Estimating Daytime Ecosystem Respiration to Improve Estimates of Gross Primary Production of a Temperate Forest

Jinwei Sun¹,²*, Jiabing Wu¹, Dexin Guan¹*, Fuqi Yao², Fenghui Yuan¹, Anzhi Wang¹, Changjie Jin¹

¹. State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P.R. China, 2. Changjiang River Scientific Research Institute, Wuhan, P.R. China

*sunjinwei615@126.com (JS); dxguan@iae.ac.cn (DG)

Abstract

Leaf respiration is an important component of carbon exchange in terrestrial ecosystems, and estimates of leaf respiration directly affect the accuracy of ecosystem carbon budgets. Leaf respiration is inhibited by light; therefore, gross primary production (GPP) will be overestimated if the reduction in leaf respiration by light is ignored. However, few studies have quantified GPP overestimation with respect to the degree of light inhibition in forest ecosystems. To determine the effect of light inhibition of leaf respiration on GPP estimation, we assessed the variation in leaf respiration of seedlings of the dominant tree species in an old mixed temperate forest with different photosynthetically active radiation levels using the Laisk method. Canopy respiration was estimated by combining the effect of light inhibition on leaf respiration of these species with within-canopy radiation. Leaf respiration decreased exponentially with an increase in light intensity. Canopy respiration and GPP were overestimated by approximately 20.4% and 4.6%, respectively, when leaf respiration reduction in light was ignored compared with the values obtained when light inhibition of leaf respiration was considered. This study indicates that accurate estimates of daytime ecosystem respiration are needed for the accurate evaluation of carbon budgets in temperate forests. In addition, this study provides a valuable approach to accurately estimate GPP by considering leaf respiration reduction in light in other ecosystems.
Introduction

Leaf respiration can account for up to 25% or higher proportions of daily photosynthesis [1–3] and therefore plays an important role in the estimation of gross primary production (GPP). In previous studies, leaf respiration in light is considered to be the same magnitude as respiration at night [4], while further studies found that leaf respiration is inhibited in light. The extent of light inhibition of leaf respiration ranges from 25% to 100% [5–8]. Traditionally, ecosystem respiration ($R_e$) was estimated using simple functional relationships associated with temperature and soil water availability [9–12]. However, this method is limited because inhibition of leaf respiration in light ($R_L$) is not considered [5, 13–14]. Therefore, $R_e$ and GPP are overestimated when daytime $R_e$ is estimated from nighttime $R_e$ [15–17]. Accounting for leaf respiration inhibition in the daytime could therefore markedly improve the accuracy of ecosystem GPP estimation [8, 17–18].

Although previous studies investigated the difference between $R_L$ and leaf respiration in darkness ($R_D$), to our knowledge, few researchers have incorporated this difference into ecosystem-level studies. Tingey et al. (2007) reported that daytime $R_e$ values of Douglas fir seedlings were 28% and 16% lower under ambient and elevated CO$_2$ concentrations, respectively, compared with the values obtained when light inhibition of leaf respiration was ignored [19]. In an old beech forest, GPP calculated after taking light inhibition of $R_e$ into account was only 76% of the value obtained when light inhibition was not considered [16]. Although the above studies estimated $R_e$ or GPP more accurately by applying the daytime leaf respiration reduction to the canopy level [16, 19], their estimates did not consider the attenuation of radiation in the canopy. Additionally, in a study conducted by Bruhn et al. (2011), the low photosynthetically active radiation (Q) levels used in the Kok method were frequently acquired in early morning or at nightfall and were very limited in number after data screening [16]. This limitation occurred because friction velocity ($u^*$) is generally low at those times, while the data screening standard is $u^*>0.5$. Some reports state that the degree of light inhibition varies with Q [17, 20–21], but failure to consider Q differences in the canopy leads to an error in canopy respiration estimation and consequently, the evaluation of $R_e$ and GPP. Radiation in a layered canopy should be estimated when the daytime inhibition at the leaf and canopy level needs to be assessed because radiation is attenuated with increasing canopy depth. Wohlfahrt et al. (2005) divided the canopy of a mountain meadow into a statistically sufficient number of layers and produced a more accurate calculation of GPP at the canopy level, showing that GPP declined by 11% to 13% (using a low estimate of leaf respiration inhibition), and by 13% to 17% (using a high estimate of leaf respiration inhibition) [17]. In this study, the estimate of dark respiration was limited because daytime inhibition of leaf-level respiration was provided as a range of values based on the scattered results of several published studies reviewed by Wohlfahrt et al. (2005) [17]. There are no studies in forest ecosystems that have provided estimates as detailed as those of Wohlfahrt et al. (2005) [17].
In our study, we estimated leaf dark respiration using the Laisk method [22] and analyzed the $R_L$ values under different $Q$ levels for dominant tree species of a mixed temperate forest. Measurements were conducted on potted seedlings of dominant tree species to determine the effect of light inhibition on dark respiration of individual species. The reduction in $R_e$ was estimated by separating the canopy into 20 layers to calculate GPP based on eddy covariance measurements and a multilayer model. The specific aims of the present study were to: (1) assess the leaf respiration reduction in light of specific species under different $Q$ levels, (2) evaluate spatial differences in $Q$ using a radiative transfer model and estimate leaf respiration reduction in light relative to darkness at the canopy level, and (3) evaluate the impacts of leaf respiration reduction in light on canopy respiration, $R_e$, and GPP.

