Rapid response of mesophyll conductance to light availability allows shade leaves to take advantage of sunflecks

Courtney E. Campany1, Belinda Medlyn1, Mark Tjoelker1, Susanne von Caemmerer2, Remko A. Duursma1.

1 Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW, Australia

2 Plant Science Division, Research School of Biology, The Australian National University, Canberra, ACT, Australia

*Corresponding author*: Courtney Campany E: [courtneycampany@gmail.com](mailto:courtneycampany@gmail.com)

# Summary

* Light gradients within tree canopies play a major role in the distribution of resources that define photosynthetic capacity of individual leaves. The transient nature of light availability within a canopy, however, complicates our ability to assess the contribution of shade leaves to canopy carbon gain.
* Leaf gas exchange was coupled with online carbon isotope discrimination to measure photosynthesis (A), stomatal conductance (gs) and mesophyll conductance (gm) of sun and shade leaves in *Eucalyptus tereticornis* trees grown in climate controlled whole tree chambers.
* Reduction in A, Vcmax, Jmax and leaf nitrogen were detected in shade leaves, however, they maintained consistently higher gs than sun leaves. When light intensity was increased for shade leaves gs was higher in shade than in sun leaves, gm increased compared to the low light environment and A in shade leaves was greater than sun leaves at the same PPFD.
* Here we show that dynamic physiological responses of shade leaves to altered light environments has important implications for upscaling leaf level measurements and predicting whole canopy carbon gain. We argue that the rapid response of gm with light allows shade leaves to "lie in wait" for sunflecks. Evidence that gm not only complicates including gmm in process based models of canopy carbon gain.

## Key words

photosynthesis, stomatal conductance, mesophyll conductance, shade, leaf optimal behavior

# Introduction

Understanding and predicting carbon (C) uptake in forest ecosystems is crucially important for assessing the impacts of environmental change. Specifically, this involves accurate upscaling of leaf level measurements to predict whole canopy function. Due to the costs and limitations of efficient light harvesting, plants cannot expose all leaves to full sun (Niinemets, 2010), making simple upscaling problematic. Incident PPFD declines exponentially with cumulative leaf area index, creating a steep light gradient from the canopy top to bottom (Monsi & Saeki, 2005). Consequently, leaves modify structural and functional properties to efficiently use light (Vogelman *et al.*, 1996; Niinemets & Valladares, 2004) as changing irradiance strongly affects rates of photosynthesis (A) (Evans, 1995). To estimate whole canopy C gain it is thus necessary to account for the non-linear response of A to light by distinguishing between shaded and sunlit leaves (De Pury & Farquhar, 1997; Linderson *et al.*, 2012).

The distribution of resources required for A, including leaf nitrogen (N) and supply of water, are also partially defined by canopy light gradients. As A has a saturating response with light and a the maximum rates depends on N, it has been argued that leaf N should be proportional to PPFD along the canopy gradient to maximize canopy C gain at a given total canopy N (Field, 1983; Field & Mooney, 1986; Peltoniemi *et al.*, 2012). Acclimiation of chlorophyll per unit N, chlorophyll a:b ratios, electron transport capacity per unit chlorophyll, and ratios of electron transport capacity to Rubisco activity can also occur in response to changes in irradiance (Evans & Poorter, 2001). Sun leaves frequently experience greater water limitations in the upper canopy, despite effective vascular systems developed for high radiation loads and transpiration (Sellin *et al.*, 2008; Niinemets, 2012). Higher rates of A and stomatal conductance (gs) can only be sustained if the hydraulic conductance (K) is also large enough to avoid low leaf water potentials (Hubbard *et al.*, 2001). Optimal photosynthetic N investment in the upper canopy will be wasted if hydraulic supply is insufficient (Niinemets, 2012; Peltoniemi *et al.*, 2012), thus K should also be higher in the upper canopy to supply sunlit leaves with sufficient water (Burgess *et al.*, 2006; Sellin & Kupper, 2007 ; Sellin *et al.*, 2008).

During A, CO2 diffuses from the atmosphere through stomata (gs), intercellular air space and into the chloroplast for fixation by Rubisco (mesophyll conductance, gm) (Tazoe *et al.*, 2011). Stomatal conductance should be efficiently distributed within a canopy to utilize supplies of light, N and water to maximize A (Peltoniemi *et al.*, 2012). This is because stomata are hypothesized to exhibit an optimal behaviour to maximize C gain while simultaneously minimizing water loss through transpiration (Cowan & Farquhar, 1977). Mesophyll conductance can also impose limitations on A as large gs (Warren, 2008; Ubierna & Marshall, 2011), reducing the efficiency of leaf N use in A (Niinemets, 2007). Variation in photosynthetic capacity between sun and shade leaves has been proposed to be due to differences in gm (Piel *et al.*, 2002; Warren *et al.*, 2007), yet the trade-offs that constrain this diffusion pathway are yet to be explicitly quantified. Stomatal and mesophyll conductance should not be considered independent of each other (Griffiths & Helliker, 2013), but a lack empirical data currently hinders are ability to interpreting their coupled responses to A across sun and shade leaves.

Additionally, assessing shade leaf behaviour is made difficult with accounting of short term light fluctuations within a canopy, via sunflecks. How shade leaves utilize sunflecks for short term C gain depends on the combined response time of gs and gm and the underlying photosynthetic biochemistry acclimated to a low light environment (Pearcy, 1990; Tausz *et al.*, 2005). For example, at short timescales the utilization of sunflecks is limited by delayed responses of stomata, effectively limiting the maximum assimilation rate that can be achieved (Vico *et al.*, 2011; Way & Pearcy, 2012). Mesophyll conductance has been shown to respond to environmental factors (e.g. CO2, temperature or VPD) across short time scales, possibly faster than gs (Flexas *et al.*, 2008 and references therin), yet short term response to light availabiity are unclear. For example, gm was found to be independent of light intensity in wheat leaves (Tazoe *et al.*, 2009) but was responsive to light in tobacco (Flexas *et al.*, 2007). Anatomical parameters which regulate gm with changing irradiance such as chloroplast surface area (Terashima *et al.*, 2006) and mesophyll thickness (Boardman, 1977) are also unlikely to adapt during short light fluctuations. The physiological behaviour of shade leaves to maximize C gain must be assessed as both a degree of acclimation to local irradiance and as a potential response to transitory light availability.

Climate warming may also potentially affect the physiological behaviour of leaves within a canopy. This is because leaves can be exposed to different heat, water and high light stresses as temperature and vapour pressure deficit (VPD) scale positively with canopy light availability (Baldocchi *et al.*, 2002; Niinemets & Valladares, 2004; Niinemets, 2007). How these stresses affect the diffusion of CO2, through either gs or gm will have implications for upscaling A for sun and shade leaves. Additionally, light saturated rates of A are limited by the maximum rate of Rubisco carboxylation (Vcmax) or the maximum rate of photosynthetic electron transport (Jmax) across a range of temperatures, yet their temperature dependencies are not the same (Farquhar *et al.*, 1980; Medlyn *et al.*, 2002). How these parameters are differentially affected by warming may impact constraints of N distribution and leaf photosynthetic capacity across light gradients. The impacts of warming on plant physiological processes are obviously vast, yet differentiating their impacts on leaf physiology within a canopy will be essential to evaluate whole tree responses to a changing climate.

In this study we use *Eucalyptus tereticornis* Sm. trees, planted in climate controlled whole tree chambers with ambient and elevated temperature (+3°C) treatments, to empirically evaluate the distribution of resources and leaf physiological behaviour of sun and shade leaves. Our hypotheses are as follows:

1. If whole tree canopies are optimized for C gain, then leaf N, K and photosynthetic capacity are predicted to be higher in sun leaves compared to shade leaves.

2. Stomatal conductance is proportional to A across sun and shade leaves under similar leaf VPD and gm scales positively with photosynthetic capacity.

3. As shade leaves are constrained by their underlying biochemistry and slow physiological responses, increases in A following sunfleck simulations were not expected to reach levels of full sun leaves.

4. The effects of climate warming were predicted to be greater in sun than shade leaves, seen as a decrease in gs and leaf C gain during summer months.

# Materials and Methods

## Whole tree chamber experimental design

Twelve *Eucalyptus tereticornis* seedlings, chosen from a single local Cumberland plain cohort, were planted in March 2013 into 12 whole-tree chambers (WTC) at the Hawkesbury Forest Experiment site near Richmond, NSW, Australia. Each chamber has a height of 9 m and seedlings were grown for 15 months. A detailed description of the WTC operation and design is available in (Barton *et al.*, 2010). Six chambers were set to match outside ambient air temperatures (AT) while the remaining 6 experienced an elevated air temperature October 2013 to the end of the experiment trees were watered every 15 days with the mean monthly rainfall amount for Richmond, NSW. In February 2013 half of the chambers (3 each of AT and ET) were subjected to a drought treatment by withholding watering. Due to a limited range of data for the drought treatment only well-watered trees are reported, which reduces the sample size of WTC (n=6) after the initiation of this treatment.

Before seedlings were planted into each chamber they were maintained under well watered conditions in 35 L pots and kept inside each chamber. This allowed for seedlings to gain sufficient biomass before planting while also allowing them to acclimate to chamber temperature treatments. Seedlings were planted into each chamber after mean seedling height reached 100 cm. The top soils at this site, used in both pots and chambers, are an alluvial formation of low-fertility sandy loam soils with low organic matter and low water holding capacity. A root exclusion barrier extended from chamber walls to the hard layer (ca. 1 m) and roots were allowed to grow freely below the barrier. After 2 months, floors were installed 45 cm from the soil surface. This enabled chamber fluxes of CO2 and H2O from the whole tree canopy to be monitored (for methods see Barton et al. (2010)). Leaf gas exchange measurements were initiated in October 2013 when trees had both ample height growth and canopy development for realistic canopy light gradients to be measured. At this point, trees under AT treatment had a mean diameter of 28.2±1.1 mm, height of 348±15.1 cm and an estimated leaf area of 3.9±0.1 m2. For ET treatments trees had a mean diameter of 34.1±2.1 mm, height of 418.3±23.1 cm and an estimated leaf area of 6.2±0.2 m2.

## Leaf gas exchange, online carbon isotope discrimination and mesophyll conductance

Leaf gas exchange measurements were performed monthly through to the final harvest. Measurements were taken on a representative sun and shade leaf for each tree during each measurement campaign. The newest fully expanded leaf from the stem apex was chosen for gas exchange measurements and sun leaves were measured in the upper third of the canopy. Here, shade leaves are defined as inner-canopy leaves developing in a low light enivironment. In order to minimize any confounding effects of leaf age, shade leaves were measured first in the lower canopy then gradually higher during each campaign. As shade leaves most likely developed slower this assured that older leaves in the lower canopy were avoided. The nearest leaf on each branch was sampled for measurement of predawn leaf water potential.

Prior to gas exchange measurements photosynthetic photon flux density (PPFD) was recorded at the individual leaf level and at the canopy position for each selected leaf. A hand-held quantum sensor (LI-COR, Lincoln, NE, USA) was used to record leaf level PPFD to ensure that chosen leaves were positioned in the desired light environment, either full sun or full shade. A ceptometer (AccuPAR LP-80, Decagon Devices, Pullman, USA) was then used to measure integrated PPFD at the canopy height of each chosen leaf type. Each ceptometer reading integrated an array of 80 sensors over a total length of 84 cm. Five ceptometer readings were recorded within the canopy at the same height and clsoe to each selected leaf. The mean of these readings was assumed to represent the local light environment of full sun and shade leaves for each tree. All measurements of PPFD and gas exchange were performed on sunny days between 10:00-14:30 h.

