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Short- and long-term responses of leaf day respiration to elevated atmospheric CO$_2$

Running title: CO$_2$ response of leaf respiration in the light

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The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plphys/pages/General-Instructions) is Xiao Ying Gong.
AUTHOR CONTRIBUTIONS
X.Y.G. designed and planned the research; Y.R.S. and Y.N.X. performed the experiment, Y.R.S. and W.T.M. analyzed the data and wrote the first draft, and all authors discussed the results and implications and contributed to the revision.

ABSTRACT
Evaluating leaf day respiration rate ($R_L$), which is believed differ from that in the dark ($R_{Dk}$), is essential for predicting global carbon cycles under climate change. Several studies have suggested that atmospheric CO$_2$ impacts $R_L$. However, the magnitude of such an impact and associated mechanisms remain uncertain. To explore the CO$_2$ effect on $R_L$, wheat ($Triticum aestivum$) and sunflower ($Helianthus annuus$) plants were grown under ambient (410 ppm) and elevated (820 ppm) CO$_2$ mole fraction ([CO$_2$]). $R_L$ was estimated from combined gas exchange and chlorophyll fluorescence measurements using the Kok method, the Kok-Phi method, and a revised Kok method (Kok-$C_c$ method). We found that elevated growth [CO$_2$] led to an 8.4% reduction in $R_L$ and a 16.2% reduction in $R_{Dk}$ in both species, in parallel to decreased leaf N and chlorophyll contents at elevated growth [CO$_2$]. We also looked at short-term CO$_2$ effects during gas exchange experiments. Increased $R_L$ or $R_L/R_{Dk}$ at elevated measurement [CO$_2$] were found using the Kok and Kok-Phi methods, but not with the Kok-$C_c$ method. This discrepancy was attributed to the unaccounted changes in $C_c$ in the former methods. We found that the Kok and Kok-Phi methods underestimate $R_L$ and overestimate the inhibition of respiration under low irradiance conditions of the Kok curve, and the inhibition of $R_L$ was only 6%, representing 26% of the apparent Kok effect. We found no significant long-term CO$_2$ effect on $R_L/R_{Dk}$, originating from concurrent reduction in $R_L$ and $R_{Dk}$ at elevated growth [CO$_2$], and likely mediated by acclimation of nitrogen metabolism.

Keywords: leaf day respiration, Kok method, atmospheric CO$_2$, photosynthesis, climate change, carbon balance, mesophyll conductance, chlorophyll fluorescence
INTRODUCTION

Terrestrial vegetation assimilates ca. 120 pg carbon via photosynthesis but releases about half of assimilated carbon via respiration (Gifford, 2003; Dusenge et al., 2019). The balance between plant respiration and photosynthesis is therefore essential for plant productivity and global carbon balance. Despite considerable variations depending on N fertilisation and climatic conditions, the ongoing increase in atmospheric CO₂ mole fraction ([CO₂]) promotes leaf photosynthesis and primary production, which is referred to as the “CO₂ fertilization effect” (Drake et al., 1997; Cramer et al., 2001). Although the CO₂ fertilization effect on biomass (but not necessarily yield) is evident from greenhouse and field experiments (Ainsworth and Long, 2005; Norby et al., 2005; Walker et al., 2021), the response of plant respiration to [CO₂] is rather uncertain, limiting our ability to predict future climate change-driven modifications of plant physiology.

The respiratory response is complicated by the fact that leaf respiration takes place not only in darkness (the respiration rate is denoted as $R_{Dk}$), but also in the light. In illuminated leaves, respiration is referred to as "respiration in the light" or "day respiration" (denoted as $R_L$; here we refer to CO₂ evolution rather than O₂ consumption). Leaf respiration has been shown to be partially inhibited by the light although the magnitude of inhibition varies broadly, with reported $R_L/R_{Dk}$ values ranging from 0.2 to 1.3 (Ayub et al., 2011; Griffin and Turnbull, 2013; Crous et al., 2017; Gong et al., 2018; Way et al., 2019). Given the longer light periods during the growing season and higher temperature during the day than at night in most ecosystems, $R_L$ is a key component of plant- and community-scale carbon budgets (Atkin et al., 2007; Gong et al., 2017). Experimental results revealed that the inhibition of respiration by light (i.e., 1-$R_L/R_{Dk}$) also occurs at the stand scale (Gong et al., 2017). Neglecting respiration inhibition might have led to considerable errors in estimated gross primary production (Wehr et al., 2016; Gong et al., 2017). Furthermore, the response of $R_L$ to environmental cues are essential to predict carbon balance, carbon use efficiency and improve land surface models (Wehr et al., 2016; Atkin et al., 2017; Tcherkez et al., 2017b; Keenan et al., 2019).

So far, there is no consensus on the response of $R_L$ to long-term [CO₂] increase. Some studies have shown that $R_L$ is stimulated by elevated growth [CO₂] (Wang et al., 2001; Shapiro et al., 2004; Crous et al., 2012; Griffin and Turnbull, 2013), and this effect may be related to higher carbohydrate concentrations in leaves (Rogers et al., 2004; Gong et al., 2017). Also, increased
leaf respiration at elevated $[\text{CO}_2]$ has been suggested to be associated with a larger mitochondrial number per mesophyll cell (Griffin et al., 2001), indicating cellular and transcriptional (gene regulation) mechanisms of respiratory control (Leakey et al., 2009). Other studies have reported a decrease in $R_L$ in plants grown under elevated $[\text{CO}_2]$ compared with that grown under ambient $[\text{CO}_2]$ (Ayub et al., 2011; Ayub et al., 2014).

The decrease in $R_L$ at elevated $[\text{CO}_2]$ has been suggested to be linked to either photorespiration or nitrogen metabolism. Under elevated CO$_2$, there is a reduction in photorespiration rate (and the rate of oxygenation of RuBP, $v_o$), and this could cause an alteration in $R_L$, as suggested by results obtained on short-term changes in respiratory metabolism under varying CO$_2$ mole fraction. In effect, using $^{13}\text{C}$-enriched substrates to trace decarboxylation processes, Tcherkez et al. (2008) found that decarboxylation decreased when leaves were exposed to elevated $[\text{CO}_2]$ for short periods. Likewise, results obtained using the Kok method suggested there was a linear relationship between photorespiration rate and $R_L$ (Griffin and Turnbull, 2013). However, the mechanism behind this relationship is still unclear. In particular, the Kok effect itself has been shown not to be fully caused by changes in respiration rate (Gauthier et al., 2020) and thus, the relationships between photorespiration and Kok method-based $R_L$ are presently uncertain. In addition, $R_L$ has been reported to either decrease (Pinelli and Loreto, 2003; Tcherkez et al., 2008; Griffin and Turnbull, 2013), increase (Yin et al., 2020; Fang et al., 2022) or remain unaffected (Sharp et al., 1984; Tcherkez et al., 2012), in the short-term using gas-exchange experiments at elevated $[\text{CO}_2]$. Thus, conclusions drawn from short-term changes in $R_L$ caused by instantaneous elevation of $[\text{CO}_2]$ might not be relevant to long-term changes in $R_L$.

