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Vertical variation in photosynthetic parameters in two different tropical forest ecosystems

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Contents

Abstract

Forests contribute to the carbon balance as the largest vegetative sink for atmospheric carbon (CO_2). Anthropogenic emissions are counteracted by carbon sequestration in trees, but nutrients could be limiting photosynthesis and the effect could possibly be not as large as believed. In tropical forests, phosphorus (P) is only available from weathered bedrock and is thereby in an imbalance with the rising levels of carbon and nitrogen in the atmosphere. If P is limiting carbon uptake in tropical forests, global carbon cycle models are likely overestimating uptake by forests. Another overestimation might be to only conduct photosynthesis measurements on sunlit leaves of the canopy and take this as an overall canopy average, whilst a vertical profile in photosynthesis is very likely. Our study was conducted on two sites of the Amazonian rain forest in French Guiana. Photosynthesis and dark respiration (Rd) was measured of 120 trees in 12 plots per site. The plots were situated along a geographical gradient (at top, slope and bottom) to cover a large variety in soil P concentration. We derived the photosynthetic parameters V_{cmax} and J_{max} from the photosynthesis measurements using the Farquhar model (Farquhar *et al*., 1980). The measurements were performed at two different height levels in the canopy to investigate the vertical profile. In this study we aimed to relate the spatial and vertical variability to parameters such as leaf P concentration, leaf height, light availability, the specific leaf area and the chlorophyll content (SPAD). Soil P concentrations were correlated with the leaf P concentrations, which indicates P uptake from the soil is limited. There were significant vertical differences in the leaves in V_{cmax}, J_{max}, R_d and leaf P concentrations. We conclude that P limits the photosynthetic capacity in our study areas and vertical profiles of photosynthesis should be taken into account when estimating carbon uptake by a tropical forest ecosystem.

Keywords: *leaf phosphorus, photosynthetic capacity*, *P imbalance, vertical profile*

Abstract in layman's terms

Forests are the biggest vegetative sink for atmospheric carbon (CO_2). Human-induced CO_2 emissions are taken up by trees, but other nutrients could be limiting photosynthesis and the effect could possibly be not as large as believed. In tropical forests, phosphorus (P) is only available from the bedrock and is thereby in an imbalance with the rising levels of carbon (C) and nitrogen in the atmosphere. If P is limiting photosynthesis in tropical forests, models of global C uptake are likely overestimating uptake by forests. Another overestimation might be to only measure photosynthesis on the highest, sunlit leaves and take this as an overall average of the forest, whilst vertical differences in photosynthesis are common. Our study was therefore conducted on two sites of the Amazonian rain forest in French Guiana. Photosynthesis and respiration was measured of 120 trees in 12 plots per site. The plots were situated on top, on the slopes and on the bottom of hills to cover a large variety in soil P concentration. Photosynthesis of multiple trees in every plot was determined using the Farquhar model (Farquhar *et al*., 1980). Other measured parameters were leaf P concentration, leaf height, light availability, the specific leaf area and the chlorophyll content. On every tree we performed multiple measurements at two different levels, on top of the tree and at the lowest part of the tree, to detect vertical differences. Soil P concentrations were correlated with the P concentrations in the leaves, which indicates P uptake from the soil is limited. There were significant vertical differences in the leaves in photosynthesis, respiration and leaf P concentration. We conclude that P limits photosynthesis in our study areas and vertical differences of photosynthesis should be taken into account when estimating carbon uptake by a tropical forest ecosystem.

Keywords: *leaf phosphorus, photosynthetic capacity*, *P imbalance, vertical profile*

1. Introduction

Forests contribute to the global carbon balance as they can function as a carbon sink (Grace *et al.*, 1995 and Phillips *et al.*, 1998). A balance between carbon uptake and release is maintained by the gas exchange of trees (Valentini *et al.*, 2000 & Myneni *et al.*, 2001). Through photosynthesis, trees remove carbon (C) from the atmosphere and store it in above and below ground plant tissues and soil (Eliasch, 2008), a process also known as carbon sequestration. When growing, forests generally have higher carbon sequestration rates than other vegetation types (Houghton, 2007). Recent anthropogenic emissions and deforestation have increased the concentration of atmospheric carbon (CO_2). The climate has since changed more rapidly, with global warming, ice diminishing, sea level rise and the increase of extreme events (IPCC, 2014) and thus an increased risk on human welfare. In turn, these effects can lead to changes in forest characteristics, but are yet uncertain (Eliasch, 2008).

To mitigate further warming, a better understanding of the global system is necessary. Forests cover 42 million km², which is 30% of the land surface (Bonan, 2008) and absorb about 2.4 billion tonnes of C each year, which is one-third of the gross primary production (GPP) (Pan *et al*., 2011). Of all carbon present in vegetation, 77 percent is stored in forests and 39 percent of all carbon present in soils is stored in the soils of forests (Eliasch, 2008). Tropical rainforest alone can contain at least four times more carbon per hectare than cropland (Houghton, 1999), rendering them the most productive systems in the world. Due to deforestation, lack of data and commonly used assumptions, there are nonetheless large uncertainties regarding the carbon balance of tropical forests.

Models have been created to study how further increases in CO_2 will influence the earth's temperature. Every emission scenario predicts an increase in extreme events (IPCC, 2014). Models predict that a considerable proportion of atmospheric CO_2 may be incorporated in land ecosystems, counteracting carbon emissions (Hungate *et al.*, 2003). Effects of increasing atmospheric CO_2 on temperate and boreal forests are well researched. Most carbon uptake estimates could possibly be overestimated because these models often do not take limiting nutrients such as nitrogen into account (Hungate *et al.*, 2003 and Peñuelas *et al*., 2013).

To be able to take up carbon, 16 other nutrients are essential (Stevenson *et al.,* 1999), with nitrogen (N) and phosphorous (P) as the two most important. Carbon can directly be replenished from the atmosphere to the plant, as CO_2 enters the leaves during photosynthesis. Nitrogen can be replenished in the soil, as atmospheric nitrogen (N_2) can be fixed by N-fixing free-living heterotrophic soil bacteria and converted into amino acids and ammonium (NH_4^+) (Vitousek *et al*., 1997; Larcher,

2003 and Van Der Heijden *et al*., 2008). These compounds are then available for plants to take up. Plant material contains quite large quantities of N (10-50 g kg^{-1} of dry matter), incorporated in proteins, nucleic acids and secondary compounds (Larcher, 2003). A considerable amount (20-30%) of the total leaf N is part of the RuBisCo proteins in C_3 plants (Feller et al., 2008), for C_4 plants this is only 5-9% of the total leaf N. RuBisCo (Ribulose-1,5-bisphosphate carboxylase/oxygenase) is a photosynthetic enzyme involved in the first major step of carbon fixation (Cooper, 2000). Overall, N influences many aspects of plant physiology and photosynthesis, including production of ATP and NADPH (Marschner, 1995).

In contrast to C and N, P is not present in the atmosphere and is only available in small quantities in the soil, if not fertilised, where P is released from the bedrock and from decomposed material. Plants take up only a small amount (≤ 10 g kg^{-1} dry matter) of P (Larcher, 2003), but it plays a role in an array of processes (Vance *et al*., 2003), including photosynthesis, respiration, energy generation, nucleic acid synthesis, glycolysis, membrane synthesis and stability, enzyme activation and inactivation, redox reactions, signalling, carbohydrate metabolism, and N fixation.

The availability of C and N is high and increasing due to human activities such as CO_2 emission from fossil fuels and N deposition by fertilisation (Grace *et al.*, 1995, Peñuelas *et al*., 2013). Primary production in most temperate and boreal forests is limited by N (Vitousek & Howarth, 1991). This N is largely used in capturing energy through photosynthesis (Evans, 1989), with a strong correlation between the N concentration in the plants and their photosynthetic capacity (Field & Mooney, 1986). The organic material in temperate and boreal forests have high C:N ratios and low N:P ratios (Vitousek, 1982 and Melillo & Gosz, 1983), although these ratios shift when there is dominance of symbiotic nitrogen fixers or when there are large anthropogenic inputs. Fertilisation with N decreases C:N ratios (Miller *et al*., 1976) and leads to increased NPP (net primary productivity) in temperate (Mitchell & Chandler, 1939 and Miller, 1981) and boreal forests (Ågren, 1983; van Cleve & Zasada, 1976; van Cleve *et al*., 1983 and Bonan, 1990).

Tropical forests are wet and have high temperatures, which makes them likely to have high rates of N fixation (Stewart *et al.*, 1978 & Cleveland *et al.*, 1999). Moreover, because they were not affected by ice ages for many millions of years, they have accumulated very large N stocks. The large N stocks and optimal conditions for N fixation and decomposition and N mineralization explain why NO_3^- availability is high in most tropical forests (Vitousek *et al.* 1986). Generally, N is thus not limiting growth in tropical forests, C:N ratios are low and N:P ratios are very high compared to other biomes

(Vitousek, 1984; Vitousek & Sanford, 1986 and Jordan, 1985). In contrast, two fertilisation studies did identify N as a growth-limiting factor (Vitousek *et al.*, 1987 and Tanner *et al.*, 1990). This could be due to the fact that the tropical forests in these studies developed on sandy soils (spodosols and arenosols), which have a low N retention capacity (Cuevas & Medina, 1986, 1988; Vitousek & Sanford, 1986 and Vitousek *et al.*, 1988).

With increasing CO_2 concentrations and N deposition, but marginal P inputs in natural ecosystems, there is a shift from the original C:N:P balance towards a strong P imbalance (Peñuelas *et al.*, 2012). Limitation by P and other elements can occur, sometimes together with N limitation (Vitousek & Howarth, 1991). Many studies have been conducted on forests in temperate and boreal climates, but little is known about the gas exchange and photosynthetic capacity of tropical rainforests and their nutrient limitations (Carswell *et al.*, 2000 and Vitousek & Howarth, 1991), though they contain about half of the carbon present as biomass in the world's terrestrial ecosystems. Pan *et al.* (2011) estimated a global forest sink of 2.4 ± 0.4 petagrams of carbon per year, but with large uncertainties in the tropical regions. One study demonstrated in a tropical forest on an oxisol that, by adding P, root systems grew larger, which was not the case when adding N (Cuevas & Medina, 1988).

Within this framework, this study is committed to clarify potential nutrient limitations on photosynthesis in tropical forests and to enhance carbon uptake estimates by performing a study on one of the world's largest carbon sinks, the Amazon forest.

In this research, the Farquhar *et al*. (1980) model is used to better understand photosynthesis responses to nutrient availability, which is a realistic leaf-level photosynthesis model. It is the most commonly used model because it allows us to make quantitative links between leaf biochemistry and gas exchange kinetics. The photosynthetic rate (A) or carbon assimilation rate is determined by light, CO_2 and RuBisCO activity. In ambient conditions, assimilation is limited by either the kinetics of RuBisCo or by electron transport (Denning, 1993). The model of Farquhar *et al*. (1980) calculates the photosynthetic rate as the minimum of those two possible limitations,

$$A = \min \{A_J, A_V\} - R_d$$

with subtracting R_d, the dark respiration, as it is a loss of carbon. A_V is the RuBisCo-limited photosynthetic rate and A_J the electron-transport limited rate. With this model, the photosynthetic parameters V_{cmax} and J_{max} can be calculated. These parameters can in turn be linked to P availability. V_{cmax} and J_{max} have also been linked to driving factors of photosynthesis and leaf properties (Walker

et al., 2014). Here we will also discuss its relation with the specific leaf area (SLA), the chlorophyll content (SPAD index) and the light availability (Dawkins' crown illumination index).

If the soil limits the availability of P, P becomes a limiting factor for carbon assimilation. The availability of this nutrient is a determining factor for the growing capacity of the trees. Limited P availability should become visible with a comparison between the P concentration in the soil and the P concentration in the leaves, as a positive correlation. *Our first hypothesis states that different P concentrations in the soil will be correlated with differences in leaf P concentration.*

Tropical rainforests have a complex canopy structure which leads to a broad variation in light environment (Montgomery & Chazdon, 2001). Up till now most models estimate photosynthesis based on only sunlit top leaves. These models likely overestimate photosynthesis, because it has been shown that strong vertical gradients of stomatal conductance and maximum photosynthetic rate exist (Roberts *et al.*, 1990 & McWilliam *et al.*, 1996). Carswell *et al*. (2000) has performed a study on vertical variation in leaf N in the Amazon and concluded that the photosynthetic capacity can be represented as an average of the total vertical profile. In this study we will look for vertical differences. A vertical profile in leaf P concentration is expected.

This availability of P is expected to have its effect on the photosynthetic rate of the canopy. More light in the higher canopy regions results in higher allocation of nutrients to the top leaves to optimise their photosynthesis. Increases in photosynthetic capacity with increasing canopy height is shown to have strong correlations with leaf N (Carswell *et al.*, 2000). *If N is not limiting, but P is, our second hypothesis states that V_{cmax} and J_{max} increase with increasing leaf height and that leaf height is strongly correlated with leaf P.* This also implies that the top leaves will not be representative for the whole tree, but that an average of the total vertical profile is to be preferred.

