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Canopy position influences the degree of light suppression of leaf respiration in abundant tree genera in the Amazon Forest

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Abstract

Leaf respiration in the dark (R_{dark}) and light (R_{day}) is poorly characterized in diverse tropical ecosystems, and little to no information exists on the degree of light suppression in common tree species within the Amazon basin, and their dependences upon plant functional traits and position within the canopy. We quantified R_{dark} and apparent R_{day} using the Kok method and measured key leaf traits in 26 tree species distributed in three different crown positions: canopy, lower canopy, and understory. We found that canopy trees had significantly higher rates of R_{dark} and R_{day} than trees in the understory. The difference between R_{dark} and R_{day} (the light suppression of respiration) was greatest in the understory (68 ± 9%, 95% CI) and lower canopy (49 ± 9%, 95% CI) when compared to the canopy (37 ± 10%, 95% CI). We found that R_{day} was significantly and strongly correlated with R_{dark} (r^2 = 0.76). R_{dark} had a significant relationship to leaf mass area (LMA, r^2 = 0.26), and phosphorus (P, r^2 = 0.18) but no

significant relationship with nitrogen, sugars, and starch. Our results highlight the importance of including representation of the light suppression of leaf respiration in terrestrial biosphere models and also of accounting for vertical gradients within forest canopies and connections with functional traits.

1 Introduction

Autotrophic respiration is one of the processes that strongly regulates~~contribute to~~ both terrestrial and global carbon balances (Tang et al., 2019). However, large uncertainties remain in the magnitude of biological and environmental controls over tropical autotrophic respiration, largely due to limited observational studies requiring advanced technologies (*e.g.* portable photosynthesis systems) deployed to logistically challenging field sites. In tropical forests, limited estimates suggested that only 30% of the carbon fixed by photosynthesis is allocated to the formation of new tissues (biomass), with the rest being respired back into the atmosphere (Chambers et al., 2004; Malhi et al., 2014). Previous work has estimated that tropical in forests, autotrophic respiration contributes between 20 to 29 Mg C ha year^{-1}, with leaf respiration being the major, but highly uncertain contributor at an estimated 32-56% of the total (Chambers et al., 2004; Metcalfe et al., 2010; Malhi, 2012; Malhi et al., 2014). Both field observations and modeling development of autotrophic respiration is substantially less studied than photosynthesis (Huntingford *et al.*, 2017). This might be explained by the difficulty in measuring foliar CO_2 release (Meir et al., 2001; Chambers et al., 2004; Heskel, 2018) due to the lower fluxes and higher signal to noise ratio when compared to measurements of photosynthesis (Tcherkez et al., 2017a, 2017b).

~~In addition, l~~Leaf respiration can be substantially inhibited by light (Kok, 1948, 1949) and this metabolism is dynamic and complex, and regulated by changes in enzyme activities and interactions with photosynthesis, photorespiration, and other pathways such as nitrate assimilation (Tcherkez et al., 2017a, 2017b). For example, (Heskel and Tang, 2018) determined that when the inhibition of leaf respiration in a temperate forest by light is not accounted for in land models, an overestimation of net primary productivity (NPP) occurs. In addition, current leaf respiration models generally do not take into account changes in the vertical gradient of light availability within the forest, which is known to greatly influence plant metabolism~~-~~ (Weerasinghe et al., 2014; Heskel and Tang, 2018). Respiratory rates have also been associated with morphological and nutrient variation of leaves, such as leaf mass per area, ~~and~~ nitrogen and phosphorus content (Meir et al., 2001; Atkin et al., 2015; Crous et

al., 2017; Rowland et al., 2017), and can be regulated by the availability of respiratory substrates, such as non-structural carbohydrates including soluble sugars and starch (Collalti et al., 2019).

~~Light is considered a limiting resource in tropical forests (Wu et al., 2016) and it varies as a function of height in the canopy. For this reason, trees modify their resource capture and use strategies according to light availability, reflecting changes in their morphological and nutrient composition, due to the high competition for light (Wright et al., 2004). Studies such as those by (Kosugi et al., 2012; Weerasinghe et al., 2014; Heskel and Tang, 2018) reported a large effect of leaf position within the canopy on leaf respiration. However, little to no information exists on the magnitudes of leaf respiration in common tree species within the Amazon basin, and their dependence upon position within the canopy.~~

The difference between apparent respiration in the light (R_{day}) and respiration in the dark (R_{dark}) is known as the light suppression of leaf respiration. ~~Several hypotheses have been advanced to explain the phenomena ; enzyme deactivation by light which reduces the flux of carbon into the TCA cycle, CO_2 refixation by photosynthesis, and interactions with other biochemical pathways during the day (Tcherkez and Ghashghaie, 2017; Tcherkez et al., 2017a; Gauthier et al., 2020).~~ The two main approaches used to estimate R_{day} and consequently light suppression are, the $^{13}CO_2$ isotopic method **(Loreto et al., 1999, 2001; Gong et al., 2018)** and the Kok method (Kok, 1948, 1949). Th<u>is</u>~~is~~ isotopic method assumes that in an atmosphere of $^{13}CO_2$, all $^{12}CO_2$ detected is from respiration, and is considered to be the most accurate method (Tcherkez et al., 2017a). <u>The Kok method uses the abrupt change in the gradient of the initial slope of the response of photosynthesis to irradiance. Regression photosynthesis against irradiance points above the Kok kink results in a shallower initial slope where the y intercept provides an estimate of R_{light} (Farquhar & Busch, 2017) (Tcherkez et al., 2017a, 2017b; Way et al., 2018; Yin et al., 2020).</u>~~The Kok method uses the abrupt change in the gradient of the initial slope of the response of photosynthesis to irradiance. Regression photosynthesis against irradiance points above the Kok kink results in a shallower initial slope where the y intercept provides an estimate of R_{day} (Farquhar & Busch, 2017) (Farquhar and Busch, 2017; Tcherkez et al., 2017a, 2017b; Way et al., 2018; Yin et al., 2020).~~

It is known that the Kok method can underestimate rates of light respiration (Gong et al., 2018; Way et al., 2018; Yin et al., 2020), but on the other hand is the most practical

method to apply in logistically challenging field conditions (Tcherkez et al., 2017a). The Kok "effect" has been described as due to not only changes in respiratory rates with light, but also other physiological and biochemical process (Gauthier et al., 2020; Yin et al., 2020). At least three phenomenon can explain the Kok "effect", as decrease in the photochemical efficiency, refixation of CO_2 by photosynthesis, and a decrease of mitochondrial respiration due to light inhibition (Yin et al., 2020). CO_2 reassimilation has been widely discussed, but there are still disagreements regarding its importance in the Kok "effect" (Buckley et al., 2017; Farquhar and Busch, 2017).

Light is considered a limiting resource in forests understory varying as a function of height in the canopy (Mulkey et al., 1996). For this reason, trees modify their resource capture and use strategies according to light availability, reflecting changes in their morphological and nutrient composition, due to the high competition for light (Wright et al., 2004). Studies such as those by (Kosugi et al., 2012; Weerasinghe et al., 2014; Heskel and Tang, 2018) reported a large effect of leaf position within the canopy on leaf respiration. However, little to no information exists on the magnitudes of leaf respiration in common tree species within the Amazon basin, and their dependence upon position within the canopy.

Given the great biodiversity of tropical forests **(Cardoso et al., 2017; ter Steege et al., 2020)**, the scarcity of leaf respiration measurements in the tropics, and the known high sensitivity of leaf respiration to canopy position ~~(~~(Griffin et al., 2002)~~REF)~~, there is an urgent need to quantify both R_{dark} and the suppression of respiration that occurs in the light. In particular, ~~-~~we need in situ measurements to know how it varies across common tree species, as a function of height within the forest.

~~canopy..~~

Due to the great importance of the Amazon rainforest in the global climate context, this study aims to quantify the ~~*biosphere-atmosphere CO_2 fluxes resulting*~~R_{dark} and R_{day} from leaf autotrophic respiration~~,~~; the component that most contributes to total autotrophic respiration, and is the most sensitive to environmental change (Chambers et al., 2004; Malhi et al., 2014; Cavaleri et al., 2017). Thus, the questions that guided this work were: **i**) Does the Kok method, which can be applied in the field, compare quantitatively to $^{13}CO_2$ labeling method?; **ii**) What are the R_{dark} and R_{day} leaf respiration rates of common "Terra-firme" tree species in the Central Amazon basin and what is the degree of light suppression?; **iii**) Does canopy position influence the variation of leaf respiration rates and light suppression? and **iv**)

What are the relationships between respiratory rates and leaf functional traits? In the laboratory we compared estimates of R_{dark} and R_{day}, using two methods~~:~~; the $^{13}CO_2$ method based on a portable photosynthesis system coupled to a cavity ringdown isotopic analyzer for $^{13}CO_2$ and $^{12}CO_2$, and the Kok method. In the field, we used the Kok method to measure R_{dark} and R_{day} in 26 trees occupying three different canopy positions including the canopy, lower canopy, and understory where we also measured key leaf traits.

2 Material and methods

Determination of leaf dark adapted R_{dark} and R_{day} using the Kok and $^{13}CO_2$ methods

For all gas exchange measurements, branches were cut and recut in a bucket with water to restore hydraulic conductivity (Weerasinghe et al., 2014; Albert et al., 2018). For all leaf gas exchange measurements, the leaf temperature was set (T_{block}-) at 31 ± 1 ºC (Slot and Winter, 2017) and the air flow rate through the chamber was maintained at 300 µmol s^{-1} (Crous et al., 2012; Weerasinghe et al., 2014; Heskel and Tang, 2018) and the reference CO_2 concentration maintained at 400 ppm~~(Crous et al., 2012; Weerasinghe et al., 2014; Heskel and Tang, 2018)~~. For the Kok method, the respiratory CO_2 flux of the sampled leaves was measured using a portable photosynthesis system (Li-6400XT, Li-Cor®, Lincoln, USA). Following the introduction of a leaf into the chamber with 100 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR), the two IRGAs were matched. Net photosynthetic assimilation values (A_{net}) were subsequently recorded for PAR values of 100, 90, 80, 70, 60, 50, 40, 30, 20, 15, 10 and 5 µmol m^{-2} s^{-1} (with IRGA matching before each recording). Following this, the light source was switched off (0 µmol m^{-2} s^{-1}) and the leaf was allowed 10 minutes to acclimate in the dark before matching the IRGAs and recording the dark adapted respiration rate (R_{dark}). Due to the interactions that occur in the presence of light, the Kok method (Kok, 1948) was used to estimate the "apparent" respiratory rates in the light. The Kok method consists of determining R_{day} at low irradiance, due to the "break" that occurs in the light curve near the light compensation point. In this method, R_{day} is estimated as the intercept, on the "y" axis, of the net photosynthesis rate as a function of PAR, at low light intensity. For each leaf, a linear regression using at least three points between 5 and 20 PAR was performed to estimate R_{day}. With data from the dark adapted measurement of R_{dark} and the "apparent" estimate of light respiration (R_{day}), was used to calculate the light suppression, according to **Equation 1**:

Eq. 1. $Suppression\,(\%) = \left[\frac{(R_{dark} - R_{day})}{R_{dark}}\right] * 100$

For the $^{13}CO_2$ method (Loreto et al., 2001), two modifications were made to the portable photosynthesis system including 1) a 400 ppm atmosphere of 99% $^{13}CO_2$ (Cambridge Isotopes) was delivered to the leaf chamber and 2) a fraction (50 ml/min) of the air exiting the leaf chamber was routed to a cavity ringdown isotopic analyzer for CO_2 (G2131-i, Picarro) which measured the concentration of $^{13}CO_2$ and $^{12}CO_2$ exiting the leaf chamber. This method assumes that the leaf uses the $^{13}CO_2$ atmosphere for photosynthesis with any $^{12}CO_2$ detected deriving from leaf respiration (Loreto et al., 2001). The experiment was carried out with a tropical tree species in the family Chrysobalanaceae growing near the laboratory and exposed to natural sunlight for 6-7 hours per day. To compare the Kok and $^{13}CO_2$ methods for determining R_{day}, R_{dark}, and the degree of suppression by light (%), both methods were performed on six different leaves from the same individual, but each on a different branch.