The decrease of $R_L$ at elevated $[\text{CO}_2]$ has also been suggested to be linked to nitrogen metabolism. It has been observed in many free air CO$_2$ enrichment (FACE) experiments that elevated $[\text{CO}_2]$ reduces leaf N content, which is accompanied by a down-regulation of photosynthetic capacity (Long et al., 2004; Ainsworth and Long, 2005). It is believed that elevated $[\text{CO}_2]$ inhibits N assimilation in leaves via the potential link between photorespiration and nitrate assimilation (Bloom et al., 2010; Bloom et al., 2014; Busch et al., 2018). Given that N assimilation in leaves is energy demanding and thus a driving factor for leaf respiration (Amthor, 2000; Reich et al., 2008), it would be important to know whether $[\text{CO}_2]$ affected $R_L$ and $R_{Dk}$ via
leaf N content. All in all, the response of $R_L$ to elevated $[CO_2]$ appears to be highly variable and mechanisms behind are unclear.

Another uncertainty associated with $R_L$ and how it varies is technological. In fact, there are several methods to estimate $R_L$, but none of them can measure $R_L$ directly (for a review see (Tcherkez et al., 2017b)). The Kok method (Kok, 1949) and the Laik method (Laisk, 1977), the two most commonly used methods, require manipulation of net $CO_2$ assimilation rates ($A$) at low irradiances ($I_{inc}<150 \mu mol \, m^{-2} \, s^{-1}$) (Kok) or low $CO_2$ (Laisk). Another method, the $^{13}C$ isotopic disequilibrium method, uses two $CO_2$ sources with different $\delta^{13}C$ values to disentangle $R_L$ and photosynthesis under physiologically relevant environmental conditions without the need to manipulate $A$ (Gong et al., 2015; Gong et al., 2018). The $^{13}C$ disequilibrium method is valuable since it does not require the use of low irradiance or low $CO_2$ and can be performed at any $CO_2$ mole fraction, and therefore, is suitable to study $CO_2$ effects on $R_L$. It is, however, technically demanding (isotopic $CO_2$ sources, mass spectrometers). The Laik method is, by definition, not suitable for studying $CO_2$ effects because it manipulates $[CO_2]$ at sub-ambient levels. So far, the response of $R_L$ to $[CO_2]$ has mainly been estimated using the Kok method. However, as mentioned above, the Kok method has been questioned since the Kok effect is not exclusively caused by a decrease in respiration rates (Gauthier et al., 2020). Several studies showed that the Kok method has conceptual uncertainties (Farquhar and Busch, 2017; Tcherkez et al., 2017a; Tcherkez et al., 2017b; Yin et al., 2020). First, the Kok method assumes a constant photochemical efficiency of PS II ($\Phi_2$) along the $A$-$I_{inc}$ curve (i.e. the Kok curve, see Theory). To address this issue, Yin et al. (2009) suggested to use measured $\Phi_2$ to improve the $R_L$ estimation. Second, the Kok method usually disregards variation in chloroplastic $[CO_2]$ ($C_c$) along the $A$-$I_{inc}$ curve, which could bias the estimates of $R_L$ according to recent studies based on model analysis (Farquhar and Busch, 2017; Yin et al., 2020). Estimating $C_c$ along the $A$-$I_{inc}$ curve requires measurements of mesophyll conductance ($g_m$). Measuring $g_m$ is challenging and this is particularly true when measurements are performed at low irradiance (Pons et al., 2009; Gu and Sun, 2014; Gong et al., 2015). So far, the uncertainty associated with $C_c$ has not been fully solved.

Taken as a whole, neither long-term nor short-term responses of $R_L$ to $CO_2$ mole fraction are well-known, and technologies used to measure $R_L$ may be problematic. Here, we intend to address the following questions: 1) how do short-, medium- and long-term $CO_2$ enrichment
affect $R_L$ in C$_3$ leaves? 2) do the original- and revised Kok methods provide similar estimations of $R_L$? To this end, we combine gas exchange and chlorophyll fluorescence (ChF) measurements to study the response of $R_L$ of wheat (*Triticum aestivum* L.) and sunflower (*Helianthus annuus* L.) plants grown under ambient (410 ppm) and elevated [CO$_2$] (820 ppm). We assessed the medium-to-long term CO$_2$ response (days to months) by comparing parameters of plants at different growth [CO$_2$], and the short-term CO$_2$ response (minutes) by measuring the same leaves at 410 and 820 ppm of [CO$_2$]. We compared $R_L$ estimated by the Kok method, the Yin method (i.e. the Kok-Phi method) and a revised Kok method (i.e. the Kok-$C_c$ method) which takes the influence of $\Phi_2$ and $C_c$ into account.

**RESULTS**

**Effects of growth CO$_2$ on photosynthetic parameters and leaf traits**

Growth at elevated [CO$_2$] led to a reduction in net CO$_2$ assimilation ($A$) for both species, when $A$ values were compared at the same intercellular CO$_2$ concentration ($C_i$) levels (Fig. 1AB). Sunflower plants grown at elevated [CO$_2$] exhibited lower $E$ and $g_{sw}$ compared with that grown at ambient CO$_2$ (Fig. 1DF). This effect on water vapour exchange was minor in wheat (Fig. 1CE). In order to assess the long-term growth CO$_2$ effect on common grounds, gas exchange parameters of leaves were compared at their respective growth [CO$_2$] (indicated by the subscript ‘growth’). Net CO$_2$ assimilation rate ($A_{growth}$), intrinsic water-use efficiency ($iWUE_{growth}$) and leaf carbon-use efficiency ($CUE_L$) of plants grown under elevated [CO$_2$] were significantly higher than those of plants grown under ambient [CO$_2$] in both species (Table 1). Averaged across species, growth at elevated [CO$_2$] led to 5.6% reduction in $A_{max}$, 7.9% reduction in $V_{cmax}$, and 8.0% in $J$, indicating a decline in photosynthesis capacity. The ratio of $g_{sc}$ to $g_m$ was not significantly affected by growth [CO$_2$] or species. $R_{Dk}$ of both species was lower at elevated [CO$_2$] but this decrease differed between species (20% for wheat and 11% for sunflower).