Variation between regions could be possible due to differences in soil, altitude and climate (Kitayama & Aiba, 2002). The two hypotheses from above are therefore tested at two different forests differing in soil texture and annual rainfall In both sites a P gradient is expected to be present. The photosynthetic capacity and P availability of these two sites (Nouragues and Paracou, both in French Guiana) are compared.

2. Material and methods

2.1 Study area

Fieldwork was carried out in French Guiana, South America, where samples were taken at two different sites, named Nouragues and Paracou. Mean daily temperatures fluctuate around 26 °C with minima around 21 °C and maxima around 32 °C (Janssens *et al*., 1998). Data collection occurred in the summer of 2015, during the wet season.

Nouragues is a dense evergreen rain forest that is undisturbed for hundreds of years since the Amerindians left the place over 200 years ago. The Nouragues Research Station (4°50'N and 52°42'W) is located near the Arataye river (Poncy *et al*., 1998) and is managed by The National Center for Scientific Research (CNRS). This research station, created in 1987, is built next to a granite hill, a so-called inselberg. Annual rainfall is 2760 mm with around 70 mm in dry season from September to November and more than 300 mm in January and during wet season from April to June (Chave *et al*., 2001). The average daily temperature is 26 °C (Poncy *et al*., 1998). The soil is an oxisol, a typical acid soil type for tropical rain forests (FAO-UNESCO, 2005). The soil has a measured average pH of 4, with a minimum of 2.9 and a maximum of 4.3 (this thesis). The forest is a typical lowland wet tropical rain forest (Poncy *et al*., 1998). There is a very rich plant biodiversity, with over 1200 species of angiosperms, with a diversity index (number of species per ha) between 160 and 260 species. (Chave *et al*., 2001; Poncy *et al*., 1998).

The lowland tropical forest of Paracou (5°18' N, 52°55' W) near Sinnamary, is a typical Guianan rain forest (Aubry-Kientz *et al.*, 2015). It is an experimental site managed by CIRAD who are researching sustainable development of tropical regions. Mean annual rainfall is 2980 mm, with most of the precipitation during the long rainy season from mid-March to mid-June (Wagner *et al.*, 2011). The soil is a well-drained oxisol on Precambrian bedrock (Janssens *et al*., 1998) with a measured average pH of 4.2, with a minimum of 3.9 and a maximum of 4.9 (this thesis).

At each site twelve plots were set up from which four were situated on top of a hill, four at the bottoms and four on the slopes of the hills. In appendix A there is a map of French Guiana where the two sites are indicated (Figure A1) and two maps are shown wherein the plots are indicated for both Nouragues (Figure A2) and Paracou (Figure A3). This division in hill, bottom and slope was made to cover the whole landscape wherein a moisture gradient could be found due to water runoff towards the lower parts. With runoff, erosion and sedimentation the probability increases that a phosphorus (P) gradient was established from P poor on the top plots to rich in the bottom plots. The size of the

plots was fifty by fifty meters wherein all the trees were identified at species level and labelled, whereas measurements were carried out in a 20x20m core to avoid border effects. A difficulty in choosing the plots was the enormous biodiversity. Every plot was different because it was virtually impossible to find plots of similar species composition. In each plot the three biggest trees were selected, as they likely dominated the functioning of the plot. These three trees cover most of the plot with their root system reaching for nutrients in the nutrient-poor environment and they intercept most sunlight at the top of the canopy. In addition, two additional smaller trees were also selected. In all plots the selected species included both common and rare species. A list with the measured tree species and their families can be found in Table B1 and Table B2 in appendix B for Nouragues and Paracou respectively.

2.2 Leaf data collection

To study the vertical profile of the photosynthesis of the trees, we selected branches at two different levels in the canopy: one on top and one at a lower level of the tree, later referred to as top and down leaves, respectively. Climbers collected the branches of the chosen trees and measured its exact height with a laser rangefinder (Forestry Pro, Nikon Vision co., Tokyo, Japan). Branches were selected with many mature leaves, to have as many data as possible and yet avoid differences due to possible leaf age effects on the nutrient allocation and photosynthesis. Branches were immediately put in buckets and recut under water to avoid cavitation before they were transported to the field lab. Only fully developed leaves were used for further analyses.

In Nouragues, the light regime of every measured tree was determined using the Dawkins' crown illumination index (Dawkins and Field, 1978). Figure 1 shows a schematic example used in Jennings *et al*. (1999). This index describes a tree's light environment based on a five-point scale with (1) receiving no direct light; (2) crown lit only from side; (3) partial (10–90%) vertical illumination; (4) full vertical illumination; (5) crown fully exposed to lateral and vertical light. Because it was difficult to estimate the index from the ground, the climbers determined in which class the tree of the collected branch belonged. This data was not available for Paracou.

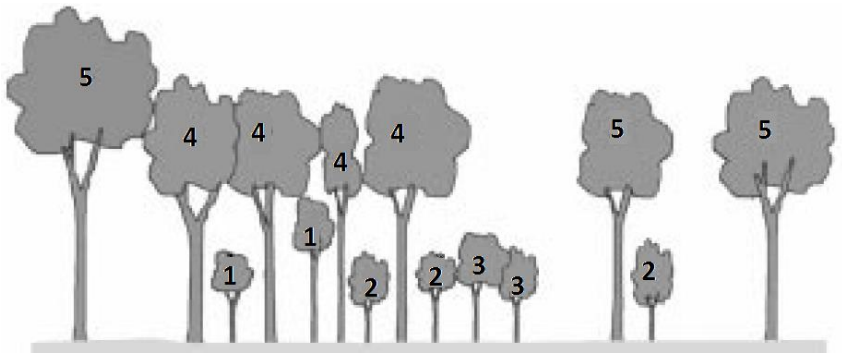


Figure 1. Example of the Dawkins' crown illumination index (Jennings et al., 1999).

2.3 Photosynthesis and dark respiration

2.3.1 LI-6400XT

Gas exchange measurements were carried out using a portable photosynthesis system (LI-6400XT, LI-COR, Lincoln, Nebraska USA), further referred to as LI-6400XT. A leaf cuvette of 2x3 cm was connected to an infrared gas analyser or IRGA (Figure 2). The LI-6400XT measures differences of H_2O and CO_2 concentration between the in-chamber and pre-chamber conditions. Photosynthesis and transpiration are calculated based on the differences between these concentrations, the air flow rate and the exposed leaf surface, following equations derived from Von Caemmerer and Farquhar (1981) and can be found in Appendix C. Air flows through the LI-6400XT to the chamber with the leaf and the reference chamber (Figure 3). The incoming air can be conditioned in several ways. The vapour pressure deficit (VPD) can be controlled by drying the air with a desiccant. The LI-6400XT calculates the VPD by using the leaf temperature, as the maximum amount of water in the air changes with temperature. The amount of CO_2 entering the leaf chamber can be fixed and changed by scrubbing the CO_2 out of the incoming air and subsequently injecting the chosen amount of CO_2. The photosynthesis measurements were carried out between 9h and 16h. Per branch we aimed to measure photosynthesis on three leaves.

Figure 2. Pictures of a leaf in the chamber of the Li-6400XTP, connected to the IRGA.

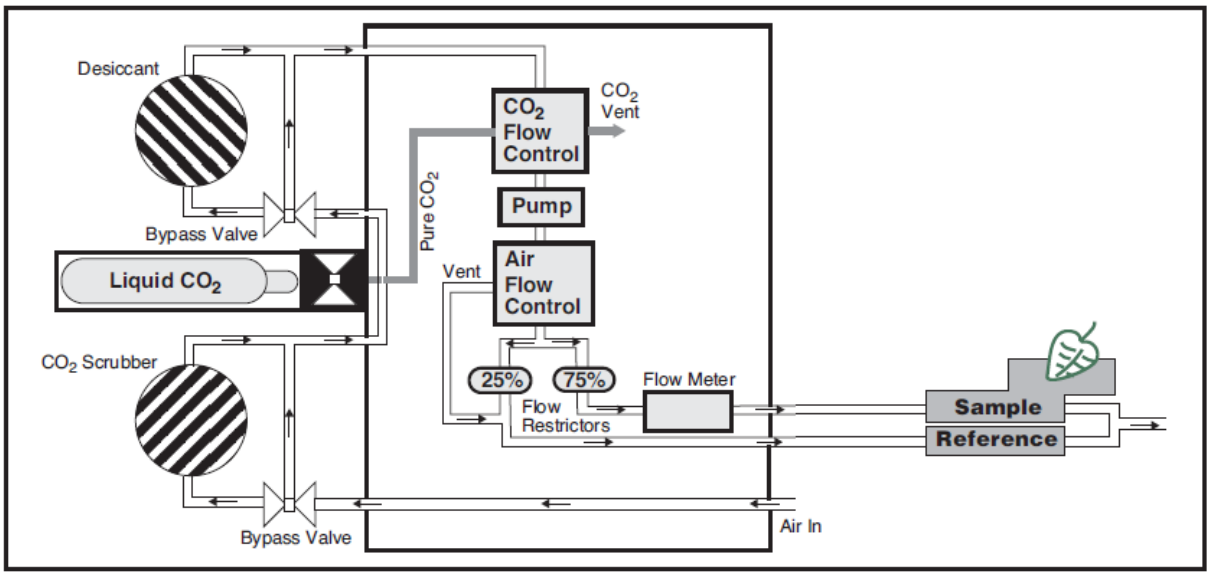


Figure 3. Schematic drawing of the flow through the Li-6400XTP (LI-COR manual 2012).

2.3.2 Photosynthesis and dark respiration

Photosynthesis of the leaves was measured at various CO_2 concentrations, which resulted in an A/C_i curve (assimilation as a function of internal CO_2 concentration). CO_2 concentrations were changed stepwise from ambient concentration (390 ppm) down to 50 ppm and then returning to ambient level and increasing to a saturating CO_2 concentration of 2000 ppm. The photosynthetically active radiation inside the chamber (PARi) was controlled and a distinction of this photosynthetic photon flux density (PPFD) was made for top (1300 µmol photons m^{-2} s^{-1}) and down leaves (500 µmol photons m^{-2} s^{-1}). Leaf temperature was controlled at 30 °C and VPD at 1.7 kPa to simulate realistic conditions.

To measure dark respiration (R_d), the branches were hidden under a cover, so no light could penetrate near the leaves, for 30 minutes. We carried out 5 consecutive measurements of Rd on 1 leaf per branch.

2.3.3 Farquhar model

A/C_i curves were fitted with the Farquhar *et al*. (1980) biochemical model (Appendix D), as defined by De Pury and Farquhar (1997). Net carbon assimilation is given by the following equation

$$A = \min\{A_j, A_v\} - R_d$$

where A_v is the Rubisco limited rate of photosynthesis, A_j is the electron transport limited rate of photosynthesis and R_d the dark respiration. The model enables determination of V_{cmax} and J_{max}, both parameters of photosynthesis. V_{cmax} (µmol m^{-2} s^{-1}) is the maximum rate of carboxylation and is determined by A_v and is the first part of the A/C_i curve (Figure 4), where C_i is low and limited by Rubisco activity. J_{max} (µmol m^{-2} s^{-1}) is the potential rate of electron transport and is determined by A_j and is the second part of the A/C_i curve (Walker *et al.,* 2014). Both A_j and A_v are modelled as functions of the intercellular CO_2 partial pressure. In Figure 4 a representative example is given of an A/C_i curve, with a V_{cmax} of 27.737 µmol m^{-2} s^{-1} and a J_{max} of 40.487 µmol m^{-2} s^{-1}. The full line is A_v, which determines V_{cmax} and the dashed line represents A_j, which determines J_{max}. The measured R_d of this particular tree was 0.66 µmol m^{-2} s^{-1}.

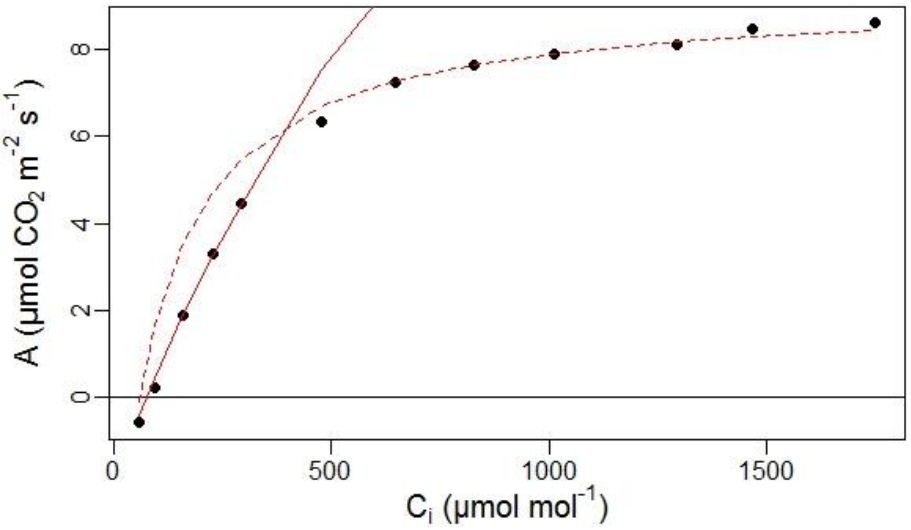


Figure 4. Representative example of an A/C_i curve fitted with the Farquhar (1980) model. The full line is A_v, which determines V_{cmax} and the dashed line represents A_j, which determines J_{max}. This particular one was of a top leaf of the Lueheopsis rugose of the Malvaceae family in plot T4 in Paracou. The calculated V_{cmax} is 27.737 µmol m^{-2} s^{-1} and J_{max} is 40.487 µmol m^{-2} s^{-1}.