Leaf level gas exchange was measured with a standard 6 cm2 leaf chamber using a portable gas exchange system (LI-6400, LI-COR, Lincoln, NE, USA). This system was coupled with a tunable diode laser (TDL; TGA100,Campbell Scientific, Inc., Logan, UT, USA) for concurrent measurements of online C isotope discrimination. The CO2 in the leaf chamber was set at ambient atmospheric [CO2] (400 ppm) with a flow rate of 200 mols s-1. Two identical gas exchanges systems were run simultaneously, one in each of a randomly chosen WTC for each temperature treatment. Leaf temperatures were controlled at the current AT or ET chamber air temperature. PPFD in the chamber was set to match the individual light environment of each leaf type (explained above). Sun flecks were then simulated for shade leaves by increasing the chamber PPFD to the light environment of the full sun leaf in the same tree. The maximum sunfleck response was then recorded once shade leaves re-stabilized in the leaf cuvette (ca. 25 min).

Once the chamber environment for leaves was stable, for each leaf measurement, the sample and reference gas lines were diverted to the TDL via T-junctions inserted into the reference gas tube and match valve outlet. These gases were dried by passing through napion gas dryers in the respective gas lines, and then 12CO2 and 13CO2 concentrations were measured for each gas by the TDL. Reference, sample and 2 calibration gases were run on alternating 80 s loops (20 s each), one for each paired WTC leaf, for a total of 12 min. This allowed for 4-5 measurements per leaf and data were averaged over the last 10 s of reference and samples gases for calculations. The two calibration gases were drawn from compressed air tanks (330 and 740 ppm CO2) in order to correct for gain drift of the TDLAS on each measurement cycle. Photosynthesis, gs, transpiration, VPD and intercellular [CO2] were auto logged every 15 s for each gas exchange system over the same 12 min interval.

Using online C isotope discrimination measurements, the difference between the observed discrimination and what is predicted for light saturated gas exchange is proportional to gm (Griffiths & Helliker, 2013). First, leaf discrimination was calculated by comparing the isotopic composition of the reference gas entering the chamber (13Ce) with the sample gas (13Co) such that:

(1)

where Rs is the isotopic ratio of the sample and Rstnd is the isotopic ratio of the standard Vienna Pee Dee Belemnite. Next, the observed discrimination (obs) is calculated from Evans et al. (1986):

(2)

where:

(3)

and is the ratio of the CO2 entering the well mixed leaf cuvette to the CO2 draw down by the leaf.

Second, C isotope discrimination during C3 photosynthesis () is the resultant discrimination from CO2 diffusion from the atmosphere to the site of carboxylation, consisting or a series of fractionation steps described in (Evans *et al.*, 1986). In this experiment, a modified form of this equation presented in (Evans & Von Caemmerer, 2013) with ternary effect corrections by (Farquhar & Cernusak, 2012) was used such that:

(4)

where o is the observed discrimination and i, gm , e and f are the contributions to fractionation if Ci = Cc, gm, respiration and photorespiration, respectively. The equations for each are as follows:

(5)

(6)

(7)

(8)

where the different fractionation factors include; diffusion through water (ai, 1.8‰), Rubisco carboxylation (b, 29‰), the photorespiratory fractionation (f, 16.2‰) and the combined fractionation through the boundary layer and the stomata (a'). a' is defined by:

(9)

where Cs is the CO2 partial pressure at the leaf surface, ab is the fractionation from boundary layer diffusion (2.9‰) and a is the fractionation due to diffusion in air (4.4‰) (Evans *et al.*, 1986). Ca and Ci are the atmospheric and intercellular partial pressures and is the compensation point in the absence of mitochondrial respiration in the light (Rd). In this experiment both and Rd were derived using a standard Arrhenius function with parameters for *Eucalyptus globulis* from (Crous *et al.*, 2012). The ternary effect corrections (t) are described by:

(10)

where E denotes the transpiration rate and is the total conductance to CO2 diffusion to both the boundary layer and stomatal conductance.

The CO2 diffusion from the intercellular airspace to the chloroplast, gm, is given by its relationship to the CO2 assimilation rate (A) by:

(11)

where Cc is the chloroplast CO2 partial pressure. Once gm was calculated Cc and the drawdown of CO2 from the intercellular air spaces to the site of carboxylation were then estimated using Equation 11. Examples of this approach to measure gas exchange and C isotope discrimination are presented in (Evans & Von Caemmerer, 2013). The variation in o between sun and shade leaves and the simulated sunfleck where then compared as a function of Ci:Ca.

## Biochemical parameters of photosynthesis

Photosynthetic CO2 response (ACi) curves were measured at 25 °C for one sun and shade leaf for each chamber prior to the initiation of the drought treatment. Each ACi curve began at the reference [CO2] of 400 l l-1 and then consisted of 12 additional steps from [CO2] of 50 to 1800 l l-1 at 25 °C at saturating light (1800 mols m-1 s-1). From these curves the photosynthetic parameters, Jmax and Vcmax, were quantified using the biochemical model of (Farquhar *et al.*, 1980) and fit with the 'plantecophys' package (Duursma, 2014) in R (R Development Core Team, 2011).

## Leaf chemistry and hydraulic parameters

Following gas exchange measurements each leaf was collected, measured for leaf water potential (explained below), scanned for leaf area, dried to constant mass and then weighed. These leaves were then milled and analyzed for leaf N content and 13C. Leaf samples were analysed on a Delta V Advantage coupled to a Flash HT and Conflo IV (Thermo Fisher Scientific, Bremen, Germany) in dual-reactor setup. Samples were flash combusted at 1000°C and converted to CO2 and N2 and then subjected to stable isotope ratio mass spectrometry. Leaf N is reported on an area basis (Na) and isotopic signatures are reported relative to the VPDP scale.

Predawn () and midday () leaf water potentials (MPa) were measured for sun and shade leaves during each gas exchange campaign using a PMS 1505D pressure chamber (PMS Instruments, Albany, OR, USA). Predawn leaf water potential was measured before sunrise on the same day as gas exchange measurements. Leaves used for gas exchange were immediately sampled for once measurements were completed. All leaves were detached and immediately stored inside foil covered bags before water potential measurements were performed. Leaf water potential and transpiration (E, mmol-2 s-1) from gas exchange were then used to calculate leaf-specific hydraulic conductance (K) through the equation:

(12)

Leaf level instantaneous transpiration efficiency (ITE) was calculated as leaf photosynthesis divided by transpiration. The g1 parameter was estimated from ITE to VPD response curves by fitting a rearranged optimal stomatal conductance model for ITE (Medlyn *et al.*, 2011) using non-linear regression, where k=0.5 (see Duursma *et al.*, 2013).

## Data analysis

Differences in experimental parameters to either the warming treatment or leaf type were analysed by mixed-effects models in R (R Development Core Team, 2011) with WTC as a random effect. Explained variance (R2) of mixed models were computed as in (Nakagawa & Schielzeth, 2013). Confidence intervals (95 %) of mixed effect linear models of A as a function of different physiological parameters were generated using bootstrapping methods with 999 simulations using the bootMer function in the 'lme4' package (Bates *et al.*, 2013). For non-linear relationships, confidence intervals were estimated by fitting a generalized additive model to the data with the 'mgcv' package, using WTC as a random effect. Results were considered significant when P ≤ 0.05.

# Results

## Leaf resource distribution

Across all measurement campaigns PPFD was significantly different between sun and shade leaves (P<0.001) and PPFD was reduced by 78% in the shade (Figure 2). Leaf-specific hydraulic conductance was similar across sun and shade leaves (Table 1). This was because , (Table 1) and E (Table 2) did not differ between leaf types. Leaf Na was approximately 20% higher in sun leaves than in shade leaves (Table1). Leaf mass per area was not different between leaf types. No effect of the warming treatment was detected with PPFD, , , K, E, Narea or LMA either within or across leaf types.

## Photosynthetic capacity and leaf photosynthesis rates

The photosynthetic parameters Jmax and Vcmax were both significantly higher in sun than shade leaves (Table 1), as esimated from ACi curves from each chamber (Figure 3a). Within leaf types, no effect of the warming treatment was detected on either parameter. Within individual chambers Vcmax was positively related to leaf Na across leaf types and temperature treatments (P = 0.01, Figure 3b). As gm (results below) is implicit in the estimates of Jmax and Vcmax with conventional ACi curves, we also simulated ACc curves to determine if treatment differences in ACi parameters where instead the result of differences in gm. When accounting for the effect of gm, chloroplastic Vcmax and Jmax were still higher in sun leaves (Figure S1).

Mean leaf photosynthesis rates were significantly higher in sun compared to shade leaves (+23%), when measured at their local light environment (Table 2). Additionally, leaf Na was positively related to A across gas exchange campaigns and leaf types (P < 0.001, Figure 3c). Following an increase in available light, A of shade leaves was significantly greater than both shade leaves at low light and sun leaves (P < 0.001). No effect of the warming treatment was detected on rates of A of sun leaves or shade leaves at low or high light. Photosynthesis within leaf types and treatments was similar through time and across the range of leaf temperatures measured (Figure S2a).

## Stomatal conductance and leaf water use efficiency

Mean gs was significantly higher in shade compared to sun leaves (+18%) under their local light environment (P = 0.004). In sun leaves, A was postively correlated with gs, however, gs and A were not correlated in shade leaves under low light (Figure 4a). Following an increase in available light, gs of shade leaves was significantly greater than both shade leaves at low light and sun leaves (Figure 5a). With increased light availability, increases in A and gs in shade leaves exhibited similar behavior as observed in sun leaves.

Leaf ITE was significantly greater in sun leaves than in shade leaves at low light (+21%, P = 0.001). Following an increase in available light, ITE of shade leaves did not differ from shade leaves at low light and was still significantly lower than sun leaves (P < 0.001). Instantaneous transpiration efficiency in sun leaves was reduced in the warming treatment but no effect was noted in shade leaves with low or high light (Table 2). For all leaf types and light treatments there was a strong response of ITE to VPD, and individual data points broadly corresponded to response curves from the optimal WUE model (Figure 6a). The mean estimated g1 for sun leaves was 1.51±0.11 and for shade leaves with low and high light was 2.59±0.12 and 2.74±0.04. Within leaf types and light treatments the response of VPD to leaf temperature was similar across all measurement campaigns (Figure S3a)

Bulk-leaf 13C, as an index of integrated water use efficiency (Marshall *et al.*, 2007), was significantly lower in shade leaves compared to sun leaves by ca. 2‰ (Table 1). No effects of the warming treatment on leaf 13C were detected. Leaf 13C and Narea were positively correlated for all leaves (P<0.001, Figure 6b), with less negative 13C and higher N investment in sun leaves.

## Leaf carbon isotope discrimination and mesophyll conductance

The observed carbon isotope discrimination was positively correlated with Ci:Ca for all leaf types (P < 0.001) with larger o detected for sun leaves and shade leaves at high light (Figure 7). Carbon isotope discrimination associated with gm accounted for the majority of o (69.7±0.4%) and varied little across measurement temperatures, leaf types, or warming treatments. No differences in gs were detected with the warming treatment within leaf types and gs did not vary significantly across measurement campaigns (Figure S2b).

Mean gm was higher in sun compared to shade leaves (+27%) under their local light environment (P < 0.001). Following an increase in available light, gm of shade leaves was significantly greater than both shade leaves at low light and sun leaves (Table 2). Mesophyll conductance was nearly proportional to A within leaf types and light treatments (Figure 5b,c). Photosynthesis scaled positively with increases in gm for all leaves and the large increases in gm in shade leaves under high light resulted in the highest rates of A (Figure 4b). No differences in gm were detected with the warming treatment within leaf types. Additionally, gm did not vary significantly across measurements campaigns (Figure S3b) and only a weak negative relationship with increasing leaf temperature was detected (Figure S2b).