Field Study Site

Field data of 26 trees was collected at the Tropical Silviculture Experimental Station (E.E.S.T - ZF2), managed by the National Institute for Amazon Research (INPA), located approximately 60 km NW of Manaus, Brazil. The ZF-2 has an area of approximately 21,000 ha adjacent to extensive areas of undisturbed tropical forest. Data was collected in a permanent plot installed in 1996, known as North–South transect (2°35'40.3"S, 60°12'28.7"W), located at km 33 of the local road, covering an area of 5 ha (20m x 2,500m) (Araújo et al., 2020). The predominant vegetation in the reserve is ~~of the type~~type of Dense Tropical Rainforest of "Terra Firme" with a great diversity of woody and herbaceous species. The North-South transect presents in the plateau areas a predominance of species belonging to the families Lecythidaceae, Sapotaceae, Burseraceae, and Fabaceae. Among these families, the most common species belonged to the genera *Eschweilera, Pouteria, Protium, Swartzia,* **and** *Inga (Carneiro, 2004).*

Tree Species selection in the field

We sampled 26 trees (**Table 1**), all located along the North-South Transect, between 200 and 400 meters, in plateau areas. Access to the canopy leaves was obtained by a tree climber who scaled trees with a pole pruner and removed a branch. Thus, the trees were selected taking into consideration: i) ecological aspects, with a focus on highly abundant tree

species in the Amazon basin (Ter Steege et al., 2013; Cardoso et al., 2017) and ii) practical, related to climber access to the individual's branches. The classification of crown illumination index was performed according to (Clark and Clark, 1992). In the 26 individuals studied, crowns were distributed in different classes (see **Table 1**). For analysis ~~they~~ eight trees were classified in understory, nine in the lower canopy and nine in the canopy group~~s~~. The ones with low, medium and high lateral light ~~was~~were associated to understory trees, the ones with some overhead light are lower canopy and trees with full overhead light belong to the canopy.

Determination of leaf R_{dark} and R_{day} using the Kok method in the field

The measurements were made in 2019 during three different field campaigns, in June/July, September and November. Data was collected between 8 am and 2 pm Local Time. The measurements were made using mature, fully expanded leaves with a good visual aspect. A fully expanded leaf per tree was selected for each field campaign. Due to the method of access to the canopy, the orientation for obtaining branches was not standardized and branches were collected on positions North, South, East, and West of the crown.

~~**Determination of leaf R_{dark} and R_{day} using the Kok and $^{13}CO_2$ methods**~~

~~**For all gas exchange measurements, branches were cut and recut in a bucket with water to restore hydraulic conductivity (Weerasinghe et al., 2014; Albert et al., 2018). For all leaf gas exchange measurements, the leaf temperature was maintained at 31 ± 1 ºC (Slot and Winter, 2017) and the air flow rate through the chamber was maintained at 300 µmol s^{-1} and the reference CO_2 concentration maintained at 400 ppm (Crous et al., 2012; Weerasinghe et al., 2014; Heskel and Tang, 2018). For the Kok method, the respiratory CO_2 flux of the sampled leaves was measured using a portable photosynthesis system (Li-6400XT, Li-Cor®, Lincoln, USA). Following the introduction of a leaf into the chamber with 100 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR), the two IRGAs were matched. Net photosynthetic assimilation values (A_{net}) were subsequently recorded for PAR values of 100, 90, 80, 70, 60, 50, 40, 30, 20, 15, 10 and 5 µmol m^{-2} s^{-1} (with IRGA matching before each recording). Following this, the light source was switched off (0 µmol m^{-2} s^{-1}) and the leaf was allowed 10 minutes to acclimate in the dark before matching the IRGAs and recording the dark respiration rate (R_{dark}). Due to the interactions that occur in the presence of light, the Kok method (Kok, 1948)**~~

~~was used to estimate the "apparent" respiratory rates in the light. The Kok method consists of determining R_{day} at low irradiance, due to the "break" that occurs in the light curve near the light compensation point. In this method, R_{day} is estimated as the intercept, on the "y" axis, of the net photosynthesis rate as a function of PAR, at low light intensity. For each leaf, a linear regression using at least three points between 5 and 20 PAR was performed to estimate R_{day}. With data from dark respiration R_{dark} and the "apparent" estimate of light respiration (R_{day}), was used to calculate the light suppression, according to Equation 1:~~

~~Eq. 1.~~ $$Suppression(\%) = \left[\frac{(R_{dark} - R_{day})}{R_{dark}}\right] * 100$$

~~For the $^{13}CO_2$ method (Loreto et al., 2001), two modifications were made to the portable photosynthesis system including 1) a 400 ppm atmosphere of 99% $^{13}CO_2$ (Cambridge Isotopes) was delivered to the leaf chamber and 2) a fraction (50 ml/min) of the air exiting the leaf chamber was routed to a cavity ringdown isotopic analyzer for CO_2 (G2131-i, Picarro) which measured the concentration of $^{13}CO_2$ and $^{12}CO_2$ exiting the leaf chamber. This method assumes that the leaf uses the $^{13}CO_2$ atmosphere for photosynthesis with any $^{12}CO_2$ detected deriving from leaf respiration (Loreto et al., 2001). The experiment was carried out with a tropical tree species in the family Chrysobalanaceae growing near the laboratory and exposed to natural sunlight for 6-7 hours per day. To compare the Kok and $^{13}CO_2$ methods for determining R_{day}, R_{dark}, and the degree of suppression by light (%), both methods were performed on six different leaves from the same individual, but each on a different branch.~~

Leaf traits

Leaves used for gas exchange measurements and others were collected and obtained six discs of known diameter (1.7 cm). These were dried in an oven at 65 ℃ for 72 hours until ~~the~~ constant mass was obtained and was calculated the leaf mass per area (LMA g m^{-2}). For leaf nitrogen and phosphorus content, leaves were collected, following the same drying process and subsequently the leaf samples were ground in a Wiley mill. The nitrogen (N) content was determined by the Kjeldahl method and the phosphorus content (P) obtained by colorimetry and the absorbance readings made at 660 nm in a spectrophotometer using ammonium molybdate and 3% ascorbic acid. For nonstructural carbohydrates, the leaf samples were collected only in the first campaign (June/July), kept, and transported for 3

hours in a box with ice with a temperature between 0-4 ℃ to the laboratory in Manaus, Brazil (consistent with guidelines from **(Landhäusser et al., 2018)**~~Landhausser et al. 2018~~). In the laboratory, they were placed in microwaves for 90 seconds and dried in an oven for 72 hours at 65 ℃. The processed leaf samples were ground in a ball mill and sent to the Pacific Northwest National Laboratory in Washington state, USA for the quantification of non-structural carbohydrates including sugars, starch and total carbohydrates (NSC) as previously described (Dickman et al., 2019; Zhang et al., 2021).

Data analysis

All statistical analysis was performed using R version 3.6.0 (R Development Core Team, 2019) and IGOR Pro, version 6.37 (WaveMetrics, Inc., United States). ~~A statistical~~To compare the methods used to estimate ~~comparison of~~ R_{day}, R_{dark}, and the degree of suppression by light (%) obtained from the Kok and $^{13}CO_2$ methods, were ~~was~~ performed a non-parametric t-test to compare the two methods (n = 4-6). In the field study, ~~T~~to verify the effect of canopy position on the studied variables, we performed a Kruskal-Wallis test, considering significant differences if $p \leq 0.05$. To access the relations between R_{dark} and other variables (*i.e* LMA, N and P content, sugars, starch and total NSC concentrations), we used linear regression analysis and a Spearman correlation matrix (**Supplementary Figure S1**) to see the correlations for all the data (n = 77). All the results are presented as mean ± 95% CI.

3 Results

Comparison between Kok and $^{13}CO_2$ methods for estimating R_{day} and the degree of light suppression

In order to validate the Kok method for the determination of R_{day} in the remote central Amazon field site, an inter-comparison between Kok and $^{13}CO_2$ methods for estimating R_{day} and the degree of light suppression was performed at the National Institute for Amazon Research (INPA) campus in Manaus, Brazil by taking advantage of a cavity ringdown CO_2 isotope spectrometer that we interfaced to the portable photosynthesis system supplied with $^{13}CO_2$ (see Material and methods). Due to its proximity to the laboratory, we utilized a tree in the family Chrysobalanaceae and observed statistically identical mean values of dark respiration between the methods ($p = 0.94$, n = 6). The mean values of dark adapted R_{dark} and~~determined using the Kok and~~ $^{13}CO_2$ methods was 0.67 ± 0.17 and 0.68 ± 0.27 µmol CO_2

m^{-2} s^{-1} (95% CI), respectively (**Figure 1A**). Both Kok and $^{13}CO_2$ methods also observed that estimates of R_{day} were lower than R_{dark} (**Figure 1B**). The mean R_{day} values, determined by the Kok method was 0.36 ± 0.17 µmol CO_2 m^{-2} s^{-1} and the $^{13}CO_2$ method 0.27 ± 0.03 µmol CO_2 m^{-2} s^{-1}. Thus, statistically identical mean values of R_{day} were observed between the methods ($p = 0.73$, $n = 4$). Finally, the percentage of leaf dark respiration suppression by light was also statistically equal between the Kok and $^{13}CO_2$ methods ($p = 0.41$, $n = 4$), with 44 ± 17% (Kok) and 54 ± 17% ($^{13}CO_2$) (**Figure 1C**).

Effect of crown position on respiratory rates and degree of light suppression in a central Amazon forest transect

Given the favorable comparison between the Kok and $^{13}CO_2$ methods for estimation of R_{day} and the light suppression of R_{dark}, we utilized the Kok method to determine these leaf respiratory rates in an established north-south forest transect with identified species in the central Amazon. **Figure 2** summarizes the leaf respiration measurements from 26 individuals across 18 common species in the Amazon forest, and grouped according to canopy position (understory, lower canopy, and canopy). The mean values of R_{dark} observed among the 26 individuals ranged from 0.19 ± 0.04 µmol CO_2 m^{-2} s^{-1} to 0.90 ± 0.26 µmol CO_2 m^{-2} s^{-1} (95% CI), whereas mean estimated values of R_{day} using the Kok method showed reduced values ranging from 0.01 ± 0.01 to 0.69 ± 0.27 µmol CO_2 m^{-2} s^{-1}. Thus, the light suppression of R_{dark} ranged between 17 ± 6% to 95 ± 4%. Moreover, the pattern of reduced R_{day} relative to R_{dark} was observed for each of the 26 individuals. Overall, a general pattern emerged that higher rates of R_{dark} and R_{day} were associated with lower values of light suppression of R_{dark}. Moreover, lower rates of R_{dark} and R_{day} were associated with higher values of light suppression of R_{dark}. Relative to canopy species, understory species tended to have lower rates of R_{dark} and R_{day} but higher values of light suppression of R_{dark}.

