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Day and night respiration of three tree species in a temperate forest of northeastern China

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Leaf day respiration is one of the most fundamental plant physiological processes and plays a vital role in the plant carbon cycle. However, day respiration is inherently complex and difficult to measure. In this study, the Kok method and the Laisk method were used to measure leaf day respiration on saplings of one evergreen conifer species (*Pinus koraiensis*) and two deciduous broadleaved species (*Tilia amurensis* and *Fraxinus mandshurica*) in a temperate forest of northeastern China. Results show that discrepancy between the corrected day respiration values estimated by the Kok and Laisk methods was only 4% for the three tree species. On average, day respiration was 55.9% and 52.6% lower compared to night respiration for the three tree species, as measured by the Kok and the Laisk method, respectively. Day respiration of the evergreen conifer species estimated by the Kok method was 31.7% lower, while that estimated by the Laisk method was 36.8% lower than that of the deciduous broadleaved species. Night respiration of the evergreen conifer trees was 40.7% lower than those obtained for the deciduous broadleaved trees. Day respiration rate was positively correlated with night respiration rate. Notably, day respiration rate decreased with increased photosynthetic photon flux density, and even a small amount of light significantly inhibited leaf day respiration in all the three species.

Keywords: Dark Respiration, Deciduous Broadleaved Tree, Evergreen Conifer Tree, Gross Primary Production, Light Inhibition, Temperate Forest

Introduction

Dark respiration (non-photorespiratory respiration), which occurs both in light and in darkness, has a critical function in modulating the carbon balance of plants and terrestrial ecosystems. Ryan (1991) showed that the proportion of carbon consumption by respiration is nearly 70% of the total photosynthetic carbon fixation, and leaf respiration accounts for approximately 50% of total plant respiration (Poorter et al. 1991). At the ecosystem level, CO_2 emission from plant respiration accounts for 30-70% of the total ecosystem CO_2 exchange (Amthor 2000). Plant respiration releases 60 Gt C yr^{-1} into the atmosphere (Schimel 1995). Therefore, understanding the carbon cycle of terrestrial ecosystems requires a more thorough understanding of the characteristics of leaf respiration.

Due to difficulty in directly measuring leaf day respiration (R_L), it is often assumed to be equal to night respiration (Poorter et al. 1990, 1992, Collier et al. 1992, Atkin et al. 1996). However, Kok (1948) found that net CO_2 assimilation rate (A_n) rapidly decreased near the light compensation point, leading to the conclusion that leaf respiration may vary with light intensity. Indeed, McCashin et al. (1988) found that $^{14}CO_2$ production during tricarboxylic acid cycle is approximately 20% lower in light than in darkness, which indicates that day respiration is inhibited in light. Subsequent studies confirmed that day respiration rates are lower than night respiration rates. Using stable $^{12}C/^{13}C$ isotope techniques, Tcherkez et al. (2005) found that two main processes (glycolysis and the Krebs cycle) of day respiration are strongly inhibited in illuminated leaves of French bean. Thus, plant respiration in light is overestimated if R_L is assessed using data obtained during nighttime (Graham 1980, Sharp et al. 1984, Kirschbaum & Farquhar 1987, Kromer 1995, Atkin et al. 1997, Hoefnagel et al. 1998, Ayub et al. 2011).

Plant respiration is a critical component of the gross primary production (GPP). Therefore, it is important to take into account the inhibitory effect of light on leaf respiration in order to obtain sound estimates of GPP. Wohlfahrt et al. (2005) estimated a reduction of 11-17% in GPP due to light inhibition in a mountain meadow, as compared to GPP model based on night respiration. Janssens et al. (2001) proposed that assessing day respiration by extrapolating night respiration results in an overestimation of ~15% of the total ecosystem respiration. Bruhn et al. (2011) recently reported that GPP estimation after taking into account light inhibition on leaf respiration for the average day is 76% of the GPP estimation taken without consideration of reduction in leaf respiration due to illumination. Thus, accurate estimates of the GPP requires the inhibition of leaf dark respiration by light to be considered.

The Kok and Laisk methods are the two main methods used to estimate leaf day respiration. Kok (1948) analyzed the response of net photosynthesis rate to low light intensities and showed that the slope abruptly increases when the light intensity declines to the light compensation point - a phenomenon now known as the "Kok effect". Extrapolation of the linear section of the light response curve before the slope change to a light intensity of zero gives an estimate of R_L. This technique has been widely used to analyze leaf respiration (Sharp et al. 1984, Villar et al. 1994, Bruhn et al. 2011). Laisk (1977) proposed a different R_L estimation method which was later extended by Brooks & Farquhar (1985). Such method uses the response of A_n to a series of low intercellular CO_2 concentrations (C_i) with two or more varying light intensities. Then, the CO_2 compensation point (at which the CO_2 released from photorespiration equals the CO_2 uptake by photosynthesis) and R_L are estimated by calculating the intersection points of the A_n-C_i curves under different light intensities.