## Variation in intercellular and chloroplastic CO2 concentrations

More open stomata in shade leaves under low and high light lead to significant increases in Ci compared to sun leaves (Figure 8a). The drawdown of CO2 from intercellular spaces to the chloroplast, Ci-Cc, measures the coordination between gm and A (Caemmerer & Evans, 2014). This drawdown was similar between sun and shade leaves at their local light environment and increased marginally in shade leaves at high light (Figure 8c). This was the result of the proportional relationship between gm and A across all leaves. The CO2 drawdown from Ca to Ci and Ci to Cc were both relatively stable across the range of temperatures measured and gas exchange campaigns (Figure S3c and S2c, respectively).

# Discussion

Here we show that leaves within canopies of *Eucalyptus tereticornis* trees maintain the ability to upregulate A beyond the limitations of their local light environment. More open stomata and rapid increases in gm allowed shade leaves to readily utilize increases in light availability, leading to rates of leaf C gain exceeding sun leaves. This finding is unexpected because although we know shade leaves experience interchanging periods of sun and shade (Pearcy, 1990), a lack of empirical data within tree canopies still impedes our ability to predict whole canopy C gain. This dynamic behaviour of shade leaves complicates optimizations of canopy C uptake and accounting for the response of leaf physiology to transient light dynamics is an essential need in future studies. Additionally, with measurements recorded across a large natural range of temperatures we were unable to detect any effects of a +3 °C warming treatment on leaf physiology.

## Resource distribution and photosynthetic capacity

The allocation of Na constrains photosynthesis rates and is thus a key trait in determining the relative contribution of individual leaves to canopy C gain. Decreasing light availability should decrease the investment into photosynthetic enzyme within a canopy (Mooney and Gulmon 1979). As a result, acclimation of photosynthetic capacity to irradiance is typically reflected in the key photosynthetic biochemical parameters Vcmax and Jmax (Farquhar *et al.*, 1980). Our data agree with these conventional conclusions as the distribution of Na , both measures of photosynthetic capacity and A were all reduced in shade leaves.

Photosynthesis is also limited by the ability to supply water to the upper canopy. Ultimately, the ability of a trees hydraulic architecture to supply water to foliage across increasing pathlengths affects productivity and survival (Sellin *et al.*, 2008). Using a two-leaf model, Peltoniemi et al. (2012) theorizes that optimal N distribution will be proportional to light distribution only if leaf K is also optimally distributed. In this study, variation in leaf N distribution and A rates were not associated with subsequent changes in leaf K between sun and shade leaves. As both sun and shade leaves were acclimated to their local light environment this sub-optimal distribution of water supply has the potential to diminish the C return for the whole canopy.

Unexpected higher rates of gs in shade leaves compared to sun leaves led to decreased ITE throughout the experiment. Additionally, consistently higher leaf 13C in shade leaves suggests that this pattern was prevalent across the leaf lifespan. From a canopy perspective this pattern in water use efficiency initially appears to be detrimental to C gain as A in sun leaves was characterized by low rates of gs and low Ci. Relative to the differences in photosynthesis rate between leaf types the higher rates of gs in shade leaves appear to exhibit waste water loss. As whole canopy C gain integrates the efficiency of all leaves, this begs the question of why shade leaves maintained a lower ITE compared to sun leaves.

## Physiological behaviour of sun and shade leaves

The pattern of wasteful water use in shade leaves is important as we hypothesized that gs and A would be proportional across sun and shade leaves. In sun leaves, A and gs were strongly correlated, exhibiting behaviour broadly consistent with optimal stomatal theory. However, lower rates of A in shade leaves were not coupled with decreases in gs, explaining the observed decreases in WUE. This is significant as optimal stomatal behaviour has been reported across a wide range of ecosystems and plant functional types; however, empirical data is often collected only on sun leaves (see Prentice *et al.*, 2014; Lin *et al.*, 2015). As a result, the often used economic framework of balancing costs of using water versus N allocation to predict A (Wright *et al.*, 2003) may break down when considering all leaves within a tree canopy.

It is possible that reducing stomatal response time, by sustaining higher gs, is a strategy to take advantage of high light quickly in shade leaves (Tausz *et al.*, 2005). Evidence from this study supports this hypothesis, as shade leaves increased A equivalent or even outperforming sun leaves under identical light intensity. Transipiration-induced cooling in shade leaves, by keeping stomata open, has also been suggested as an effective strategy to reduce sunfleck induced thermal load (Schymanski *et al.*, 2013). This is because rapid increases in leaf temperature with sunflecks have been shown to inhibit C gain (Leakey *et al.*, 2003). However, this response likely occurs at already high air temperatures and may not explain the observed rates of gs in shade leaves across the large range of temperatures included in this study. How prevalent each of these strategies are within tree canopies is still unknown, yet empirical studies assessing photosynthetic responses to sunflecks generally focus on seedlings (Küppers & Schneider, 1993; Pepin & Livingston, 1997; Leakey *et al.*, 2002) and understory plants (Chazdon & Pearcy, 1991; Allen & Pearcy, 2000; Brantley & Young, 2009). Thus, our findings highlight a critical need for empirical measurements of shade leaves under dynamic light environments in order to accurately scale C gain from leaf to canopy (see De Pury & Farquhar, 1997).

We found that A and gm scaled positively across leaf types and, suprisingly, increased proportionately when light intensity was increased in shade leaves. Research has suggested that aquaporins can facilitate increases in the CO2 permeability of the cell membranes resulting in rapid modulation of gm (Hanba *et al.*, 2004; Heinen *et al.*, 2009; Li *et al.*, 2014). This provides a potential explanation for the observed rapid increases in gm but the impacts of aquaporins on gm are untested in leaves of tree species. Our findings support a growing wealth of evidence that gm is highly variable and can respond to environmental variables (Flexas *et al.*, 2008). Here we provide empirical data showing gm not only varies within a canopy but the up-regulation of gm plays a critical role in the photosynthetic response of shade leaves to sunflecks.

If shade leaves "lie in wait" for sunflecks then perhaps we should consider an alternate leaf economic strategy to maximize C gain, beyond conventional trade-offs associated with canopy resource distribution. If the valuation of sunflecks as a C resource is large enough then costs of sub-optimal stomatal behaviour could be offset over the leaf lifespan. For example, the potential C gain in leaves which experience high amounts of sunflecks may be large enough to tolerate excess losses from transpiration. However, accounting for the heterogeneous nature of light within a canopy remains a current challenge for empirical and modelling studies. Thus, models which predict leaf photosynthesis from N distribution within a canopy will be flawed unless inclusion of canopy light extinction and the integration of sunflecks on shade leaves are included (De Pury & Farquhar, 1997).

## Conclusions

Here we show that dynamic physiological responses of shade leaves to altered light environments has important implications for upscaling leaf level measurements to the canopy. Although the distribution of resources, such as N and water, define leaf photosynthetic capacity it is the physiological behaviour of individual leaves which actually determine C gain. These findings suggest that current theories of leaf optimal behaviour should be extended to include dynamic light environments, which will have important implications for process based models that predict canopy C gain from rates of leaf photosynthesis. Furthermore, the dynamic nature of gm cannot be simply parameterized in tree growth models and possibly should be excluded until it can be represented properly. Additional empirical data across multiple tree species are needed to determine both the mechanisms and the capacity of gm to rapidly increase CO2 drawdown. To improve our ability to predict whole canopy C gain future research should prioritize the incorporation of both sun and shade leaf physiology, which may be optimized differently.

# List of Tables

**Table 1**. *Eucalyptus tereticornis* leaf traits and the distribution of resources between full sun and shade leaves under ambient and elevated temperature treatments. Leaf mass per area, leaf Na, 13C, pd, l and K values represent treatment mean (± 1 standard error) across measurement campaigns (n=6). Values of Vcmax and Jmax are treatment mean (± 1 standard error) from ACi curves measured in each chamber at saturating light. Units of LMA and leaf Narea are g m2, K is mmol m-2 s-1 MPa, WP is MPA and 13C is ‰. Different letters represent significant differences between treatments. The P value represents the overall difference between leaf types and warming treatments.

**Table 2**. Responses of leaf level gas exchange parameters of *Eucalyptus tereticornis* trees between full sun and shade leaves under ambient and elevated temperature treatments. Each value reflects the mean (± 1 standard error) for each treatment across all gas exchange campaigns (n=6). Units for A and E are mol m-2 s-1, for gs and gm are mol m-2 s-1 and for VPD are kPa. Different letters represent significant differences between treatments. The P value represents the overall difference between leaf types and both light and warming treatments.

# List of Figures

**Figure 1**. Daily maximum and minimum temperature (a) and total daily PPFD (b) for each chamber across the experiment duration.

**Figure 2**. Local light environment for sun and shade leaves for each gas exchange campaign. Means ± 1 standard error represent 1 m integrated PPFD, measured with a ceptometer, at the canopy height of each selected leaf.

**Figure 3**. (a) ACi curves for sun and shade leaves at elevated (ET) and ambient (AT) temperature treatments. ACi curves were measured once on all trees, before the initiation of the drought treatment, at 25°C and at saturating light (1800 mols m-1 s-1). (b) The relationship between Vcmax and mean leaf Na for each chamber, including sun leaves and shade leaves at low light. (c) The relationship between A and leaf Na for all each sun and shade leaf. For (b,c) the dashed line represents the significant linear model fit for all leaves with a marginal and conditional R2 of 0.28 and 0.35 for (b), and 0.24 and 0.33 for (c).

**Figure 4**. The response of A to gs (a) and gm (b) for sun leaves and shade leaves at both low and high light with elevated and ambient temperature treatments. The response of shade leaf physiology to high light was recorded once both A and gs were stable in the gas exchange leaf cuvette (ca. 25min). Lines represent either smoothed regressions from a generalized additive model fit (a) or linear model fits (b). Grey areas are approximately 95% confidence intervals from the mean.

**Figure 5**. The mean ± 1 standard error of gs (a), gm (b) and A (c) of sun leaves and shade leaves at both low and high light with standard errors.

**Figure 6**. (a) Response of ITE to leaf vapor pressure deficit (VPD) for sun leaves and shade leaves at both low and high light with elevated and ambient temperature treatments. (b) The relationship between leaf 13C and leaf Na for sun leaves and shade leaves at low light. For (a) VPD is the leaf to air pressure difference inside the gas exchange cuvette and lines represent predictions from the optimal ITE model with a g1 value for each leaf type and treatment. For (b) the dashed line represents the significant linear model fit for all leaves with a marginal and conditional R2 of 0.41 and 0.45, respectively.

**Figure 7**. Relationship between the observed discrimination of 13CO2 and measured Ci/Ca for sun leaves and shade leaves at both low and high light. The solid line represents the theoretical line for C3 plants from (Evans *et al.*, 1986).

**Figure 8**. The mean ± 1 standard error of intercellular CO2 concentration (a), CO2 concentration in the chloroplasts (b) and CO2 drawdown from substomatal cavities to sites of carboxylation of sun leaves and shade leaves at both low and high light.

**Figure S1**. Photosynthetic CO2 response (ACc) curves for sun and shade leaves at elevated and ambient temperature treatments. Cc values were predicted with gm, thus curves represent chloroplastic photosynthetic parameters at 25°C and at saturating light (1800 mols m-1 s-1).

**Figure S2**. Response of A (a), gm (b) and Ci-Cc to leaf temperature for sun leaves and shade leaves at low and high light. Shaded symbols represents each monthly measurement campaign.

**Figure S3**. Response of VPD (a), gs (b) and Ca-Ci to leaf temperature for sun leaves and shade leaves at low and high light. Shaded symbols represents each monthly measurement campaign.

