken for each P_N–C_a or R_D–C_a curve. Leaf temperature and relative air humidity were kept at 25 °C and 60–65 %, respectively. All measurements were made from 10:00 to 15:00, on 3 to 30 August 2006. Long-term effect of EC on the leaf R_D of *F. denudata* and *P. cathayanna*, which were exposed to EC for 5 months, was also investigated.

Data analysis: Relations between R_D and C_a were fitted by a quadratic function that was used to predict the R_D values at usually adopted CO_2 concentrations (350, 500, 700, and 1 000 µmol mol^{-1}) in the research field of EC. *T*-test was applied to check whether long-term effects of EC on R_D of poplar and bamboo leaves were statistically significant.

Results and discussion

Bamboo and poplar showed different responses to series of CO_2 concentrations (Fig. 1*A*) although their climax both appeared at C_a = 400 µmol mol^{-1}. R_D of bamboo dropped from 4.41±0.66 to 0.55±0.50 µmol(CO_2) m^{-2} s^{-1} with the increase of C_a from 0 to 400 µmol mol^{-1}, and then promptly increased to 7.2±1.5 µmol(CO_2) m^{-2} s^{-1} with C_a elevated to 1 500 µmol mol^{-1}. However, R_D of poplar dropped from 1.68±0.10 to 0.74±0.55 µmol(CO_2) m^{-2} s^{-1}, and after the minimum it rose to 5.21±0.39 µmol(CO_2) m^{-2} s^{-1}, following the corresponding C_a with bamboo. The two R_D–C_a curves were not the same, but their horizontal coordinate at the apex point were both at 400 µmol(CO_2) mol^{-1}. However, with the help of quadratic function (Table 1), the lowest R_D of bamboo and poplar was 0.010 µmol(CO_2) m^{-2} s^{-1} at 631 µmol(CO_2) mol^{-1} and 1.046 µmol(CO_2) m^{-2} s^{-1} at 413 µmol(CO_2) mol^{-1}, respectively. We would take the predicted R_D for further discussion in this paper because the two functions conformed well to observed data (Table 1) and enabled calculate R_D (Fig. 2) at various CO_2 concentrations. During the past several decades, CO_2 concentrations were usually set at ambient (approx. 350 µmol mol^{-1}) to double ambient (approx. 700 µmol mol^{-1}) level (Raghavendra and Vani 1989, Shaish *et al.* 1989), or 500–1 000 µmol mol^{-1} in relevant studies (Nijs *et al.* 1988, Bunce and Ziska 1996). Thus we will mainly discuss the predicted R_D at corresponding C_a that was usually adopted by the mentioned scientists (Fig. 1*B*).

Table 1. Simulated R_D–C_a curves by quadratic function and its parameters and ANOVA analysis. *dfs are 2 and 5 for regression and residuals, respectively; each value applied to estimate function parameters is the mean of 4 replications.

Plant	Quadratic function	r^2	F	sig.
bamboo	$R_D = 8.716\times10^{-6}C_a^2 - 0.011C_a + 3.481$	0.9563	54.73	0.0004
poplar	$R_D = 3.632\times10^{-6}C_a^2 - 0.003C_a + 1.665$	0.9914	288.17	<0.0001

The quadratic function predicted that R_D (Fig. 1*B*) values of bamboo were 0.879±0.725, 0.417±0.213, 0.412±0.358, and 1.712±0.745 μmol(CO_2) m^{-2} s^{-1} at concentrations 350, 500, 700, and 1 000 μmol(CO_2) mol^{-1}, respectively. Those of poplar at corresponding CO_2 concentrations were 1.048±0.460, 0.417±0.213, 1.322±0.500, and 1.712±0.745 μmol(CO_2) m^{-2} s^{-1}, respectively. This indicated that R_D of bamboo was reduced when CO_2 concentration increased from ambient to double ambient while that of poplar was stimulated by EC. R_D of both bamboo and poplar were stimulated by EC from 500 μmol mol^{-1} to 1 000 μmol mol^{-1}. These different responses of different species to EC might explain various effects of EC reported in other studies. At least, this could be employed for understanding short-term (direct) effects of EC on leaf R_D.