Based on the aforementioned methods, a number of experiments have been performed to study the degree of respiration inhibition by light in different plant species. Villar et al. (1994, 1995) found that the mean inhibition of R_L is 55% for two woody species, and that the light inhibition of respiration is greater in mature leaves (81%) than in young leaves (36%). Several studies further explored the variation in R_L associated with physiolo-

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gical factors, including photosynthetic rate, specific leaf area, leaf age and relative growth rate (Reddy et al. 1991, Villar et al. 1995, Atkin et al. 1997, Reich et al. 1998), as well as environmental factors, namely, CO_2 concentration, temperature, and drought (Bunce & Ziska 1996, Nakaji et al. 2001, Peisker & Apel 2001, Vose & Ryan 2002, Warren & Adams 2002, Tingey et al. 2007, Crous et al. 2011, Zou et al. 2011). However, our understanding of the inhibition of plant day respiration is still limited compared with that of night respiration, and the degree of light inhibition and the many variables that may influence it are not clear. This is particularly true for trees of temperate forests, in spite of their fundamental contribution to the global carbon budget.

In this study, the Kok and Laisk methods were used to estimate the leaf day respiration for three dominant tree species (*Pinus koraiensis, Tilia amurensis* and *Fraxinus mandshurica*) in the Changbai mountain forest, which is a typical temperate natural forest in northeastern Asia. The major aim was to explore leaf respiration and the light inhibition degree of day respiration of evergreen conifer and deciduous broadleaved species in a temperate forest. Our findings may contribute to a better understanding of the biological processes of plant photosynthesis and respiration for the selected species, as well as to facilitate the accuracy of GPP estimates in temperate forests.

Materials and Methods

Experimental field and plant materials

The experiments were performed in the Changbai Mountain region of the Jilin province (northeastern China - 42° 24′ N, 128° 06′ E; elevation: 738 m a.s.l.). This region has a humid subalpine climate, and the growing season is from May to September. The average annual temperature is 3.6 °C and the total precipitation 695 mm. The soil is classified as dark brown forest soil.

Five-year-old saplings of three main tree species (*P. koraiensis, T. amurensis*, and *F. mandshurica*) were used in the experiment. The mean height of the potted saplings was 1.5 m, and its averaged basal diameter was about 2 cm. Data were collected from leaves of two deciduous broadleaved species (*T. amurensis*, and *F. mandshurica*) and two-years-old leaves of an evergreen conifer species (*P. koraiensis*).

Measurements and data processing

A portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA) was used to measure the net photosynthetic rate (A_n) of foliage under different photosynthetic photon flux density (PPFD) values and CO_2 concentration levels. A 2×3 cm cuvette was used in the gas-exchange measurements, with the flow rate set to 500 µmol s^{-1}. During the measurements, leaf temperature and relative humidity were controlled at approx. 25 °C and 60%, respectively. Measurements were taken from 08:00 to 12:00 on sunny days from July to September. For each treatment condition, three to four fully expanded and healthy representative leaves were randomly selected on saplings and considered as replicates. The sampled leaves were treated for each series gradient of PPFD values or CO_2 concentrations under controlled optimal conditions using the portable photosynthesis system.

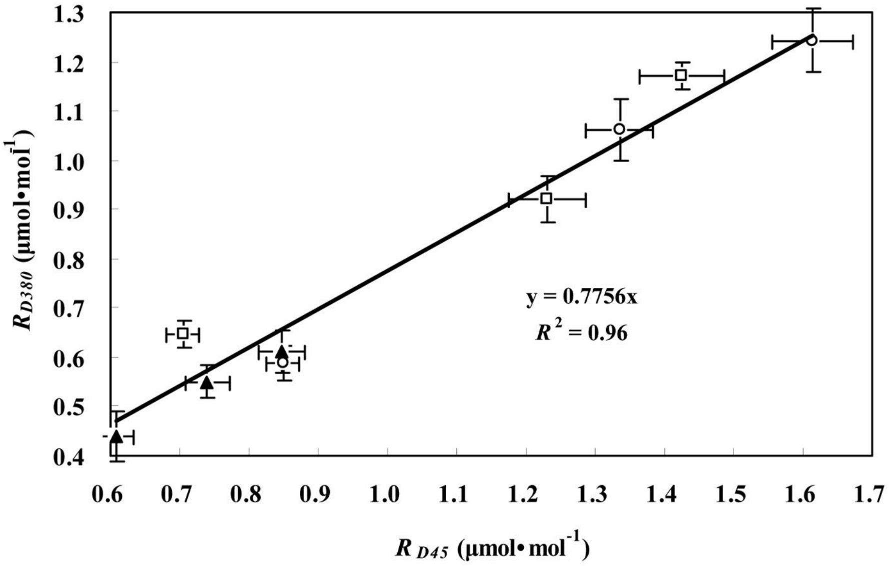

Fig. 1 - Relationship between R_{D45} and R_{D380} for the three tree species considered. R_{D45} and R_{D380} represent the leaf respiration in darkness measured at CO_2 concentrations of 45 µmol mol^{-1} and 380 µmol mol^{-1}, respectively, for *P. koraiensis* (black triangles), *T. amurensis* (white squares) and *F. mandshurica* (white circles). Data are presented as mean ± SD.