Figure 3 shows the mean A_{net} versus PAR leaf gas exchange response curves for species in the canopy (**Figure 3A**), lower canopy (**Figure 3B**), and understory (**Figure 3C**). All three canopy positions showed the classic Kok effect where a sudden change in the gradient of the initial slope of net photosynthesis (A_{net}) occurred at low PAR intensities (0-15 µmol m^{-2} s^{-1}) which corresponded to the light compensation point (1-6 µmol photons m^{-2} s^{-1}). When the mean rate of R_{dark} and estimates of R_{day} and the percent light suppression of R_{dark} was obtained for each of the three canopy positions, a large effect of canopy position was

observed. R_{dark} was the highest in canopy trees (0.50 ± 0.07 µmol of CO_2 m^{-2} s^{-1}) and lowest in the understory trees (0.25 ± 0.03 µmol of CO_2 m^{-2} s^{-1}) with the lower canopy trees with intermediate values of R_{dark} (0.36 ± 0.04 µmol of CO_2 m^{-2} s^{-1}) (**Figure 3D**). A similar pattern was observed for estimates of R_{day} using the Kok method with canopy trees showing the highest rates (0.32 ± 0.07 µmol of CO_2 m^{-2} s^{-1}), understory trees showing the lowest rates (0.10 ± 0.02 µmol of CO_2 m^{-2} s^{-1}), and intermediate rates in lower canopy trees (0.18 ± 0.03 µmol of CO_2 m^{-2} s^{-1}) (**Figure 3E**). Thus, there was a decrease in both R_{dark} and R_{day} with depth into the canopy. In contrast, the light suppression of R_{dark} showed a different trend with respect to canopy position with canopy trees showing the lowest values (37 ± 10%), understory trees showing the highest values (68 ± 9 µmol of CO_2 m^{-2} s^{-1}), and lower canopy trees showing intermediate values (50 ± 5 µmol of CO_2 m^{-2} s^{-1}) (**Figure 3F**). Thus, while R_{dark} and R_{day} decreased with depth into the forest canopy, the light suppression of R_{dark} increased. These trends were found to be statistically significant ($p < 0.001$, n = 74 - 770~~1~~).

Relationships between respiratory rates and leaf traits

~~While t~~The mean values of leaf mass area (LMA) ~~for canopy trees decreased from a maximum in~~were 99 ± 5 g m^{-2} in canopy trees~~(99 ± 5~~, 88 ± 4 g m^{-2} in lower canopy trees and 86 ± 2 g m^{-2} in the understory trees. There were~~this trend was not~~ no statistically difference ($p = 0.069$, n = 77) between the groups (**Supplementary Figure S2A**). For N and P leaf content (**Supplementary Figures S2 B,C**), no clear variation with canopy position was observed ($p = 0.29$; $p = 0.28$, n = 77). We also observed no statistical difference in concentration of sugars ($p = 0.13$, n = 26) and total NSC ($p = 0.19$, n = 26; **Supplementary Figures S2D-F**). Also, for starch there was no statistical difference between the groups (**Supplementary Figures S2E;** $p = 0.47$, n = 26), but the mean values for the canopy (0.68 ± 0.19%) was higher when compared to the lower canopy (0.44 ± 0.11%) and understory (0.40 ± 0.14%).

The relationship between R_{day} and R_{dark} (**Figure 4A**) shows a strong positive correlation ($r^2 = 0.76$, $p < 0.001$). The R_{day} x Light suppression had a different trend and significant relationship ($p < 0.001$), with a strong explanation ($r^2 = 0.59$, $p < 0.001$), demonstrating that higher values of daytime respiration have lower values of light suppression (**Figure 4B**).

When assessing the relationship between R_{dark} and leaf mass per area (LMA), we found 26% of explanation ($p < 0.001$) (**Figure 5A**). For R_{dark} and N content the relationship is non-significant ($r^2 = 0.01$, $p = 0.37$; **Figures 5B, C**) while the relationship between R_{dark} and P is

significant (r^2 = 0.18, $p < 0.001$). In addition, no significant correlations were observed between R_{dark} and any of the components of NSCs (**Figures 5D-F**), including sugars (r^2 = 0.053, p = 0.26), starch (r^2 = 0.00053, p = 0.91), and total NSC (r^2 = 0.047, p = 0.29).

4 Discussion

Here we observed that using Kok method we can have an accurate estimate of apparent light respiration when compare with $^{13}CO_2$ labelling (**Figure 1**). It is important to highlight in this case that $^{13}CO_2$ labelling also does not consider the internal recycling of CO_2. In the field work, our results highlight the influence of the crown position in the rates of R_{day} and R_{dark}. The physiological variables related to ~~photosynthesis and~~ respiration in the dark and in the light, differ according to the crown position (**Figures 3D, E**). In addition to physiological variables, light suppression was also influenced by crown position (**Figure 3F**), with higher values in the understory and lower values in the canopy trees. R_{dark} has a strong relationship with R_{day} (**Figure 4A**) and medium correlation with leaf mass area (**Figure 5A**), which indicates that the higher respiration is associated with higher LMA. Trees in the canopy have higher LMA (**Supplementary Figure S2A**), which may be associated with the plant's strategies for using light. In this study, no relationships were found for N (**Figure 5B**) and carbohydrates (**Figures 5 D-F**). Otherwise, relationships with P content were significant (**Figure 5C**). All these results are very important and have great implications on dynamics models of carbon assimilation in tropical forests.

Crown position and the effect on leaf respiration and light suppression of R_{dark}

Leaf respiration is affected by the availability of light (Meir et al., 2001; Kosugi et al., 2012; Asao et al., 2015; Araki et al., 2017), as the microenvironment along the vertical gradient of the forest is different, especially when comes to temperature and light (Mulkey et al., 1996; Marenco et al., 2014; Rey-Sánchez et al., 2016). The need for light varies continuously between species and influences their life strategies, directing the investment of their resources, whether for growth or survival (Poorter and Bongers, 2006). These results demonstrate the importance of the vertical gradient in the physiological characteristics of the leaves (Kosugi et al. 2012, Weerasinghe et al. 2014, Heskel and Tang 2018).

The relationship between higher respiration rates and the availability of light may be related to a higher demand for energy for possible repairs due to photodamage (Weerasinghe et al., 2014; Santos et al., 2018). In contrast, the lower values in the understory, indicate that

these trees do not require as much energy. This promotes a positive carbon balance under light-limited conditions, a typical characteristic of understory species that invest in survival, waiting for the moment of increased resources to invest in growth (Poorter and Bongers, 2006; Lambers et al., 2008). Light suppression of R_{dark} is higher in the understory trees (**Figure 3F).** However, most studies have not observed differences in light suppression when assessing the effect of upper and lower position of the same canopy (Weerasinghe et al. 2014, Heskel and Tang 2018). This divergence between studies can be explained using different species in different crown positions, since the studies mentioned worked with the intraspecific variation of the individuals.

The rate of light suppression of respiration and the canopy position had an different behavior when compared to respiratory rates in light and in the dark. Suppression is higher on species in the understory and is lower in species in the canopy (**Figure 3F; Figure 4B**). Respiratory activity throughout the day is regulated by the demand for energy and structural carbohydrates during the photosynthesis process (Lambers et al., 2008; Tcherkez and Ghashghaie, 2017; Collalti et al., 2019). Thus, suppression rates are lower in species that have greater metabolic activity. Higher irradiance results in greater demand for respiratory products, such as ATP, NADH, and carbon skeletons, reflecting higher rates of respiration in the presence of light (Weerasinghe et al. 2014), and this also might vary in function of temperature (Way et al., 2018). To elucidate which factors influences the variation in light suppression, it is necessary to know the effect of temperature and irradiance on leaf respiration (Atkin et al., 2000; Lambers et al., 2008; Way et al., 2015, 2018).

The rate of leaf respiratory inhibition can vary under conditions of water availability (Crous et al., 2012; Turnbull et al., 2017) and depending on the age of the soils, being higher in old and P-deficient soils (Atkin et al., 2013). In temperate forests, the inhibition of respiration varies seasonally (Heskel et al., 2014; Heskel and Tang, 2018), as they have defined seasons. In addition, apparent suppression may vary depending on the method of estimating daytime respiration (Way et al., 2018; Keenan et al., 2019). Knowing the light suppression of respiration in different ecosystems allows inferring carbon use efficiency (CUE). However, specific studies are necessary, since suppression varies in each type of ecosystem (Atkin et al., 2013; Turnbull et al., 2017).

In the context of climate change, a higher occurrence of extreme events is expected, which will cause an increase in the frequency of natural disturbances in the forest and,

consequently, might benefit individuals from the understory, promoting and an increase in their respiratory rates, as evidenced by this work. Emission of respiratory CO_2 is higher in canopy trees being two times higher than those in the understory. Species in the understory have slower growth, which is why they invest more in the carbon economy, whereas canopy species have more light and higher temperatures available, respires more, emitting more amount of atmospheric CO_2 through the forest.

Although the Amazon rainforest is of great importance for the global carbon cycle, the quantification of light suppression of respiration in this biome is still limited. Inhibition of leaf respiration strongly influences the respiration estimates of the ecosystem and when not considered in the models, they cause overestimations and, consequently, underestimate the NPP (Kroner and Way, 2016; Wehr et al., 2016; Keenan et al., 2019). Studies that estimated autotrophic respiration of the ecosystem in the Amazon rainforest (Chambers et al., 2004; Malhi et al., 2014), considered the inhibition of light respiration through correction factors obtained in studies not developed in the Amazon.

Relationships between respiratory rates and leaf traits

Canopy trees, which have a relatively exposed canopy, have higher values of respiration, leaf mass per area, and nitrogen rates (Asao et al., 2015; Araki et al., 2017; Rowland et al., 2021). Those individuals in the canopy invest in a smaller area and higher mass to protect them from damage by excessive irradiance, while those in the understory invest in a specific leaf area for light interception (Wright et al., 2004; Poorter and Bongers, 2006; Lambers et al., 2008). Respiration in the dark correlates with the levels of phosphorus (**Figure 5C**). Respiratory substrates participate in nitrogen assimilation pathways and phosphorus, present in the ATP molecule, the main respiratory product (Tcherkez et al., 2017a; Tcherkez and Ghashghaie, 2017).

Levels of nitrogen and phosphorus prove to be important for improve modeled carbon fluxes and dynamics in the forest (Zhu et al., 2019) and also can help to understand the response of plants to global warming (Tang et al., 2018). In this study no relationships were found for N (**Figure 5B**), but for P we found a significant relationship (**Figure 5C**). A similar result was observed by (Meir et al., 2001). It is known that Amazonian soils are limited by this nutrient (Quesada et al., 2010), and this nutrient is linked to R_{dark} through multiple processes, is essential for the formation of proteins, nucleic acids, phosphate trioses and ADP

phosphorylation (Tcherkez and Ghashghaie, 2017). On the respiratory process it can restrict glycolysis and function of the electron transport chain (Atkin et al., 2015; Rowland et al., 2017; Tcherkez and Ghashghaie, 2017).