2.4 Further leaf analyses

After measuring photosynthesis on a leaf, the chlorophyll content was approximated using a chlorophyll meter (SPAD 502 Plus, Spectrum Technologies, Inc., Aurora, Illinois, USA). The meter must be clamped over the leaf and measures light transmission at two wavelengths to determine the greenness and the thickness of the leaf. This provides a logarithmic index of the chlorophyll content (from -9.9 to 199.9) in less than 2 seconds. Afterwards the leaf was put in a separate paper bag which was labelled, ready for further analysis.

We calculated the specific leaf area (SLA, cm^2 g^{-1}) for every leaf of which we measured photosynthesis. This was done by photographing the leaf on a white background with a ruler to have a scale. These pictures were analysed with the software Image J (Version 1.4.3.67, Broken Symmetry Software, Bethesda, Maryland, USA) to calculate the leaf surface area (cm^2). These leaves were dried for 48h at 70 °C and then weighed. The surface area divided by this dry mass resulted in the SLA.

In order to determine the stoichiometry, the dried leaves were grinded and sent to the Autonomous University of Barcelona (UAB, Barcelona, Spain) for further analyses. The macronutrients in the samples were determined using a plasma mass spectrometer (7500 Series IPC-MS, Agilent

Technologies, Santa Clara, California, USA). First 0.25 g of every sample was digested with 5 ml HNO_3 concentrate in a microwave (UltraWAVE, Milestone S.r.l., Sorisole, Bergamo, Italy). After digestion, the supernatants were analysed with the plasma mass spectrometer. Concentrations were retrieved as a mass percentage. This percentage was divided by 100 and multiplied by the SLA to calculate the leaf P concentration per leaf area (g P m^{-2} leaf).

2.5 Soil data collection

Per plot five soil samples were taken following a dice design. On each of these places, a sample was taken from the top 15 cm (depth A, 0-15 cm) and the next 15 cm (depth B, 15-30cm) of the soil. Afterwards pH was measured, soil moisture was determined and nutrient extractions were performed.

To measure pH, 10 g of moist soil was added to a 25 mL 1.0 M KCl solution. The tubes with the reagents were shaken for an hour at 160 strokes per minute. Afterwards the tubes were left alone for an hour and only then the electrode was put in the supernatant and stirred until the pH value was stabilized. Soil moisture (%) was determined by the difference in wet and dry weight. To measure the dry weight, the samples were first put in an oven of 105 °C for 24 hours.

To determine the bioavailable Phosphorus the Olsen P method was used. Bioavailable phosphate (PO_4^{3-}) was measured, using the protocol described in Olsen (1954). This method is used worldwide and commonly used in other studies (Wang *et al*., 2006; Simfukwe *et al*., 2011; Li *et al*., 2015). The method is based on the use of 0.5 M $NaHCO_3$ at pH 8.5. This solution was added to 30 g of every soil sample and this mixture was shaken for 30 minutes. The next step was the molybdenum blue method; colouring the extract with a molybdate solution which can be measured with a spectrophotometer at 882 nm. The choice to use the Olsen P method for the present acidic soils is justified, it has been shown that it is effective in acidic circumstances (Fixen and Grove, 1990).

2.6 Statistical analyses

The statistical analyses were carried out using the open source statistical program R (R Development Core Team, 2013). With this program regression and correlation analyses were done and graphs were made. T-tests were performed on parametric data and Tukey's test to test multiple comparisons in a single-step. Non-parametric alternatives were the Wilcoxon signed-rank test and the pairwise Wilcoxon test. For comparing averages, ANOVA was carried out or a non-parametric alternative, the Spearman's rank correlation.

A model II regression was used for large datasets when the parameters on both x- and y-axis had a standard error. For bivariate normal data, a major axis (MA) regression was used if both variables were expressed in the same physical units and if the error variances of the variables were equal. If the variables are not expressed in the same physical units or the error variances on the two axes differ, two alternative methods were available. Standard major axis regression (SMA) was used only when there was a significant correlation supporting the hypothesis. If the distribution was not bivariate normal, if the relationship was linear, the ordinary least squares (OLS) was used to estimate the parameters of the regression line.

3. Results

3.1 Relation between soil P and leaf P

The range of soil P we measured in the plots was rather low (Figure 5), with an average value of 2.3 µg g^{-1} soil in Nouragues and 2.2 µg g^{-1} soil in Paracou. Soil P was not significantly different between the sites. The bottom plots at both sites reached higher values compared to the top and slope plots. In Paracou, this increase was significant compared to the top ($p < 0.01$) and slope plots ($p < 0.001$). This difference was not significant in Nouragues. The differences in soil P were not induced by a soil moisture gradient ($p > 0.05$).

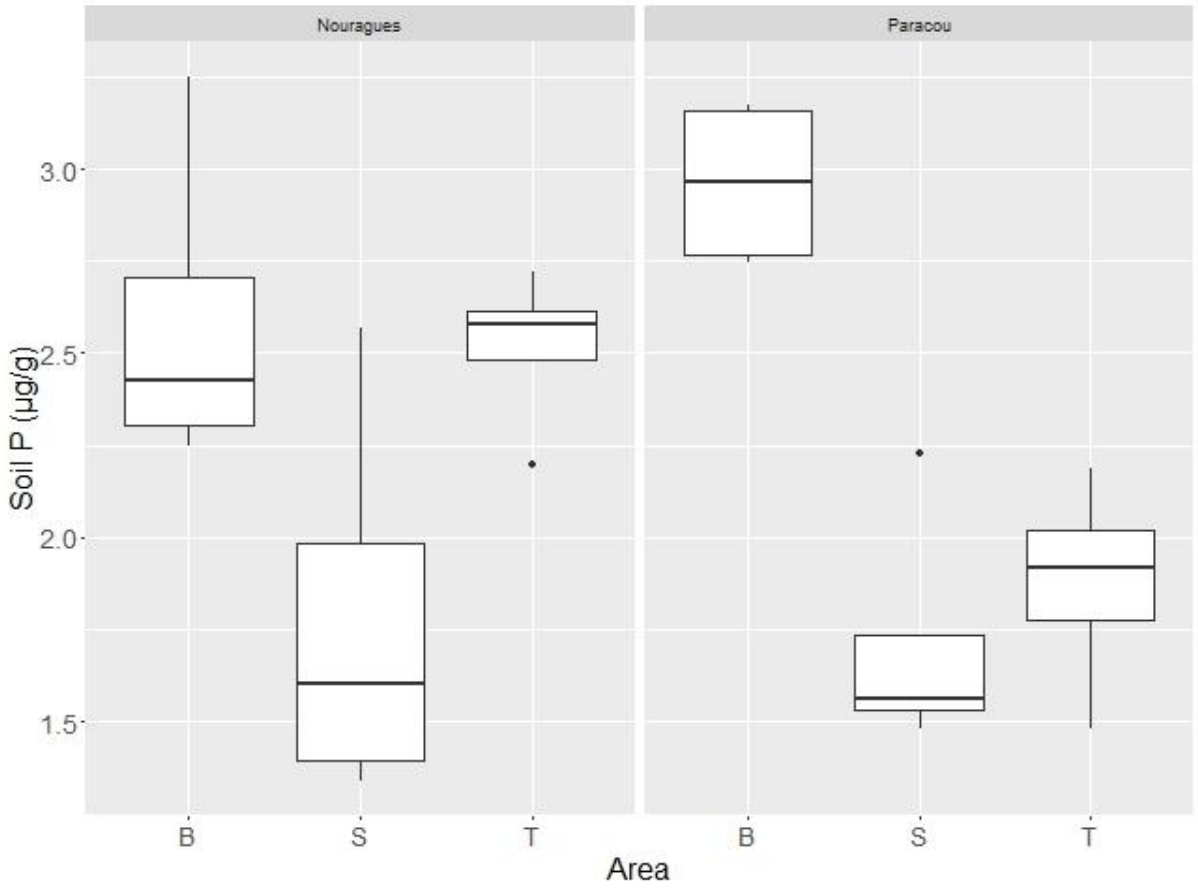


Figure 5. Soil phosphorus (P) content per type of plot (Bottom (B), Slope (S), Top (T)). In Paracou, the bottom plots contained significantly more soil P than the top ($p < 0.01$) and slope plots ($p < 0.001$). In Nouragues there were no such significant differences ($p > 0.05$).

Although the plots were low in soil P, a correlation existed between soil P and leaf P (Figure 6). Leaf P was positively correlated with soil P of the upper soil layer (depth A, 0-15 cm) ($p < 0.001$, Figure 6). A similar correlation with the soil P content of depth B (15-30 cm, data not shown) was not found.

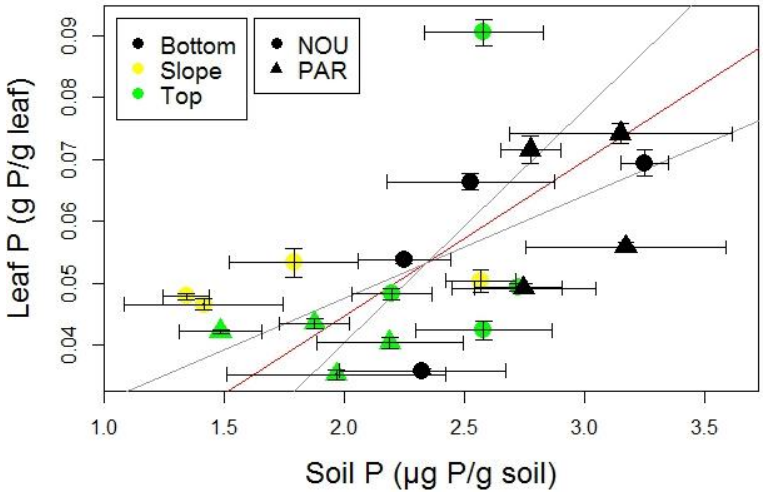
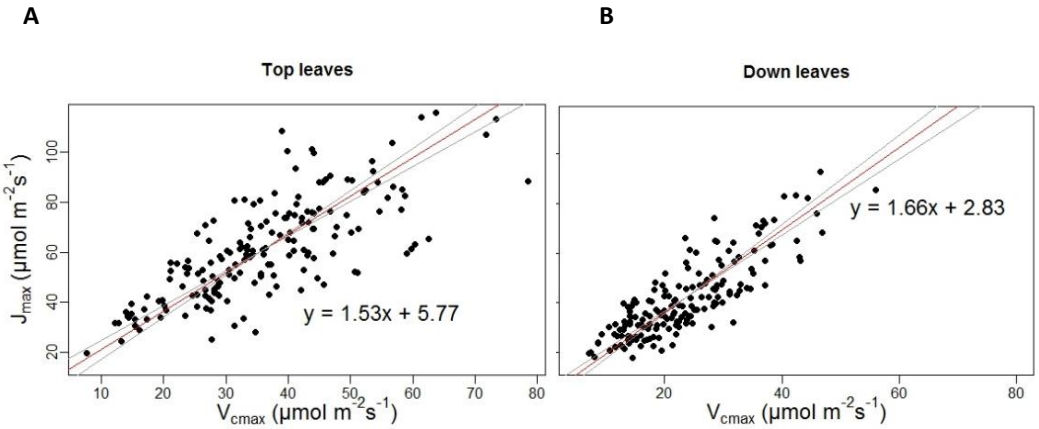


Figure 6. Correlation between soil P (depth A: 0-15 cm) and leaf P for Nouragues (●) and Paracou (◢). Colours represent the different plot types: bottom, slope and top. For Paracou no leaf P data were available for the slope plots.

3.2 Photosynthetic parameters V_{cmax} and J_{max}, and R_d

Figure 7 presents the relations between V_{cmax}, J_{max} and R_d. A positive correlation was shown between V_{cmax} and J_{max} for both top (A) and down (B) leaves. The regressions were significantly different between top and down leaves ($p < 0.05$). The same was true for V_{cmax} in relation to R_d (C for top leaves and D for down leaves) and J_{max} vs. R_d (E for top leaves and F for down leaves).