Materials and Methods

2.1 Ethics Statement

The field studies were conducted at the Changbai Mountains National Nature Reserve in northeastern China. This is a practice base for the researchers of the Chinese Academy of Sciences. The experiments conducted in this study did not involve any protected animals or plants, and this study was permitted by the Station of Changbai Mountain Forest Ecosystem, Chinese Academy of Science.

2.2 Site description and materials

The National Natural Conservation Park of Changbai Mountain (42°24′N, 128°06′E; 738 m elevation) is situated in the temperate continental climatic zone [23]. The annual mean air temperature is 3.6 °C, and mean precipitation is 695.3 mm according to meteorological records from 1982 to 2003. The soil is classified as dark brown forest soil. The growing season extends from May to September. The dominant tree species of this ecosystem are *Pinus koraiensis*, *Tilia amurensis*, *Fraxinus mandshurica*, *Acer mono*, and *Quercus mongolica*.

The experiment was performed in openings in a temperate broad-leaved Korean pine mixed forest, and four dominant tree species (*T. amurensis*, *F. mandshurica*, *A. mono*, and *P. koraiensis*) were selected. Four potted seedlings were selected for each tree species. For sampling purposes, four fully expanded and healthy representative leaves were randomly selected from each seedling and all measurements were averaged. The four mean values of each tree species were used as replicates for statistical analysis.

2.3 Measurement and estimation of leaf respiration

Leaf respiration of mature trees could not be measured directly considered the height of the trees, and therefore leaf respiration of seedlings of the dominant tree species was measured. Leaf respiration in light was estimated using the Laisk method by evaluating the net photosynthetic rate ($A_n$) at a series of low
intercellular CO₂ concentrations (cᵢ) under different Q levels. Photosynthetic measurements were conducted using an open-mode portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA), and Aₐ was calculated on an area basis. To determine the effect of light intensity on daytime respiration, Aₐ was measured at seven Q levels (50, 100, 150, 210, 300, 600, and 800 μmol·m⁻²·s⁻¹) and CO₂ concentrations (150, 120, 90, 70, 60, 50, and 40 μmol·mol⁻¹). Linear regressions of Aₐ versus cᵢ were performed for each Q level. The linear regressions crossed at a point under each pair of Q values, and the Aₐ coordinate of this point represented the Rₐ that corresponded to the average of these two Q values.

Seedling Rₐ was measured following 20 min of dark acclimation. During all measurements, the leaf temperature was maintained at approximately 25 °C, relative humidity at approximately 60%, and the flow rate was set at 500 μmol·s⁻¹. The measurements were performed from 08:00 to 12:00 a.m. on sunny days.

2.4 Ecosystem CO₂ flux measurements

2.4.1 Instruments
The carbon dioxide flux over the forest site was continuously measured at a height of 40 m using the eddy-covariance (EC) technique. The flux system included a triaxial sonic anemometer (CSAT3, Campbell Inc., USA) and a fast-response open-path CO₂/H₂O infrared gas analyzer (LI-7500, LI-COR, USA). Soil temperature was measured using multilevel thermocouple probes, and sensors were placed at 5, 10, 20, 50, and 100 cm below the soil surface. Air temperature was measured using HMP-45C sensors located at 32 and 60 m and recorded using a data logger (CR5000, Campbell Inc., USA).

2.4.2 Flux calculations and corrections
Fluxes were calculated online at 30-min intervals based on the time series of vertical velocity (w′) and CO₂ concentration (c′) fluctuations according to Reynolds decomposition: Fₓ = w′c′. A coordinate rotation was applied to force the average vertical wind speed to zero and to align the horizontal wind to mean wind streamlines based on Wilczak et al. (2001) [24]. CO₂ eddy fluxes were corrected for density effects using the WPL correction based on Webb et al. (1980) [25]. The underestimation caused by sensor line averaging, spatial separation, and high frequency losses was compensated by physically sound spectra correction following Moore (1986) [26].

Spikes and gaps in the archived records of EC measurements are inevitable because the sonic anemometer and infrared gas analyzer are sensitive to precipitation. In addition, power failures occur, and sensor calibration and maintenance are required. The data set coverage was approximately 78% for the above canopy EC system after testing for stationary and integral turbulence statistics using the method introduced by Foken and Wichura (1996) [27]. Linear interpolation was used to fill small gaps in data (<2 h) and the strategies proposed by Falge et al. (2001) [28] were used to fill large data gaps. When >30%
of the data were missing for one day, only the total daily flux was calculated based on the relationship between CO$_2$ flux processes and environmental variables [29].

2.5 Components of GPP

GPP is calculated by subtracting $R_e$ from NEE. $R_e$ includes mainly soil respiration ($R_s$), stem respiration ($R_{st}$), and canopy leaf respiration ($R_c$). Soil, stem and canopy leaf respiration (ignoring the reduction in light) were calculated using an exponential function [30]:

$$ R = a \times e^{b \cdot T} \tag{1} $$

where $R$ is the respiration of the soil, stem, and canopy, and $T$ is the temperature of these components. The parameters were obtained from the results presented in Wang et al. (2010) [30], who conducted research at this site (Table 1). There were small differences in stem respiration between the broad-leaved deciduous species (Table 1), and stem respiration of A. mono was used as the average value for the other three broad deciduous species. Light inhibition was not accounted for in the estimate of $R_c$ when equation (1) was used. Therefore, the estimated $R_c$ was higher than the true canopy leaf respiration in light and consequently, GPP was overestimated.