The relations of R_{dark} and N and P levels are higher in areas with soils with lower P concentration (Crous et al., 2017; Rowland et al., 2017). This can be explained by the high respiratory cost for investment in leaf nutrients. Saturated photosynthesis is less sensitive than respiration at low concentrations of P in the soil (Crous et al., 2017; Rowland et al., 2017). However, (Meir et al., 2001), when comparing the behavior of dark leaf respiration in the Jaru forest in Rondônia and in a forest in Cameroon, observed that in Brazil (Jaru), LMA explained 20% of the variation of R_{dark}. In Brazil, the best predictor variables were LMA and P content. The relationships between the main characteristics of the leaf (N, P, and LMA or SLA) with respiration shows a significant biogeographic variation (Atkin et al., 2015; Crous et al., 2017; Rowland et al., 2017).

Although some studies report a strong relationship between respiratory rates and nitrogen content, the characteristics of the study site, such as soil fertility, must be considered (Crous et al., 2017; Rowland et al., 2017). As for respiration responses, biogeographic variation is large, which indicates that soil fertility has a strong influence on these responses, being inversely proportional to the availability of P in the soil (Atkin et al., 2015; Crous et al., 2017; Rowland et al., 2017). These results are relevant to the modeling of the carbon cycle in tropical forests, as they support the hypothesis that nutrient limitations affect photosynthetic and respiratory rates (Lambers et al., 2008; Peng et al., 2021) at different scales.

Non-structural carbohydrates are important for plant metabolism since they provide carbon skeletons for plant metabolism (*e.g* growth, reproduction, protection, defense) and are substrate for respiration process (Hartmann and Trumbore, 2016; Landhäusser et al., 2018; Dickman et al., 2019). Here in this study, we found no relationship between soluble sugar concentration and respiratory rates (**Figure 5D**), where higher respiratory demand shows a lower concentration of sugars. For starch concentrations, the relationship with respiration was also not evident (**Figure 5E**). Asao and Ryan, (2015) report that changes in carbohydrate levels did not directly affect the responses of photosynthesis and respiration in trees under branch girdling. However, (Collalti et al., 2019) state that the variation in plant respiration is dependent on the demand for substrates, demonstrating that respiratory rates vary depending on the need of the plant, varying between photoassimilates products or investment in biomass.

The variation within canopy position did not affect sugar, starch and total NSC concentration (**Supplementary Figures S2 D-F**). Leaves more exposed to sun usually have higher levels of soluble sugars when compared to shade leaves (Weerasinghe et al., 2014; Dayer et al., 2021). Starch is a reserve carbohydrate and not a fast consumption like sugars (Dickman et al. 2019), this perhaps can explain why there is higher concentration of starch and lower concentration of sugars in canopy trees. Other explanation is because our samples were collected during the early morning and because leaf NSCs peak of accumulation occurs at midday (Dickman et al., 2019; Gersony et al., 2020) we did not verify the effect of canopy position on NSCs concentrations.

5 Conclusion

Light suppression of leaf respiration varies according to the degree of canopy position. So, trees from the canopy had higher rates of light and dark respiration than trees from the understory. In the opposite light suppression is higher in the understory and lower on canopy trees. In addition, leaf respiration shows a positive and strong relationship with LMA and P content. These changes in physiology as a function of light availability have a great importance in carbon assimilation and can influence drought responses (Rowland et al., 2021). When the suppression is not considered in the models, it may cause an underestimation of NPP. Furthermore, knowledge about the impact of canopy position on inhibition of respiration will allow the refinement of the parameterization of climate models, as canopy trees have lower suppression and, consequently, have higher exchange of CO_2 with the atmosphere than the understory.

Conflict of interest

None declared.

Supplementary data

Supplementary data is available at …. Online.

Author Contribution

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NGEE – Tropics.

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Data Availability Statement

References

Albert, L. P., Wu, J., Prohaska, N., de Camargo, P. B., Huxman, T. E., Tribuzy, E. S., et al. (2018). Age-dependent leaf physiology and consequences for crown-scale carbon uptake during the dry season in an Amazon evergreen forest. *New Phytol.* 219, 870–884. doi:10.1111/nph.15056.

Araki, M. G., Gyokusen, K., and Kajimoto, T. (2017). Vertical and seasonal variations in temperature responses of leaf respiration in a Chamaecyparis obtusa canopy. *Tree Physiol.* 37, 1269–1284. doi:10.1093/treephys/tpx012.

Araújo, R. F., Chambers, J. Q., Celes, C. H. C., Muller-landau, H. C., Santos, A. P. F., Emmert, F., et al. (2020). Integrating high resolution drone imagery and forest inventory

to distinguish canopy and understory trees and quantify their contributions to forest structure and dynamics. 1–16. doi:10.1371/journal.pone.0243079.

Asao, S., Bedoya-Arrieta, R., and Ryan, M. G. (2015). Variation in foliar respiration and wood CO<inf>2</inf> efflux rates among species and canopy layers in a wet tropical forest. *Tree Physiol.* 35, 148–159. doi:10.1093/treephys/tpu107.

Asao, S., and Ryan, M. G. (2015). Carbohydrate regulation of photosynthesis and respiration from branch girdling in four species of wet tropical rain forest trees. *Tree Physiol.* 35, 608–620. doi:10.1093/treephys/tpv025.

Atkin, O. K., Bloomfield, K. J., Reich, P. B., Tjoelker, M. G., Asner, G. P., Bonal, D., et al. (2015). Global variability in leaf respiration among plant functional types in relation to climate and leaf traits. *New Phytol.* 206, 614–636. doi:10.1111/nph.13253.

Atkin, O. K., Evans, J. R., Ball, M. C., Lambers, H., and Pons, T. L. (2000). Leaf respiration of snow gum in the light and dark. Interactions between temperature and irradiance. *Plant Physiol.* 122, 915–923. doi:10.1104/pp.122.3.915.

Atkin, O. K., Turnbull, M. H., Zaragoza-Castells, J., Fyllas, N. M., Lloyd, J., Meir, P., et al. (2013). Light inhibition of leaf respiration as soil fertility declines along a post-glacial chronosequence in New Zealand: An analysis using the Kok method. *Plant Soil* 367, 163–182. doi:10.1007/s11104-013-1686-0.

Buckley, T. N., Vice, H., and Adams, M. A. (2017). The Kok effect in Vicia faba cannot be explained solely by changes in chloroplastic CO 2 concentration. *New Phytol.* 216, 1064–1071. doi:10.1111/nph.14775.

Cardoso, D., Särkinen, T., Alexander, S., Amorim, A. M., Bittrich, V., Celis, M., et al. (2017). Amazon plant diversity revealed by a taxonomically verified species list. *Proc. Natl. Acad. Sci. U. S. A.* 114, 10695–10700. doi:10.1073/pnas.1706756114.

Carneiro, V. M. C. (2004). Composição florística e análise estrutural da floresta primária de terra firme na bacia do Rio Cuieiras, Manaus-AM. *Diss. Mestr.*, 77.

Cavaleri, M. A., Coble, A. P., Ryan, M. G., Bauerle, W. L., Loescher, H. W., and Oberbauer, S. F. (2017). Tropical rainforest carbon sink declines during El Niño as a result of reduced photosynthesis and increased respiration rates. *New Phytol.* 216, 136–149. doi:10.1111/nph.14724.

Chambers, J. Q., Tribuzy, E. S., Toledo, L. C., Crispim, B. F., Higuchi, N., Dos Santos, J., et al. (2004). Respiration from a tropical forest ecosystem: Partitioning of sources and low carbon use efficiency. *Ecol. Appl.* 14, 72–88. doi:10.1890/01-6012.

Clark, D. A., and Clark, D. B. (1992). Life History Diversity of Canopy and Emergent Trees in a Neotropical Rain Forest. *Ecol. Monogr.* 62, 315–344.

Collalti, A., Tjoelker, M. G., Hoch, G., Mäkelä, A., Guidolotti, G., Heskel, M., et al. (2019). Plant respiration: Controlled by photosynthesis or biomass? *Glob. Chang. Biol.* 23. doi:10.1111/gcb.14857.

Crous, K. Y., O'Sullivan, O. S., Zaragoza-Castells, J., Bloomfield, K. J., Negrini, A. C. A., Meir, P., et al. (2017). Nitrogen and phosphorus availabilities interact to modulate leaf trait scaling relationships across six plant functional types in a controlled-environment

study. *New Phytol.* 215, 992–1008. doi:10.1111/nph.14591.

Crous, K. Y., Zaragoza-Castells, J., Ellsworth, D. S., Duursma, R. A., Löw, M., Tissue, D. T., 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, 966–981. doi:10.1111/j.1365-3040.2011.02465.x.

Dayer, S., Murcia, G., Prieto, J. A., Durán, M., Martínez, L., Píccoli, P., et al. (2021). Non-structural carbohydrates and sugar export in grapevine leaves exposed to different light regimes. *Physiol. Plant.* 171, 728–738. doi:10.1111/ppl.13258.

Dickman, L. T., McDowell, N. G., Grossiord, C., Collins, A. D., Wolfe, B. T., Detto, M., et al. (2019). Homoeostatic maintenance of nonstructural carbohydrates during the 2015–2016 El Niño drought across a tropical forest precipitation gradient. *Plant Cell Environ.* 42, 1705–1714. doi:10.1111/pce.13501.

Farquhar, G. D., and Busch, F. A. (2017). Changes in the chloroplastic CO2 concentration explain much of the observed Kok effect: a model. *New Phytol.* 214, 570–584. doi:10.1111/nph.14512.

Gauthier, P. P. G., Saenz, N., Griffin, K. L., Way, D., and Tcherkez, G. (2020). Is the Kok effect a respiratory phenomenon? Metabolic insight using 13C labeling in Helianthus annuus leaves. *New Phytol.* doi:10.1111/nph.16756.

Gersony, J. T., Hochberg, U., Rockwell, F. E., Park, M., Gauthier, P. P. G., and Holbrook, N. M. (2020). Leaf carbon export and nonstructural carbohydrates in relation to diurnal water dynamics in mature oak trees1[OPEN]. *Plant Physiol.* 183, 1612–1621. doi:10.1104/pp.20.00426.

Gong, X. Y., Tcherkez, G., Wenig, J., Schäufele, R., and Schnyder, H. (2018). Determination of leaf respiration in the light: comparison between an isotopic disequilibrium method and the Laisk method. *New Phytol.* 218, 1371–1382. doi:10.1111/nph.15126.

Griffin, K. L., Turnbull, M., and Murthy, R. (2002). Canopy position affects the temperature response of leaf respiration in Populus deltoides. *New Phytol.* 154, 609–619. doi:10.1046/j.1469-8137.2002.00410.x.

Hartmann, H., and Trumbore, S. (2016). Understanding the roles of nonstructural carbohydrates in forest trees - from what we can measure to what we want to know. *New Phytol.* 211, 386–403. doi:10.1111/nph.13955.