Leaf chlorophyll content was significantly lower at elevated [CO$_2$] compared with ambient [CO$_2$]. Similarly, elevated [CO$_2$] led to 6.7% reduction (averaged across species) in nitrogen elemental content (N%) and 12% reduction in nitrogen content per surface area (N$_{area}$) on average, but the effect of CO$_2$ was not significant at a $p$-level of 0.05. SLA was significantly different between species but not affected by growth [CO$_2$] (Table 1).
CO₂ response of Rₗ estimated by different methods

Φ₂, Cᵦ, and γ, the key parameters associated with assumptions in both original and revised Kok methods, were found to decrease along the Kok curve in all species and treatments (Fig. 2). With the increase of Iinc from 40 to 100 μmol m⁻² s⁻¹, Φ₂ decreased by 3.1% for wheat and 2.4% for sunflower and this trend was not substantially influenced by growth [CO₂] (long term effect) and measurement [CO₂] (short-term effect). A short-term CO₂ effect on γ was detected, i.e., γ decreased more strongly at measurement [CO₂] of 410 ppm (by 5.2%) than that at measurement [CO₂] of 820 ppm (by 3.0%, averaged across species) with the increase in Iinc (Fig. S1). That is, under our conditions, terms (γfₐₐₚΦ₂ρ₂α) in Eqn 3 and (γfₐₐₚρ₂α) in Eqn 4 were not constant along a Kok curve, causing errors in Rₗ estimated by the Kok and the Kok-Phi method, respectively.

Applying the Kok, the Kok-Phi and the Kok-Cᵦ method, A was plotted against Iinc, Φ₂Iinc, and γΦ₂Iinc, respectively (Fig. 3). Both growth [CO₂] and measurement [CO₂] had impacts on A-Iinc and A-Φ₂Iinc response curves (Fig. 3A-D). As a result, growth at elevated [CO₂] led to a significant decrease in RₗKok and RₗKok-Phi. The same was true for RₗKok-Cᵦ on average but it was only significant with a p-value of 0.06 (Fig. 4, Table 2). There was a clear, although statistically insignificant (p>0.05), tendency for elevated measurement [CO₂] to increase both RₗKok and RₗKok-Phi (Fig. 3 and Fig. 4) in both species. By contrast, A-γΦ₂Iinc curves obtained under different measurement [CO₂] seemed to coincide perfectly (Fig. 3EF), in agreement with the insignificant effect of measurement [CO₂] on RₗKok-Cᵦ.

CO₂ response of Rₗ/RDk estimated by different methods

We found no significant long-term CO₂ effect on Rₗ/RDk estimated via all three methods (Table 3). There is a tendency that Rₗ/RDk of wheat increased with the growth [CO₂] for all three methods (comparing acO₂-410 and eCO₂-820), while that tendency was not found in sunflower. That is, the long-term CO₂ effect on Rₗ/RDk is not conclusive. Under elevated measurement [CO₂], significant increases in RₗKok/RDk and RₗKok-Phi/RDk were observed, but this short-term response was not observed using the Kok-Cᵦ method (Fig. 5). These results indicate that short-term CO₂ effect on RₗKok and RₗKok-Phi could result from a technical bias simply due to neglecting the change in Cᵦ along the Kok curve.

When pooling all data across species and treatments together, RDk was positively correlated to RₗKok (r²=0.82, p<0.05), RₗKok-Phi (r²=0.82, p<0.05) and RₗKok-Cᵦ (r²=0.77, p<0.05) (Fig. 6).
These linear regressions yielded an average $R_{L, Kok}/R_{Dk}$ of $0.78\pm0.04$ (SE), $R_{L, Kok-Phi}/R_{Dk}$ of $0.85\pm0.04$, and $R_{L, Kok-Cc}/R_{Dk}$ of $0.94\pm0.04$. That is, the Kok-Cc method showed a small light-induced inhibition of respiration, of 6% only, thus much lower than inhibition values from the other two methods (22%, 15%).

**DISCUSSION**

Growth at elevated CO$_2$ leads to reduction in $R_L$

This study showed that $R_L$ of plants grown at elevated [CO$_2$] was lower than that at ambient [CO$_2$], and this result was confirmed by all three methods: Kok, Kok-Phi and Kok-Cc. On average, elevated [CO$_2$] led to an 8.4% reduction in $R_L$, regardless of the method. This is in agreement with previous findings that leaf $R_L$ of plants grown at elevated [CO$_2$] is lower (Ayub et al., 2014) despite opposite findings (Wang et al., 2001, Shapiro et al., 2004).

Interestingly, although a long-term CO$_2$ effect on $R_L$ was evident, elevated [CO$_2$] had no influence on the $R_L/R_{Dk}$ ratio, because $R_{Dk}$ was also significantly lower at elevated [CO$_2$]. Similar to our study, there was no significant long-term CO$_2$ effect on $R_L/R_{Dk}$ in Sydney blue gum (Eucalyptus saligna) (Ayub et al., 2011; Crous et al., 2012). However, Wang et al. (2001), Shapiro et al. (2004) and (Gong et al., 2017) found that non proportional changes in $R_L$ and $R_{Dk}$ led to higher $R_L/R_{Dk}$ ratio in common cocklebur (Xanthium strumarium) leaves and sunflower stands grown at elevated [CO$_2$]. By contrast, $R_L/R_{Dk}$ was reduced by elevated growth [CO$_2$] in wheat because $R_L$ declined (Ayub et al., 2014) or $R_{Dk}$ increased (Griffin and Turnbull, 2013). Presumably, variations in the response to growth CO$_2$ between species and conditions might be linked to differences in nutrient content, metabolism, protein content, etc. which are all related to respiration.

The long-term response of $R_L$ to CO$_2$ is associated with changes in leaf N status

Leaf N has long been suggested to be a key parameter influencing respiration rate, and used to estimate leaf respiration in vegetation models (Atkin et al., 2017). In our study, the reduction in $R_L$ and $R_{Dk}$ was associated with a decrease in N$_{area}$ and chlorophyll content, suggesting that leaf N effectively drives the respiration rate. Nitrate reduction and maintenance of proteins are energy consuming (Wullschleger et al., 1997). Lower N content implies lower energy requirements and thus lower growth and maintenance respiration.
It has often been found in FACE or growth cabinet experiments that leaf N content was lower at elevated [CO₂]. This has been explained by different mechanisms. For example, elevated [CO₂] was shown to cause a decrease in stomatal conductance of leaves, leading to decreasing transpiration rates (Ainsworth and Rogers, 2007) and thus, lower transpiration-driven mass flow of soil N to roots and stems (so-called transpiration mechanism (McGrath and Lobell, 2013; Feng et al., 2015)). Another mechanism is associated with photorespiration. Generally N assimilation is believed to be lower due to lower photorespiration (Bloom et al., 2010), which is accompanied by the reduced reductant supplied via photorespiration at elevated [CO₂] (Taub and Wang, 2008). Furthermore, a ‘dilution effect’ could occur whereby N uptake does not increase proportionally to the increase of biomass at elevated [CO₂] (Feng et al., 2015).