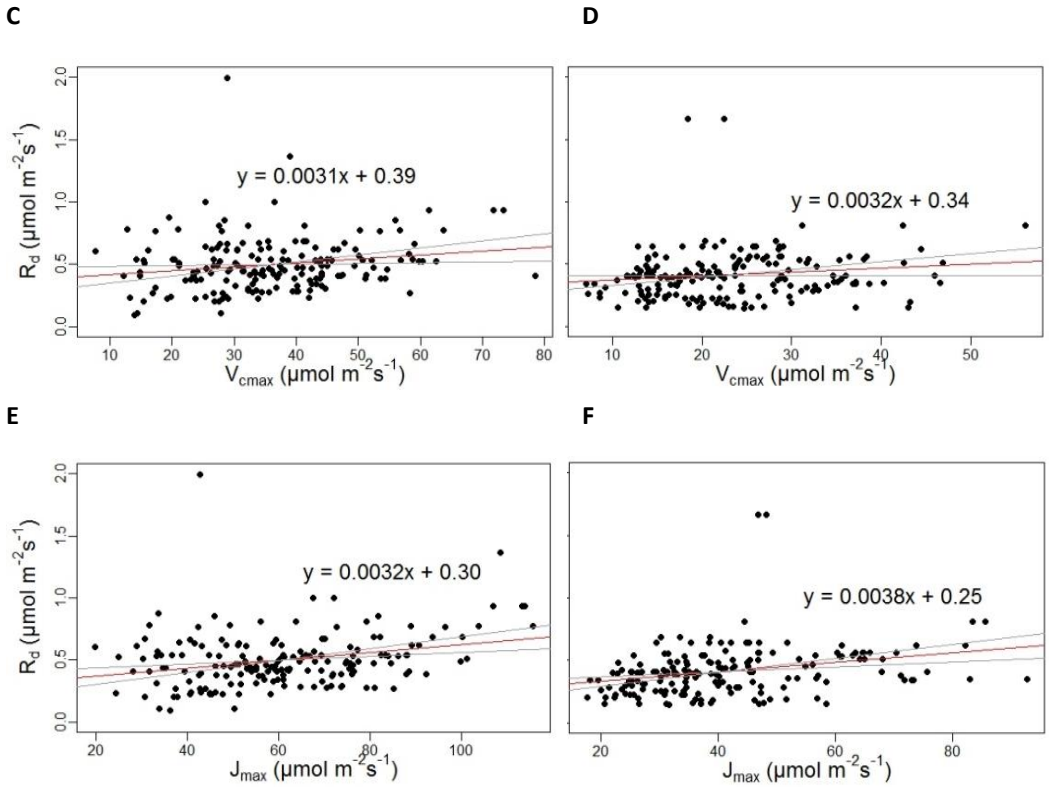


Figure 7. Major axis correlations (red lines) between V_{cmax}, J_{max} and R_d shown for top leaves (A, C, E) (n = 176) and down leaves (B, D, F) (n = 178). The gray lines represent the 95% confidence interval.

3.3 Vertical profile

Differences between top and down leaves were indicated in Figure 7. Here we will look deeper into the vertical profiles and differences in V_{cmax}, J_{max}, and R_d within the canopy. Figure 8 shows significant differences between top and down leaves. The top leaves had significant higher values for V_{cmax}, J_{max} and R_d ($p < 0.001$). We compared the photosynthetic parameters and R_d of every single leaf with the true measured height at which the branches were cut. They were all positively correlated with leaf height ($p < 0.001$, Figure 9). We related these parameters also to Dawkins' five light classes as another vertical parameter. This is shown in Figure 10, J_{max} (Figure 10, B) and R_d (Figure 10, C) increased with a higher light class, but this was not the case for V_{cmax} (Figure 10, A: $p > 0.05$). Leaves in class 1 had a significantly lower J_{max} than leaves in class 3 and 5 ($p < 0.05$) and those leaves in class 5 had also a significantly higher J_{max} than those in class 2 ($p < 0.01$). Leaves in class 5 had a higher R_d value than the leaves in all other classes ($p < 0.05$).

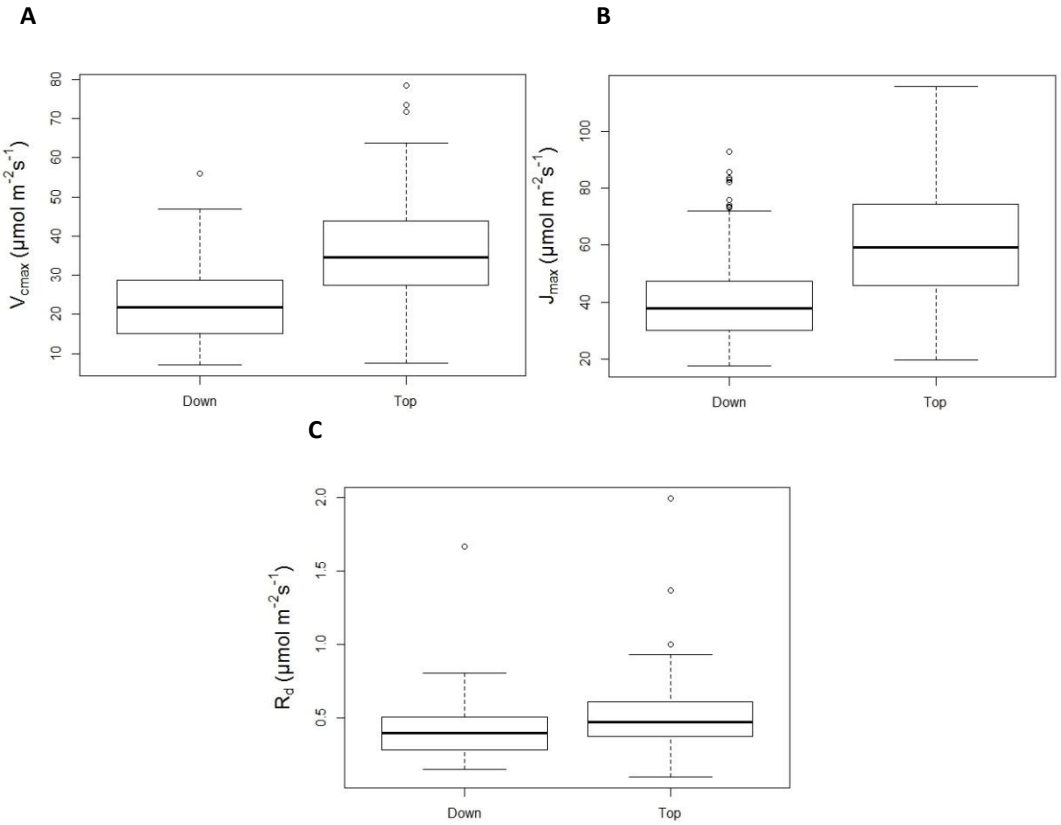


Figure 8. Differences between top and down leaves in A) V_{cmax}, B) J_{max} and C) R_d (all $p < 0.001$).

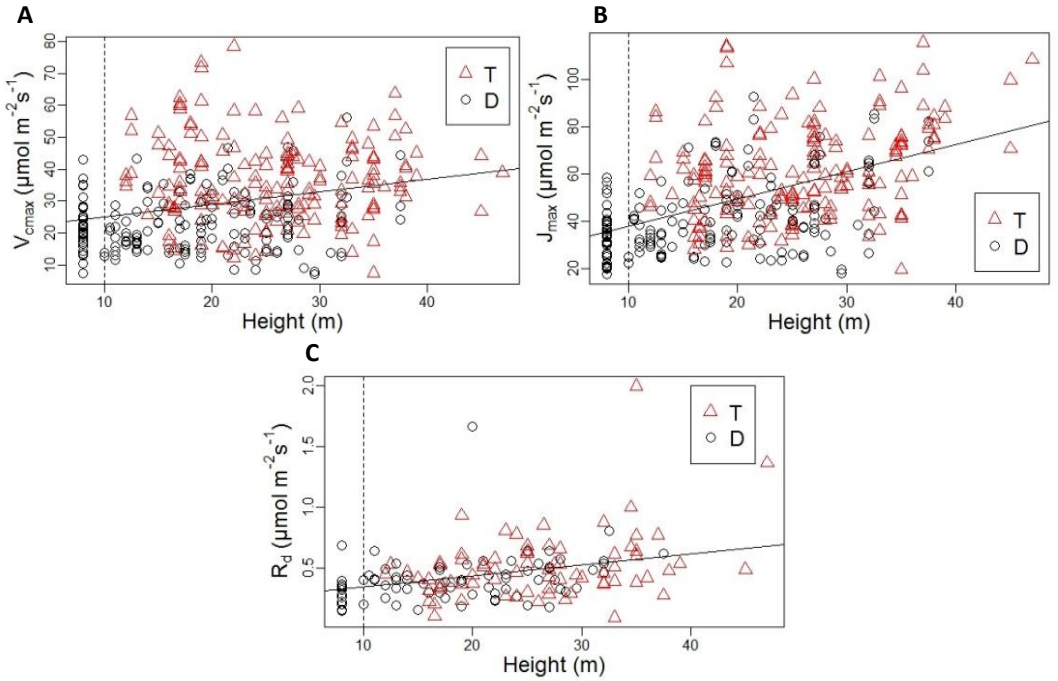


Figure 9. Correlations (all $p < 0.001$) between the photosynthetic parameters and leaf height with A) V_{cmax}, B) with J_{max} and C) with R_d. A distinction was made between top (Δ) and down (o) leaves. The dotted line represents the limit below which branch height could not be measured (<10m).

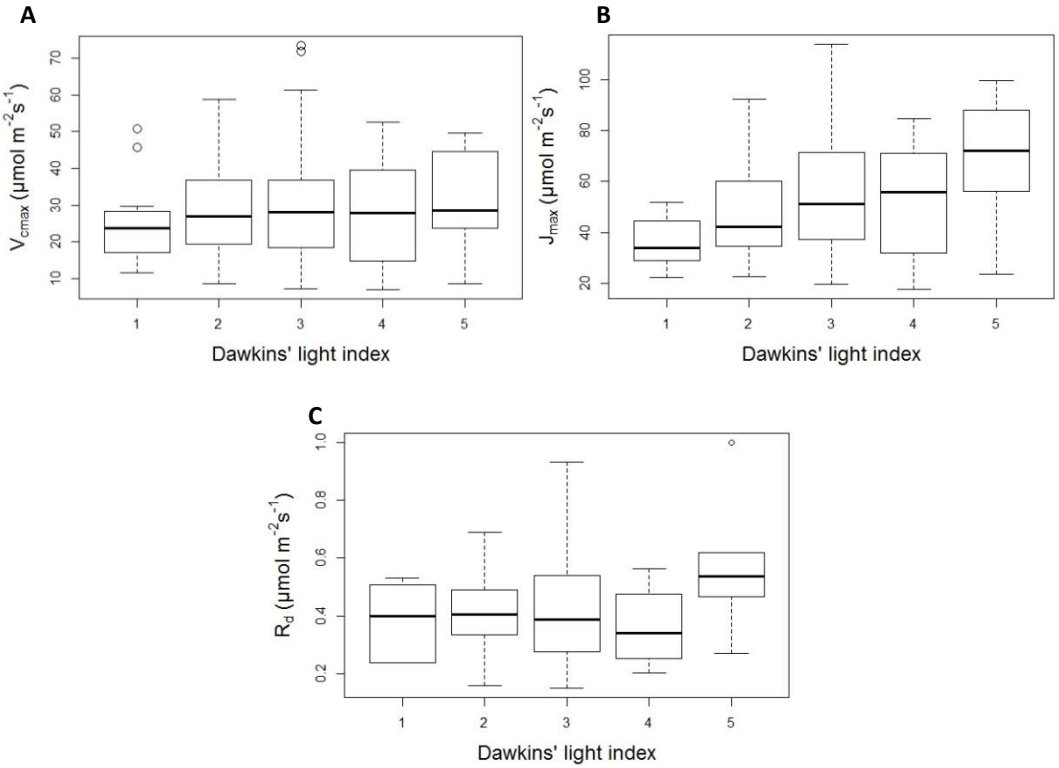
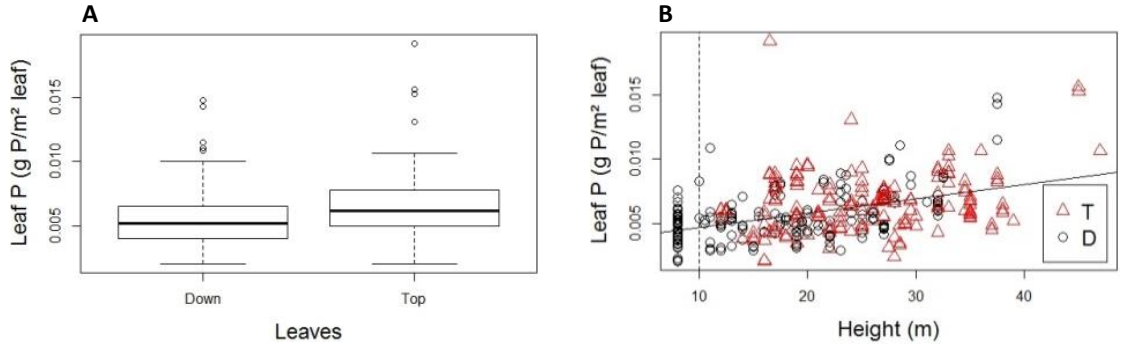


Figure 10. Differences between the photosynthetic parameters in different light classes according to Dawkins' light index. A) V_{cmax} did not significantly differ between the light classes (p > 0.05). B) J_{max} had a trend to go higher with more light availability. Leaves in class 1 had a significant lower J_{max} than in classes 3 and 5 (p < 0.05). The leaves in class 5 had also a significantly higher J_{max} than in class 2 (p < 0.01). C) R_d was significantly higher in leaves with class 5 than in every other class (p < 0.05).