As for the Kok method, the sampled leaves were exposed to a range of PPFD values (150, 120, 90, 60, 50, 40, 30, 20, 10, 5, and 0 µmol m^{-2} s^{-1}) at a normal ambient CO_2 concentration (approximately 380 µmol mol^{-1}). As for the Laisk method, each selected leaf was exposed to a series of CO_2 concentrations (150, 120, 90, 70, 60, 50, and 40 µmol mol^{-1}), and PPFD set at 50, 100, and 150 µmol m^{-2} s^{-1}, respectively.

To compare R_L and night respiration (R_D), R_D measurements were conducted after 20 min of dark acclimation at CO_2 concentration of 45 and 380 µmol mol^{-1} in order to determine differences in respiration under low and ambient CO_2 condition. To determine the effect of light intensity on day respiration, seven PPFD levels (50, 100, 150, 210, 300, 600, and 800 µmol m^{-2} s^{-1}) and CO_2 concentration series (150, 120, 90, 70, 60, 50, and 40 µmol mol^{-1}) were set for leaves of *P. koraiensis* in August and September. A_n was measured under different CO_2 concentrations for each PPFD level. For each level of PPFD the linear regression of A_n over C_i was performed. R_L was estimated to be the value of the intercepted point of two regression lines established at each two ambient PPFD values. The measured data were then used to obtain R_L values by the Laisk method, and each R_L value corresponded to the averaged value of the two PPFD values. Additionally, determination for leaf nitrogen content was based on the Kjeldahl nitrogen method (Domini et al. 2009).

In the Kok method, the curve departed from a straight line when PPFD decreased to light compensation point. R_L was obtained by extrapolating the line to zero while ignoring the points below the light compensation point. Day respiration estimated by the Kok method was corrected based on Kirschbaum & Farquhar (1987). In the Laisk method, linear regressions of the A_n-C_i curves were obtained at three light levels, and the leaf dark respiration was estimated by the value of the point of intersection of those lines in the A_n coordinate. However, the Laisk method required the measurement to be carried out at very low CO_2 concentration. To determine the effect of low CO_2 concentrations on leaf respiration, the CO_2 concentration was controlled at 45 or 380 µmol mol^{-1} during R_D measurements for the three tree species. Fig. 1 shows the positive relationship between R_{D45} and R_{D380}. The rates of R_D at low CO_2 concentration were always higher than the corresponding values at high CO_2 concentration. Their relationship was expressed by a linear equation following the suggestion of Villar et al. (1994, 1995 - eqn. 1):

$$R_{D380}=0.7756\cdot R_{D45}$$

(R^2 = 0.96, P < 0.01) where R_{D380} and R_{D45} represent R_D in the ambient CO_2 concentration of 380 and 45 µmol mol^{-1}, respectively. R_{D380} was about 23% lower than R_{D45} ($P < 0.05$). Therefore, the respiration rate measured at low CO_2 concentration was generally overestimated. A correction for leakage was necessary because it is hard to avoid CO_2 exchange or leakage between the infrared gas analyzer (IRGA) chamber and the surrounding air (Pons & Welschen 2002, Shapiro et al. 2004, Flexas et al. 2007, Rodeghiero et al. 2007). We corrected for CO_2 leakage by diffusion in the Laisk method based on the following equation (eqn. 2) which was derived from the instruction manual of Li-Cor Inc (2004):

$$\frac{K}{u_i}=\frac{C_S-C_R}{C_A-C_S}$$

where K is the CO_2 diffusion flow rate (µmol s^{-1}), and u_i is the flow rate into the leaf chamber (µmol s^{-1}). C_S, C_R and C_A indicates sample, reference and ambient CO_2 concentration (µmol mol^{-1}), respectively.

Statistical analysis was performed using SPSS® version 17.0 (SPSS, Chicago, IL, USA). Differences among the three species analyzed were tested by one-way ANOVA (Duncan test) for the parameter of R_L estimated independently by the Kok and the Laisk methods, R_D, and degree of light inhibition of R_L. Student's *t*-test was used to evaluate the differences between R_L estimated by the two above methods used, as well as the differences between R_L and R_D for the three tree species. Relationships were fitted with linear or polynomial functions which provided a simple and good description of the phenomenon. All the tests were based on a significance level of 0.05.