2.6 Estimation of the reduction in canopy leaf respiration

The reduction in canopy leaf respiration in light relative to darkness ($F$) was estimated as follows:

$$ F = \frac{R_{CL}}{R_{CD}} = \frac{1}{\text{LAI}(y,m)} \left[ \sum_{i=1}^{5} \sum_{l=l_i}^{l'-l_i+1} \frac{\text{LAI}(y,m,r)}{l'-l_i+1} \sum_{s=1}^{2} \frac{R_{L}}{R_{D}} Q(s)f(s) \right], \tag{2} $$

where $m$ (1 to 5) represents the month of the growing season from May to September, respectively, and $y$ (1 to 3) represents the year from 2003 to 2005, respectively. LAI ($y$, $m$) is the canopy LAI determined by the month ($m$) and year ($y$), which is shown in Fig. 1. The values $i=1$ to 5 represent the five dominant tree species, respectively; $l$ represents the layer number from the top of the canopy for a tree species; $l_i$ represents the first layer number, and $l'$ represents the last layer number; $l'-l_i+1$ is the total number of layers per tree species; LAI ($y$, $m$, $r$) represents the LAI determined in a specific month and $r$ represents the leaf biomass percent of a given species. The radiation absorption of a multilayer model [31] was used to estimate the $Q$ in each canopy layer. Total canopy height ranged from 7 to 27 m based on field survey data collected near the flux tower. The range in tree height, leaf biomass, and the leaf biomass percent of the five dominant tree species are shown in Table 2 [32]. To apply the multilayer model to the mixed temperate forest, the canopy was divided into 20 layers. The thickness in each canopy layer was 1 m and the LAI was LAI/20. Sunlit and shaded leaves were treated separately for each canopy layer. Sunlit leaves receive both diffuse and
direct beam radiation, whereas shaded leaves only receive diffuse radiation. The leaf angle distribution was assumed as a spherical distribution. Direct beam and diffuse radiation were calculated using the exponent profile method. The detailed radiation transmission model for the canopy is described in Appendix S1. The two values of $s$ represent the cases of sunlit ($s=1$) and shaded leaves ($s=2$); $Q(1)$ is the radiation received by sunlit leaves and $f(1)$ is the fraction of sunlit leaf area; $Q(2)$ and $f(2)$ represent the radiation received by shaded leaves, and the fraction of the shaded leaf area, respectively. The ratio of canopy leaf respiration in light ($R_{cL}$) to canopy leaf respiration in darkness ($R_{cD}$) of sunlit and shaded leaves of each tree species was determined in each canopy sublayer, and $f_{sl}$ in each canopy layer is equal to the fraction of direct beams reaching that particular layer [33]:

$$f_{sl} = \exp(-k_p \cdot \xi),$$

where $\xi$ is the LAI accumulated from the top of the canopy and $f_{sh} = 1 - f_{sl}$.

The canopy leaf respiration estimation assumes that temperature, humidity, wind velocity, and CO$_2$ concentration are horizontally uniform in the canopy and

| Parameter          | $\alpha$ | $\beta$ |
|--------------------|----------|---------|
| Stem respiration   | $Pinus$ koraiensis | 0.414 | 0.096 |
|                    | $Tilia$ amurensis  | 0.523 | 0.081 |
|                    | $Quercus$ mongolica | 0.665 | 0.097 |
| Leaf respiration   | $Fraxinus$ mandshurica | 0.408 | 0.114 |
|                    | $Pinus$ koraiensis | 0.250 | 0.035 |
|                    | $Tilia$ amurensis  | 0.232 | 0.042 |
|                    | $Quercus$ mongolica | 0.238 | 0.087 |
|                    | $Fraxinus$ mandshurica | 0.141 | 0.098 |
| Soil respiration   |          | 0.640 | 0.101 |

Table 1. Parameters of the temperature response in equation (2) for soil respiration and stem and leaf respiration of the dominant tree species ($P. koraiensis$, $T. amurensis$, $Q. mongolica$, and $F. mandshurica$) (µmol·m$^{-2}$·s$^{-1}$) (Wang et al., 2010).
that leaf biomass is uniformly distributed in the layers occupied by a given tree species. The mean discrepancy of air temperature between the top and bottom of the forest canopy was only 0.47 °C based on our measurements collected at the tower during the growing season from 2003 to 2005. The photosynthetically active radiation at the bottom of the canopy was attenuated by approximately 79%–94% compared with the top of the canopy under a leaf area index range of 2 to 6. Therefore, the temperature variation in the canopy was not considered. In the $R_{L}/R_{D}$ estimation, the $R_{L}/R_{D}$ values, calculated as a function of $Q$, for the five dominant tree species, were obtained by measurements detailed in section 2.3, and the $R_{L}/R_{D}$ value of $Q. mongolica$ was taken as the mean value of the other three broadleaved tree species.