Heskel, M. A. (2018). Small flux, global impact: Integrating the nuances of leaf mitochondrial respiration in estimates of ecosystem carbon exchange. *Am. J. Bot.* 105, 815–818. doi:10.1002/ajb2.1079.

Heskel, M. A., Bitterman, D., Atkin, O. K., Turnbull, M. H., and Griffin, K. L. (2014). Seasonality of foliar respiration in two dominant plant species from the Arctic tundra: Response to long-term warming and short-term temperature variability. *Funct. Plant Biol.* 41, 287–300. doi:10.1071/FP13137.

Heskel, M. A., and Tang, J. (2018). Environmental controls on light inhibition of respiration and leaf and canopy daytime carbon exchange in a temperate deciduous forest. *Tree Physiol.* 38, 1886–1902. doi:10.1093/treephys/tpy103.

Huntingford, C., Atkin, O. K., Martinez-De La Torre, A., Mercado, L. M., Heskel, M. A., Harper, A. B., et al. (2017). Implications of improved representations of plant respiration in a changing climate. *Nat. Commun.* 8. doi:10.1038/s41467-017-01774-z.

Keenan, T. F., Migliavacca, M., Papale, D., Baldocchi, D., Reichstein, M., Torn, M., et al. (2019). Widespread inhibition of daytime ecosystem respiration. *Nat. Ecol. Evol.* 3, 407–415. doi:10.1038/s41559-019-0809-2.

Kok, B. (1948). A critical consideration of the quantum yield of Chlorella photosynthesis. *Enzymologia* 13, 407–415.

Kok, B. (1949). On the interrelation of respiration and photosynthesis in green plants. *Biochim. Biophys. Acta* 3, 625–631.

Kosugi, Y., Takanashi, S., Yokoyama, N., Philip, E., and Kamakura, M. (2012). Vertical variation in leaf gas exchange parameters for a Southeast Asian tropical rainforest in Peninsular Malaysia. *J. Plant Res.* 125, 735–748. doi:10.1007/s10265-012-0495-5.

Kroner, Y., and Way, D. A. (2016). Carbon fluxes acclimate more strongly to elevated growth temperatures than to elevated CO2 concentrations in a northern conifer. *Glob. Chang. Biol.* 22, 2913–2928. doi:10.1111/gcb.13215.

Lambers, H., Chapin, S., and Pons, T. (2008). *Plant Physiological Ecology*. doi:10.1017/CBO9781107415324.004.

Landhäusser, S. M., Chow, P. S., Dickman, L. T., Furze, M. E., Kuhlman, I., Schmid, S., et al. (2018). Methods paper Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. 1764–1778. doi:10.1093/treephys/tpy118.

Loreto, F., Delfine, S., and Di Marco, G. (1999). Estimation of photorespiratory carbon dioxide recycling during photosynthesis. *Aust. J. Plant Physiol.* 26, 733–736.

Loreto, F., Velikova, V., and Di Marco, G. (2001). Respiration in the light measured by 12CO2 emission in 13CO2 atmosphere in maize leaves. *Aust. J. Plant Physiol.* 28, 1103–1108. doi:10.1071/pp01091.

Malhi, Y. (2012). The productivity, metabolism and carbon cycle of tropical forest vegetation. *J. Ecol.* 100, 65–75. doi:10.1111/j.1365-2745.2011.01916.x.

Malhi, Y., Farfán Amézquita, F., Doughty, C. E., Silva-Espejo, J. E., Girardin, C. A. J., Metcalfe, D. B., et al. (2014). The productivity, metabolism and carbon cycle of two lowland tropical forest plots in south-western Amazonia, Peru. *Plant Ecol. Divers.* 7, 85–105. doi:10.1080/17550874.2013.820805.

Marenco, R. A., Antezana-vera, S. A., Romenya, P., Angelo, M., Camargo, B., Oliveira, M. F. De, et al. (2014). Fisiologia de espécies florestais da Amazônia : fotossíntese , respiração e relações hídricas. *Acta Amaz.*, 786–789.

Meir, P., Grace, J., and Miranda, A. C. (2001). Leaf respiration in two tropical rainforests: Constraints on physiology by phosphorus, nitrogen and temperature. *Funct. Ecol.* 15, 378–387. doi:10.1046/j.1365-2435.2001.00534.x.

Metcalfe, D. B., Meir, P., Aragão, L. E. O. C., Lobo-do-Vale, R., Galbraith, D., Fisher, R. A.,

et al. (2010). Shifts in plant respiration and carbon use efficiency at a large-scale drought experiment in the eastern Amazon. *New Phytol.* 187, 608–621. doi:10.1111/j.1469-8137.2010.03319.x.

Mulkey, S. S., Kitajima, K., and Wright, S. J. (1996). Plant physiological ecology of tropical forest canopies. *Trends Ecol. Evol.* 11, 436–437. doi:10.1016/0169-5347(96)10043-4.

Peng, Y., Bloomfield, K. J., Cernusak, L. A., Domingues, T. F., and Colin Prentice, I. (2021). Global climate and nutrient controls of photosynthetic capacity. *Commun. Biol.* 4, 1–9. doi:10.1038/s42003-021-01985-7.

Poorter, L., and Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology* 87, 1733–1743. doi:10.1890/0012-9658(2006)87[1733:LTAGPO]2.0.CO;2.

Quesada, C. A., Lloyd, J., Schwarz, M., Patiño, S., Baker, T. R., Czimczik, C., et al. (2010). Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences* 7, 1515–1541. doi:10.5194/bg-7-1515-2010.

Rey-Sánchez, A. C., Slot, M., Posada, J. M., and Kitajima, K. (2016). Spatial and seasonal variation in leaf temperature within the canopy of a tropical forest. *Clim. Res.* 71, 75–89. doi:10.3354/cr01427.

Rowland, L., Antonio, C. L., Oliveira, R. S., Bittencourt, P. R. L., Giles, A. L., Coughlin, I., et al. (2021). The response of carbon assimilation and storage to long-term drought in tropical trees is dependent on light availability. 43–53. doi:10.1111/1365-2435.13689.

Rowland, L., Zaragoza-Castells, J., Bloomfield, K. J., Turnbull, M. H., Bonal, D., Burban, B., et al. (2017). Scaling leaf respiration with nitrogen and phosphorus in tropical forests across two continents. *New Phytol.* 214, 1064–1077. doi:10.1111/nph.13992.

Santos, V. A. H. F. dos, Ferreira, M. J., Rodrigues, J. V. F. C., Garcia, M. N., Ceron, J. V. B., Nelson, B. W., et al. (2018). Causes of reduced leaf-level photosynthesis during strong El Niño drought in a Central Amazon forest. *Glob. Chang. Biol.* 24, 4266–4279. doi:10.1111/gcb.14293.

Slot, M., and Winter, K. (2017). In situ temperature response of photosynthesis of 42 tree and liana species in the canopy of two Panamanian lowland tropical forests with contrasting rainfall regimes. *New Phytol.* 214, 1103–1117. doi:10.1111/nph.14469.

Tang, X., Fan, S., Zhang, W., Gao, S., Chen, G., and Shi, L. (2019). Global variability in belowground autotrophic respiration in terrestrial ecosystems. *Earth Syst. Sci. Data* 11, 1839–1852. doi:10.5194/essd-11-1839-2019.

Tang, Z., Xu, W., Zhou, G., Bai, Y., Li, J., Tang, X., et al. (2018). Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to productivity in China's terrestrial ecosystems. *Proc. Natl. Acad. Sci. U. S. A.* 115, E6095–E6096. doi:10.1073/pnas.1808126115.

Tcherkez, G., Gauthier, P., Buckley, T. N., Busch, F. A., Barbour, M. M., Bruhn, D., et al. (2017a). Leaf day respiration: low CO2flux but high significance for metabolism and carbon balance. *New Phytol.* 216, 986–1001. doi:10.1111/nph.14816.

Tcherkez, G., Gauthier, P., Buckley, T. N., Busch, F. A., Barbour, M. M., Bruhn, D., et al.

(2017b). Tracking the origins of the Kok effect, 70 years after its discovery. *New Phytol.* 214, 506–510. doi:10.1111/nph.14527.

Tcherkez, G., and Ghashghaie, J. (2017). *Plant Respiration : Metabolic Fluxes and Carbon Balance*. Cham, Switzerland doi:10.1007/978-3-319-68703-2.

Ter Steege, H., Pitman, N. C. A., Sabatier, D., Baraloto, C., Salomão, R. P., Guevara, J. E., et al. (2013). Hyperdominance in the Amazonian tree flora. *Science (80-.).* 342. doi:10.1126/science.1243092.

ter Steege, H., Prado, P. I., Lima, R. A. F. d., Pos, E., de Souza Coelho, L., de Andrade Lima Filho, D., et al. (2020). Biased-corrected richness estimates for the Amazonian tree flora. *Sci. Rep.* 10, 1–13. doi:10.1038/s41598-020-66686-3.

Turnbull, M. H., Ogaya, R., Barbeta, A., Peñuelas, J., Zaragoza-Castells, J., Atkin, O. K., et al. (2017). Light inhibition of foliar respiration in response to soil water availability and seasonal changes in temperature in Mediterranean holm oak (Quercus ilex) forest. *Funct. Plant Biol.* 44, 1178–1193. doi:10.1071/FP17032.

Way, D. A., Aspinwall, M. J., Drake, J. E., Crous, K. Y., Campany, C. E., Ghannoum, O., et al. (2018). Responses of respiration in the light to warming in field-grown trees: a comparison of the thermal sensitivity of the Kok and Laisk methods. *New Phytol.* 222, 132–143. doi:10.1111/nph.15566.

Way, D. A., Holly, C., Bruhn, D., Ball, M. C., and Atkin, O. K. (2015). Diurnal and seasonal variation in light and dark respiration in field-grown Eucalyptus pauciflora. *Tree Physiol.* 35, 840–849. doi:10.1093/treephys/tpv065.

Weerasinghe, L. K., Creek, D., Crous, K. Y., Xiang, S., Liddell, M. J., Turnbull, M. H., et al. (2014). Canopy position affects the relationships between leaf respiration and associated traits in a tropical rainforest in Far North Queensland. *Tree Physiol.* 34, 564–584. doi:10.1093/treephys/tpu016.

Wehr, R., Munger, J. W., McManus, J. B., Nelson, D. D., Zahniser, M. S., Davidson, E. A., et al. (2016). Seasonality of temperate forest photosynthesis and daytime respiration. *Nature* 534, 680–683. doi:10.1038/nature17966.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi:10.1038/nature02403.

Yin, X., Niu, Y., van der Putten, P. E. L., and Struik, P. C. (2020). The Kok effect revisited. *New Phytol.* 227, 1764–1775. doi:10.1111/nph.16638.

Zhang, P., Mcdowell, N. G., Zhou, X., Wang, W., Leff, R. T., Pivovaroff, A. L., et al. (2021). Declining carbohydrate content of Sitka-spruce treesdying from seawater exposure Research Article. 1682–1696. doi:10.1093/plphys/kiab002.