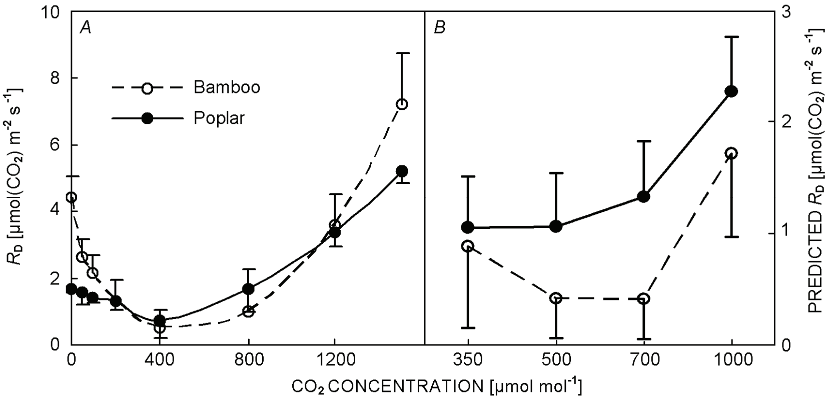

Fig. 1. Dark respiration rate (R_D)–atmospheric CO_2 concentration (C_a) curves (*A*) and predicted R_D (*B*) by quadratic function at usually applied CO_2 concentration. In *A*, each point is the mean of four replicates and the horizontal bar shows S.E. Bamboo and poplar showed their minimum R_D between 350–700 μmol mol^{-1} of CO_2. Either the increase or the decrease of CO_2 concentration will enhance R_D of leaves. The shape of the two lines and their trough points may determine whether R_D will be enhanced by elevated CO_2. In *B*, R_D is reduced when bamboo leaves are exposed to 500 and 700 μmol mol^{-1} CO_2. R_D of poplar leaves is not affected significantly by the elevation of CO_2 from 350 to 500 and 700 μmol mol^{-1} CO_2. R_D of both the species was significantly stimulated by 1 000 μmol mol^{-1} CO_2 comparing to 350 μmol mol^{-1} CO_2.

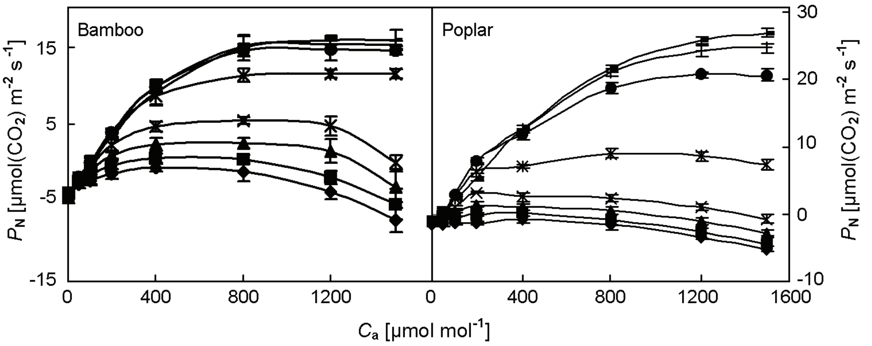

Fig. 2. Responses of net photosynthetic rate (P_N) of bamboo and poplar leaves to CO_2 at eight levels of photosynthetically active radiation (PAR) [μmol m^{-2} s^{-1}]: – 1 500, | 1 200, ● 800, ∗ 300, × 100, ▲ 50, ■ 20, ♦ 0. P_N drops to below zero with the elevation of CO_2 concentration under low PAR (≤100). The increase of leaf R_D can account for the decrease of P_N. Different from the P_N–C_a curves measured under PAR of less than 100 μmol m^{-2} s^{-1}, P_N does not drop with the elevation of CO_2 concentration when PAR is higher than 100 μmol m^{-2} s^{-1}.

Table 2. Summary of *t*-test of long-term effect of elevated CO_2 concentration on leaf dark respiration rate (R_D). Means ± S.E.