2.7 Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS, Chicago, IL, USA). Student’s $t$-test was used to evaluate the differences between $R_{L}$ and $R_{D}$ for the dominant tree species. Relationships were fitted with polynomial functions that provided simple and well-fitting descriptions of the phenomena. All tests were based on a significance level of 0.05.

Results

3.1 Light inhibition of leaf respiration

The $R_{L}$ values were lower (Student’s $t$-test, $P<0.05$) than the $R_{D}$ values for the dominant species. $R_{L}$ declined exponentially with increasing $Q$ levels, and the relationships between $R_{L}$ and $Q$ fit the following exponential equation:

$$R_{L} = a \cdot e^{b \cdot Q}$$  \hspace{1cm} (4)

These relationships were significant at $P<0.05$, and the values of $a$ and $b$ are listed in Table 3. Similarly, $R_{L}/R_{D}$ decreased exponentially with increasing $Q$ levels and the variation in $R_{L}/R_{D}$ with $Q$ is presented in Fig. 2 for the four dominant tree species.

Table 2. Tree height, canopy thickness and leaf biomass of five dominant tree species ($P. koraiensis$, $T. amurensis$, $Q. mongolica$, $F. mandshurica$, and $A. mono$) in the study forest (Shi et al., 2010).

| Species          | Tree height range (m) | Leaf biomass (t × ha$^{-1}$) | Percentage of leaf |
|------------------|-----------------------|------------------------------|-------------------|
| Pinus koraiensis | 9–22                  | 3.13                         | 55.3%             |
| Tilia amurensis  | 9–22                  | 1.07                         | 18.9%             |
| Quercus mongolica| 9–25                  | 0.91                         | 16.1%             |
| Fraxinus mandshurica| 15–27                | 0.42                         | 7.4%              |
| Acer mono        | 7–17                  | 0.13                         | 2.3%              |

doi:10.1371/journal.pone.0113512.t002
3.2 Estimation of canopy leaf respiration

Canopy leaf respiration without correction for light inhibition \( (R_c) \) was estimated using equation (1), and the corrected canopy leaf respiration \( (R_c^*) \) was calculated by accounting for the reduction in leaf respiration during the daytime based on equation (2). The diurnal variation in \( Q, R_c \), and \( R_c^* \) is shown in Fig. 3. At night, \( R_c \) was equal to \( R_c^* \), whereas \( R_c^* \) was lower than \( R_c \) in the day owing to light inhibition. The discrepancy between \( R_c \) and \( R_c^* \) initially increased with \( Q \) and reached its highest value \( (2.23 \text{ \mu mol m}^{-2} \text{s}^{-1}) \) at midnight when \( Q \) was highest. The discrepancy decreased with decreasing \( Q \) during the afternoon. The diurnal variation is readily explained by equation (2) and (4).

The effects of seasonal variation on \( R_c \) and \( R_c^* \) are shown in Fig. 4. Monthly \( R_c \) varied from 2.45 g C m\(^{-2}\) month\(^{-1}\) to 112.48 g C m\(^{-2}\) month\(^{-1}\) and \( R_c^* \) ranged from 1.96 g C m\(^{-2}\) day\(^{-1}\) to 88.07 g C m\(^{-2}\) day\(^{-1}\) from 2003 to 2005. The highest \( R_c \) and \( R_c^* \) values occurred in July and August, and the lowest in January and December. The discrepancy between \( R_c \) and \( R_c^* \) reached its highest value in August in all three years. The cumulative values of \( R_c \) and \( R_c^* \) in each year from 2003 to 2005 are shown in Table 4. Thus, the cumulative values of \( R_c \) were 20.0%, 20.5%, and 20.6% higher than the cumulative \( R_c^* \) values in 2003, 2004, and 2005, respectively.

3.3 Effect of light inhibition on canopy and ecosystem respiration, and GPP estimation

Cumulative values of \( R_c \) and \( R_e \) with and without light-inhibition correction showed similar seasonal variation over the three years (Fig. 5). These values were low in winter and high over the period from June to August in each year. The average difference between the corrected and uncorrected \( R_c \) values is 61.79 g C m\(^{-2}\), which is equivalent to the \( R_c \) overestimation for the three years. The annual sums of components of \( R_c \) (i.e., \( R_o, R_c^*, R_s, \) and \( R_l \), \( R_e^* \), NEE, and GPP are summarized in Table 4 for the three years. The value of \( R_c/R_e \) range from 28.6% to 30.1%, whereas the value of \( R_e^*/R_e \) range from by 25.0% to 26.3%, i.e., accounting for daytime canopy respiration inhibition resulted in an \( R_c/R_e \) reduction of 3.7% (Student’s \( t \)-test, \( P<0.05 \)). The \( R_c \) was 5.2%, 5.4%, and 5.1% higher than \( R_c^* \), and GPP was 4.5%, 4.7%, and 4.4% higher than GPP \( ^* \) from 2003 to 2005, respectively, owing to daytime canopy light inhibition.