3.4 Photosynthesis in relation to leaf stoichiometry

Leaf P (P_{mass}, g P g^{-1} leaf) did not differ between top and down leaves (p > 0.05, data not shown) and no correlations were found with the exact leaf height, nor with Dawkins' light index (data not shown). In contrary, leaf P (P_{area}, g P m^{-2} leaf) was significantly different between top and down leaves (p < 0.001, Figure 11A). P_{area} increased with increasing height in the canopy (p < 0.001, Figure 11B), and with Dawkins' light index, where P_{area} was higher in almost every higher class (p < 0.05, Figure 11C). Only P_{area} in class 2 was not significantly higher than in class 1 (p > 0.05).



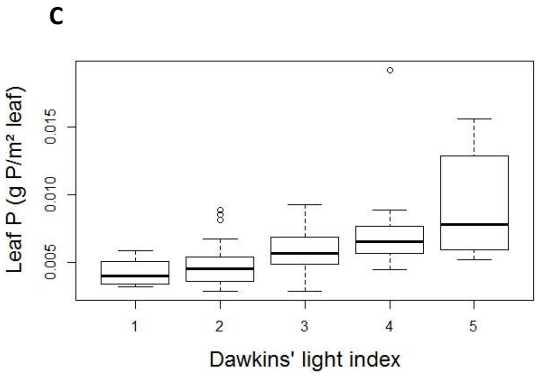


Figure 11. P_{area} (g m^{-2}) plotted against A) top and down leaves (p < 0.001). B) Height, for both top (Δ) and down (o) leaves (p < 0.001). The dotted line represents the limit below which branch height could not be measured (<10m). C) Light class of the leaf, where P_{area} was significantly higher in every higher light class (p < 0.05) except for class 1 and 2 (p > 0.05).

In Figure 12 P_{area} is plotted against the photosynthetic parameters (V_{cmax}: A and J_{max}: B) and R_d (C) for both top and down leaves. There were no significant results for the top leaves (p > 0.05), but for the down leaves all correlations were significant (p < 0.01).

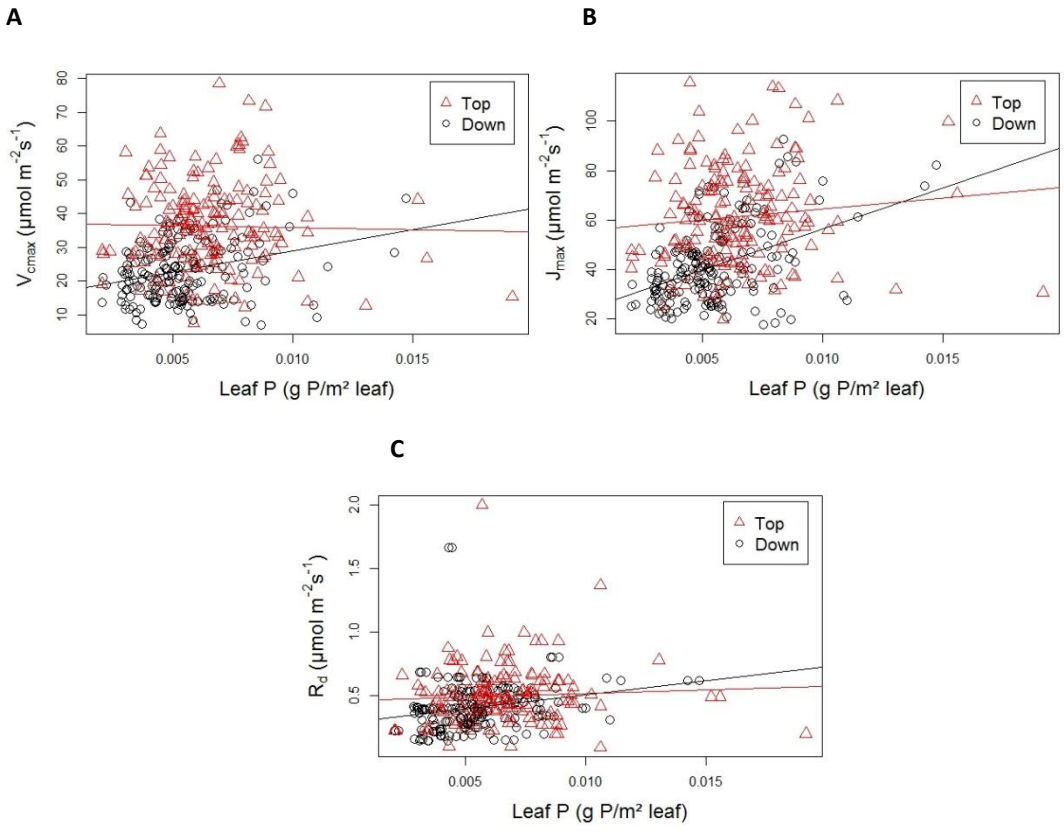


Figure 12. P_{area} plotted against A) V_{cmax} B) J_{max} and C) R_d. All correlations were significant for the down (o) leaves (p < 0.01), but not for the top (Δ) leaves (p > 0.05).

In the canopy there was a vertical reduction in multiple parameters (Figure 8, 9 and 11). The calculated % reduction between top and down leaves for P_{area} was plotted against the % reduction in V_{cmax} and J_{max} from the top leaves to the down leaves (Figure 13). Both correlations were positive and significant ($p < 0.05$). R_d was not incorporated here because calculation of reduction through the canopy was not possible due to only one measurement per tree.

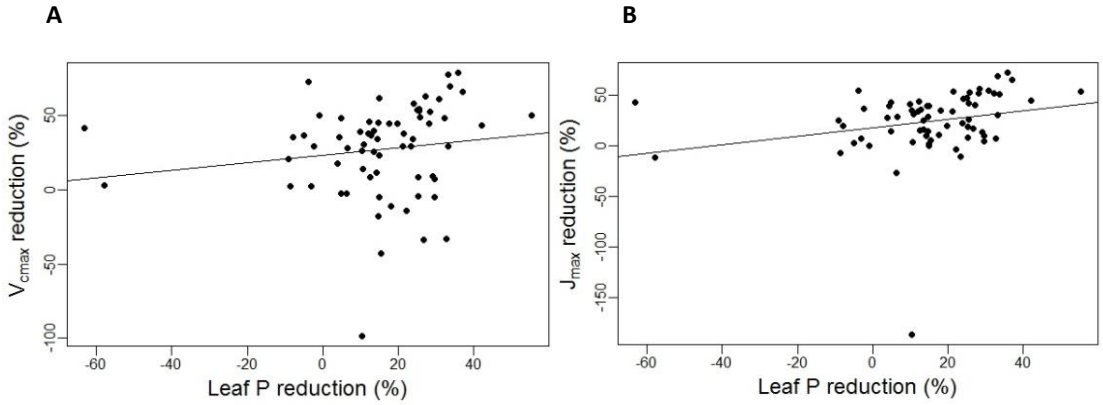


Figure 13. Reduction in the canopy of P_{area} was positively correlated with the reduction in A) V_{cmax} and B) J_{max}.

3.5 Other leaf parameters and its features

The SLA increased significantly ($p < 0.0001$) from higher to lower leaves (Figure 14). In other words, the leaves were thicker on top of the canopy and thinner at the lower parts.

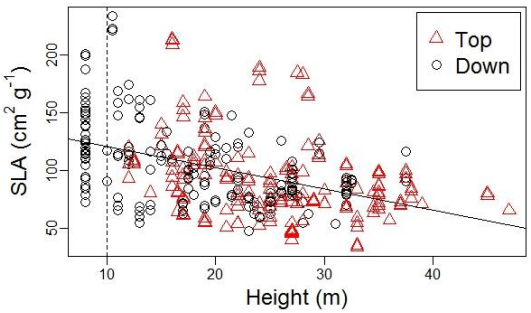


Figure 14. SLA declined significantly with leaf height ($p < 0.0001$) for both top (Δ) and down (o) leaves. The dotted line represents the limit below which branch height could not be measured (<10m).

Compared to the leaf P content, SLA decreased significantly ($p < 0.0001$) with increased P_{area}, but increased significantly ($p < 0.0001$) with increased P_{mass} (Figure 15).

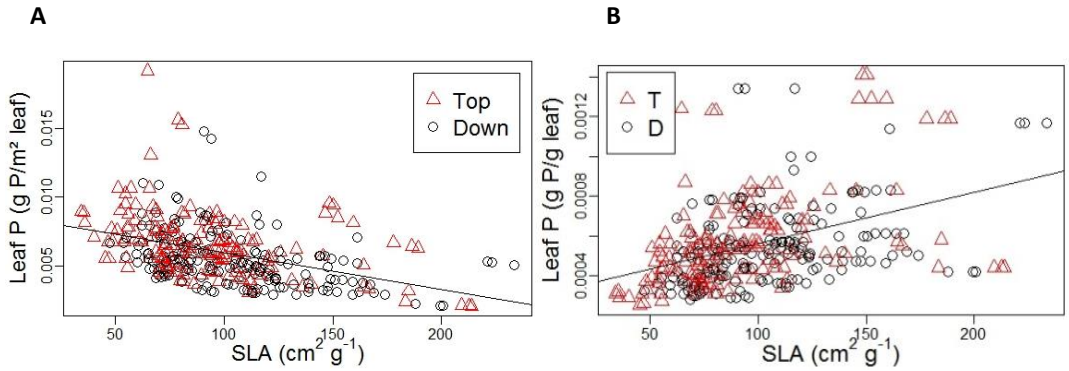


Figure 15. A) SLA was negatively correlated to P_{area} ($p < 0.0001$). B) SLA was positively correlated with P_{mass} ($p < 0.0001$) for both top (Δ) and down (o) leaves.

This correlation with the available leaf P was also visible when SLA was correlated to the photosynthetic parameters (Figure 16). V_{cmax} ($p < 0.01$), J_{max} ($p < 0.0001$) and R_d ($p < 0.0001$) decreased significantly with increasing SLA.

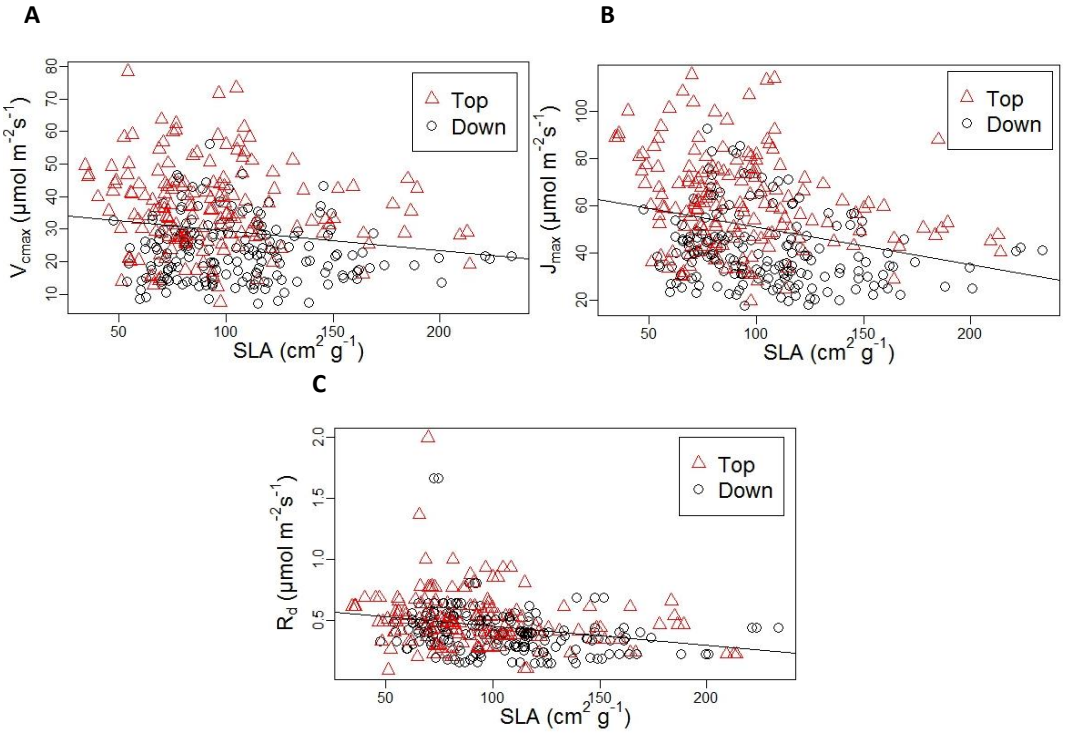


Figure 16. For both top (Δ) and down (o) leaves A) V_{cmax} ($p < 0.01$), B) J_{max} ($p < 0.0001$) and C) R_d ($p < 0.0001$) were negatively correlated with SLA.