Zhu, Q., Riley, W. J., Tang, J., Collier, N., Hoffman, F. M., Yang, X., et al. (2019). Representing Nitrogen, Phosphorus, and Carbon Interactions in the E3SM Land Model: Development and Global Benchmarking. *J. Adv. Model. Earth Syst.* 11, 2238–2258. doi:10.1029/2018MS001571.

~~Zhang P, McDowell NG, Wang W, Leff RT, Pivovaroff AL, Zhang H, Chow PS, Ward ND,~~

Indivero J, Zhou X, Bailey VL. Declining carbohydrate content of Sitka spruce trees dying from seawater exposure. Plant Physiology in pre

Figures:

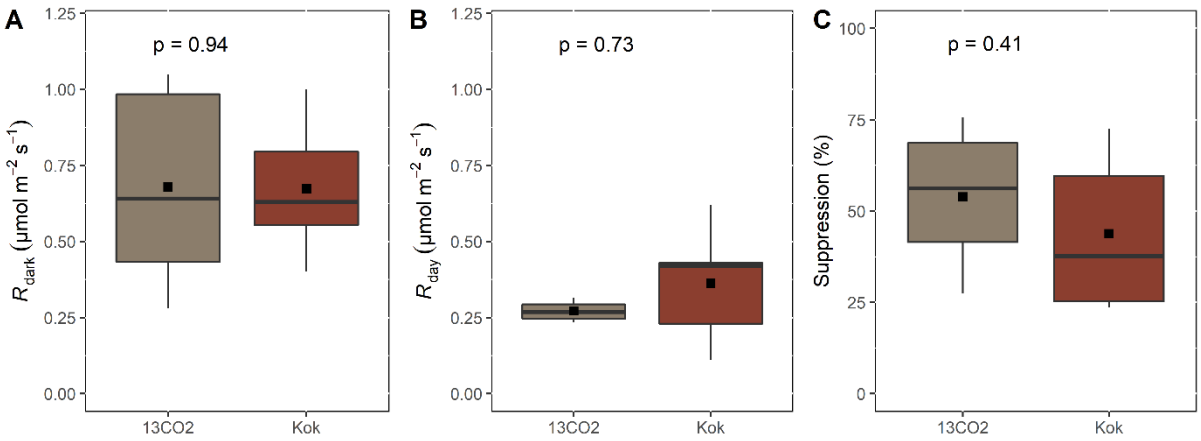


FIGURE 1. Comparison between Kok and $^{13}CO_2$ method for estimating leaf respiration rates in the dark (R_{dark}, **A**); in the light (R_{day}, **B**), and the suppression of R_{dark} (Suppression, **C**). The line inside the box indicates the median of the data, the colored bar represents the 50% variation of the data, the vertical lines represent the 90 % of the variation, and the square point inside the colored box represents the mean. (n =4-6). The test used was non-parametric t-test.

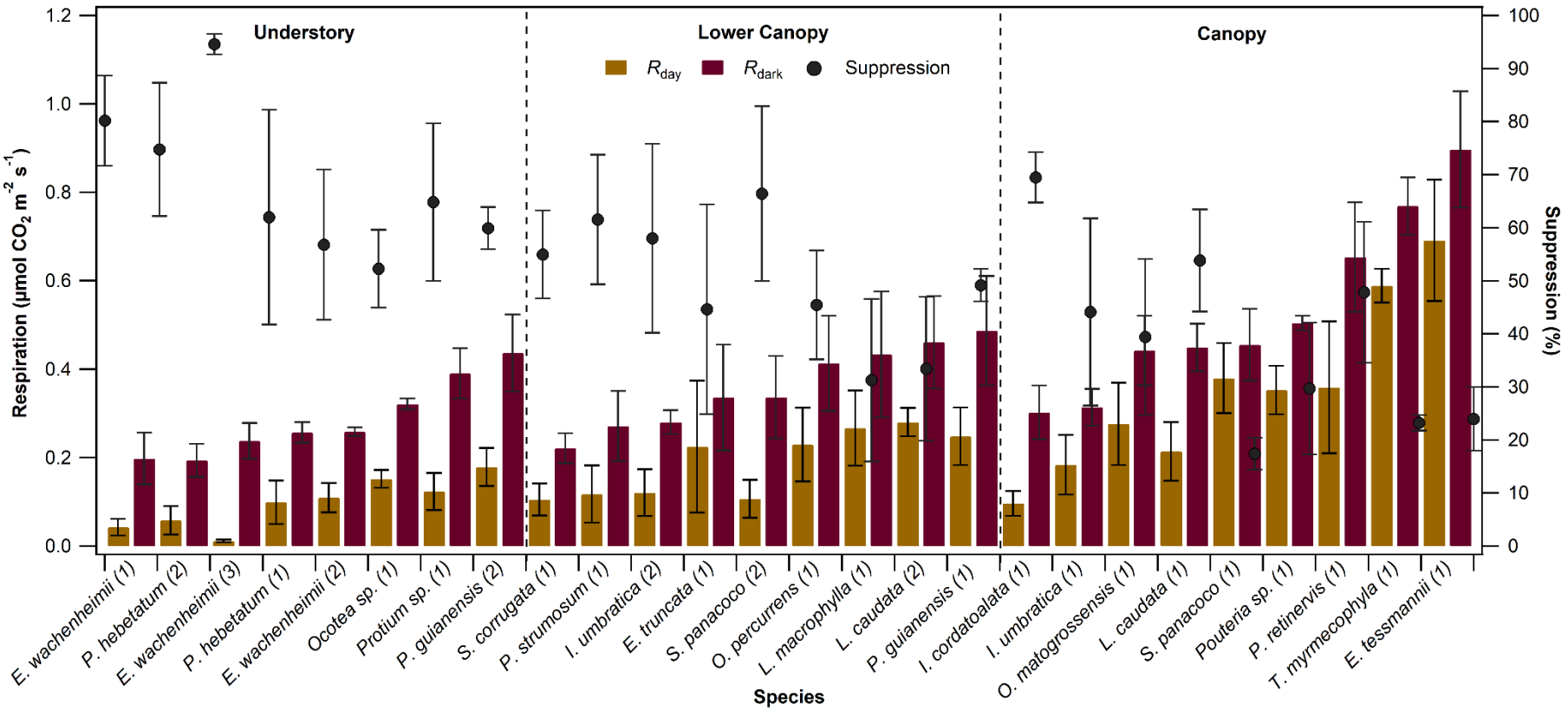


FIGURE 2. Respiration rate measured in the light (R_{day}, brown bars) and in dark adapted leaves (purple bar), and light suppression (black point) for each individual studied. The number after the specie name represent the number of individuals per specie studied. Values are mean ± standard error (n = 2-3). Data are organized in function of crown position, u~~U~~nderstory, l~~L~~ower canopy and c~~C~~anopy.

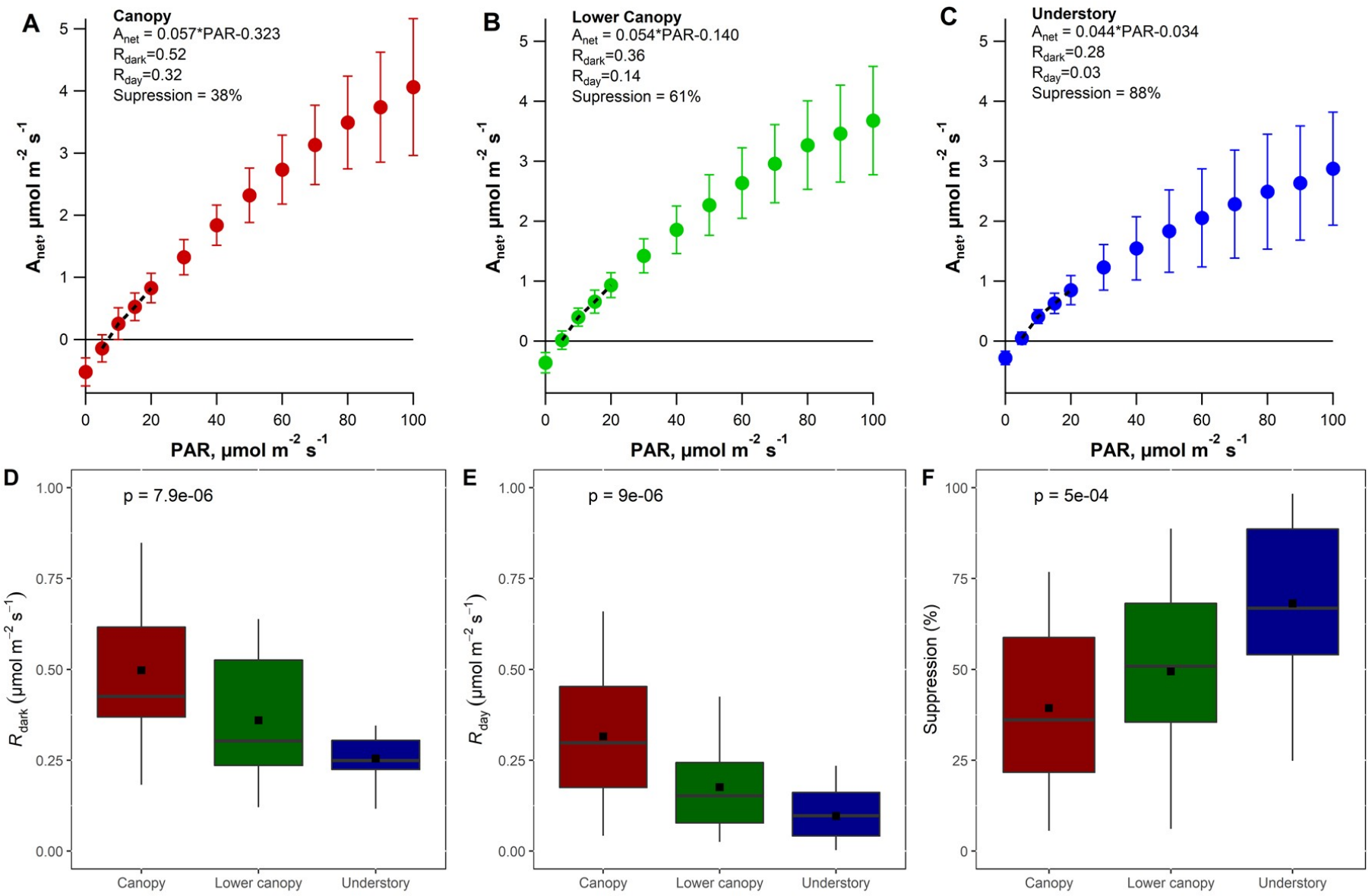

A
Canopy
A_{net} = 0.057*PAR-0.323
R_{dark}=0.52
R_{day}=0.32
Supression = 38%
B
Lower Canopy
A_{net} = 0.054*PAR-0.140
R_{dark}=0.36
R_{day}=0.14
Supression = 61%
C
Understory
A_{net} = 0.044*PAR-0.034
R_{dark}=0.28
R_{day}=0.03
Supression = 88%
A_{net}, µmol m^{-2} s^{-1}
PAR, µmol m^{-2} s^{-1}
D
p = 7.9e-06
E
p = 9e-06
F
p = 5e-04
R_{dark} (µmol m^{-2} s^{-1})
R_{day} (µmol m^{-2} s^{-1})
Suppression (%)
Canopy
Lower canopy
Understory

FIGURE 3. Light response curve showing the Kok effect for each class of crown position. **A** - canopy, **B** - lower canopy, and **C** - understory, showing linear regression for points above the abrupt change in the quantum yield (QY) of net CO_2 assimilation. Dotted lines show points 5 to 20, that was used to obtain apparent light respiration (below), estimated using the Kok method. Data are averages of leaves measured during 2019 in three different campaigns. Points are the average, and the vertical line represents the standard error. (understory, n = 24; lower canopy, n = 27; canopy, n = 26). The second part of the figure represents boxplots of **D** - respiration rates in the dark (R_{dark}), **E** - apparent respiration rates in the light (R_{day}), and **F -** apparent suppression of light respiration relative to the dark. The line inside the box indicates the median of the data, the colored bar represents the 50% variation of the data, the vertical lines represent the nineteenth percent of the variation, and the square point inside the colored box represents the mean (understory, n = 24; lower canopy, n = 27; canopy, n = 24-26). p values are shown for the non-parametric, Kruskal-Wallis test.