### Table 3. Parameter values of \( a \) and \( b \) in the functions listed in equation (5) for leaf respiration in light \( (R_L) \), and photosynthetically active radiation \( (Q) \) of four dominant tree species \( (P. koraiensis, T. amurensis, F. mandshurica, \) and \( A. mono) \).

| Species           | Pinus koraiensis | Tilia amurensis | Fraxinus mandshurica | Acer mono |
|-------------------|------------------|-----------------|----------------------|-----------|
| \( a \)           | 0.6672           | 0.7832          | 0.8808               | 0.8811    |
| \( b \)           | −0.0043          | −0.0032         | −0.0039              | −0.0031   |

doi:10.1371/journal.pone.0113512.t003
Figure 2. Variation in the ratio of $R_L$ to $R_D$ for four main tree species ($P. koraiensis$, $T. amurensis$, $F. mandshurica$, and $A. mono$) with different photosynthetically active radiation ($Q$) values. All relationships were significant at $P<0.05$.

doi:10.1371/journal.pone.0113512.g002
Discussion

4.1 Leaf respiration reduction by light

Leaf-level $R_L/R_D$ declined exponentially with increasing $Q$, indicating that $R_L$ is inhibited by light. Although this type of inhibition has been widely reported, the reason remains unclear [8, 34–35]. Some studies suggest that light inhibition is merely a phenomenon caused by internal CO$_2$ re-fixation [36–37]. More studies have shown that leaf dark respiration is clearly inhibited by light [38–42]. This has been attributed to increased amounts of ATP and NADPH in response to increasing $Q$ levels [38], and the inhibition of respiratory enzymes (pyruvate dehydrogenase and isocitrate dehydrogenase) with increasing ATP and NADPH levels [4, 39–42]. In addition, leaf respiration in light was lower than in darkness even if the refixed respiratory CO$_2$ was considered [43]. Further, the Krebs cycle, one of main metabolic pathways of leaf dark respiration, was reduced by 95% in light [13]. The mechanism causing light inhibition of respiration is likely related to inhibition of specific enzymes but further investigation of the mechanism is beyond the scope of this work.

4.2 Effect of environmental factors on leaf respiration and GPP estimation

Light inhibition of $R_L$ ranged from 10% to 98% in this study, which reflects $R_L$ variation in the different species and developmental stages. In addition, $R_L$ and the effect of light inhibition on this variable vary with environmental factors, for example, light inhibition of $R_L$ decreased with increasing nitrogen availability [44–45], increasing temperature [8, 46], and under elevated CO$_2$ [45, 47] and well-watered conditions [48]. These studies indicate that research focusing on the effect...
Figure 4. Seasonal trends in monthly canopy respiration rate per unit ground area with and without light inhibition correction from 2003 to 2005.

doi:10.1371/journal.pone.0113512.g004
of light inhibition on leaf respiration under different environmental conditions has increased in recent years. GPP overestimation was quantified based on the light inhibition of $R_L$, which was determined by the ecosystem species composition and LAI at certain developmental stages in the current study. Environmental factors such as temperature, precipitation, ambient CO$_2$ concentration, and nitrogen deposition were not taken into account in the present study; nevertheless, a basic and important method to quantify the GPP overestimation is provided. Based on this study, the GPP overestimation can be evaluated more accurately if the above-mentioned environmental factors are considered.

4.3 Effect of canopy structure on light inhibition and GPP estimation

Leaf area distribution within the canopy changes as forests grow and develop. GPP overestimation in this study (4.6%) was lower than that (>20%) in an old beech forest ecosystem [16]. The discrepancy may result from the in-canopy Q value, which was affected by LAI. In this study, the entire canopy was divided into multiple layers, which avoided the overestimation of light on leaves and consequently, the decrease in $R_c$ also avoided the overestimation. In a study conducted by Bruhn et al. (2011), the Q value above the canopy was used to represent the value of the entire canopy, ignoring light attenuation in the canopy [13]. The $R_{ail}/R_{aD}$ value increased with increasing LAI at each Q level (Fig. 6). The results indicate that LAI is an important factor that affects canopy-level respiration reduction in light. In comparison, small reductions in $R_c$ may occur in ecosystems characterized by high LAI values as a result of the self-shading effect on Q.

In addition, canopy structure impacts the canopy respiration ratio (as a component of ecosystem respiration), which may affect GPP overestimation. Model simulation results suggest that GPP overestimation in our study was approximately 4.6%. The GPP overestimation value reported by Wohlfahrt et al. (2005) was 11–13% [17] under a high light inhibition scenario which is similar to the light inhibition used in our study. In the present study, the forest ecosystem $R_c/R_{a}$ was 29%, compared with 42% in a mountain meadow ecosystem [17]. Differences in the $R_c/R_{a}$ values may result in the discrepancy in GPP overestimation between these two ecosystems. The variation in the discrepancy between GPP and GPP’ increased exponentially with increasing $R_c/R_{a}$ values (Table 4).

| Year | $R_c$ | $R_c^*$ | $R_c^*/R_{a}$ | $R_{aD}$ | $R_{aL}$ | NEE | $R_{st}$ | $R_s$ | $R_e$ | $R_e^*$ | $R_e^*/R_{a}$ | GPP | GPP’ | GPP/\text{GPP’} |
|------|-------|---------|----------------|---------|---------|-----|---------|-------|-------|---------|----------------|-----|-------|----------------|
| 2003 | 361.08 | 300.89  | 83.3%          | 256.71  | 593.65  | –188.54 | 1211.44 | 1151.25 | 127.52 | 1399.98 | 1339.79 | 95.6% |
| 2004 | 376.22 | 312.25  | 83.0%          | 267.47  | 606.44  | –168.08 | 1250.13 | 1186.16 | 116.46 | 1418.21 | 1354.24 | 95.5% |
| 2005 | 358.89 | 297.70  | 83.0%          | 255.15  | 638.99  | –185.49 | 1253.03 | 1191.84 | 91.37  | 1438.52 | 1377.33 | 95.8% |