The SLA was also negatively correlated with the Dawkins' Light index (Figure 17). Most correlations were significant ($p < 0.05$), except between classes 1 and 2, between 3 and 4 and between 4 and 5.

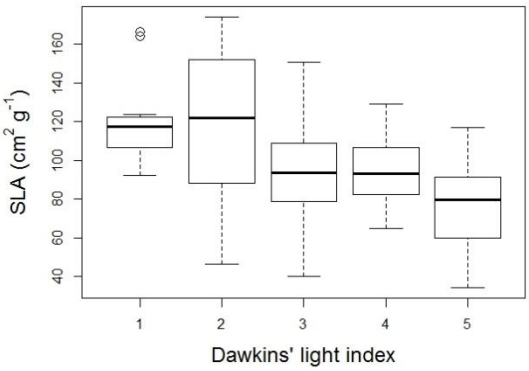


Figure 17. SLA declined significantly (p < 0.05) with increased Dawkins' light index.

The chlorophyll content of the leaves (SPAD) was plotted against leaf P content in Figure 18. P_{mass} was negatively correlated (p < 0.0001) with SPAD, but P_{area} and SPAD were not correlated (p > 0.05). SPAD was also not correlated with leaf height, nor any of the photosynthetic parameters (data not shown). SPAD declined significantly (p < 0.0001) with higher SLA (Figure 19).

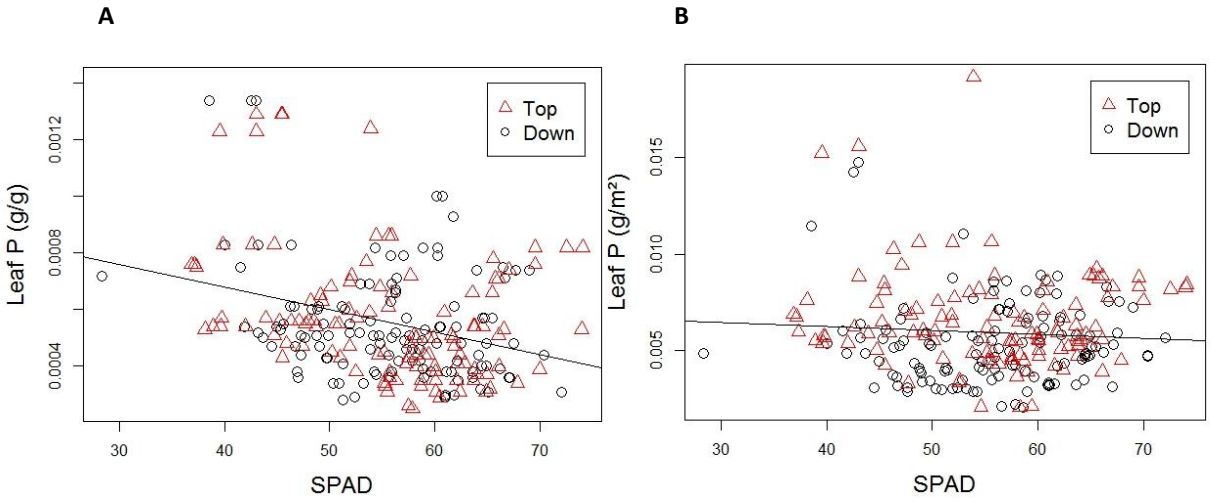


Figure 18. The leaf chlorophyll content (SPAD) for both top (Δ) and down (o) leaves plotted against A) P_{mass}, which declined with higher SPAD (p < 0.0001) and B) P_{area}, which did not correlate with SPAD.

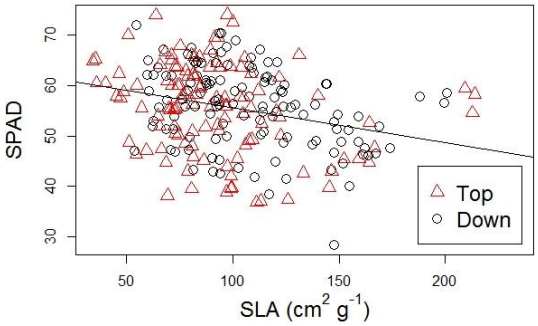


Figure 19. For both top (Δ) and down (o) leaves, SPAD declined significantly (p < 0.0001) with higher SLA.

3.6 Horizontal spatial variation

The two sites, Nouragues and Paracou, differed significantly from each other if we look at the V_{cmax} values ($p < 0.05$) (Figure 20). In Paracou higher values were reached than in Nouragues. The other photosynthetic parameters did not differ significantly ($p > 0.05$). Also leaf P and SLA did not differ between the two sites (data not shown). Ratios of J_{max} and V_{cmax} were significantly higher ($p < 0.0001$) in Nouragues than in Paracou (Figure 20, D).

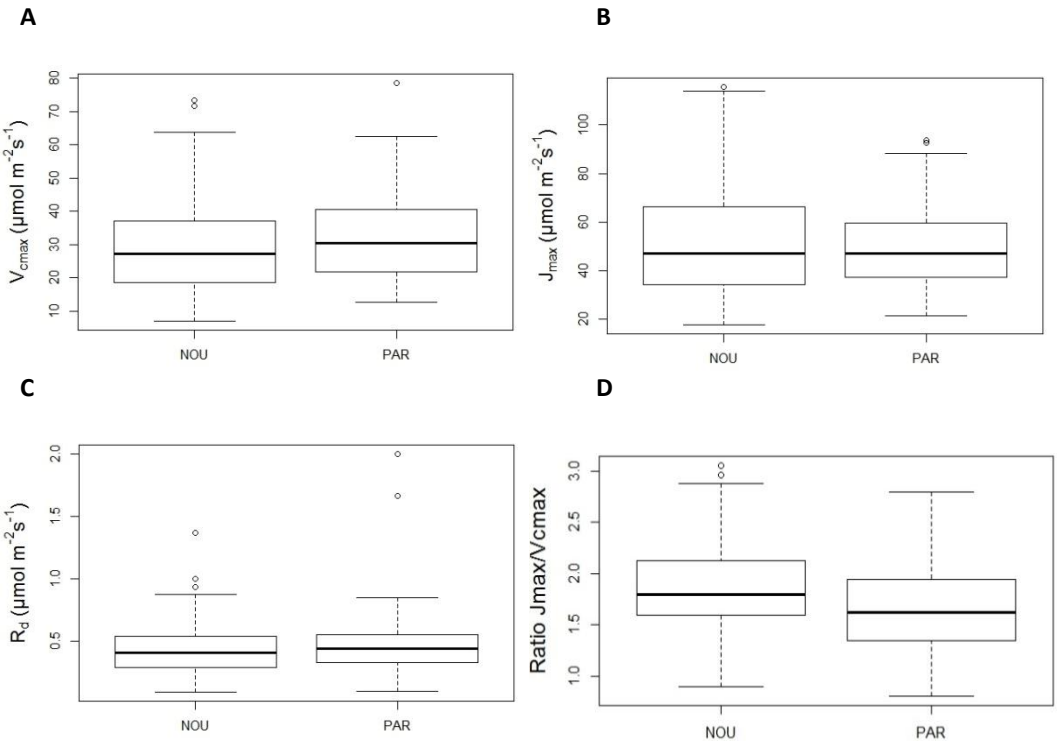


Figure 20. The photosynthetic parameters compared between the two sites Nouragues and Paracou for A) V_{cmax}, which was significantly higher ($p < 0.05$) in Paracou, B) J_{max}, which was the same in both sites and C) R_d, which was also the same in both sites. D) Ratio J_{max}/V_{cmax} for both sites. Nouragues had a higher ratio than Paracou ($p < 0.05$).

In Figure 21, the relations between P_{area} and the photosynthetic parameters were compared between Paracou and Nouragues. Leaf P and V_{cmax}, J_{max} and R_d respectively were all positively correlated, although all relations differed significantly for the two sites ($p < 0.05$). For P_{mass} there were no significant differences ($p > 0.05$) between the sites (data not shown).

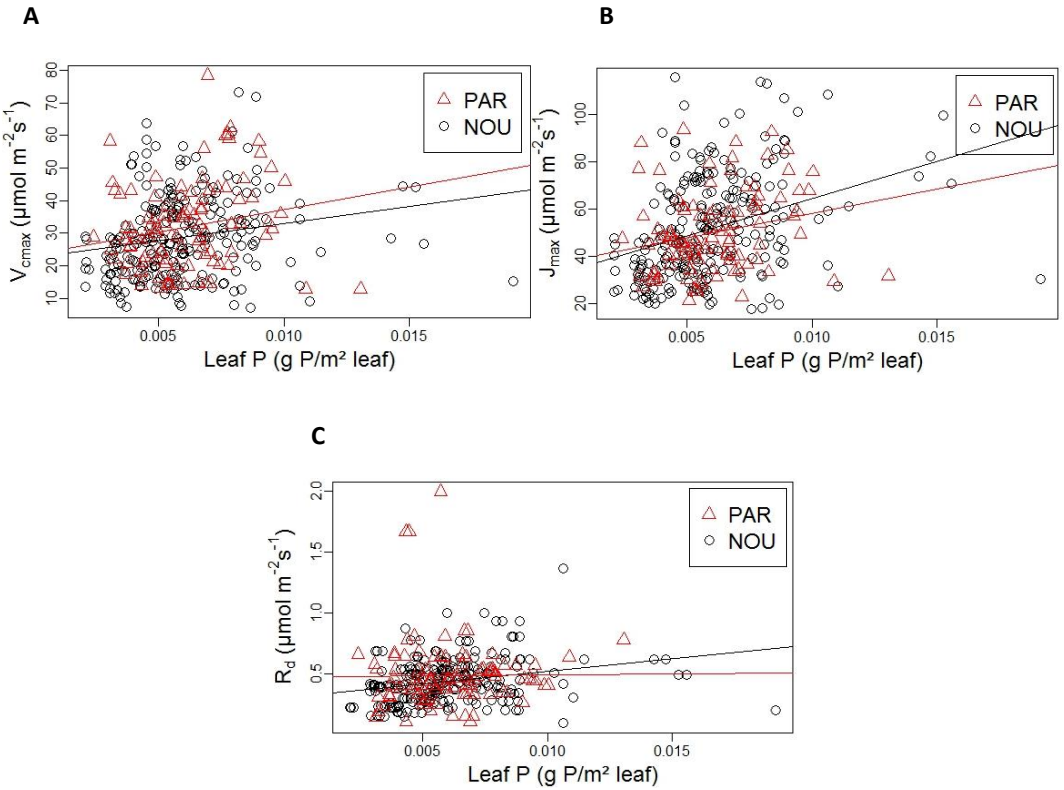


Figure 21. For Paracou (Δ) and Nouragues (o) separately, P_{area} was plotted against the photosynthetic parameters. The regressions between the sites were all significantly different ($p < 0.05$).

4. Discussion

4.1 Carbon uptake limited by available soil P

The available soil P was, with an average of 2.2 µg g^{-1} in Paracou and 2.3 µg g^{-1} in Nouragues, very low. These soil P values are, compared to other studies in tropical regions, rather low (Kitayama & Aiba, 2002), but much lower than other studies in other tropical regions where concentrations can be hundred times higher (Cleveland *et al*., 2002). Reasons for these differences can be effects by substrates, temperatures and altitudes (Kitayama & Aiba, 2002). It is common in highly weathered lowland rain forest soils to have an impoverishment of bioavailable P (Vitousek & Sanford, 1986 and Tiessen *et al*., 1994). There are better alternatives than the Olsen P method for determination of acid soil P concentration, e.g. the Bray P method, which we also carried out. However, when comparing results of both, there was not much difference. We have chosen to work with Olsen P, so our results are comparable with universal soil P maps. Fixen and Grove (1990) has shown that the Olsen P method is also effective in acidic circumstances. Bottom plots reached higher soil P concentrations than other plots (Figure 5), as expected, though not due to differences in soil moisture. Plots were chosen to have variation in soil P, so we succeeded in that objective.

Trees growing on soils with a P deficiency, can experience limitation of carboxylation in photosynthesis (Kitayama & Aiba, 2002). Carboxylation capacity may often be controlled by leaf N (Evans, 1989). Nitrogen data were not yet available for this study, but N should not be limiting photosynthesis. Possible different concentrations in the soil should not affect the concentration of N in the leaves (Janssens *et al.*, 2010). In such acid soils (average pH of 4 in Nouragues and 4.2 in Paracou) with small P quantities, carboxylation can be controlled by leaf P (Raaimakers *et al*., 1995), given that the rate of RuBisCo regeneration is controlled by P (Jacob & Lawlor, 1992). A correlation has been found between the soil P content and the leaf P content (Figure 6), which is a first indication that the soil P availability is limited and could have an effect on the carbon uptake of the trees in both Nouragues and Paracou.