The decreased leaf N content at elevated [CO₂] has also consequences on photosynthetic capacity (i.e. \( V_{cmax} \)). It was reported that species grown under elevated [CO₂] had lower maximum apparent carboxylation velocity (\( V_{cmax} \)) and carboxylation efficiency (Ainsworth and Long, 2005). Finally, elevated [CO₂] significantly increased CUE\(_L\) by enhancing photosynthetic rate and reducing dark respiration. Gong et al. (2017) reported that CUE of sunflower stands was higher at 200 ppm growth [CO₂] than that of 1000 ppm growth [CO₂]. This results thus could not be explained by the response of CUE\(_L\) itself since at the leaf level, CUE\(_L\) likely increased at elevated growth [CO₂]. We speculate that the reduction of whole plant CUE in their study was mainly due to enhanced respiration of heterotrophic organs or exudation.

**Changes in \( \Phi_2 \) and \( C_c \) are involved in the Kok effect and impact on \( R_L \) estimates**

Our study found a short-term CO₂ effect on \( R_L \) estimated using the Kok and Kok-Phi method, but no effect using the Kok-C\(_c\) method. In fact, both Kok and Kok-Phi methods showed an increase in \( R_L \) when measured at elevated [CO₂]. This short-term response was in agreement with the finding of Yin et al. (2020) and Fang et al. (2021), but is not supported by the findings of other studies (Tcherkez et al., 2008; Griffin and Turnbull, 2013). We believe discrepancies in short-term CO₂ effect on \( R_L \) is mostly associated with methodological differences. As shown in the *Theory* section, the classical Kok method has conceptual uncertainty with the assumption that \( \Phi_2 \) remains constant across the Kok curve. This assumption must be rejected as \( \Phi_2 \) decreases with increasing \( I_{inc} \) (Fig. 2). However, this short-term CO₂ effect on \( R_L \) cannot be explained by changes in \( \Phi_2 \) because (i) the decrease in \( \Phi_2 \) along Kok curve was similar at both measurement
[CO₂] and (ii) the effect persisted when the Kok-Phi method was used to account for variation in Φ₂.

Another assumption that has been made for both the Kok and Kok-Phi method is that γ (determined by Γ*/Cc) remains constant throughout the Kok curve. This assumption has also been challenged in recent model analyses (Buckley et al., 2017; Farquhar and Busch, 2017), but the question is how to quantify the change in Cc as this requires gm estimates. Here, we used species-specific gsc/gm ratios to calculate Cc, suggesting that Cc and γ decreased with increasing Iinc. Importantly, measurement CO₂ influenced the trend of γ with increasing Iinc, which might be the origin of this short-term CO₂ effect on RL Kok and RL Kok-Phi. When changes in γ (or Γ*/Cc) are accounted for, the apparent short-term effect of CO₂ on RL, as found with the Kok and Kok-Phi methods, became insignificant (see also Fig. 3, 4).

Kok- and Kok-Phi based estimates of RL suppression are overestimates

The inhibition of RL by light is supported by biochemical evidence. Utilizing ¹³C labelling, flux calculations suggest that decarboxylation rates associated to glucose catabolism and activation of malic enzyme increase with decreasing irradiance in the irradiance region where the Kok effect occurs (Gauthier et al., 2020). Recently, how much of the Kok effect is associated with respiration has been under debate (Farquhar and Busch, 2017; Gauthier et al., 2020; Yin et al., 2020). Indeed, the methods used in the present study show different levels of inhibition of respiration by light. The average RL/RDk was 0.74 for the Kok method, 0.81 for the Kok-Phi method and 0.93 for the Kok-Cc method. That is, the change in Φ₂, γ (or Γ*/Cc), and real light inhibition of RL explained c. 27, 46 and 27% of the apparent Kok effect (i.e. the apparent 26%-inhibition of RL found with the classical Kok method), respectively. This is in agreement with the results of previous model analyses which show that the Kok effect is not purely respiratory (Farquhar and Busch, 2017; Yin et al., 2020), and both the Kok method and the Kok-Phi method underestimated RL and overestimated the inhibition of RL (Yin et al., 2020).

The real light inhibition of RL (as revealed by the Kok-Cc method) was only 6%, which is close to the mean inhibition of 8% of several herbaceous species determined using the ¹³C disequilibrium method (Gong et al., 2018) and the mean inhibition of 10% in wheat leaves determined using a nonrectangular hyperbolic model to interactively solve gm and RL (Fang et al.,...
In line with these results, a break point in the linear section of the photosynthetic response curve could hardly be seen in the Kok-Cc plots (Fig. 3EF).

The Kok-Cc method developed here requires $g_{sc}/g_m$ to estimate $C_c$ along a Kok curve since $C_c$ cannot be directly measured. Estimating $g_m$ under low light remains technically very challenging. We used species specific $g_{sc}/g_m$ values measured under the growth condition to estimate $g_m$ at each step of Kok curves. Similar approach has been applied to estimate $C_c$ to improve the Laisk method (Gong et al., 2018; Way et al., 2019). These calculations assume that $g_{sc}/g_m$ was the same under the measurement condition of the Kok method and the growth condition. In another word, $g_{sc}$ and $g_m$ should decrease similarly with the decrease of PPFD. This assumption is supported by experimental results (Flexas et al., 2008; Drouthe et al., 2011; Xiong et al., 2015). Estimating $g_m$ from species specific $g_{sc}/g_m$ ratio is supported by the robust relationship between $g_{sc}$ and $g_m$ observed in different species under manipulated CO$_2$, irradiance, and drought stress (Flexas et al., 2008; Ma et al., 2021; Gong et al., 2022). Although the $g_{sc}/g_m$ ratio estimated here could have a certain level of uncertainty due to methodological issues associated with $g_m$ estimation (Pons et al., 2009; Gu and Sun, 2014; Gong et al., 2015), $R_L$ _Kok-Cc_ was not very sensitive to $g_{sc}/g_m$. Importantly, the factor that directly influences $R_L$ _Kok-Cc_ estimation is the decreasing rate of $\gamma$ with the increase of $I_{inc}$ ($d \gamma/d I_{inc}$) but not absolute values of $g_m$ or $C_c$. Varying $g_{sc}/g_m$ by $\pm 0.4$ or assuming a constant $g_m$ has little effect on $d \gamma/d I_{inc}$ and a negative $d \gamma/d I_{inc}$ was evident in all cases (Fig. S1, S2). In effect, our sensitivity tests showed that varying $g_{sc}/g_m$ by $\pm 0.4$ has a minor influence on both $R_L$ estimates and the CO$_2$ effect (Fig. S3). However, $R_L/R_{Dk}$ is sensitive to small variations in $R_L$ and thus is affected by $g_{sc}/g_m$ (Fig. S4). Adjusting $g_{sc}/g_m$ ($\pm 0.4$ units) leads to changes of mean light inhibition from 4% to 10%. These results highlighted that accounting for $d \gamma/d I_{inc}$ is essential for estimating $R_L$ (Farquhar and Busch, 2017), and the uncertainty associated with the accuracy of $d \gamma/d I_{inc}$ is much less than assuming a constant $\gamma$ along a Kok curve. The Kok-Cc based estimates of $R_L$ suppression could be further improved if a new method is developed to precisely estimate $g_m$ at very low light. Taken as a whole, neither the Kok nor Kok-Phi method seem suitable to quantify the inhibition of respiration by light (as also suggested by Yin et al. 2020 and Tcherkez et al. 2017a, 2017b), and the inhibition of $R_L$ at the operating PPFDs of this study should be lower than 10%. 