Table 4. Cumulative annual gross primary production and its components with (indicated by ‘*’) and without light inhibition correction (GPP, gross primary production; $R_c$, canopy leaf respiration; $R_{aD}$, stem respiration; $R_{aL}$, soil respiration; $R_e$, ecosystem respiration; and NEE, net ecosystem CO$_2$ exchange, all units of GPP and its components with and without correction are g C m$^{-2}$ year$^{-1}$).

doi:10.1371/journal.pone.0113512.t004
A greater amount of leaf respiration will be ignored if the effect of light inhibition is not taken into account in ecosystems with a high \( R_c/R_e \) value. Based on the above discussion, large reductions in GPP may be expected in ecosystems with high proportions of canopy respiration relative to ecosystem respiration, such as a Finland agricultural ecosystem where the \( R_c/R_e \) value reached 50% [49]. In contrast, there is a lower GPP reduction in ecosystems with lower \( R_c/R_e \) values, such as a mixed temperate forest with an \( R_c/R_e \) value of 31% to 33% [30, 50], and other forest ecosystems where \( R_c/R_e \) was less than 31% [11, 51–53].

### 4.4 Impact of leaf respiration differences between seedlings and trees on GPP estimation

The measurements of mature trees are usually conducted on cut branches because of the difficulty associated with direct measurements. In this study, leaf respiration
of seedlings were used as a proxy for leaf respiration of trees because of the long duration required when estimating leaf respiration in light when using the Laisk method. It was not feasible to measure leaf dark respiration in vitro in mature trees because detached leaves do not maintain their physiological activity because the water supply is disrupted. However, numerous studies have indicated that many biological processes change with increases in tree age [54–56]. Leaf respiration and the effect of light inhibition on leaf respiration may differ between seedlings and mature trees. The accuracy of results may be affected should this difference be ignored. Therefore, differences in leaf respiration characteristics between seedlings and trees should be analyzed in future studies.
Conclusions
Increasing attention is being given to understand light inhibition on leaf respiration, but few studies have quantified the impact of light inhibition on GPP estimation. Our results demonstrated that inhibition of leaf respiration during the day is species-specific. Light inhibition of leaf respiration increased exponentially with increasing light intensity for the dominant tree species of a mixed mature temperate forest in northeast China. Canopy respiration and GPP were overestimated across the three years of the study by approximately 20.4% and 4.6%, respectively, when leaf respiration reduction in light was ignored. It is important that leaf respiration reduction by light is taken into account when estimating the GPP of the ecosystem with high LAI. In the present study, numerous factors that may influence leaf respiration reduction were considered to provide accurate GPP estimates. Therefore, this study provides important methodological approaches that can be applied to other ecosystems with different species. Collectively, these results are vital to make predictions about how leaf respiration reduction by light will impact ecosystem carbon measurements.

Supporting Information
Table S1. Nomenclature.
doi:10.1371/journal.pone.0113512.s001 (DOCX)

Appendix S1. A brief description of the model of solar radiation transmission through the canopy.
doi:10.1371/journal.pone.0113512.s002 (DOCX)

Acknowledgments
This study was financially supported by the National Science Foundation of China (no. 41375119), and the State Key Laboratory of Forest and Soil Ecology (No. LFSE2013-11). The authors are deeply grateful to the staff of the National Forest Ecosystem Research Station of Changbai Mountain for their assistance in the maintenance of instruments and collection of field data.

Author Contributions
Conceived and designed the experiments, JS DG F. Yao. Performed the experiments, JS JW F. Yuan. Analyzed the data, JS DG F. Yao. Contributed reagents/materials/analysis tools, F. Yuan AW CJ. Wrote the manuscript, JS JW.

References
1. Atkin OK, Scheurwater I, Pons TL (2007) Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures. New Phytol 174(2): 367–380.
2. Poorter H, Remkes C, Lambers H (1990) Carbon and nitrogen economy of 24 wild species differing in relative growth rate. Plant Physiol 94(2): 621–627.

3. Ryan MG, Hubbard RM, Clark DA, Sanford Jr RL (1994) Woody-tissue respiration for Simarouba amara and Minquartia guianensis, two tropical wet forest trees with different growth habits. Oecologia 100(3): 213–220.

4. Graham D (1980) Effects of light on “dark” respiration. Academic Press, New York The biochemistry of plants 2: 525–579.

5. Kirschbaum MU, Farquhar GD (1987) Investigation of the CO₂ dependence of quantum yield and respiration in Eucalyptus pauciflora. Plant Physiol 83(4): 1032–1036.

6. Avelange MH, Thiéry JM, Sarrey F, Gans P, Rébéillé F (1991) Mass-spectrometric determination of O₂ and CO₂ gas exchange in illuminated higher-plant cells. Planta 183(2): 150–157.

7. Nunes-Nesi A, Sweetlove LJ, Fernie AR (2007) Operation and function of the tricarboxylic acid cycle in the illuminated leaf. Physiol Plantarum 129(1): 45–56.