Species	CO_2 concentration	R_D	*t*-test *t*	*df*	*p*
bamboo	350	1.066±0.141	4.298	6.000	0.005
	700	0.432±0.043			
poplar	350	1.449±0.241	–0.646	6.000	0.542
	700	1.817±0.516			

Effects of EC on leaf R_D had been sorted into long-term (indirect) and short-term (direct) effects (Amthor 1991, Jach and Ceulemans 2000). To compare them, we investigated the R_D of controlled treatments exposed to corresponding CO_2 concentration for 5 months (Table 2) as well as for a short-term as above. R_D of bamboo leaves was significantly reduced by the long-term effect, whereas that of poplar was slightly stimulated. These effects were in accordance with the short-term effects analyzed in the first paragraph.

Stimulation effects of much higher CO_2 concentrations (>400 µmol mol^{-1}) on R_D are seen from the measurements of P_N–PAR curves where PAR was less than 400 µmol m^{-2} s^{-1} (Fig. 2). The P_N–PAR curves of both species fitted a quadratic shape when PAR was 0, 20, 50, and 100 µmol m^{-2} s^{-1} because there was a sharp drop with EC. P_N of bamboo and poplar leaves did not drop with CO_2 concentration when PAR was higher than 300 µmol mol^{-1} (Fig. 2). Kitaya *et al.* (2003) found similar results on *Ceratophyllum demersum* at PAR of 1 000 µmol m^{-2} s^{-1}. The declining trend of P_N which was detected when CO_2 concentration was 300 µmol mol^{-1} under 400 µmol m^{-2} s^{-1} of PAR could also be found in an earlier paper of Caemmerer and Farquhar (1981). The above facts indicated that PAR (or PPFD) is an important factor affecting the response of gas exchange to CO_2. The decrease of P_N might be caused by stimulation of R_D affected by EC, but whether P_N would drop or not might depend on the PAR intercepted by leaves, plant species, or their ecological and physiological types.

Plants had been categorized into C_3, C_4, and CAM plants on the basis of their carboxylation pathways or into sun and shade plants according to their photon requirement. Each one of C_3, C_4, or CAM plants is distinct from the other two types by CO_2 compensation and saturation concentrations, carboxylation efficiency, compensation and saturation irradiance, quantum yield, *etc.* Sun and shade plants can be discriminated by their photon requirement. They are also different in response to irradiance and CO_2 concentration. Leaf characteristics in gas exchange might be determined by plant species, ecological and physiological types, and their P_N and R_D would respond dissimilarly to CO_2 concentration.

That R_D–C_a curve conformed well to quadratic function might be an inherent property of leaf R_D. Leaves or plants had shown their great adaptation to environment in many aspects during the long evolution history. For instance, by comparing leaves collected around 1987 from central England to those specimens collected over the past two centuries before 1987, Woodward (1987) reported that the number of stomata per area of leaf has declined steadily by about 40 % during the past two centuries. The decrease is attributed to the 60 µmol mol^{-1} increase of atmospheric CO_2 during the period. Thus plants react to the changing atmospheric CO_2 concentration around them by changing their photosynthetic and respiration characters. Hence CO_2 concentration is an important environmental factor that affects leaf R_D. In order to keep their P_N at the highest level, leaves might adjust their R_D to the minimum when leaves are exposed to a certain CO_2 concentration for a long time. Thus, any variation of CO_2 concentration would cause the R_D to rise. If so, the various effects caused by EC could be interpreted as the appearance of the minimum R_D of different plants at different CO_2 concentration. That is to say, since the reported minimum R_D of experiment species has appeared at CO_2 concentrations ranging from 350 to 700, positive, negative, or no effect could be certainly found within that range. And if the inherent property was true, when the CO_2 concentration continued to rise, especially where CO_2 concentration is much higher than doubled, R_D must be promptly enhanced with the enrichment of CO_2. It was true at least when the CO_2 concentration was less than 1 500 µmol mol^{-1} in our experiment.

Along with the progress of studies on R_D response to EC, scientists are being more aware of the mechanism of leaf R_D and its change when exposed to EC. Although various effects of EC have been detected, R_D–C_a curve offers an important way to explain these effects, at least for short-term effects. Perhaps, before we know why leaf responded to EC differently, we should know first how it responded to a series of CO_2 concentration. We hope our results can shed light on the understanding of the leaf R_D response to EC using R_D–C_a curves; this has been ignored by scientists for decades. To understand the various effects of EC on R_D, further study can check the differences between plant species, the surroundings they live in, and the evolution of leaf dark respiration with the increasing CO_2 concentration over the geologic time.

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