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Conclusions and Perspectives

This study showed that elevated growth [CO$_2$] reduced $R_L$ and $R_{DK}$ likely as a result of decreasing leaf N status and chlorophyll content. We found no significant long-term CO$_2$ effect on $R_L/R_{DK}$, indicating a concurrent response of $R_L$ and $R_{DK}$ to elevated growth [CO$_2$], mediated by the adjustment of nitrogen metabolism in leaves. These results shed light into the incorporation of $R_L$ into the carbon cycling models. We revisited the theoretical basis of the Kok method, revised Kok methods and discussed their respective limitations. Using Kok and Kok-Phi methods, we found that $R_L$ were stimulated by short-term CO$_2$ enrichment, while the effect was not supported by the data of the Kok-C$_c$ method. We attributed this short-term CO$_2$ effect to methodological uncertainty associated with unaccounted changes in $\gamma$ (or $\Gamma^*/C_c$) along a Kok curve. Accounting for those effects, we found that the Kok and Kok-Phi method underestimate $R_L$ and overestimate the inhibition of respiration under low irradiance conditions of the Kok method, and the inhibition of $R_L$ is only 6±4%, which represents 26% of the Kok effect (i.e. of the apparent inhibition of $R_L$ found using the classical Kok method). Although the Kok-C$_c$ method has less theoretical uncertainty and is thus in principle more reliable, we are aware that all three methods have operating PPFD much lower than usual, ambient irradiance encountered by plants. However, estimated $R_L$ could vary with irradiance. Earlier studies have showed a decrease of $R_L$ with the increase of operating PPFD (Brooks and Farquhar, 1985; Atkin et al., 1998; Atkin et al., 2000) by using the Laisk method which also has the uncertainty associated with the unaccounted changes in $C_c$ (Farquhar and Busch 2017). To date, the effect of irradiance on $R_L$ is poorly known and this should be addressed in subsequent studies.

MATERIALS AND METHODS

Theory

When estimating $R_L$ with the Kok method, $A$ should be measured at low irradiance, where $A$ is limited by the light-dependent electron transport rate. According to the equation of the electron transport-limited photosynthesis (Farquhar et al., 1980), $A$ at low light is described as:

$$A = J \frac{1-\Gamma^*/C_c}{4+8\Gamma^*/C_c} - R_L$$  

Eqn 1

where $J$ is the electron transport rate that is used for CO$_2$ fixation and photorespiration, $\Gamma^*$ is the $C_c$-based CO$_2$ compensation point in the absence of mitochondrial respiration (37.4 μmol mol$^{-1}$ at 25°C, (Silva-Perez et al., 2017)). According to the theoretical evaluations of Yin et al. (2011,
Eqn 1 forms the theoretical basis of the Kok method, and is useful for evaluating methodological uncertainties.

In this equation, $J$ can be replaced by $f_{aet} \Phi_2 \rho_2 a I_{inc}$, where $f_{aet}$ is the fraction of electron transport for photosynthesis, $\rho_2$ is the fraction of absorbed irradiance partitioned to PS II, $a$ is the absorptance by leaf photosynthetic pigments and $I_{inc}$ is incident irradiance (Yin et al., 2011). Here, we define the term $\frac{1-\gamma}{4+8\gamma/C_c}$ as $\gamma$, so that Equation (1) becomes:

$$A = \gamma f_{aet} \Phi_2 \rho_2 a I_{inc} - R_L$$  Eqn 2

With the Kok method, net CO$_2$ assimilation rates are plotted against $I_{inc}$ and datapoints that fall above the breakpoint are used to extrapolate $A$ up the $y$-axis and thereby estimate $R_L$. In fact, if the term $\gamma f_{aet} \Phi_2 \rho_2 a$ is assumed to be constant, thus the intercept of this linear relation provides the estimate of $R_{L, Kok}$. In terms of equation, this can be written as:

$$A = (\gamma f_{aet} \Phi_2 \rho_2 a) I_{inc} - R_{L, Kok}$$  Eqn 3

However, it has been shown that $\Phi_2$ could decrease with increasing $I_{inc}$ even within the range of low irradiance (Genty and Harbinson, 1996; Yin et al., 2020). Alternatively, $\Phi_2$ can be obtained from chlorophyll fluorescence measurements. Yin et al. (2009) thus suggested to plot $A$ against $\Phi_2 I_{inc}$ as:

$$A = (\gamma f_{aet} \rho_2 a) \Phi_2 I_{inc} - R_{L, Kok-Phi}$$  Eqn 4

The Yin et al. (2011) method can be considered as a revised Kok method with variation in $\Phi_2$ accounted for, and thus it is renamed as the 'Kok-Phi' method here to highlight the modification. This method assumes that $\gamma$ is constant across Kok curve, which is obviously not true under photorespiratory conditions, i.e. under ambient conditions where O$_2$ mole fraction is about 21% (Yin et al., 2014). Theoretically, the Kok-Phi method is applicable for measuring C$_3$ leaves at nonphotorespiratory conditions or C$_4$ leaves (Yin et al., 2011; 2020; Fang et al., 2021).

On the basis of these two methods, we propose a revised Kok method, named 'Kok-C$_c$' method, accounting for variations in $\gamma$ caused by the decrease in $C_c$ along the Kok curve. In the Kok-C$_c$ method, $A$ should be plotted against $\gamma \Phi_2 I_{inc}$, the intercept of the linear relation yields the estimation of $R_L$ ($R_{L, Kok-C_c}$):

$$A = (f_{aet} \rho_2 a) \gamma \Phi_2 I_{inc} - R_{L, Kok-C_c}$$  Eqn 5

This method requires estimates of $C_c$ at each step of the $A$-$I_{inc}$ curve (see below the section dedicated to $C_c$ estimation). It is worth noting that in practice all ‘Kok type’ methods, assume
that $R_L$ is not sensitive to changes in $C_c$ along the Kok curve, as they rely on linear extrapolations. To our knowledge, this assumption has not been verified (see Introduction).