# Tables

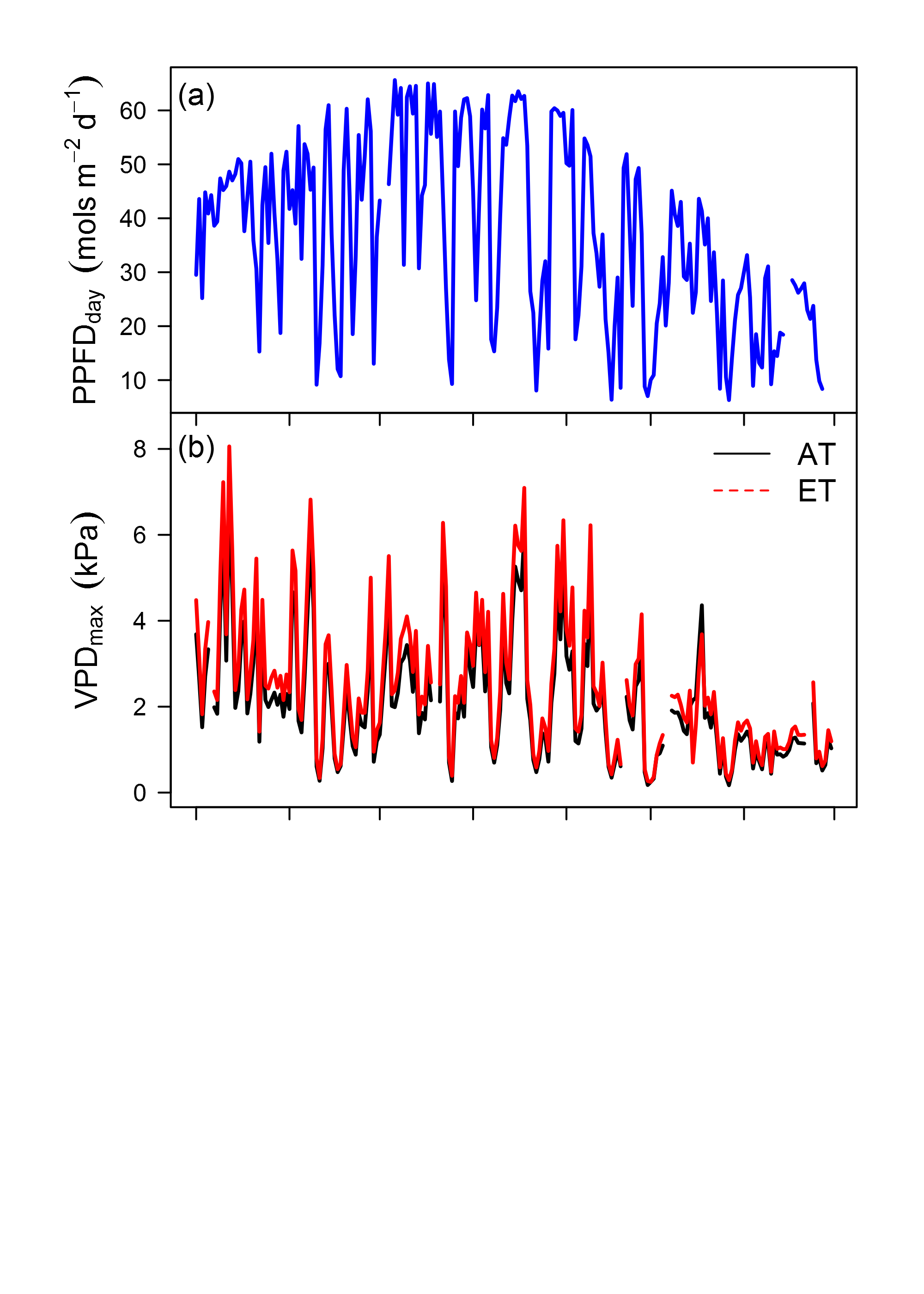
**Table 1**. *Eucalyptus tereticornis* leaf traits and the distribution of resources between full sun and shade leaves under ambient and elevated temperature treatments. Leaf mass per area, leaf Na, 13C, pd, l and K values represent treatment mean (± 1 standard error) across measurement campaigns (n=6). Values of Vcmax and Jmax are treatment mean (± 1 standard error) from ACi curves measured in each chamber at saturating light. Units of LMA and Leaf Nitrogenarea are g m2, K is mmol m-2 s-1 MPa, WP is MPA and 13C is ‰. Different letters represent significant differences between treatments. The P value represents the overall difference between leaf types and warming treatments.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Leaf** | **Temperature** | **LMA** | **Leaf Narea** | **Vcmax** | **Jmax** | **K** | **WPpre** | **WPmid** | **δ13c** |
| Sun | AT | 114.1 (4.5) a | 2.63 (0.08) b | 96.0 (6.7) b | 141.6 (7.5) b | 1.69 (0.18) a | -0.32 (0.03) a | -1.60 (0.10) a | -28.1 (0.18) b |
|  | ET | 109.9 (4.8) a | 2.60 (0.09) b | 95.5 (6.6) ab | 148.3 (11.8) b | 1.79 (0.15) a | -0.32 (0.02) a | -1.70 (0.09) a | -28.3 (0.17) b |
| Shade | AT | 118.3 (4.4) a | 2.13 (0.07) a | 73.3 (6.4) a | 102.1 (6.9) a | 1.70 (0.13) a | -0.27 (0.02) a | -1.50 (0.09) a | -29.9 (0.17) a |
|  | ET | 113.1 (4.3) a | 1.88 (0.06) a | 77.6 (4.9) ab | 106.2 (6.5) a | 1.78 (0.14) a | -0.30 (0.02) a | -1.60 (0.11) a | -30.4 (0.22) a |
| P value |  | 0.785 | 0.001 | 0.028 | 0.002 | 0.001 | 0.3486 | 0.6385 | 0.973 |

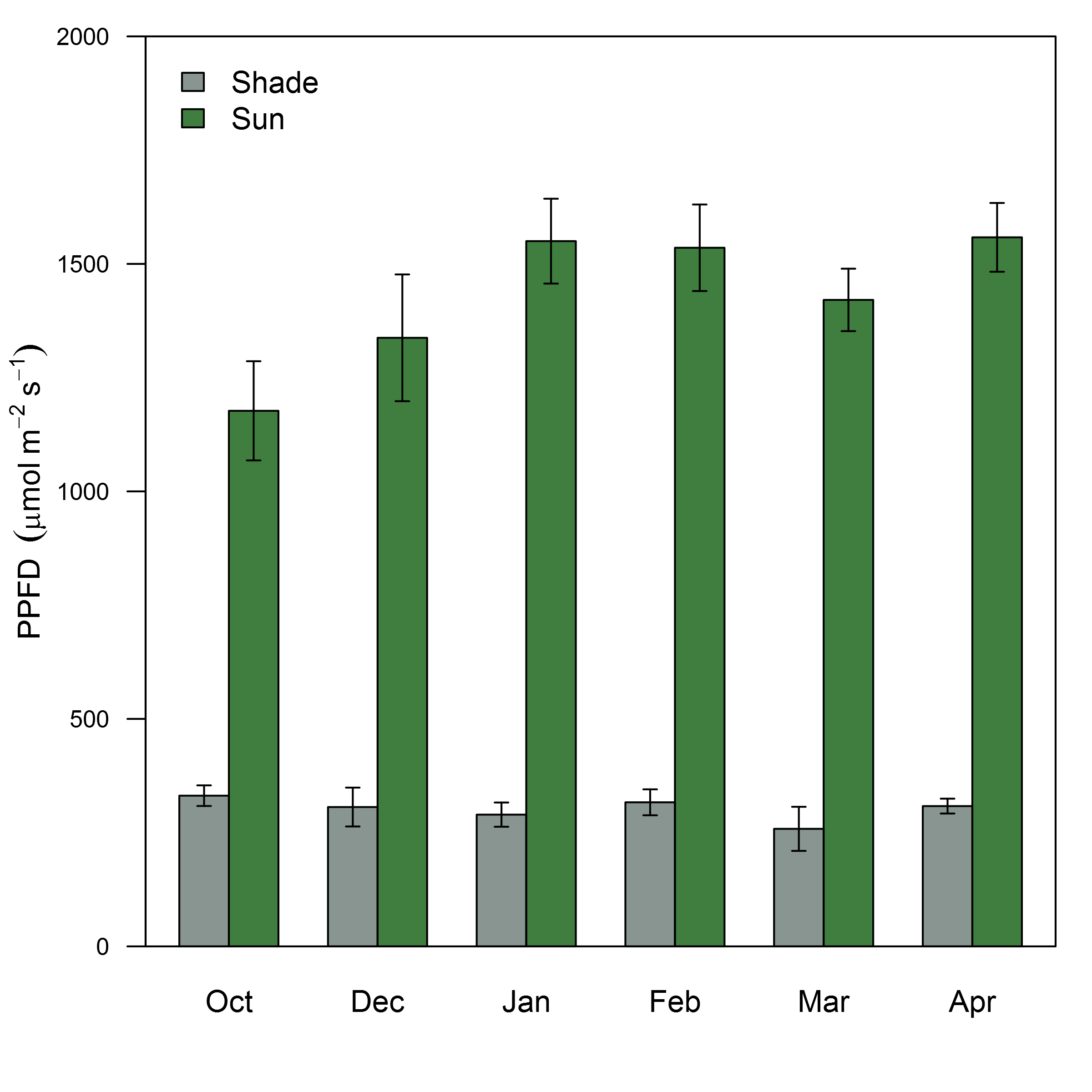
**Table 2**. Responses of leaf level gas exchange parameters of *Eucalyptus tereticornis* trees between full sun and shade leaves under ambient and elevated temperature treatments. Each value reflects the mean (± 1 standard error) for each treatment across all gas exchange campaigns (n=6). Units for A and E are mol m-2 s-1, for gs and gm are mol m-2 s-1 and for VPD are kPa. Different letters represent significant differences between treatments. The P value represents the overall difference between leaf types and both light and warming treatments.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Leaf** | **Light** | **Temperature** | **A** | **gs** | **gm** | **ITE** | **E** | **VPD** | **Ci** | **Cc** |
| Sun | High | AT | 13.5 (0.3) b | 0.122 (0.005) a | 0.163 (0.005) c | 8.26 (0.48) b | 1.78 (0.07) a | 1.60 (0.04) ab | 179.8 ( 3.2) a | 92.2 ( 2.9) a |
|  |  | ET | 13.1 (0.3) b | 0.123 (0.005) a | 0.153 (0.007) bc | 6.57 (0.39) ab | 2.21 (0.09) a | 1.90 (0.05) b | 187.9 ( 2.9) a | 92.2 ( 2.8) a |
| Shade | Low | AT | 10.4 (0.1) a | 0.150 (0.005) a | 0.117 (0.004) ab | 6.24 (0.50) a | 1.93 (0.07) a | 1.40 (0.04) a | 255.4 ( 3.8) b | 160.0 ( 4.1) c |
|  |  | ET | 10.0 (0.1) a | 0.146 (0.005) a | 0.116 (0.004) a | 5.43 (0.51) a | 2.23 (0.09) a | 1.60 (0.05) a | 253.8 ( 4.1) b | 160.3 ( 3.5) bc |
|  | High | AT | 18.1 (0.3) c | 0.255 (0.007) b | 0.184 (0.003) c | 5.85 (0.33) a | 3.42 (0.12) b | 1.40 (0.04) a | 237.4 ( 2.2) b | 137.4 ( 1.9) b |
|  |  | ET | 16.7 (0.2) c | 0.246 (0.009) b | 0.177 (0.003) c | 5.02 (0.35) a | 3.81 (0.15) b | 1.70 (0.04) ab | 238.1 ( 3.2) b | 141.7 ( 2.8) bc |
| P value |  |  | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.005 | 0.001 | 0.001 |