Results

Leaf dark respiration

Fig. 2 and Fig. 3 show the R_L values obtained for the leaves of *P. koraiensis* by the Kok and the Laisk methods, respectively. The black arrow in Fig. 2 indicates the apparent rate of day respiration (0.21 µmol m^{-2} s^{-1}). As for the Laisk method, three linear regressions of the A_n-C_i curves intersected with each other within a centralized triangle range (Fig. 3). The central point (marked with red square in Fig. 3) represents the mean value of the three intersections of the triangle extended to the A_n axis and provides the value of day respiration. The intersection of the three lines obtained using the Laisk method formed a triangle instead of being a single point. The apparent lack of convergence of the three lines might result from measurement error arising from CO_2 leak through the gaskets or from the influence of the respired CO_2 on the leaf under the gaskets. Therefore, the day respiration rates estimated by the Kok and the Laisk methods were corrected. As for the Kok method, the corrected values of day respiration (R_{LK}) were 18% higher ($P < 0.05$) than the apparent values, while for the Laisk method, the corrected values of day respiration (R_{LL}) decreased 22% ($P < 0.05$) compared with the apparent values. Tab. 1 showed the R_L values estimated by the Kok and the Laisk methods for the three tree species during the peak growing season. On average, R_{LK} values obtained for the evergreen conifer (*P. koraiensis*) were 31.7% lower ($P < 0.05$) while the R_{LL} values was 36.8% lower ($P < 0.05$) than those obtained for the deciduous broadleaved trees (*T. amurensis* and *F. mandshurica*). The difference between R_{LK} and R_{LL} values was about 4% for the three tree species.

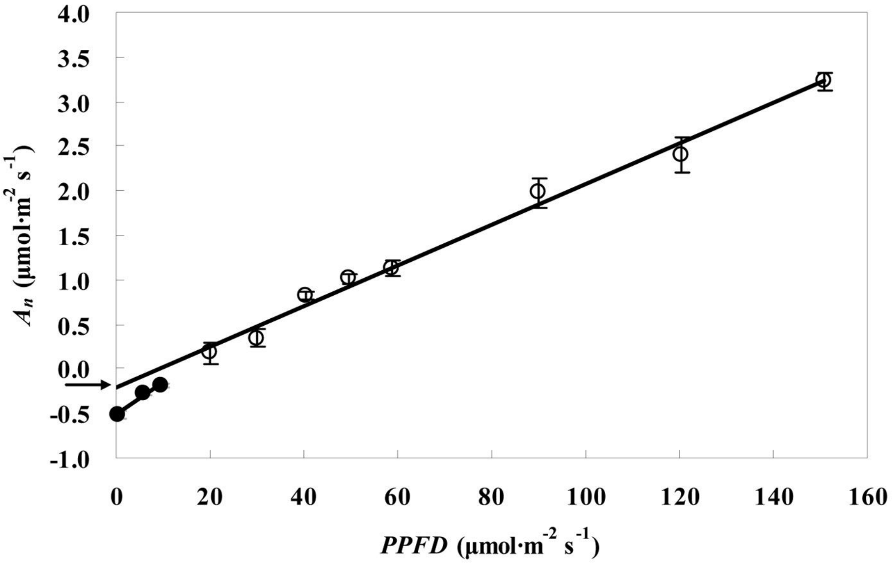

Fig. 2 - Linear relationship between the net CO_2 assimilation rate (A_n) and low PPFD values (Kok method) for *P. koraiensis* saplings in the Changbai station in July. The slope of the regression line of black circles ($R^2 = 0.98$, $P< 0.05$) is higher than that of the linear regression line of white circles ($R^2 = 0.91$, $P< 0.05$). Arrow indicates the value of day respiration. Data are presented as mean ± SD.