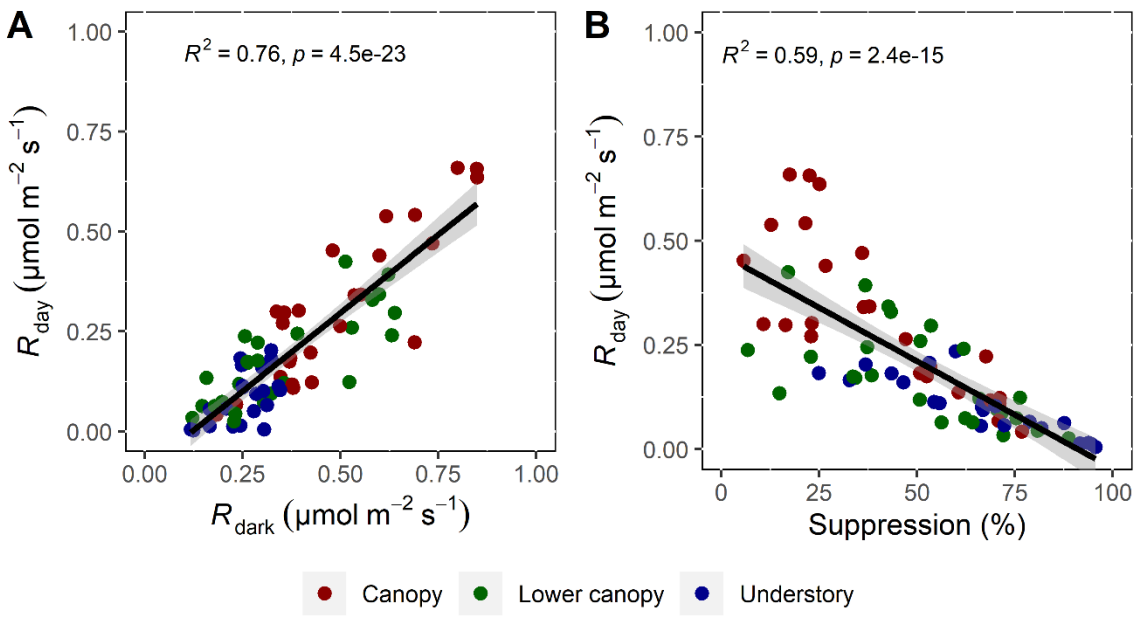


FIGURE 2. Respiration rates in the light as a function of respiration in the dark (**A**) and respiration in the light as a function of light suppression (**B**). The data come from the measurements collected in understory (blue), lower canopy (green), and canopy trees (red), n = 74-76.

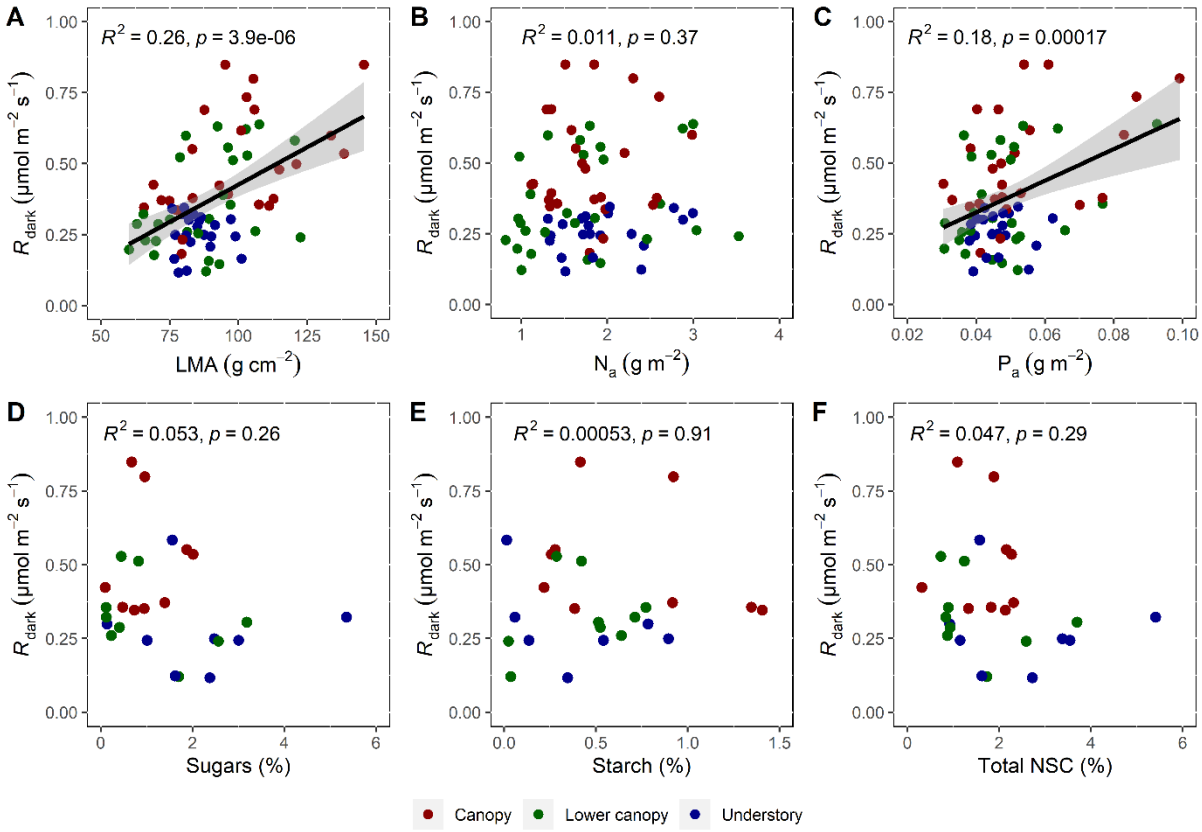


FIGURE 5 Respiration rates in the dark as a function of LMA (**A**), nitrogen (**B**), phosphorus (**C**), (n = 76~~6~~). Sugars (**D**), Starch (**E**) and Total NSC (**F**), (n = 26). The data come from the measurements collected in understory (blue), lower canopy (green), and canopy (red) trees.

TABLE 1. List of the 26 individuals studied and their corresponding scientific name, diameter at breast height (DBH), total height and crown illumination index (Clark and Clark, 1992).

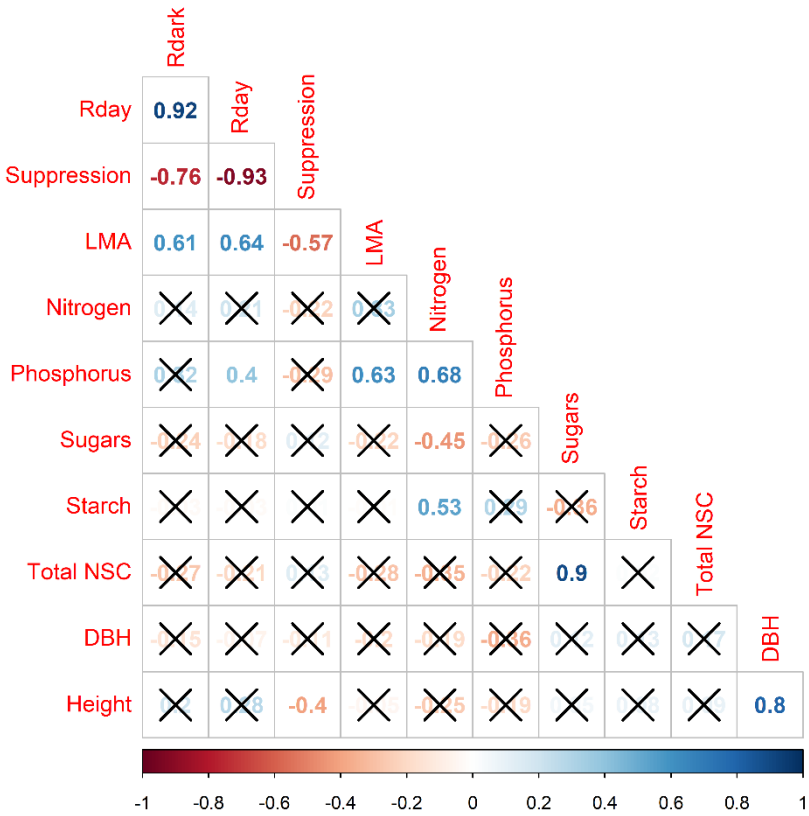
Scientific name	Family	DBH (cm)	Height (m)	Crown index*
Eschweilera wachenheimii (Benoist) Sandwith~~*Protium strumosum* Daly~~	Lecythidaceae~~Burseraceae~~	15.8~~26.5~~	13~~17~~	2~~3~~
Eschweilera wachenheimii (Benoist) Sandwith~~*Eschweilera wachenheimii* (Benoist) Sandwith~~	Lecythidaceae~~Lecythidaceae~~	23.1~~15.8~~	15~~13~~	2~~2~~
Eschweilera wachenheimii (Benoist) Sandwith~~*Eschweilera wachenheimii* (Benoist) Sandwith~~	Lecythidaceae~~Lecythidaceae~~	12.5~~23.1~~	12~~15~~	2~~2~~
Protium hebetatum Daly~~*Eschweilera wachenheimii* (Benoist) Sandwith~~	Burseraceae~~Lecythidaceae~~	13.6~~12.5~~	13.5~~12~~	2~~2~~
Ocotea sp.~~*Licania caudata* Prance~~	Lauraceae~~Chrysobalanaceae~~	14.5~~29.5~~	11~~22.5~~	2~~4~~
Protium hebetatum Daly~~*Eschweilera truncata* A.C.Sm.~~	Burseraceae~~Lecythidaceae~~	12~~24.5~~	9.5~~20~~	2~~3~~
Protium sp.~~*Inga cordatoalata* Ducke~~	Burseraceae~~Fabaceae~~	12.5~~18.7~~	11.7~~18~~	2~~4~~
Pouteria guianensis Aubl.~~*Protium hebetatum* Daly~~	Sapotaceae~~Burseraceae~~	10.4~~13.6~~	8~~13.5~~	2~~2~~
Protium strumosum Daly~~*Inga umbratica* Poepp. & Endl.~~	Burseraceae~~Fabaceae~~	26.5~~19.5~~	17~~21~~	3~~4~~
Eschweilera truncata A.C.Sm.~~*Ocotea* sp.~~	Lecythidaceae~~Lauraceae~~	24.5~~14.5~~	20~~11~~	3~~2~~
Ocotea percurrens A. Vicentini~~*Protium hebetatum* Daly~~	Lauraceae~~Burseraceae~~	12.3~~12~~	14~~9.5~~	3~~2~~
Pouteria guianensis Aubl.~~*Swartzia corrugata* Benth.~~	Sapotaceae~~Fabaceae~~	14.2~~17~~	15~~19~~	3~~4~~
Ocotea matogrossensis Vatt.~~*Ocotea percurrens* Vicentini~~	Lauraceae~~Lauraceae~~	16~~12.3~~	17~~14~~	3~~3~~
Licania macrophylla Benth.~~*Pouteria* sp.~~	Chrysobalanaceae~~Sapotaceae~~	14.8~~24.5~~	15.5~~21~~	3~~4~~
Swartzia panacoco (Aubl.) R.S. Cowan~~*Tachigali myrmecophyla* (Ducke) Ducke~~	Fabaceae~~Fabaceae~~	15.1~~17.6~~	17~~23~~	3~~4~~
Inga umbratica Poepp. & Endl.~~*Pouteria guianensis* Aubl.~~	Fabaceae~~Sapotaceae~~	14.5~~14.2~~	16.5~~15~~	3~~3~~
Licania caudata Prance~~*Swartzia panacoco* (Aubl.) R.S. Cowan~~	Chrysobalanaceae~~Fabaceae~~	11.9~~16~~	14.5~~20~~	3~~4~~
Licania caudata Prance~~*Ocotea matogrossensis* Vatt.~~	Chrysobalanaceae~~Lauraceae~~	29.5~~16~~	22.5~~17~~	4~~3~~
Inga cordatoalata Ducke~~*Licania macrophylla* Benth.~~	Fabaceae~~Chrysobalanaceae~~	18.7~~14.8~~	18~~15.5~~	4~~3~~
Inga umbratica Poepp. & Endl.~~*Swartzia panacoco* (Aubl.) R.S. Cowan~~	Fabaceae~~Fabaceae~~	19.5~~15.1~~	21~~17~~	4~~3~~
Swartzia corrugata Benth.~~*Inga umbratica~~*	Fabaceae~~Fabaceae~~	17~~14.5~~	19~~16.5~~	4~~3~~