8. Heskel MA, Atkin OK, Turnbull MH, Griffin KL (2013) Bringing the Kok effect to light: a review on the integration of daytime respiration and net ecosystem exchange. Ecosphere 4: art98.

9. Barford CC, Wofsy SC, Goulden ML, Munger JW, Pyle EH, et al. (2001) Factors controlling long-and short-term sequestration of atmospheric CO₂ in a mid-latitude forest. Science 294(5547): 1688–1691.

10. Granier A, Ceschia E, Damesin C, Dufrêne E, Epron D, et al. (2000) The carbon balance of a young beech forest. Funct Ecol 14(3): 312–325.

11. Janssens IA, Lankreijer H, Matteucci G, Kowalski AS, Buchmann N, et al. (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biol. 7(3): 269–278.

12. Valentini R, Matteucci G, Dolman AJ, Schulze E-D, Rebmann C, et al. (2000) Respiration as the main determinant of carbon balance in European forests. Nature 404: 861–865.

13. Tcherkez G, Cornic G, Bligny R, Gout Elizabeth, Ghashghaie J (2005) In vivo respiratory metabolism of illuminated leaves. Plant Physiol 138(3): 1596–1606.

14. Villar R, Held AA, Merino J (1994) Comparison of methods to estimate dark respiration in the light in leaves of two woody species. Plant Physiol 105(1): 167–172.

15. Amthor JS, Baldocchi DD (2001) Terrestrial higher plant respiration and net primary production. Terrestrial global productivity, Academic Press 33–59.

16. Bruhn D, Mikkelsen TN, Herbst M, Kutsch WL, Ball MC, et al. (2011) Estimating daytime ecosystem respiration from eddy-flux data. Biosystems 103(2): 309–313.

17. Wohlfahrt G, Bahn M, Haslwanter A, Newesely C, Cernusca A (2005) Estimation of daytime ecosystem respiration to determine gross primary production of a mountain meadow. Agr Forest Meteorol 130(1): 13–25.

18. Davidson EA, Janssens IA, Luo Y (2006) On the variability of respiration in terrestrial ecosystems: moving beyond Q₁₀. Global Change Biol 12: 154–164.

19. Tingeý DT, Lee EH, Phillips DL, Rygiewicz PT, Waschmann RS, et al. (2007) Elevated CO₂ and temperature alter net ecosystem C exchange in a young Douglas fir mesocosm experiment. Plant Cell Environ 30(11): 1400–1410.

20. Villar R, Held AA, Merino J (1995) Dark leaf respiration in light and darkness of an evergreen and a deciduous plant species. Plant Physiol 107(2): 421–427.

21. Ross IU (1981) The radiation regime and architecture of plant stands. 3. Junk Publishers, The Hague.

22. Brooks A, Farquhar GD (1985) Effect of temperature on the CO₂/O₂ specificity of ribulose-1, 5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Planta 165(3): 397–406.

23. Guan DX, Wu JB, Zhao XS, Han SJ, Yu GR, et al. (2006) CO₂ fluxes over an old, temperate mixed forest in northeastern China. Agr Forest Meteorol 137(3–4): 136–149.

24. Wilczak JM, Oncley SP, Stage SA (2001) Sonic anemometer tilt correction algorithms. Bound-Lay Meteorol 99(1): 127–150.
25. Webb EK, Pearman GI, Leuning R (1980) Correction of flux measurements for density effects due to heat and water vapour transfer. Q J Roy Meteor Soc 106(447): 85–100.

26. Moore CJ (1986) Frequency response corrections for eddy correlation systems. Bound-Lay Meteorol 37(1-2): 17–35.

27. Foken T, Wichura B (1996) Tools for quality assessment of surface-based flux measurements. Agr Forest Meteorol 78(1): 83–105.

28. Falge E, Baldocchi D, Olson R, Anthoni P, Aubinet M, et al. (2001) Gap filling strategies for defensible annual sums of net ecosystem exchange. Agr Forest Meteorol 107(1): 43–69.

29. Wu JB, Guan DX, Wang M, Pei TF, Han SJ, et al. (2006) Year-round soil and ecosystem respiration in a temperate broad-leaved Korean Pine forest. Forest Ecol Manag 223(1–3): 35–44.

30. Wang M, Guan DX, Han SJ, Wu JL (2010) Comparison of eddy covariance and chamber-based methods for measuring CO2 flux in a temperate mixed forest. Tree Physiol. 30(1): 149–163.

31. Leuning R (1995) A critical appraisal of a combined stomatal-photosynthesis model for C3 plants. Plant Cell Environ 18(4): 339–355.

32. Shi TT, Guan DX, Wang Anzhi, Wu JB, Yuan FH, et al. (2010) Modeling canopy CO2 and H2O exchange of a temperate mixed forest. J Geophys Res 115(D17) (D17117).

33. Spitters CJT (1986) Separating the diffuse and direct component of global radiation and its implications for modeling canopy photosynthesis Part II. Calculation of canopy photosynthesis. Agr Forest Meteorol 38(1): 231–242.

34. Yin XY, Sun ZP, Struik PC, Gu JF (2011) Evaluating a new method to estimate the rate of leaf respiration in the light by analysis of combined gas exchange and chlorophyll fluorescence measurements. J Exp Bot 62(10): 3489–3499.