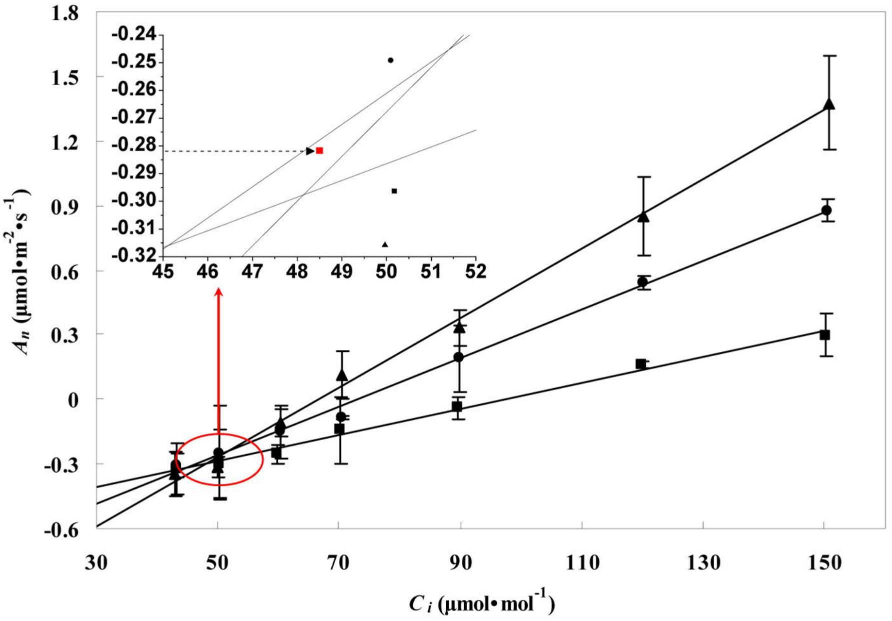

Fig. 3 - Linear regressions of the net CO_2 assimilation rate (A_n) *vs.* the intercellular CO_2 concentrations (C_i) at three PPFD values of 50 (black squares), 100 (black circles), and 150 (black triangles) µmol m^{-2} s^{-1} for leaves of *P. koraiensis* saplings in the Changbai Mountain in July (Laisk method). Day respiration value was calculated as the barycenter (the dotted arrow pointed) of the triangle formed by the intersection of the three lines. Data are presented as mean ± SD.

Also, the R_D value of the conifer was lower than those of the deciduous trees (Tab. 1), and the averaged R_D values for the conifer over the whole study period were 40.7% lower ($P < 0.05$) than those obtained for the broadleaves. As displayed in Fig. 4, a significant positive correlation was found between R_D and R_L estimated by the Kok (R^2=0.70, $P < 0.05$) and the Laisk methods (R^2=0.57, $P < 0.05$). The differences between R_L and R_D were significant for all three tree species ($P < 0.05$).

Relationship between day respiration and leaf nitrogen content

The average leaf N concentration for the evergreen conifer tree (*P. koraiensis*) was 27.1% lower ($P < 0.05$) than for the two deciduous broadleaved trees (*F. mandshurica*

Tab. 1 - Leaf day respiration R_L (R_{LK} and R_{LL}) and night respiration R_D (µmol·m^{-2}·s^{-1}) for the leaves of the three species studied (*P. koraiensis, T. amurensis* and *F. mandshurica*) from July to September 2011. Data are presented as mean ± residual standard deviation (RSD) for R_{LK} and R_{LL}, and mean ± SD for R_D. R_{LK} and R_{LL} are leaf day respiration values estimated by the Kok method and the Laisk method, respectively. (*): RSD of R_{LL} was calculated by averaging the RSD values of three linear regressions.

Species	**Month**	R_L		R_D
		R_{LK}	R_{LL}	
Pinus koriaensis	July	0.26 ± 0.0113	0.22 ± 0.0309	0.44 ± 0.1208
	August	0.28 ± 0.0267	0.42 ± 0.0395	0.61 ± 0.0737
	September	0.22 ± 0.0277	0.18 ± 0.0371	0.55 ± 0.1071
Tilia amurensis	July	0.33 ± 0.0261	0.35 ± 0.0699	0.65 ± 0.0924
	August	0.52 ± 0.0407	0.59 ± 0.0294	1.17 ± 0.1104
	September	0.31 ± 0.0599	0.29 ± 0.0472	0.92 ± 0.1269
Fraxinus mandshurica	July	0.30 ± 0.0362	0.36 ± 0.0818	0.59 ± 0.0640
	August	0.56 ± 0.0278	0.59 ± 0.0529	1.24 ± 0.1593
	September	0.31 ± 0.0256	0.33 ± 0.0680	1.06 ± 0.1467