Plant material and growth conditions
Sunflower (*Helianthus annuus* L.) and wheat (*Triticum aestivum* L.) plants were grown from seed in plastic pots with garden soil and thinned to one plant per pot. Initial nutrient composition of the garden soil (Scotts Miracle-Gro, USA) was 0.68% N, 0.27% P$_2$O$_5$, and 0.36% K$_2$O. Plants were randomly placed in two growth chambers, where CO$_2$ mole fraction was 410 ppm (ambient) and 820 ppm (elevated [CO$_2$]) respectively. In both chambers, air temperature was maintained at 25°C and the relative humidity of the air was 70% for both light and dark periods. The photosynthetic photon flux density (PPFD) was 700 μmol m$^{-2}$ s$^{-1}$ for 16-h photoperiod. All plants were watered every 2-3 days to prevent water stress. This experiment had six replicates per treatment, and in total 24 plants were used for measurements.

Gas exchange and chlorophyll fluorescence measurements
Photosynthetic gas exchange and ChF parameters were measured when there were 4 fully expanded leaves in each plant (sunflower) or tiller (wheat). Using a portable gas exchange system (LI-6800; Li-Cor Inc., Lincoln, NE, USA), measurements were undertaken on the second youngest fully developed leaves. Light response curves and ChF parameters were measured to estimate $R_L$. When stable gas exchange rates were achieved, we measured $A$ starting at 120 μmol m$^{-2}$ s$^{-1}$, and the PPFD was sequentially reduced to 100, 80, 60, 40, 20 and 0 (i.e., with light source switched off) μmol m$^{-2}$ s$^{-1}$. ChF measurements were done at PPFD of 120, 100, 80, 60 and 40 μmol m$^{-2}$ s$^{-1}$ using the multi-phase flash method. $\Phi_2$ was calculated as

$$\Phi_2 = (F_m' - F_s)/F_m' \quad \text{Eqn 6}$$

where $F_s$ is the steady-state fluorescence in the light conditions and $F_m'$ is maximal fluorescence during short saturating pulses of light. For each leaf, the irradiance response of photosynthesis rates was determined at two atmospheric [CO$_2$] (410 and 820 ppm) to assess short-term CO$_2$ response of $R_L$.

All gas exchange parameters have been corrected for the leak effect (i.e. CO$_2$ diffusion across gaskets of leaf chamber) using the measured leak coefficients of intact leaves (Gong et al., 2015; Gong et al., 2018). $R_{Dk}$ measured at 410 and 820 ppm [CO$_2$] was used to calculate the...
cuvette leak coefficient for CO$_2$ ($K_{CO2}$) with the leaf present in the leaf chamber using the equations in (Gong et al., 2015). $K_{CO2}$ was not significantly different between species and growth [CO$_2$], with a mean $K_{CO2}$ of 0.21 for wheat and 0.30 for sunflower (Fig. S5). Thereafter, the response of A to [CO$_2$] (i.e. A-C$_i$ curve) was determined under an irradiance of 700 μmol m$^{-2}$ s$^{-1}$ and varying CO$_2$, using a [CO$_2$] sequence of 410, 200, 150, 100, 50, 410, 800 and 1600 μmol mol$^{-1}$. ChF parameters were acquired at 200, 410, 800 and 1600 μmol mol$^{-1}$ CO$_2$. Leaf temperature was maintained at 25°C for all gas exchange measurements, there is thus no temperature correction needed to compare $R_L$ and $R_{DK}$.

**Estimation of day respiration and $C_e$**

For the Kok method, the data of the linear range of the $A$: $I_{inc}$ curve at PPFD levels above the Kok breakpoint (kink) were used to estimate $R_L$ according to Eqn 3. Each $A$: $I_{inc}$ curve was visually inspected to identify the irradiance at the Kok breakpoint, which was 40 μmol m$^{-2}$ s$^{-1}$ (Fig. S6). The data measured at PPFD of 120 μmol m$^{-2}$ s$^{-1}$ deviated from the linear relation (i.e. the linear domain of assimilation response curve to light between 40-100 μmol m$^{-2}$ s$^{-1}$), thus they were excluded from the dataset used for the estimation of $R_L$ via all methods. Linear regressions were performed using data of the PPFD levels of 40, 60, 80, and 100 μmol m$^{-2}$ s$^{-1}$ for all three methods, with the exception of 5 out of 45 curves in which a point that deviated from the linear relation was excluded for the estimation of $R_L$. For the Kok-Phi method, the data from the same PPFD range were used to estimate $R_L$ by plotting $A$ against $\Phi_2I_{inc}$ according to Eqn 4. We have not intensively measured $A$ at very low PPFD levels to accurately identify the breakpoint. However, the data at 40 μmol m$^{-2}$ s$^{-1}$ PPFD seem to be above the Kok breakpoint and in the linear domain of $A$: $I_{inc}$ curves. Our approach is similar to recent studies which compared the Kok and the Kok-Phi method (Yin et al., 2011; Fang et al., 2021).

Estimating $R_L$ from the Kok-$C_e$ method requires estimates of mesophyll conductance ($g_m$). According to the variable $J$ method of Harley et al. (1992), $g_m$ could be calculated as:

$$g_m = \frac{A}{c_1 - \frac{R_L}{J} + \frac{8(A+R_L)}{J-4(A+R_L)}}$$

Eqn 7

Here, we used $R_L$ estimated using the Kok-Phi method to calculate $g_m$, given that this method addresses the issue of decreasing $\Phi_2$ and provides a more reliable estimation of $R_L$, compared to the Kok method (Yin et al., 2011). Furthermore, using $R_L$ Kok or $R_L$ Kok-Phi has minor influence on
$g_{sc}/g_m$, thus should have no influence on our conclusions (see the discussion on the uncertainty associated with $g_{sc}/g_m$). We chose data in a reliable range of $dC_c/dA$ between 10 and 50 for estimating $g_m$ as suggested by Harley et al. (1992). $dC_c/dA$ was calculated as:

$$dC_c/dA = 12I^*J/(J - 4(A + R_L))^2$$

Eqn 8

Most of the data obtained with sunflower met this empirical criterion of $dC_c/dA$, while $dC_c/dA$ of wheat exceeded this range ($dC_c/dA > 100$) in most cases. Therefore, the $A-C_i$ curve-fitting method was used to estimate the $g_m$ value of each leaf in wheat. Based on the EwCB photosynthesis model (Farquhar et al., 1980), the $A-C_i$ curve fitting tool developed by Sharkey et al. (2007) was used to estimate $g_m$ by minimizing the sum of squared deviations between the observed and modelled data.