35. Hoefnagel MH, Atkin OK, Wiskich JT (1998) Interdependence between chloroplasts and mitochondria in light and the dark. BBA-Bioenergetics 1366(3): 235–255.

36. Loreto F, Velikova V, Di Marco G (2001) Respiration in the light measured by 12CO2 emission in 13CO2 atmosphere in maize leaves. Aust J Plant Physiol 28(11): 1103–1108.

37. Pinelli P, Loreto F (2003) 12CO2 emission from different metabolic pathways measured in illuminated and darkened C3 and C4 leaves at low, atmospheric and elevated CO2 concentration. J Exp Bot 54(388): 1761–1769.

38. Turpin DH, Weger HG (1990) Interactions between photosynthesis, respiration and N assimilation. In Dennis DT, Turpin DH (eds) Plant physiology, Biochemistry and Molecular Biology. Longman Scientific and Technical, Harlow, UK: 422–433.

39. Gemel J, Randall DD (1992) Light Regulation of Leaf Mitochondrial Pyruvate Dehydrogenase Complex Role of Photosynthetic Carbon Metabolism. Plant Physiol. 100(2): 908–914.

40. Holness MJ, Sugden MJ (2003) Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. Biochem Soc T. 31(6): 1143–1151.

41. Igamberdiev AU, Gardestrom P (2003) Regulation of NAD- and NADP-dependent isocitrate dehydrogenases by reduction levels of pyridine nucleotides in mitochondria and cytosol of pea leaves. BBA-Bioenergetics 1606(1): 117–125.

42. Kasimova MR, Grigiena J, Krab K, Hagedorn PH, Flyvbjerg H, et al. (2006) The free NADH concentration is kept constant in plant mitochondria under different metabolic conditions. The Plant Cell 18(3): 698–698.

43. Pärnik T, Keerberg O (1995) Decarboxylation of primary and end products of photosynthesis at different oxygen concentrations. J Exp Bot 46: 1439–1477.

44. Heskel MA, Anderson OR, Atkin OK, Turnbull MH, Griffin KL (2012) Leaf-and cell-level carbon cycling responses to a nitrogen and phosphorus gradient in two arctic tundra species. Am J Bot 99(10): 1702–1714.

45. Shapiro JB, Griffin KL, Lewis JD, Tissue DT (2004) Response of Xanthium strumarium leaf respiration in the light to elevated CO2 concentration, nitrogen availability and temperature. New phytol 162(2):377–386.
46. Zaragoza-Castells J, Sánchez-GÓmez D, Valladares F, Hurry V, Atkin OK (2007) Does growth irradiance affect temperature dependence and thermal acclimation of leaf respiration? Insights from a Mediterranean tree with long-lived leaves. Plant Cell Environ 30: 820–833.

47. Wang XZ, Lewis JD, Tissue DT, Seemann JR, Griffin KL (2001) Effects of elevated atmospheric CO₂ concentration on leaf dark respiration of *Xanthium strumarium* in light and in darkness. P Natl Acad SCI USA 98(5): 2479–2484.

48. Crous KY, Zaragoza-Castells J, Ellsworth DS, Duursma RA, Löw M, et al. (2012) Light inhibition of leaf respiration in field-grown *Eucalyptus saligna* in whole-tree chambers under elevated atmospheric CO₂ and summer drought. Plant Cell Environ 35(5): 966–981.

49. Lohila A, Aurela M, Regina K, Laurila T (2003) Soil and total ecosystem respiration in agricultural fields: effect of soil and crop type. Plant Soil 251(2): 303–317.

50. Yuste JC, Nagy M, Janssens IA, Ceulemans R (2005) Soil respiration in a mixed temperate forest and its contribution to total ecosystem respiration. Tree Physiol 25(5): 609–619.

51. Davidson EA, Richardson AD, Savage KE, Hollinger DY (2006) A distinct seasonal pattern of the ratio of soil respiration to total ecosystem respiration in a spruce-dominated forest. Global Change Biol 12(2): 230–239.

52. Guidolotti G, Rey A, D’Andrea E, Matteucci G, Angelis PD (2013) Effect of environmental variables and stand structure on ecosystem respiration components in a Mediterranean beech forest. Tree Physiol 33(9): 960–972.

53. Law BE, Ryan MG, Anthoni PM (1999) Seasonal and annual respiration of a ponderosa pine ecosystem. Global Change Biol 5(2): 169–182.

54. Kolb T, Frederiksen T, Steiner K, Skelly J (1997) Issues in scaling tree size and age responses to ozone: a review. Environ Pollut 98(2): 195–208.

55. Thomas SC, Winner WE (2002) Photosynthetic differences between saplings and adult trees: an integration of field results by meta-analysis. Tree Physiol 22(2–3): 117–127.

56. Yoder B, Ryan M, Waring R, Schoettle A, Kaufmann M (1994) Evidence of reduced photosynthetic rates in old trees. Forest Sci 40(3): 513–527.

57. Goudriaan J, Laar HHV (1994) Modelling crop growth processes. Kluwer Amsterdam Press, 341–347.

58. Goudriaan J (1977) Crop micrometeorology: a simulation study. Pudoc, Center for Agricultural Publishing and Documentation.

59. Goudriaan J, Laar HHV (1994) Modelling potential crop growth processes: textbook with exercises, 2. Kluwer Academic Pub.