	Poepp. & Endl.				
	Pouteria sp.*Protium* sp.	SapotaceaeBurseraceae	24.512.5	2111.7	42
	Tachigali myrmecophyla (Ducke) Ducke*Pouteria retinervis* T.D.Penn	FabaceaeSapotaceae	17.610.9	2316.7	44
	Swartzia panacoco (Aubl.) R.S. Cowan*Licania caudata* Prance	FabaceaeChrysobalanaceae	1611.9	2014.5	43
	Pouteria retinervis T.D.Penn*Eschweilera tessmannii* R.Kunth	SapotaceaeLecythidaceae	10.911.5	16.717	44
	Eschweilera tessmannii R.Kunth*Pouteria guianensis* Aubl.	LecythidaceaeSapotaceae	11.510.4	178	42

*Classification according to Clark & Clark (1992).

Supplementary data

FIGURE S1. Matrix of correlation between respiration in the light and leaf traits. The "x" symbol represents correlations with p-value ≥ 0.05.


Rdark
Rday
Suppression
LMA
Nitrogen
Phosphorus
Sugars
Starch
Total NSC
DBH
0.92
-0.76
-0.93
0.61
0.64
-0.57
0.63
0.68
0.4
-0.45
0.53
0.9
0.8
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1

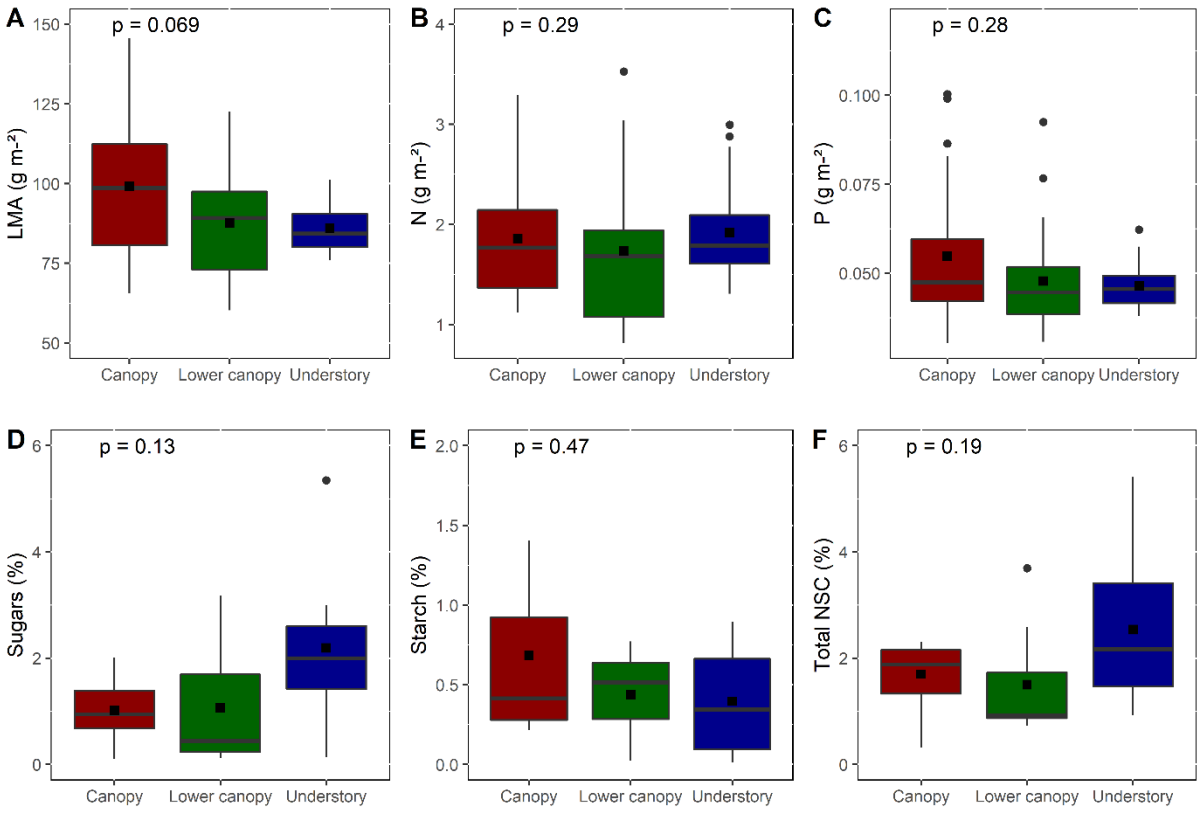

A
p = 0.069
LMA (g m-2)
B
p = 0.29
N (g m-2)
C
p = 0.28
P (g m-2)
D
p = 0.13
Sugars (%)
E
p = 0.47
Starch (%)
F
p = 0.19
Total NSC (%)
Canopy
Lower canopy
Understory

FIGURE S2. Box plots of leaf mass area (**A**), nitrogen (**B**), phosphorus (**C**), (understory, n = 24; lower canopy, n = 27; canopy, n = 26). Sugars (**D**), starch (**E**) and total NSC (**F**), (understory, n = 8; lower canopy, n = 9; canopy, n = 9). The line inside the box indicates the median of the data, the colored bar represents the 50% variation of the data, the vertical error lines represent the nineteenth percent of the variation, points are outliers (single leaves) and the square point inside the colored box represents the mean. The test used was the non-parametric, Kruskal-Wallis.

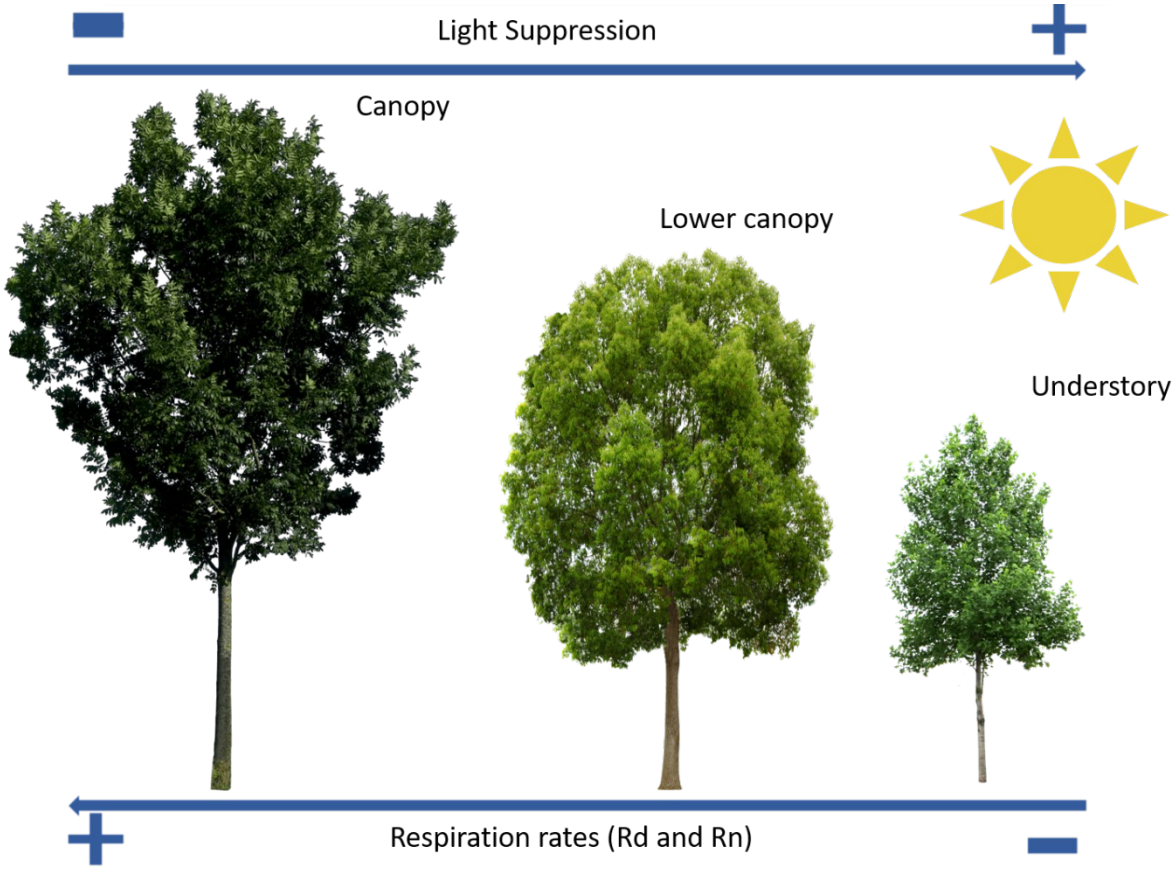


FIGURE S3 (Graphical abstract): resume of the main findings of this work. Respiration rates are higher in trees from the canopy than trees in the understory. But the light suppression had an opposite behavior, being higher in the understory trees. This may happen because of higher metabolic activity in sun exposed leaves.