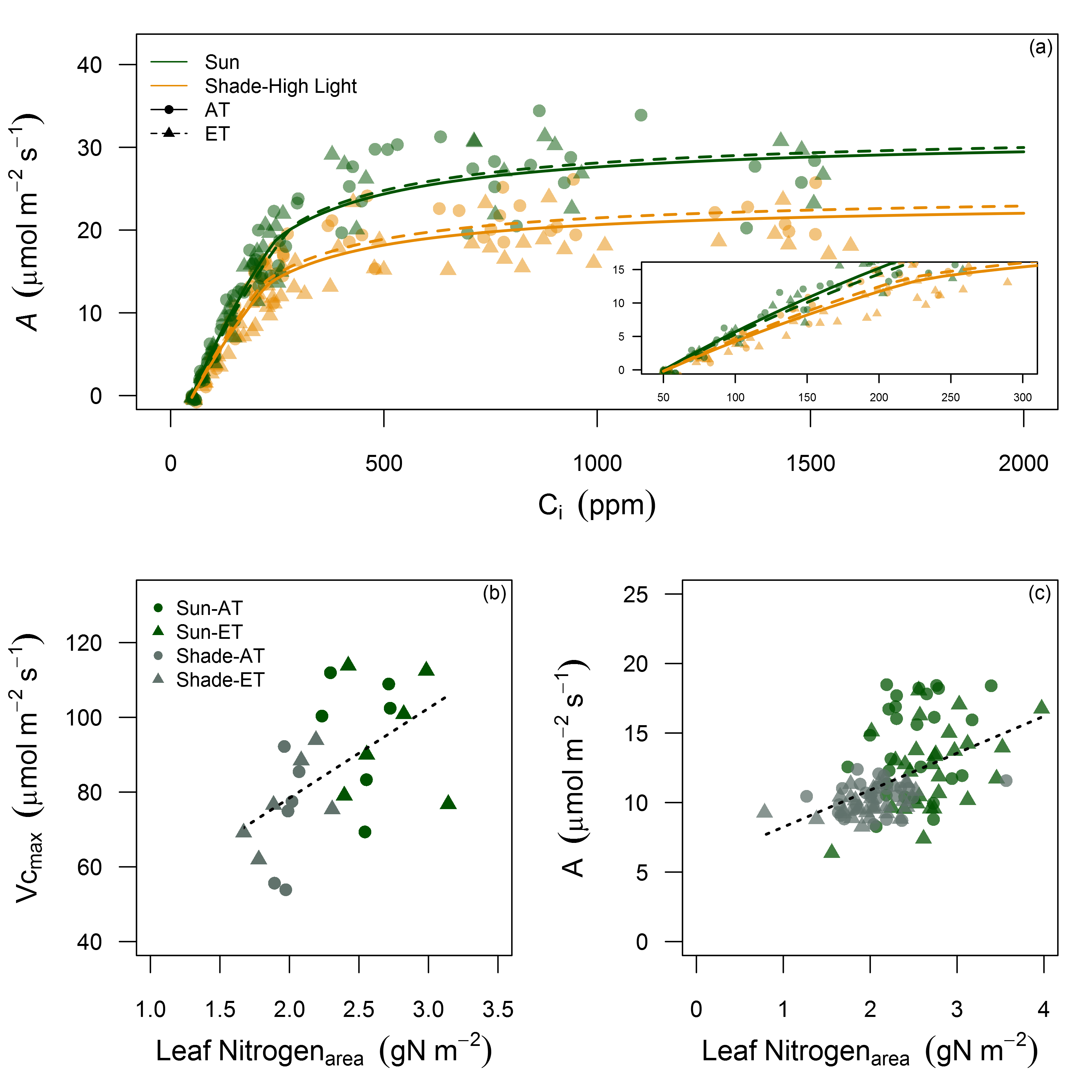
# Figures



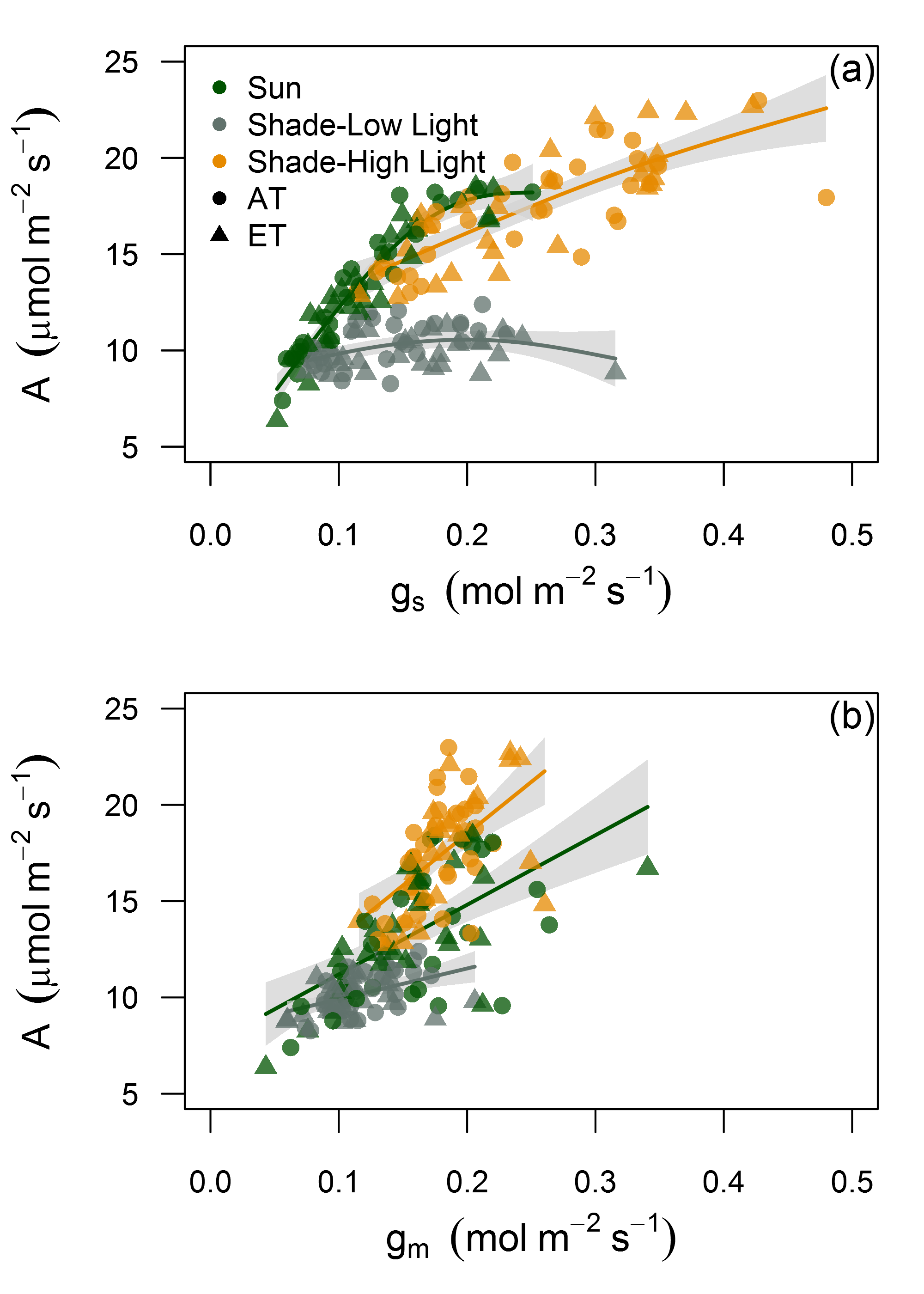
**Figure 1**



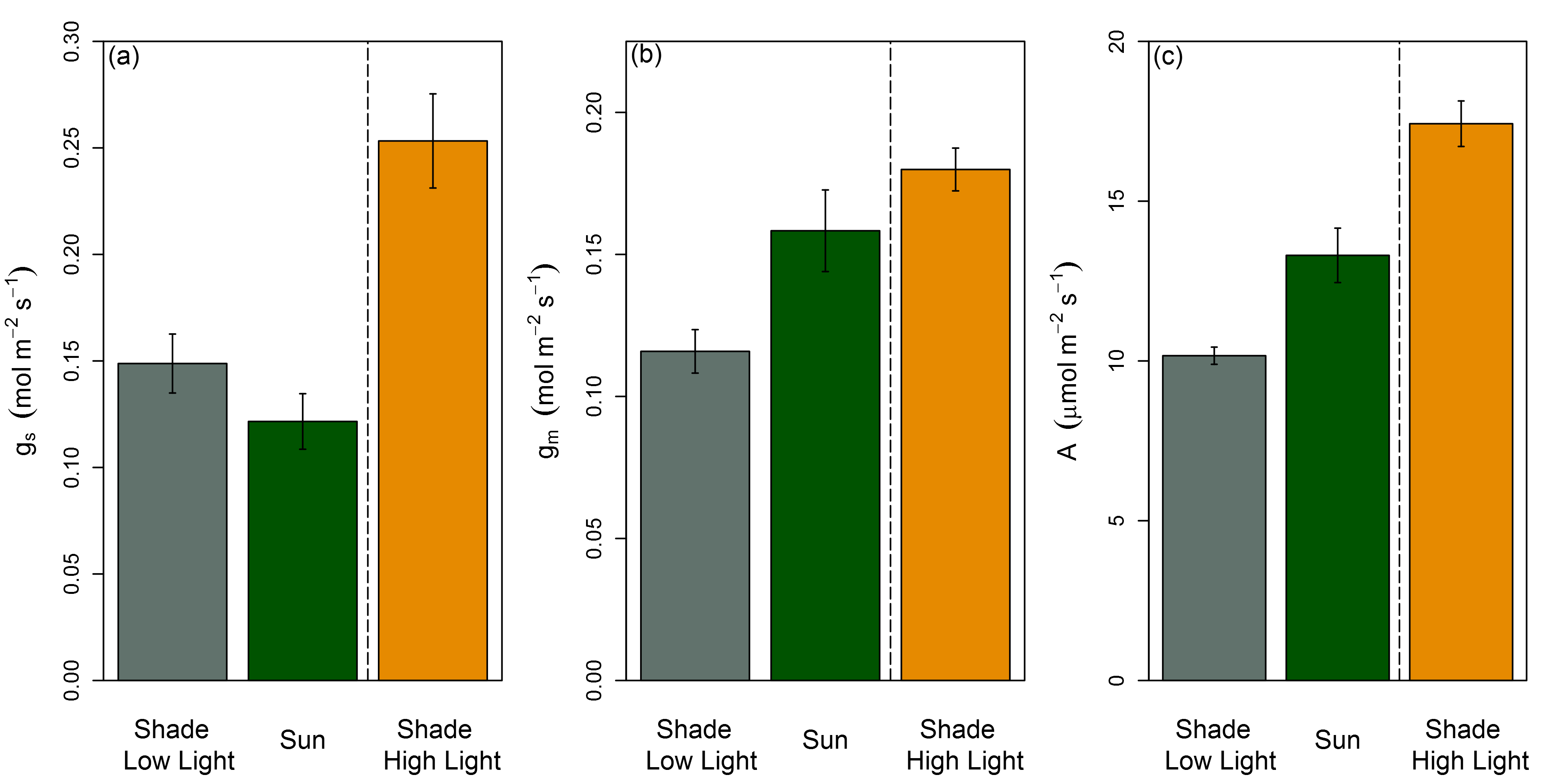
**Figure 2**.