Recently, it has been found that $g_m$ and stomatal conductance to CO$_2$ ($g_{sc}$) are strongly related (Flexas et al., 2012; Ma et al., 2021). A nearly fixed $g_{sc}/g_m$ ratio across different environments and plant functional groups was shown by Ma et al. (2021), offering a useful solution to estimate $g_m$. We first obtained species- and treatment-specific $g_{sc}/g_m$ using Equation 7 (sunflower) or curve fitting (wheat), and then $g_m$ along the Kok curve was estimated from measured $g_{sc}$ and previously estimated $g_{sc}/g_m$. $C_c$ was calculated from $g_m$ as:

$$C_c = C_i - A/g_m$$

Eqn 9

With $C_c$, $\gamma$ could be calculated and thus $R_{L,Kok-Cc}$ could be estimated by plotting $A$ against $\gamma\Phi_2I_{inc}$ using data of the PPFD range of 40-100 µmol m$^{-2}$ s$^{-1}$ according to Eqn 5. We also tested the sensitivity of $R_{L,Kok-Cc}$ to $g_{sc}/g_m$ by adjusting obtained species- and treatment-specific $g_{sc}/g_m$ ($\pm$ 0.4).

The daily carbon-use efficiency of leaves, the ratio of net carbon gain to assimilated carbon (integrated photosynthesis) was calculated as:

$$CUE_L = (\int A - \int R_{Dk})/(\int A + \int R_{L,Kok-Cc})$$

Eqn 10

Since plants were grown in controlled environments, the daily carbon fluxes were calculated as $\int A = A \times$ light hours, $\int R_{L,Kok-Cc} = R_{L,Kok-Cc} \times$ light hours, and $\int R_{Dk} = R_{Dk} \times$ dark hours.

**Plant sampling and leaf trait parameters**

After gas exchange and ChF measurements, the measured leaves were harvested. We measured leaf area and fresh weight, and the chlorophyll content (Chl) was determined by a chlorophyll meter (SPAD-502 Plus; Konica Minolta Inc., Tokyo, Japan). The chlorophyll content was
calculated from the observed SPAD values as Chl = (99 SPAD)/(144 – SPAD) (Cerovic et al., 2012). All leaves were dried at 70 °C to constant mass after drying to stop enzymatic activity at 105 °C for 1 hour. We measured dry mass of individual leaves, and then the leaves were ground with a ball mill (Tissuelyser-24, Jingxin Ltd., Shanghai, China). Leaf N content was measured using an elemental analyzer (VARIO EL III, Elementar Analysensysteme GmbH, Hanau, Germany).

Statistical analysis
Statistical analysis was performed using SPSS (v. 25.0, SPSS, Chicago, IL, USA). Leaf traits and photosynthetic parameters were analyzed with two-way ANOVAs to determine the influence of growth [CO₂], species and their interaction. Besides, ANOVAs were carried out to clarify the effect of growth [CO₂], measurement [CO₂], their interaction and species on R_L and R_L/R_Dk. A p-value lower than 0.05 is considered statistically significant.

FUNDING
This work was supported by the National Natural Science Foundation of China (NSFC 31870377, 32120103005).

DATA AVAILABILITY
All data that support the findings of this study are included in the published article and its Supplementary Information.
### TABLES

Table 1. Leaf traits and photosynthetic parameters of wheat (*T. aestivum*) and sunflower (*H. annuus*) grown under ambient or elevated CO$_2$ (aCO$_2$ or eCO$_2$). Leaf trait parameters include: specific leaf area (SLA, cm$^2$ mg$^{-1}$), leaf nitrogen content per dry mass (N%), leaf nitrogen content per area (N$_{area}$, g m$^{-2}$), chlorophyll content (Chl, g m$^{-2}$). Photosynthetic parameters include net CO$_2$ assimilation rate at the growth CO$_2$ ($A_{growth}$, μmol m$^{-2}$ s$^{-1}$), maximum CO$_2$ assimilation rate ($A_{max}$, μmol m$^{-2}$ s$^{-1}$), respiration rate in the dark ($R_{Dk}$, μmol m$^{-2}$ s$^{-1}$), intrinsic water-use efficiency ($iWUE_{growth}$, μmol mol$^{-1}$), maximum carboxylation rates by Rubisco ($V_{cmax}$, μmol m$^{-2}$ s$^{-1}$), electron transport rate ($J$, μmol m$^{-2}$ s$^{-1}$), ratio of stomatal conductance for CO$_2$ to mesophyll conductance ($g_{sc}/g_{m}$), leaf carbon-use efficiency (CUE$_L$).

Data are mean ± SE ($n$=6); significant treatment effects ($P<0.05$) tested with two-way ANOVAs are shown in bold.