4.2 Vertical profile

Ranges of the photosynthetic parameters are comparable with similar studies in the tropics (Carswell *et al.*, 2000), but smaller than studies in other regions (Xu, 2003 in an oak-grass savanna in California and Leuning, 1997 in Australia). High correlations between V_{cmax}, J_{max} and R_d were expected (Thompson *et al*., 1992; Wullschleger, 1993 and Leuning, 1997). Carswell *et al*. (2000) showed

vertical differences for V_{cmax} and J_{max}, we were able to detect similar patterns. Down leaves had significant lower V_{cmax}, J_{max} and R_d than top leaves (Figure 8). V_{cmax}, J_{max} and R_d had positive relations with the exact leaf height (Figure 9). V_{cmax} is not higher when the light availability is higher, but for J_{max} and R_d there is a trend to increase with more light availability (Figure 10). All this combined, we conclude that the photosynthetic capacity of the trees in this study increases with height. Subsequent studies in photosynthetic capacities should always take into account the vertical difference of photosynthesis within the canopy. To have a better vertical profile in our own research an extra level, e.g. "middle", would have been more precise, but due to time management, a decision had to be made between multiple layers on the one hand or more data replications from the same levels on the other hand.

This vertical profile is also visible for the leaf P concentration, though only for P_{area} (g P m^{-2} leaf) (Figure 11), not for leaf P_{mass} (g P g^{-1} leaf). A reduction in P_{area} from top to down leaves exists and is correlated with a similar reduction for V_{cmax} and J_{max} (Figure 13). P_{area} is, only for down leaves, positively related with V_{cmax}, J_{max} and R_d (Figure 12). This means that in the lower parts of the canopy, the photosynthetic capacity is limited by the amount of available P, but on top of the canopy such limitation does not exist. Trees possibly allocate the necessary P towards the higher, sunlit leaves until those are fully operational. In addition, SLA declines with height (Figure 14), which means leaves get thinner downwards, and P_{area} declines with SLA (Figure 15 A), whilst P_{mass} is positively correlated with SLA (Figure 15 B). Here it is only logical that $P_{area,}$ expressed per cm^2 leaf, is higher when the leaves are thicker. Higher leaves capture more light and have a higher metabolism, which probably results in a higher starch concentration, hence the thicker leaves. Thicker leaves need more structural components, which contains less P. This is possibly the reason why P_{mass} is lower in thicker leaves. Carswell *et al*. (2002) also found this decrease in SLA towards the top of the canopy caused either by a thickening of leaves or an increase in starch concentration. Light availability (Figure 17), V_{cmax}, J_{max} and R_d are negatively correlated with SLA (Figure 16), which is another logical consequence for thinner leaves and less photosynthetic capacity.

The chlorophyll content (SPAD) is not correlated with P_{area}, nor with leaf height, nor with any of the photosynthetic parameters. There is a negative correlation with P_{mass} (Figure 18) and SLA (Figure 19). This means thicker leaves, already shown to have a decline in P_{mass}, contain more chlorophyll.

4.3 Nouragues and Paracou compared

Rainfall, temperature and soil features like moisture content and pH were quite similar in Nouragues and Paracou. Leaf P concentrations and soil P concentrations (Figure 5) were not different between the two sites. The canopy in Paracou reached higher values for V_{cmax}, the maximum rate of carboxylation, but J_{max} and R_d are equal in both sites (Figure 20). The J_{max}/V_{cmax} ratio is hereby higher in Nouragues. Higher ratios are found where there is more light availability (Zheng *et al*., 2012) and V_{cmax} is higher in more shady areas. Light availability was not available in Paracou, so testing this hypothesis was not possible. Figure 21 shows differences between the two sites in use of leaf P, though differences are small.

Continuation of the research could be a fertilisation experiments where the plots are fertilised with N and P, to test how this will affect the photosynthetic capacity and in what way exactly. Since we showed that the top leaves might be as good as saturated already, the differences between top and down leaves could become smaller. Light availability data and more data on the slopes in Paracou will be a good addition.

5. Conclusion

We showed that P can limit photosynthesis in tropical forests. Carbon assimilation by sunlit top leaves are not limited by P availability, but in lower parts of the canopy the capacity improves with increasing P concentration. Therefore, the increasing availability of atmospheric C will not be counteracted as much as previously expected. Models of the global C cycle should take the P cycle into consideration, because P has a limiting influence on tropical systems, which is one of the largest carbon sinks in the world.

6. References

Ågren, G. I. (1983). Nitrogen productivity of some conifers. *Canadian Journal of Forest Research, 13*(3) 494-500.

Aubry-Kientz, M., Rossi, V., Wagner, F., & Hérault, B. (2015). Identifying climatic drivers of tropical forest dynamics. Biogeosciences Discussions, 12(3), 3145-3176.

Bonan, G. B. (1990). Carbon and nitrogen cycling in North American boreal forests. II. Biogeographic patterns. *Canadian Journal of Forest Research*,*20*(7), 1077-1088.

Bonan, G. B. (2008). Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *science*, *320*(5882), 1444-1449.

Bray and Kurtz (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39-45

Carswell, F. E., Meir, P., Wandelli, E. V., Bonates, L. C. M., Kruijt, B., Barbosa, E. M., ... & Jarvis, P. G. (2000). Photosynthetic capacity in a central Amazonian rain forest. *Tree Physiology*, *20*(3), 179-186.

Chave, J., Riéra, B., & Dubois, M. A. (2001). Estimation of biomass in a neotropical forest of French Guiana: spatial and temporal variability. *Journal of Tropical Ecology*, *17*(01), 79-96.

Cleve, K. V., & Zasada, J. C. (1976). Response of 70-year-old white spruce to thinning and fertilization in interior Alaska. *Canadian Journal of Forest Research*, *6*(2), 145-152.

Cleve, K. V., Oliver, L., Schlentner, R., Viereck, L. A., & Dyrness, C. T. (1983). Productivity and nutrient cycling in taiga forest ecosystems. *Canadian Journal of Forest Research*, *13*(5), 747-766.

Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O., ... & Wasson, M. F. (1999). Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global biogeochemical cycles*,13(2), 623-645.

Cleveland, C. C., Townsend, A. R., & Schmidt, S. K. (2002). Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. *Ecosystems*, *5*(7), 0680-0691.

Cooper, G. M. (2000). "10.The Chloroplast Genome". *The Cell: A Molecular Approach* (2nd ed.). Washington, D.C: ASM Press. ISBN 0-87893-106-6.

Cuevas, E., & Medina, E. (1986). Nutrient dynamics within Amazonian forest ecosystems, *68*(3), 466-472.

Cuevas, E., & Medina, E. (1988). Nutrient dynamics within Amazonian forests. *Oecologia*, *76*(2), 222-235.

Dawkins, H. C., & Field, D. R. B. (1978). *A long-term surveillance system for British woodland vegetation* (No. 634.95 D271). Commonwealth Forestry Institute, Oxford (RU).

De Pury, D., & Farquhar, G. D. (1997). Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. *Plant, Cell & Environment*, *20*(5), 537-557.

Denning, A. S. (1993). Relating surface energy budgets to the biochemistry of photosynthesis: A review for non-biologists. *Lecture Notes, Colorado State University, USA*.

Dwyer, L. M., & Stewart, D. W. (1986). Effect of leaf age and position on net photosynthetic rates in maize (*Zea mays* L.). *Agricultural and forest meteorology*, *37*(1), 29-46.

Eliasch, J. (2008). Climate change: financing global forests: the Eliasch review. Earthscan.

Evans, J. R. (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, *78*(1), 9-19.

FAO-UNESCO (2005). Soil Map of the World, digitized by ESRI. Soil climate map, USDA-NRCS, Soil Science Division, World Soil Resources, Washington D.C.

Farquhar, G. D., von Caemmerer, S. V., & Berry, J. A. (1980). A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta*,*149*(1), 78-90.

Feller, U., Anders, I., & Mae, T. (2008). Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. *Journal of experimental botany*,*59*(7), 1615-1624.

Field, C. H., & Mooney, H. A. (1986). Photosynthesis--nitrogen relationship in wild plants. In *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*. Cambridge [Cambridgeshire]: Cambridge University Press, c1986..

Fixen, P. E., Grove, J. H., & Westerman, R. L. (1990). Testing soils for phosphorus. *Soil testing and plant analysis.*, 141-180.

Grace, J., Lloyd, J., McIntyre, J., Miranda, A. C., Meir, P., Miranda, H. S., ... & Gash, J. (1995). Carbon dioxide uptake by an undisturbed tropical rain forest in southwest Amazonia, 1992 to 1993. *SCIENCE-NEW YORK THEN WASHINGTON-*, 778-778.

Hartmann, T., Mult, S., Suter, M., Rennenberg, H., & Herschbach, C. (2000). Leaf age-dependent differences in sulphur assimilation and allocation in poplar (*Populus tremula* × *P. alba*) leaves. *Journal of Experimental Botany*, *51*(347), 1077-1088.

Hikosaka, K., Terashima, I., & Katoh, S. (1994). Effects of leaf age, nitrogen nutrition and photon flux density on the distribution of nitrogen among leaves of a vine (Ipomoea tricolor Cav.) grown horizontally to avoid mutual shading of leaves. *Oecologia*, *97*(4), 451-457.

Houghton, R. A. (1999). The annual net flux of carbon to the atmosphere from changes in land use 1850–1990. *Tellus B*, *51*(2), 298-313.

Houghton, R. A. (2007). Balancing the global carbon budget. *Annu. Rev. Earth Planet. Sci.*, *35*, 313-347.

Hungate, B. A., Dukes, J. S., Shaw, M. R., Luo, Y., & Field, C. B. (2003). Nitrogen and climate change. *Science*, *302*(5650), 1512-1513.

IPCC (2014): Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jacob, J., & Lawlor, D. W. (1992). Dependence of photosynthesis of sunflower and maize leaves on phosphate supply, ribulose-1, 5-bisphosphate carboxylase/oxygenase activity, and ribulose-1, 5-bisphosphate pool size. *Plant Physiology*, *98*(3), 801-807.

Janssens, I. A., Barigah, S. T., & Ceulemans, R. (1998). Soil CO2 efflux rates in different tropical vegetation types in French Guiana. In *Annales des sciences forestières* (Vol. 55, No. 6, pp. 671-680). EDP Sciences.

Janssens, I. A., Dieleman, W., Luyssaert, S., Subke, J. A., Reichstein, M., Ceulemans, R., ... & Law, B. E. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, 3(5), 315-322.

Jennings, S. B., Brown, N. D., & Sheil, D. (1999). Assessing forest canopies and understorey illumination: canopy closure, canopy cover and other measures. *Forestry*, *72*(1), 59-74.

Jordan, C. F. (1985). Nutrient cycling in tropical forest ecosystems. John Wiley & Sons.

Keeling, H. C., & Phillips, O. L. (2007). A calibration method for the crown illumination index for assessing forest light environments. *Forest Ecology and Management*, *242*(2), 431-437.

Kitayama, K., & Aiba, S. I. (2002). Ecosystem structure and productivity of tropical rain forests along altitudinal gradients with contrasting soil phosphorus pools on Mount Kinabalu, Borneo. *Journal of Ecology*, *90*(1), 37-51.

Larcher, W. (2003). *Physiological plant ecology: ecophysiology and stress physiology of functional groups*. Springer Science & Business Media.

Leuning, R. (1997). Scaling to a common temperature improves the correlation between the photosynthesis parameters Jmax and Vcmax. *Journal of Experimental Botany*, *48*(2), 345-347.

Li, Y. K., Li, B., Guo, W. Z., & Wu, X. P. (2015). Effects of nitrogen application on soil nitrification and denitrification rates and N2O emissions in greenhouse. Journal of Agricultural Science and Technology, 17(2), 519-530.

Manual, L. I. C. O. R. (2005). Using the LI-6400 portable photosynthesis system.

Marschner, H. (1995). Functions of mineral nutrients: macronutrients. *Mineral nutrition of higher plants 2nd Edition. Academic Press, NY*, 299-312.

McWilliam, A. L. C., Cabral, O. M., Gomes, B. M., Esteves, J. L., & Roberts, J. M. (1996). Forest and pasture leaf-gas exchange in south-west Amazonia. *Amazonian Deforestation and Climate*, *1*.

Melillo, J. M., & Gosz, J. R. (1983). *Interactions of biogeochemical cycles in forest ecosystems* (pp. 177-222). John Wiley and Sons, New York.

Miller, H. G., Cooper, J. M., & Miller, J. D. (1976). Effect of nitrogen supply on nutrients in litter fall and crown leaching in a stand of Corsican pine. *Journal of Applied Ecology*, 233-248.

Miller, H. G. (1981). Forest fertilization: some guiding concepts. *Forestry*,*54*(2), 157-167.

Mitchell, H. L., & Chandler, R. F. (1939). The nitrogen nutrition and growth of certain deciduous trees of Northeastern United States, with a discussion of the principles and practice of leaf analysis as applied to forest trees. *Black rock forest bulletin*, (11.).

Montgomery, R. A., & Chazdon, R. L. (2001). Forest structure, canopy architecture, and light transmittance in tropical wet forests. *Ecology*, *82*(10), 2707-2718.