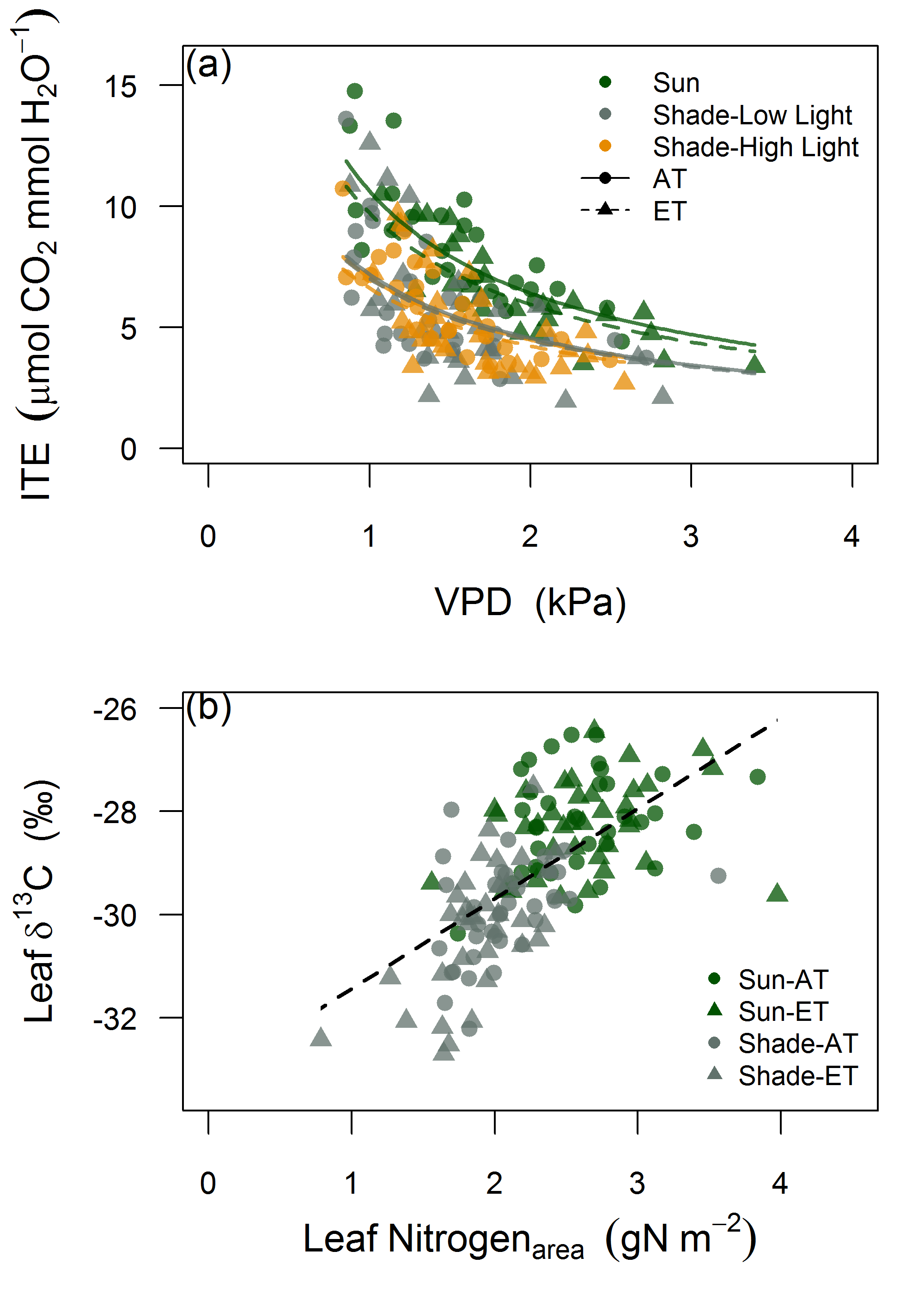
**Figure 3**.



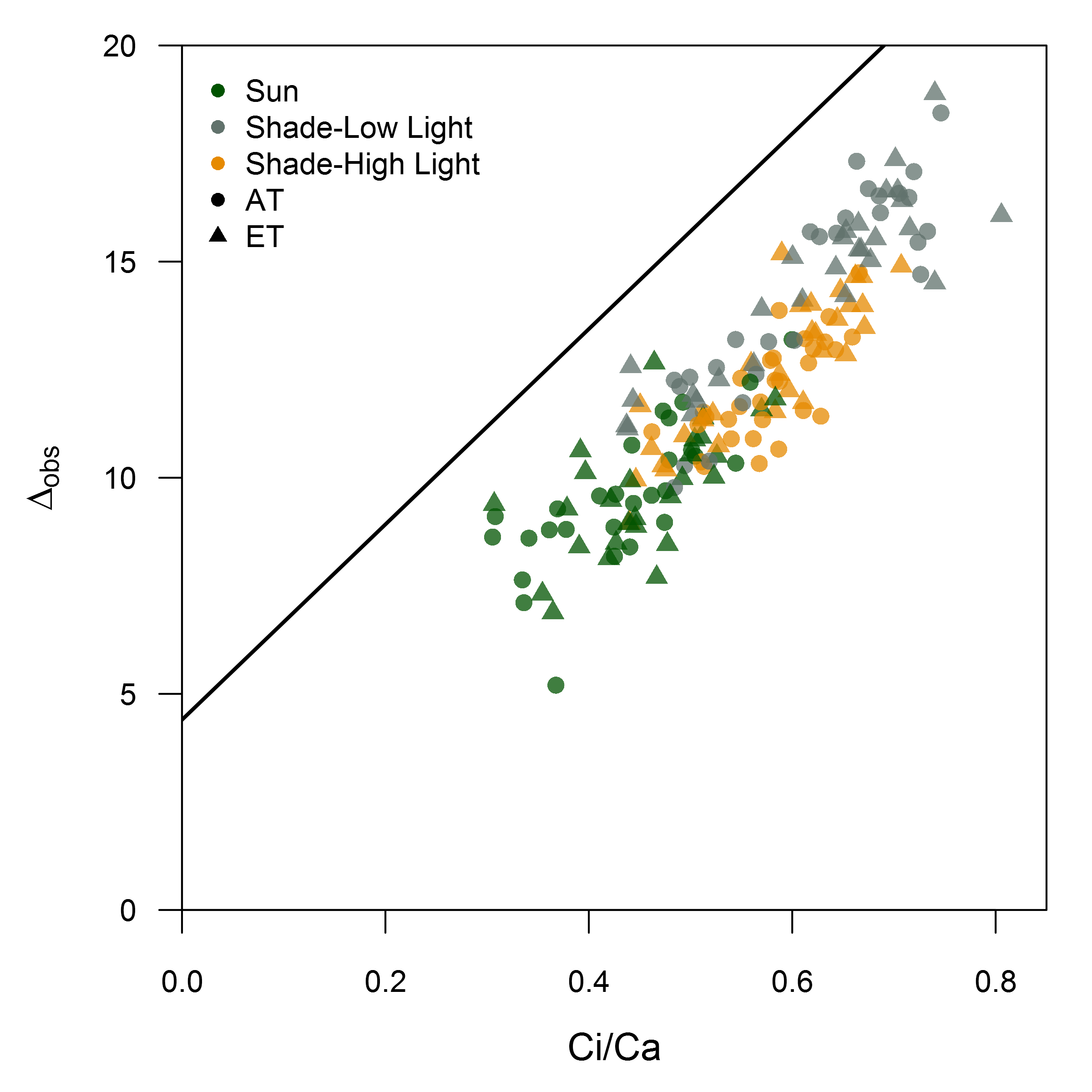
**Figure 4**.



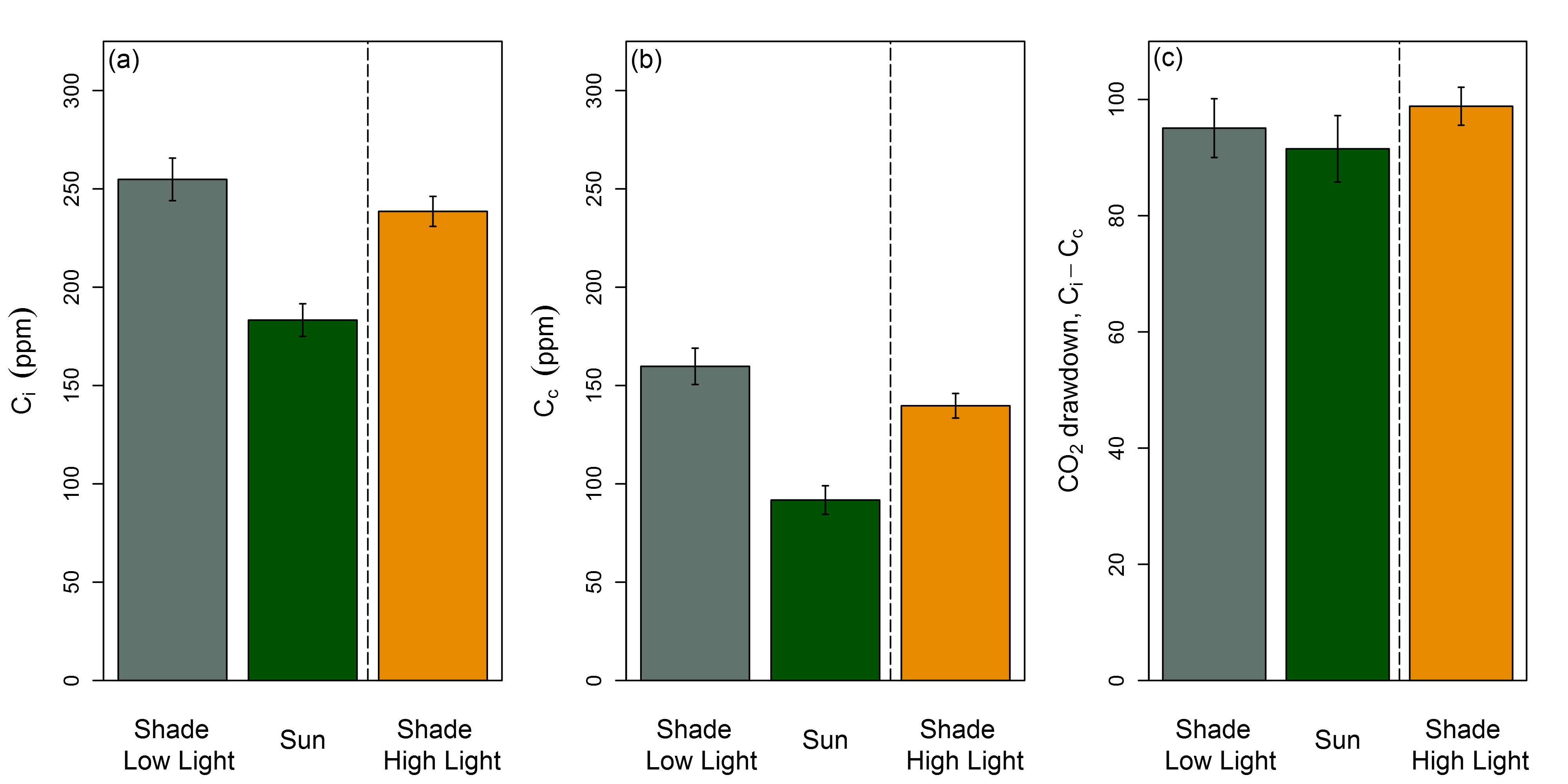
**Figure 5**.