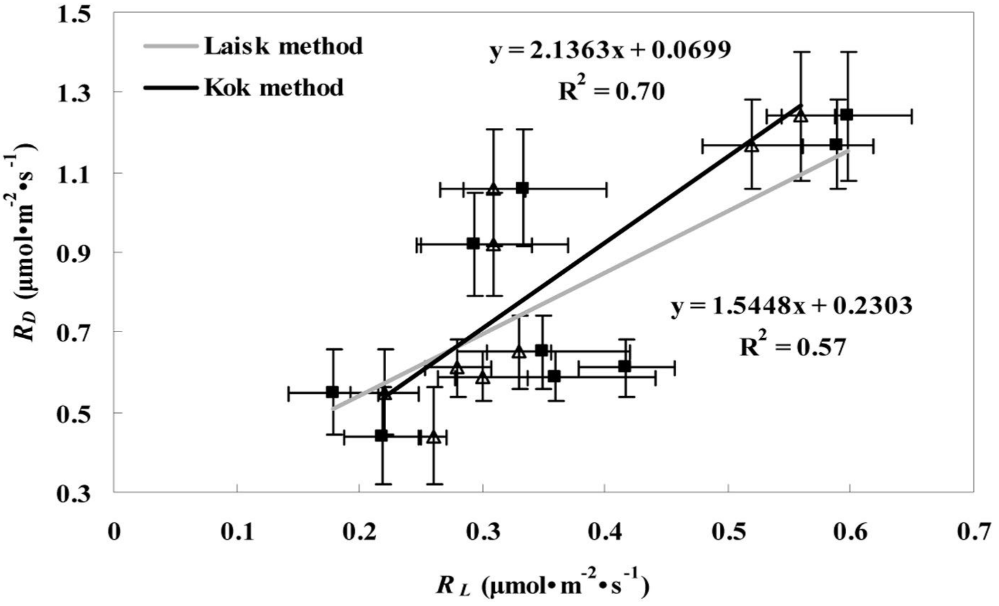

Fig. 4 - Relationship between R_D and R_L values estimated by Kok (R_{LK} - triangles) and Laisk methods (R_{LL} - squares) for the three tree species (*P. koraiensis, T. amurensis*, and *F. mandshurica*). Linear relationship estimated by the Kok and the Laisk method is represented with the black and gray line, respectively.

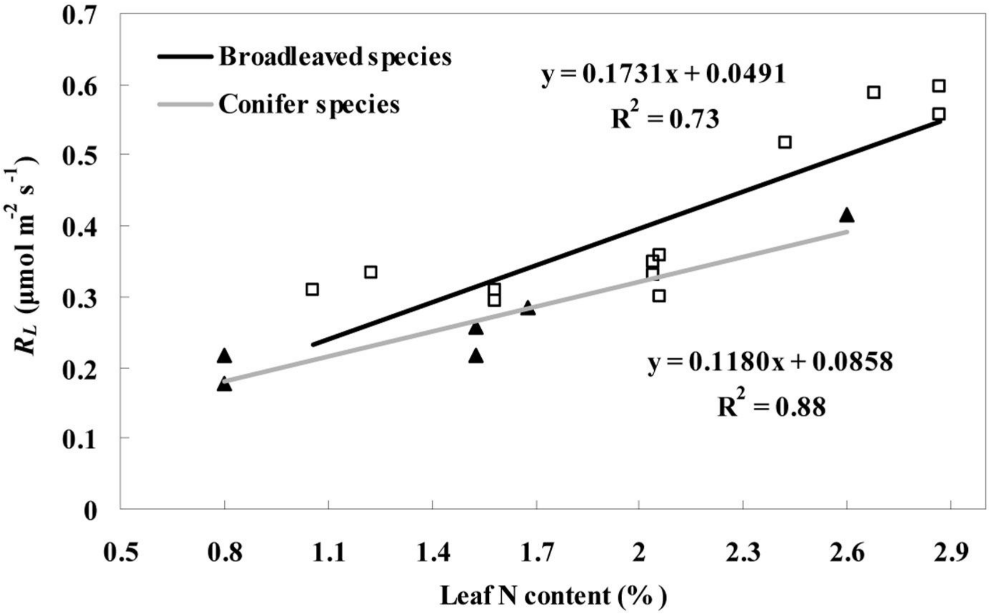

Fig. 5 - Variation in R_L with leaf nitrogen content for the two deciduous broadleaved species (white squares) and one evergreen conifer species (black triangles). The linear regression for broadleaved species and conifer species was showed with the black and gray line, respectively. R_L represents the leaf day respiration which includes the value estimated by the Kok (R_{LK}) and Laisk methods (R_{LL}) from July to September.

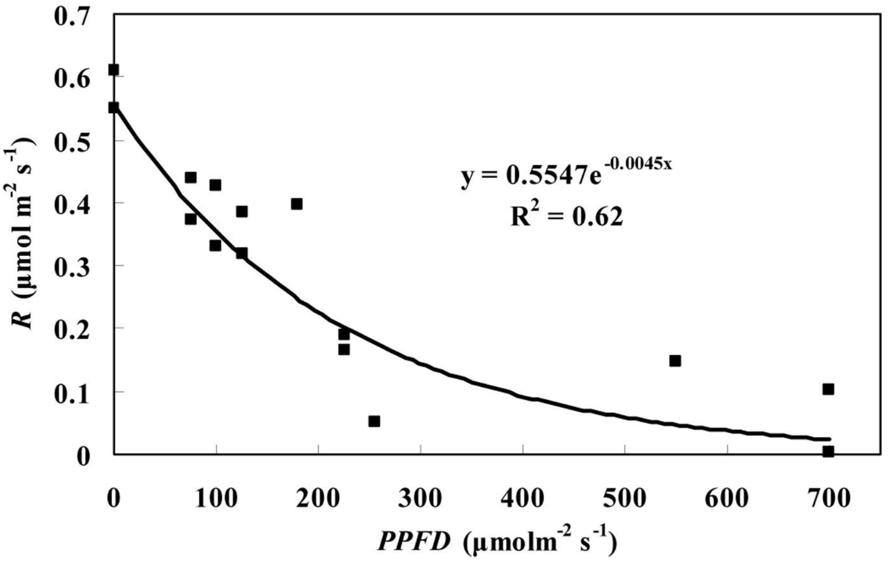

Fig. 6 - The effects of light on leaf dark respiration. Leaf dark respiration (*R*), including day respiration estimated by the Laisk method and night respiration, of *P. koraiensis* at different PPFD values in August and September.