<table>
<thead>
<tr>
<th></th>
<th><em>T. aestivum</em></th>
<th><em>H. annuus</em></th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aCO$_2$</td>
<td>eCO$_2$</td>
<td>aCO$_2$</td>
</tr>
<tr>
<td>$A_{growth}$</td>
<td>28.42±0.73</td>
<td>32.79±2.01</td>
<td>27.27±2.57</td>
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<tr>
<td>$A_{max}$</td>
<td>37.68±1.29</td>
<td>34.04±2.22</td>
<td>32.32±2.40</td>
</tr>
<tr>
<td>$R_{Dk}$</td>
<td>2.26±0.14</td>
<td>1.81±0.17</td>
<td>1.48±0.11</td>
</tr>
<tr>
<td>$iWUE_{growth}$</td>
<td>52.64±1.30</td>
<td>62.25±3.94</td>
<td>37.30±10.25</td>
</tr>
<tr>
<td>SLA</td>
<td>0.24±0.01</td>
<td>0.27±0.02</td>
<td>0.22±0.02</td>
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<tr>
<td>N%</td>
<td>6.61±0.17</td>
<td>6.39±0.11</td>
<td>3.51±0.67</td>
</tr>
<tr>
<td>N$_{area}$</td>
<td>2.70±0.06</td>
<td>2.37±0.10</td>
<td>1.53±0.18</td>
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<tr>
<td>Chl</td>
<td>0.69±0.02</td>
<td>0.56±0.03</td>
<td>0.49±0.05</td>
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<tr>
<td>$V_{cmax}$</td>
<td>160.9±3.2</td>
<td>142.7±13.0</td>
<td>123.0±11.7</td>
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<tr>
<td>$J$</td>
<td>183.4±3.3</td>
<td>160.1±9.3</td>
<td>164.2±10.4</td>
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<td>$g_{sc}/g_{m}$</td>
<td>0.95±0.03</td>
<td>1.16±0.07</td>
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<tr>
<td>CUE$_L$</td>
<td>0.89±0.01</td>
<td>0.92±0.01</td>
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Table 2. ANOVA tests for $R_L$ estimated by the Kok, Kok-Phi and Kok-$C_c$ methods. Significant treatment effects ($P<0.05$) are shown in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>$R_{L, Kok}$</th>
<th>F</th>
<th>P</th>
<th>$R_{L, Kok-Phi}$</th>
<th>F</th>
<th>P</th>
<th>$R_{L, Kok-Cc}$</th>
<th>F</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Species</td>
<td>1</td>
<td>28.36</td>
<td>&lt;0.001</td>
<td>29.74</td>
<td>&lt;0.001</td>
<td>20.06</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>Growth CO$_2$</td>
<td>1</td>
<td>6.681</td>
<td>0.013</td>
<td>5.777</td>
<td>0.021</td>
<td>3.921</td>
<td>0.055</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Measurement CO$_2$</td>
<td>1</td>
<td>1.227</td>
<td>0.275</td>
<td>1.714</td>
<td>0.198</td>
<td>0.198</td>
<td>0.658</td>
<td></td>
<td></td>
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<tr>
<td>Growth CO$_2^*$</td>
<td>1</td>
<td>0.092</td>
<td>0.763</td>
<td>0.107</td>
<td>0.745</td>
<td>0.072</td>
<td>0.790</td>
<td></td>
<td></td>
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<tr>
<td>Measurement CO$_2$</td>
<td>1</td>
<td>7.480</td>
<td>0.009</td>
<td>0.107</td>
<td>0.745</td>
<td>0.072</td>
<td>0.790</td>
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Table 3. ANOVA tests for $R_{L/Dk}$ estimated by the Kok, Kok-Phi and Kok-$C_c$ methods. Significant treatment effects ($P<0.05$) are shown in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>$R_{L, Kok}/R_{Dk}$</th>
<th>F</th>
<th>P</th>
<th>$R_{L, Kok-Phi}/R_{Dk}$</th>
<th>F</th>
<th>P</th>
<th>$R_{L, Kok-Cc}/R_{Dk}$</th>
<th>F</th>
<th>P</th>
</tr>
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<tbody>
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<td>Species</td>
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<td>2.824</td>
<td>0.101</td>
<td>1.638</td>
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<td>0.095</td>
<td>0.759</td>
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<tr>
<td>Growth CO$_2$</td>
<td>1</td>
<td>2.216</td>
<td>0.144</td>
<td>0.799</td>
<td>0.377</td>
<td>0.389</td>
<td>0.536</td>
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<tr>
<td>Measurement CO$_2$</td>
<td>1</td>
<td>7.480</td>
<td>0.009</td>
<td>0.107</td>
<td>0.745</td>
<td>0.072</td>
<td>0.790</td>
<td></td>
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<tr>
<td>Growth CO$_2^*$</td>
<td>1</td>
<td>0.328</td>
<td>0.570</td>
<td>0.441</td>
<td>0.511</td>
<td>0.243</td>
<td>0.625</td>
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FIGURE LEGENDS

Figure 1. Net CO$_2$ assimilation rate ($A$), transpiration rate ($E$) and stomatal conductance for water vapor ($g_{sw}$) in response to short-term variation of intercellular CO$_2$ concentration ($C_i$) for wheat ($T. aestivum$) and sunflower ($H. annuus$). Blue circles refer to ambient (410 $\mu$mol mol$^{-1}$) growth CO$_2$, and red squares refer to elevated (820 $\mu$mol mol$^{-1}$) growth CO$_2$. Data are shown as mean ± SE ($n=6$).

Figure 2. Photochemical efficiency of photosystem II ($\Phi_2$), chloroplastic CO$_2$ concentration ($C_c$) and $\gamma$ (the lumped parameter in Eqn 2) in response to incident irradiance ($I_{inc}$) for wheat ($T. aestivum$) and sunflower ($H. annuus$). Plants grown under ambient CO$_2$ (aCO$_2$, blue circles) or elevated CO$_2$ (eCO$_2$, red squares) were measured at gaseous conditions of 410 $\mu$mol mol$^{-1}$ (open symbols) or 820 $\mu$mol mol$^{-1}$ (closed symbols) CO$_2$ in the leaf chamber. Data are shown as mean ± SE ($n=6$).

Figure 3. Net CO$_2$ assimilation rate ($A$) in response to $I_{inc}$ (incident irradiance), $\Phi_2 I_{inc} (\Phi_2$, photochemical efficiency of photosystem II) or $\gamma \Phi_2 I_{inc} (\gamma$, the lumped parameter in Eqn 2) for wheat ($T. aestivum$) and sunflower ($H. annuus$). Data are mean ± SE ($n=6$). Meaning of symbols of different CO$_2$ treatments and measurement conditions are shown in Fig. 2.

Figure 4. Effects of growth CO$_2$ treatments (aCO$_2$ and eCO$_2$) and measurement conditions (410 and 820 ppm CO$_2$) on respiration rates in the light ($R_L$) estimated by three methods for wheat ($T.$
aestivum) and sunflower (H. annuus). \( R_L \) was measured by the Kok (A and D), Kok-\( \Phi \) (B and E) and Kok-\( C_c \) (C and F) methods. Data are mean ± SE (\( n=5-6 \)). The results of ANOVA tests are shown in Table 2.

**Figure 5.** Effects of growth \( \text{CO}_2 \) treatments (a\( \text{CO}_2 \) and e\( \text{CO}_2 \)) and measurement conditions (410 and 820 ppm) on ratio of respiration in the light to respiration in the dark (\( R_L/R_{Dk} \)) for wheat (T. aestivum) and sunflower (H. annuus). \( R_L/R_{Dk} \) was estimated by the Kok (A and D), Kok-\( \Phi \) (B and E) and Kok-\( C_c \) (C and F) methods. Data are mean ± SE (\( n=5-6 \)). The results of ANOVA tests are shown in Table 3.

**Figure 6.** Correlation between respiration in the dark (\( R_{Dk} \)) and respiration in the light (\( R_L \)). \( R_L \) was measured by the Kok (A), Kok-\( \Phi \) (B) and Kok-\( C_c \) (C) methods. The average \( R_L/R_{Dk} \) (± SE, \( n=45 \)) was calculated by pooling over the data of species (wheat and sunflower) and \( \text{CO}_2 \) treatments. Grey dashed lines give the 1:1 relationship.

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**Figure 1**
159x182 mm (9.1 x DPI)
Figure 2
159x181 mm (9.1 x DPI)
Figure 3
159x212 mm (9.1 x DPI)
Figure 4
153x100 mm (9.1 x DPI)
Figure 5

152x101 mm (9.1 x DPI)

Figure 6

159x59 mm (9.1 x DPI)