**Figure 6**.

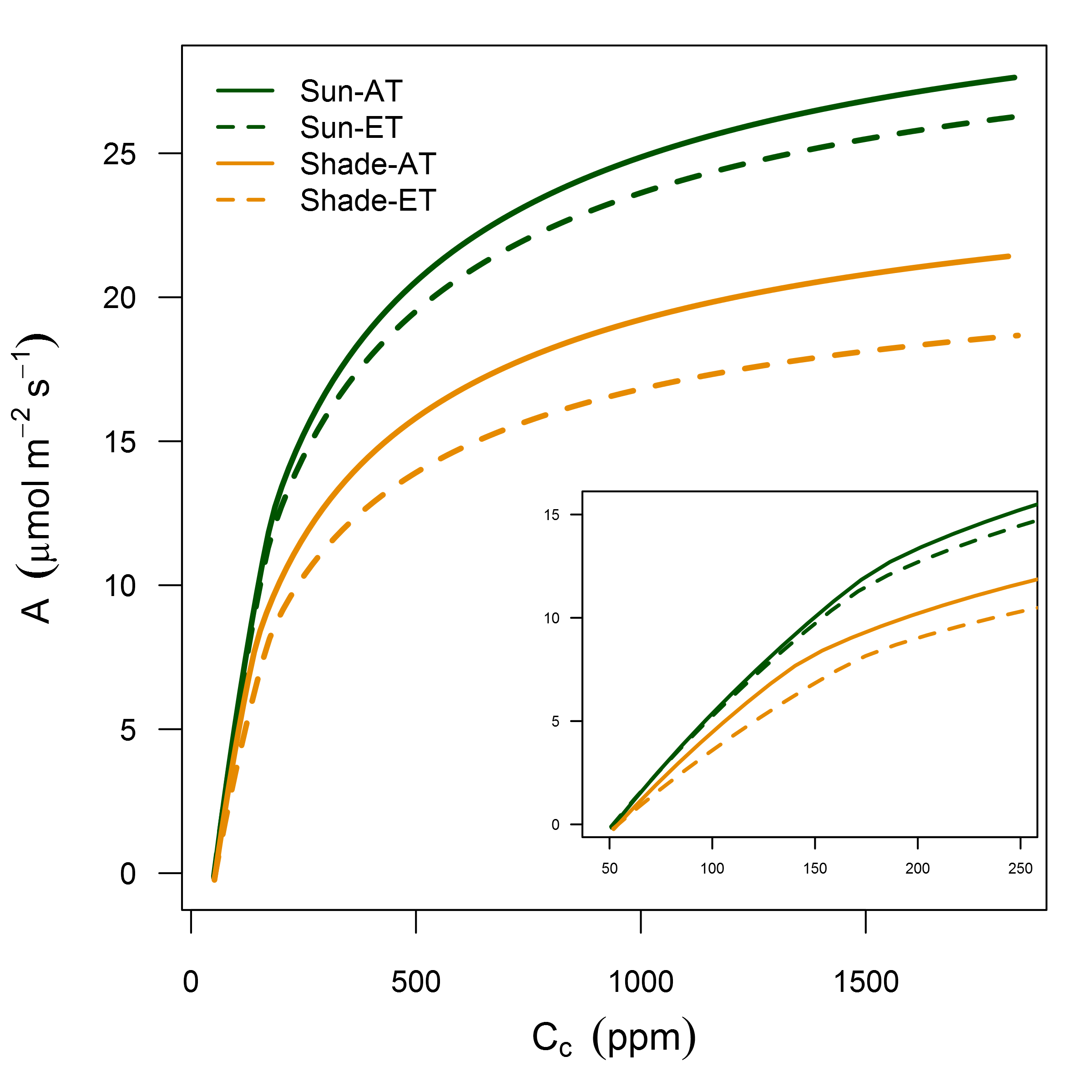


**Figure 7**.

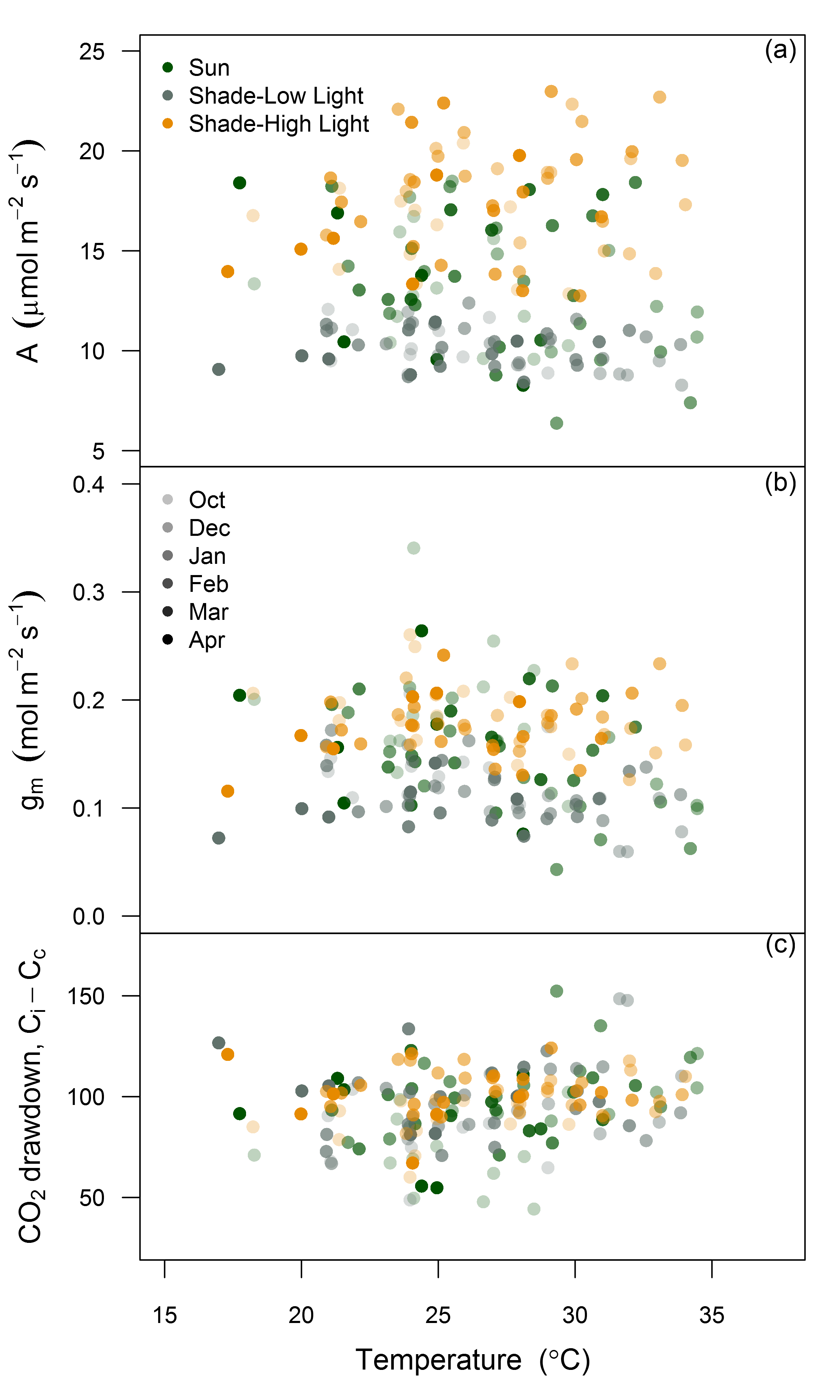


**Figure 8**.

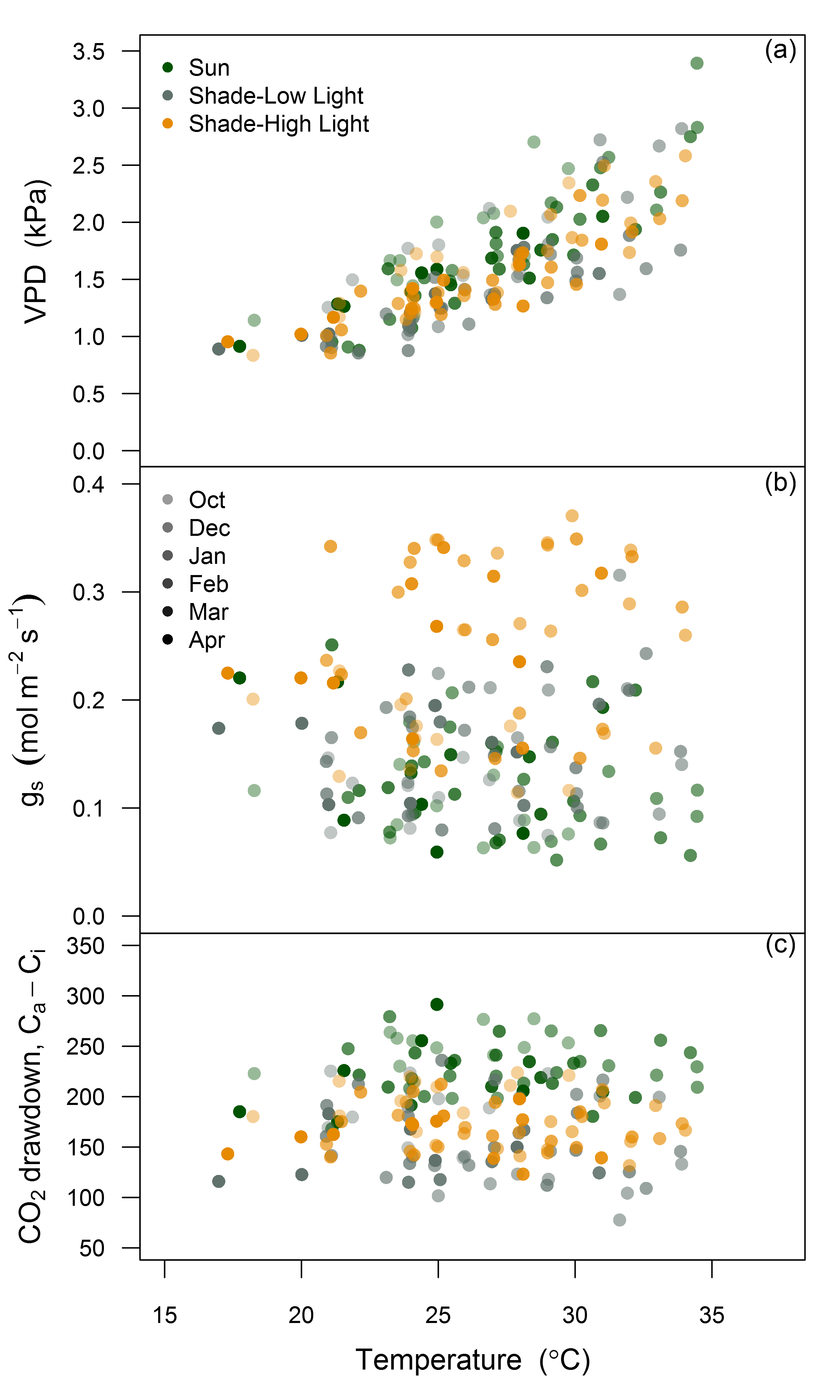
# Supporting Information



**Figure S1**.



**Figure S2**.



**Figure S3**.

# References

Allen MT**,** Pearcy RW. **2000**. Stomatal behavior and photosynthetic performance under dynamic light regimes in a seasonally dry tropical rain forest. *Oecologia* **122**: 470–478.

Baldocchi DD**,** Wilson KB**,** Gu L. **2002**. How the environment, canopy structure and canopy physiological functioning influence carbon, water and energy fluxes of a temperate broad-leaved deciduous forest—an assessment with the biophysical model CANOAK. *Tree Physiology* **22**: 1065–1077.

Barton CVM**,** Ellsworth DS**,** Medlyn BE**,** Duursma RA**,** Tissue DT**,** Adams MA**,** Eamus D**,** Conroy JP**,** McMurtrie RE**,** Parsby J***et al.*** **2010**. Whole-tree chambers for elevated atmospheric CO 2 experimentation and tree scale flux measurements in south-eastern Australia: The Hawkesbury Forest Experiment. *Agricultural and Forest Meteorology* **150**: 941–951.

Bates D**,** Maechler M**,** Bolker B**,** Walker S. **2013**. lme4: Linear mixed-effects models using Eigen and S4. *R package version* **1**.

Boardman N. **1977**. Comparative photosynthesis of sun and shade plants. *Annual review of plant physiology* **28**: 355–377.