and *T. amurensis*). Moreover, a strong positive correlation between leaf nitrogen content and day respiration was found (Fig. 5). The correlation between R_L and leaf nitrogen content is expressed as eqn. 3 for the conifer species ($R^2 = 0.88$, $P < 0.05$) and as eqn. 4 ($R^2 = 0.73$, $P < 0.05$) for the broadleaved species:

$$R_{Lec} = 0.1180 \cdot N + 0.0858$$

$$R_{Ldb} = 0.1731 \cdot N + 0.0491$$

where R_{Lec} and R_{Ldb} are the day respiration rates of the evergreen conifer species and two deciduous broadleaved trees, respectively, and *N* is the leaf nitrogen content.

Light inhibition of day respiration

The average R_L inhibition of the three tree species was found to be 55.9% and 52.6% for the Kok and Laisk methods, respectively. No significant differences were observed among the three tree species in the light inhibition on R_L. The degree of light inhibition on R_L was different under different PPFD values. Based on our data, the relationship between *R* and PPFD can be described by the following exponential equation (eqn. 5):

$$R = 0.5547 \cdot e^{-0.0045 \cdot PPFD}$$

($R^2 = 0.62$, $P < 0.05$) where *R* is the leaf dark respiration, including both leaf dark respiration in light and darkness. As shown in Fig. 6, the value of *R* for *P. koraiensis* was the highest in darkness, and then *R* decreased rapidly following an increase in the incident PPFD. The decrease rate of *R* gradually declined with PPFD increase until it remained constant.

Discussion

Uncertainties in day respiration estimate

The corrected day respiration was 18% higher and 22% lower than the apparent values obtained by the Kok and the Laisk methods, respectively. Kirschbaum & Farquhar (1987) showed that R_L measured by the Kok method is underestimated because it is assumes that the C_i values remain constant with changing PPFD. However, it has been shown that when PPFD decreases, stomatal closure is altered in response to environmental changes (Tcherkez et al. 2008), and C_i gradually increases because of the decreased stomatal conductance. Consequently, a fraction of the respired CO_2 is not promptly released and remains involved in the photosynthesis process. This results in an increase in A_n values and a decline in the slope of regression of A_n *vs*. PPFD (Kirschbaum & Farquhar 1987, Villar et al. 1994), which accounts for the underestimation of R_L in the Kok method. R_{LL} was overestimated in the Laisk method as the measurement must be conducted at low CO_2 concentration. Some studies have proposed that the overestimation of R_{LL} is caused by the CO_2 acting as an inhibitor of certain enzymes (Brooks & Farquhar 1985, Kirschbaum & Farquhar 1987, Tjoelker et al. 2001). However, a large number of studies suggest that leaf respiration changes with CO_2 possibly due to small leaks or diffusion in the gas exchange measurement systems (Amthor et al. 2001, Pons & Welschen 2002, Shapiro et al. 2004, Flexas et al. 2007, Rodeghiero et al. 2007). Therefore, in this study R_{LL} was corrected based on the method suggested by Li-Cor Inc (2004) to compensate the CO_2 leakage in the Laisk method.

Differences between dark respiration of broadleaved and conifer species

Consistent with several previous studies (Villar et al. 1995, Atkin et al. 1997), R_L was found in this investigation to be positively correlated with R_D. Both R_L and R_D of the deciduous broadleaved species were higher than those of the conifer species. In addition, Cornelissen et al. (1999) reported a growth rate of deciduous broadleaved species higher than that of the conifer species. R_L and R_D were higher in the rapidly growing tree species, likely because rapid-growth species generally require more carbon skeletons, energy and reductant-producing organelles, which were derived from the respiration process in tree species (Amthor 1989, Villar et al. 1995, Lambers et al. 1996, Atkin et al. 1997). Atkin et al. (1997) also reported that R_L and R_D were positively correlated with A_n and the relative growth rate in *Poa* species. Hence, greater values of R_L and R_D can be hypothesized to occur in fast-growing plants. Wang et al. (2001) also has suggested that higher biomass production in fast-growing species demand greater respiration production, and this requirement is fulfilled by higher R_L and R_D values. A positive correlation between R_L and leaf N concentration was also found in our study, which is consistent with several previous reports on a positive correlation between plant respiration and the leaf N concentration (Amthor 1989, Ryan 1991). Furthermore, we found that leaf N concentration in *P. koraiensis* was lower than in *F. mandshurica* and *T. amurensis*. This is consistent with previous reports by Reich et al. (1992) and Villar et al. (1995) showing that the leaf N concentration is lower in evergreen species than in deciduous species.

Light inhibition of day respiration

In this study, R_L was significantly lower than R_D for the three tree species, indicating that R_L was inhibited by light. This result is in agreement with those of several previous studies (Sharp et al. 1984, Brooks & Farquhar 1985, McCashin et al. 1988, Hanning & Heldt 1993, Tcherkez et al. 2005). Some studies have suggested that inhibition of leaf dark respiration in light is only apparent and possibly results from internal CO_2 re-fixation. Loreto et al. (2001) and Pinelli & Loreto (2003) found that R_L and R_L/R_D were inversely correlated with photosynthesis, and suggested that most of the respiratory CO_2 was re-fixed by photosynthesis. Pinelli & Loreto (2003) also reported that the sum of re-fixed and emitted $^{12}CO_2$ was close to mitochondrial respiration in the dark. However, the authors also suggested that mitochondrial respiration may be inhibited in light when the leaf is in salt- and water-stressed condition, because the sum of re-fixed and emitted $^{12}CO_2$ under the above conditions was lower than that in the dark. Furthermore, Pärnik & Keerberg (1995) stated that leaf R_L is lower than R_D in light even when the re-fixed respiratory CO_2 is taken into consideration. Additionally, many studies have suggested that the inhibition in light may be due to inhibition of respiratory enzymes by co-factors such as ATP and NADPH (Graham 1980, McCashin et al. 1988). Tcherkez et al. (2005) showed that the inhibition mainly occurred during the Krebs cycle and the inhibition in the Krebs cycle was reduced by 95% in light, while the pyruvate dehydrogenase was reduced by only 27%. The reduction in the Krebs cycle, which is one of the most important processes of leaf respiration, occurs mainly because some enzymes are inhibited by the high ratio of NADPH/NADP+ in light (Siedow & Day 2000). Likewise, it has been suggested that high NADPH/ NADP+ ratios could also inhibit isocitrate dehydrogenase in illuminated mitochondria (Igamberdiev & Gardeström 2003). Thus, there is considerable evidence supporting that day respiration is indeed inhibited in light. In this study, no significant differences were found in the light inhibition of R_L between broadleaved and conifer trees, in spite of their different photosynthetic capacities and relative growth rates. Therefore, light inhibition on R_L may not be correlated with A_n or relative growth rate. This conclusion is consistent with results reported by Atkin et al. (1997).

Our results showed that leaf dark respiration varied under different PPFD levels. The relationship between R_L and PPFD showed in Fig. 6 is consistent with that obtained in previous studies (Brooks & Farquhar 1985, Villar et al. 1994, 1995) by the Laisk method. The photosynthetic rate generally increases with increasing PPFD, but the rate of increase gradually declines. In this study, R_L was found to decrease with increasing PPFD, likely because more ATP and NADPH were available for increasing the photosynthetic rate (Turpin & Weger 1990), causing the energy demand to step up gradually from R_L to plateau (Villar et al. 1995).

Both the comparison of R_L and R_D of the three tree species and the analysis of the relationship between R and PPFD demonstrated a remarkable inhibition of the day respiration. Peisker & Apel (2001) reported that the ratio of R_L to R_D ranges from 30 to 100%. However, Hurry et al. (1996) concluded that R_L is higher in light than in darkness in rye using the ^{14}C-labeling measurement technique. Additionally, Zaragoza-Castells et al. (2007) found that R_L is inhibited in light only under certain conditions. Thus, R_L inhibition in light may not occur in all species and under all conditions. Light inhibition of R_L and the degree of inhibition of R_L may depend on the species and growth conditions. Hence, additional studies on various species and under different environmental conditions are needed to obtain a deeper understanding of the above physiological processes. Variations in R_L with change in PPFD and CO_2 concentration were analyzed in this investigation. However, many other factors affecting R_L may exist, so further studies should be carried out to fully understand the mechanism by which R_L is inhibited in light.

Conclusions

In summary, our findings revealed that R_{LK} and R_{LL} in conifer trees were lower by 31.7% and 36.8%, respectively, than those in broadleaved trees considered in this study. We detected no significant difference in light inhibition of respiration between the coniferous and the broadleaved tree species. The average R_L inhibition in the three tree species was 55.9% and 52.6% for R_{LK} and R_{LL} values, respectively, relative to R_D. R_L was found to be positively correlated with R_D in the three tree species. R_L also decreased with increasing PPFD, but the decrease rate of R_L gradually declined. These findings may be useful in estimating the component of carbon exchange and carbon budget (*e.g.*, R_e, NEE, GPP) of the forest ecosystem by evaluating the light inhibition of respiration in the representative tree species.

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