Brantley ST**,** Young DR. **2009**. Contribution of sunflecks is minimal in expanding shrub thickets compared to temperate forest. *Ecology* **90**: 1021–1029.

Burgess SSO**,** Pittermann J**,** Dawson TE. **2006**. Hydraulic efficiency and safety of branch xylem increases with height in Sequoia sempervirens (D. Don) crowns. *Plant, Cell & Environment* **29**: 229–239.

Caemmerer S**,** Evans JR. **2014**. Temperature responses of mesophyll conductance differ greatly between species. *Plant, cell & environment*.

Chazdon RL**,** Pearcy RW. **1991**. The importance of sunflecks for forest understory plants. *Bioscience*: 760–766.

Cowan IR**,** Farquhar GD. **1977**. Stomatal function in relation to leaf metabolism and environment. Symposia of the society for experimental biology.471–505.

Crous KY**,** ZARAGOZA-CASTELLS J**,** Ellsworth DS**,** Duursma RA**,** Loew M**,** Tissue DT**,** Atkin OK. **2012**. Light inhibition of leaf respiration in field-grown Eucalyptus saligna in whole-tree chambers under elevated atmospheric CO2 and summer drought. *Plant, cell & environment* **35**: 966–981.

De Pury DGG**,** Farquhar GD. **1997**. Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. *Plant Cell and Environment* **20**: 537–557.

Duursma R. **2014**. *plantecophys: Modelling and analysis of leaf gas exchange data*.

Duursma RA**,** Payton P**,** Bange MP**,** Broughton KJ**,** Smith RA**,** Medlyn BE**,** Tissue DT. **2013**. Near-optimal response of instantaneous transpiration efficiency to vapour pressure deficit, temperature and [CO 2] in cotton (Gossypium hirsutum L.). *Agricultural and forest meteorology* **168**: 168–176.

Evans JR. **1995**. Carbon fixation profiles do reflect light absorption profiles in leaves. *Functional Plant Biology* **22**: 865–873.

Evans J**,** Poorter H. **2001**. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell & Environment* **24**: 755–767.

Evans JR**,** Von Caemmerer S. **2013**. Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco. *Plant, cell & environment* **36**: 745–756.

Evans JR**,** Sharkey TD**,** Berry JA**,** Farquhar GD. **1986**. Carbon isotope discrimination measured concurrently with gas exchange to investigate CO2 diffusion in leaves of higher plants. *Functional Plant Biology* **13**: 281–292.

Farquhar GD**,** Cernusak LA. **2012**. Ternary effects on the gas exchange of isotopologues of carbon dioxide. *Plant, Cell & Environment* **35**: 1221–1231.

Farquhar GD**,** Caemmerer S von von**,** Berry JA. **1980**. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. *Planta* **149**: 78–90.

Field C. **1983**. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia* **56**: 341–347.

Field CH**,** Mooney HA. **1986**. Photosynthesis–nitrogen relationship in wild plants. On the economy of plant form and function: Proceedings of the sixth maria moors cabot symposium,‘ evolutionary constraints on primary productivity, adaptive patterns of energy capture in plants,’ harvard forest, august 1983.

Flexas J**,** DIAZ-ESPEJO A**,** GalmES J**,** Kaldenhoff R**,** Medrano H**,** RIBAS-CARBO M. **2007**. Rapid variations of mesophyll conductance in response to changes in CO2 concentration around leaves. *Plant, Cell & Environment* **30**: 1284–1298.

Flexas J**,** RIBAS-CARBÓ M**,** DIAZ-ESPEJO A**,** GalmES J**,** Medrano H. **2008**. Mesophyll conductance to CO2: current knowledge and future prospects. *Plant, Cell & Environment* **31**: 602–621.

Griffiths H**,** Helliker BR. **2013**. Mesophyll conductance: internal insights of leaf carbon exchange. *Plant, cell & environment* **36**: 733–735.

Hanba YT**,** Shibasaka M**,** Hayashi Y**,** Hayakawa T**,** Kasamo K**,** Terashima I**,** Katsuhara M. **2004**. Overexpression of the barley aquaporin HvPIP2; 1 increases internal CO2 conductance and CO2 assimilation in the leaves of transgenic rice plants. *Plant and Cell Physiology* **45**: 521–529.

Heinen RB**,** Ye Q**,** Chaumont F. **2009**. Role of aquaporins in leaf physiology. *Journal of Experimental Botany* **60**: 2971–2985.

Hubbard RM**,** Ryan MG**,** Stiller V**,** Sperry JS. **2001**. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell & Environment* **24**: 113–121.

Küppers M**,** Schneider H. **1993**. Leaf gas exchange of beech (Fagus sylvatica L.) seedlings in lightflecks: effects of fleck length and leaf temperature in leaves grown in deep and partial shade. *Trees* **7**: 160–168.

Leakey ADB**,** Press MC**,** Scholes JD. **2003**. High-temperature inhibition of photosynthesis is greater under sunflecks than uniform irradiance in a tropical rain forest tree seedling. *Plant, Cell & Environment* **26**: 1681–1690.

Leakey ADB**,** Press MC**,** Scholes JD**,** Watling JR. **2002**. Relative enhancement of photosynthesis and growth at elevated CO2 is greater under sunflecks than uniform irradiance in a tropical rain forest tree seedling. *Plant, Cell & Environment* **25**: 1701–1714.

Li G**,** Santoni V**,** Maurel C. **2014**. Plant aquaporins: roles in plant physiology. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1840**: 1574–1582.

Lin Y-S**,** Medlyn BE**,** Duursma RA**,** Prentice IC**,** Wang H**,** Baig S**,** Eamus D**,** Dios VR de**,** Mitchell P**,** Ellsworth DS***et al.*** **2015**. Optimal stomatal behaviour around the world. *Nature Climate Change*.

Linderson M-L**,** Mikkelsen TN**,** Ibrom A**,** Lindroth A**,** Ro-Poulsen H**,** Pilegaard K. **2012**. Up-scaling of water use efficiency from leaf to canopy as based on leaf gas exchange relationships and the modeled in-canopy light distribution. *Agricultural and Forest Meteorology* **152**: 201–211.

Marshall JD**,** Brooks JR**,** Lajtha K. **2007**. Sources of variation in the stable isotopic composition of plants. *Stable isotopes in ecology and environmental science*: 22–60.

Medlyn BE**,** Dreyer E**,** Ellsworth D**,** Forstreuter M**,** Harley PC**,** Kirschbaum MUF**,** Le Roux X**,** Montpied P**,** Strassemeyer J**,** Walcroft A***et al.*** **2002**. Temperature response of parameters of a biochemically based model of photosynthesis. II. A review of experimental data. *Plant, Cell & Environment* **25**: 1167–1179.

Medlyn BE**,** Duursma RA**,** Eamus D**,** Ellsworth DS**,** Prentice IC**,** Barton CVM**,** Crous KY**,** Angelis P de**,** Freeman M**,** Wingate L. **2011**. Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology* **17**: 2134–2144.

Monsi M**,** Saeki T. **2005**. On the factor light in plant communities and its importance for matter production. *Annals of Botany* **95**: 549–567.

Nakagawa S**,** Schielzeth H. **2013**. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution* **4**: 133–142.

Niinemets U. **2007**. Photosynthesis and resource distribution through plant canopies. *Plant, Cell & Environment* **30**: 1052–1071.

Niinemets Ü. **2010**. A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecological Research* **25**: 693–714.

Niinemets Ü. **2012**. Optimization of foliage photosynthetic capacity in tree canopies: towards identifying missing constraints. *Tree physiology* **32**: 505–509.

Niinemets Ü**,** Valladares F. **2004**. Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: optimality and constraints. *Plant Biology* **6**: 254–268.

Pearcy RW. **1990**. Sunflecks and photosynthesis in plant canopies. *Annual review of plant biology* **41**: 421–453.

Peltoniemi MS**,** Duursma RA**,** Medlyn BE. **2012**. Co-optimal distribution of leaf nitrogen and hydraulic conductance in plant canopies. *Tree physiology*: tps023.

Pepin S**,** Livingston NJ. **1997**. Rates of stomatal opening in conifer seedlings in relation to air temperature and daily carbon gain. *Plant, Cell & Environment* **20**: 1462–1472.

Piel C**,** Frak E**,** Le Roux X**,** Genty B. **2002**. Effect of local irradiance on CO2 transfer conductance of mesophyll in walnut. *Journal of Experimental Botany* **53**: 2423–2430.

Prentice IC**,** Dong N**,** Gleason SM**,** Maire V**,** Wright IJ. **2014**. Balancing the costs of carbon gain and water transport: testing a new theoretical framework for plant functional ecology. *Ecology letters* **17**: 82–91.

R Development Core Team R. **2011**. R: A Language and Environment for Statistical Computing (RDC Team, Ed.). *R foundation for statistical computing* **1**: 409.

Schymanski SJ**,** Or D**,** Zwieniecki MA. **2013**. Stomatal control and leaf thermal and hydraulic capacitances under rapid environmental fluctuations. *PloS one* **8**: e54231.

Sellin A**,** Kupper P. **2007**. Effects of enhanced hydraulic supply for foliage on stomatal responses in little-leaf linden (Tilia cordata Mill.). *European Journal of Forest Research* **126**: 241–251.

Sellin A**,** Õunapuu E**,** Kupper P. **2008**. Effects of light intensity and duration on leaf hydraulic conductance and distribution of resistance in shoots of silver birch (Betula pendula). *Physiologia Plantarum* **134**: 412–420.

Tausz M**,** Warren CR**,** Adams MA. **2005**. Dynamic light use and protection from excess light in upper canopy and coppice leaves of Nothofagus cunninghamii in an old growth, cool temperate rainforest in Victoria, Australia. *New Phytologist* **165**: 143–156.

Tazoe Y**,** Von Caemmerer S**,** Badger MR**,** Evans JR. **2009**. Light and CO2 do not affect the mesophyll conductance to CO2 diffusion in wheat leaves. *Journal of Experimental Botany* **60**: 2291–2301.

Tazoe Y**,** Von Caemmerer S**,** Estavillo GM**,** Evans JR. **2011**. Using tunable diode laser spectroscopy to measure carbon isotope discrimination and mesophyll conductance to CO2 diffusion dynamically at different CO2 concentrations. *Plant, Cell & Environment* **34**: 580–591.

Terashima I**,** Hanba YT**,** Tazoe Y**,** Vyas P**,** Yano S. **2006**. Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO2 diffusion. *Journal of Experimental Botany* **57**: 343–354.

Ubierna N**,** Marshall JD. **2011**. Estimation of canopy average mesophyll conductance using 13C of phloem contents. *Plant, cell & environment* **34**: 1521–1535.

Vico G**,** Manzoni S**,** Palmroth S**,** Katul G. **2011**. Effects of stomatal delays on the economics of leaf gas exchange under intermittent light regimes. *New Phytologist* **192**: 640–652.

Vogelman TC**,** Nishio JN**,** Smith WK. **1996**. Leaves and light capture: light propagation and gradients of carbon fixation within leaves. *Trends in Plant Science* **1**: 65–70.

Warren CR. **2008**. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO2 transfer. *Journal of Experimental Botany* **59**: 1475–1487.

Warren CR**,** Löw M**,** Matyssek R**,** Tausz M. **2007**. Internal conductance to CO 2 transfer of adult Fagus sylvatica: variation between sun and shade leaves and due to free-air ozone fumigation. *Environmental and Experimental Botany* **59**: 130–138.

Way DA**,** Pearcy RW. **2012**. Sunflecks in trees and forests: from photosynthetic physiology to global change biology. *Tree Physiology* **32**: 1066–1081.

Wright IJ**,** Reich PB**,** Westoby M. **2003**. Least-Cost Input Mixtures of Water and Nitrogen for Photosynthesis. *The American Naturalist* **161**: 98–111.