Myneni, R. B., Dong, J., Tucker, C. J., Kaufmann, R. K., Kauppi, P. E., Liski, J., ... & Hughes, M. K. (2001). A large carbon sink in the woody biomass of northern forests. *Proceedings of the National Academy of Sciences*, *98*(26), 14784-14789.

Ocheltree, T. W., Nippert, J. B., & Prasad, P. V. V. (2014). Stomatal responses to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance. *Plant, cell & environment*, *37*(1), 132-139.

Olsen, S. R. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate.

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., ... & Ciais, P. (2011). A large and persistent carbon sink in the world's forests. *Science*, *333*(6045), 988-993.

Peñuelas, J., Sardans, J., Rivas-ubach, A., & Janssens, I. A. (2012). The human-induced imbalance between C, N and P in Earth's life system. *Global Change Biology*, 18(1), 3-6.

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., ... & Nardin, E. (2013). Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communications*,*4*.

Phillips, O. L., Malhi, Y., Higuchi, N., Laurance, W. F., Núnez, P. V., Vásquez, R. M., ... & Grace, J. (1998). Changes in the carbon balance of tropical forests: evidence from long-term plots. *Science*, *282*(5388), 439-442.

Poncy, O., Riera, B., Larpin, D., Belbenoit, P., Jullien, M., Hoff, M., & Charles-Dominique, P. (1998). The permanent field research station 'Les Nouragues' in the tropical rainforest of French Guiana: current projects and preliminary results on tree diversity, structure, and dynamics. *MAN AND THE BIOSPHERE SERIES*, *21*, 385-410.

Raaimakers, D., Boot, R. G. A., Dijkstra, P., & Pot, S. (1995). Photosynthetic rates in relation to leaf phosphorus content in pioneer versus climax tropical rainforest trees. *Oecologia*, *102*(1), 120-125.

Roberts, J., Cabral, O. M., & De Aguiar, L. F. (1990). Stomatal and boundary-layer conductances in an Amazonian terra firme rain forest. *Journal of Applied Ecology*, 336-353.

Simfukwe, P., Hill, P. W., Emmett, B. A., & Jones, D. L. (2011). Soil classification provides a poor indicator of carbon turnover rates in soil. Soil Biology and Biochemistry, 43(8), 1688-1696.

Steffen, W., Noble, I., Canadell, J., Apps, M., Schulze, E. D., & Jarvis, P. G. (1998). The terrestrial carbon cycle: implications for the Kyoto Protocol.*Science*, *280*(5368), 1393-1394.

Stevenson, F. J., & Cole, M. A. (1999). Cycles of soils: carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley & Sons.

Stewart, W. D. P., Sampaio, M. J., Isichei, A. O., & Sylvester-Bradley, R. (1978). Nitrogen fixation by soil algae of temperate and tropical soils. *Limitations and potentials for biological nitrogen fixation in the tropics* (pp. 41-63). Springer US.

Tanner, E. V. J., Kapos, V., Freskos, S., Healey, J. R., & Theobald, A. M. (1990). Nitrogen and phosphorus fertilization of Jamaican montane forest trees. *Journal of Tropical Ecology*, *6*(02), 231-238.

Thompson, W. A., Huang, L. K., & Kriedemann, P. E. (1992). Photosynthetic response to light and nutrients in sun-tolerant and shade-tolerant rainforest trees. II. Leaf gas exchange and component processes of photosynthesis. *Functional Plant Biology*, *19*(1), 19-42.

Tiessen, H., Chacon, P., & Cuevas, E. (1994). Phosphorus and nitrogen status in soils and vegetation along a toposequence of dystrophic rainforests on the upper Rio Negro. *Oecologia*, *99*(1-2), 145-150.

Valentini, R., Matteucci, G., Dolman, A. J., Schulze, E. D., Rebmann, C. J. M. E. A. G., Moors, E. J., ... & Jarvis, P. G. (2000). Respiration as the main determinant of carbon balance in European forests. *Nature*, *404*(6780), 861-865.

Vance, C. P., Uhde-Stone, C., & Allan, D. L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*, 157(3), 423-447.

Van Der Heijden, M. G., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, *11*(3), 296-310.

Vitousek, P. (1982). Nutrient cycling and nutrient use efficiency. *American Naturalist*, 553-572.

Vitousek, P. M. (1984). Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology, 65*(1), 285-298.

Vitousek, P. M., & Sanford, R. L. (1986). Nutrient cycling in moist tropical forest. *Annual review of Ecology and Systematics*, 137-167.

Vitousek, P. M., Walker, L. R., Whiteaker, L. D., Mueller-Dombois, D., & Matson, P. A. (1987). Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. *Science*, *238*(4828), 802-804.

Vitousek, P. M., Fahey, T., Johnson, D. W., & Swift, M. J. (1988). Element interactions in forest ecosystems: succession, allometry and input-output budgets. *Biogeochemistry*, *5*(1), 7-34.

Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: how can it occur?. *Biogeochemistry*, *13*(2), 87-115.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., ... & Tilman, D. G. (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecological applications*, *7*(3), 737-750.

Von Caemmerer, S. V., & Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*,*153*(4), 376-387.

Von Caemmerer, S. (2000). *Biochemical models of leaf photosynthesis* (No. 2). Csiro publishing.

Wagner, F., Hérault, B., Stahl, C., Bonal, D., and Rossi, V. (2011). Modeling water availability for trees in tropical forests, Agr. Forest Meteorol., 151, 1202–1213, doi:10.1016/j.agrformet.2011.04.012

Walker, A. P., Beckerman, A. P., Gu, L., Kattge, J., Cernusak, L. A., Domingues, T. F., ... & Woodward, F. I. (2014). The relationship of leaf photosynthetic traits – Vcmax and Jmax – to leaf nitrogen, leaf phosphorus, and specific leaf area: a meta-analysis and modeling study. Ecology and evolution, 4(16), 3218-3235

Wang, C., Wan, S., Xing, X., Zhang, L., & Han, X. (2006). Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. Soil Biology and Biochemistry, 38(5), 1101-1110.

Wullschleger, S. D. (1993). Biochemical limitations to carbon assimilation in C3 plants—a retrospective analysis of the A/Ci curves from 109 species. *Journal of Experimental Botany*, *44*(5), 907-920.

Xu, L., & Baldocchi, D. D. (2003). Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree physiology*, *23*(13), 865-877.

Zheng, Y. U. A. N., Zhao, Z., Zhou, H., & Zhou, J. J. (2012). Effects of slope aspect and stand age on the photosynthetic and physiological char-acteristics of the black locust (*Robinia pseudoacacia* L.) on the Loess Plateau. *Pakistan Journal of Botany*, *44*(3), 939-948.

Appendix A

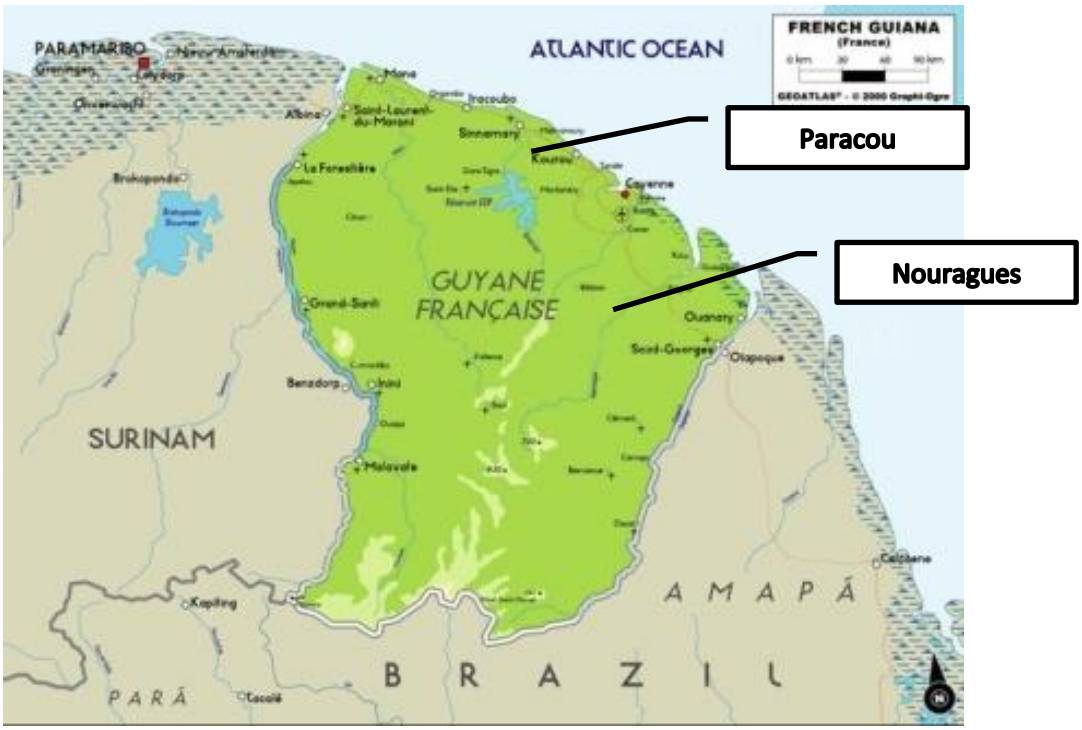


Figure A1. Situation of the two sites Paracou and Nouragues in French Guiana.

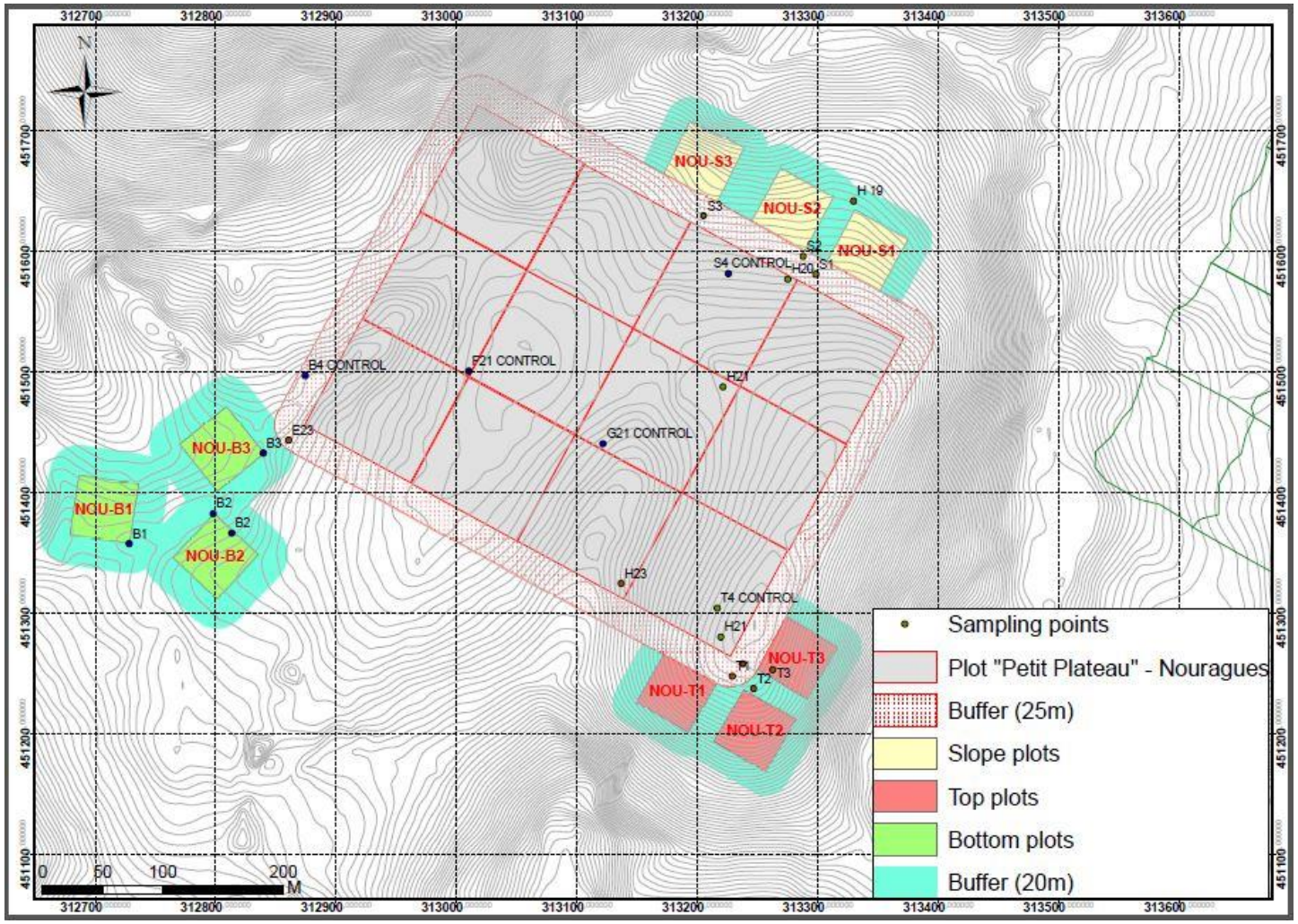


Figure A2. Plots of Nouragues. Plots T4, B4 and S4 are indicated as control plots, but this is not the case for this research.

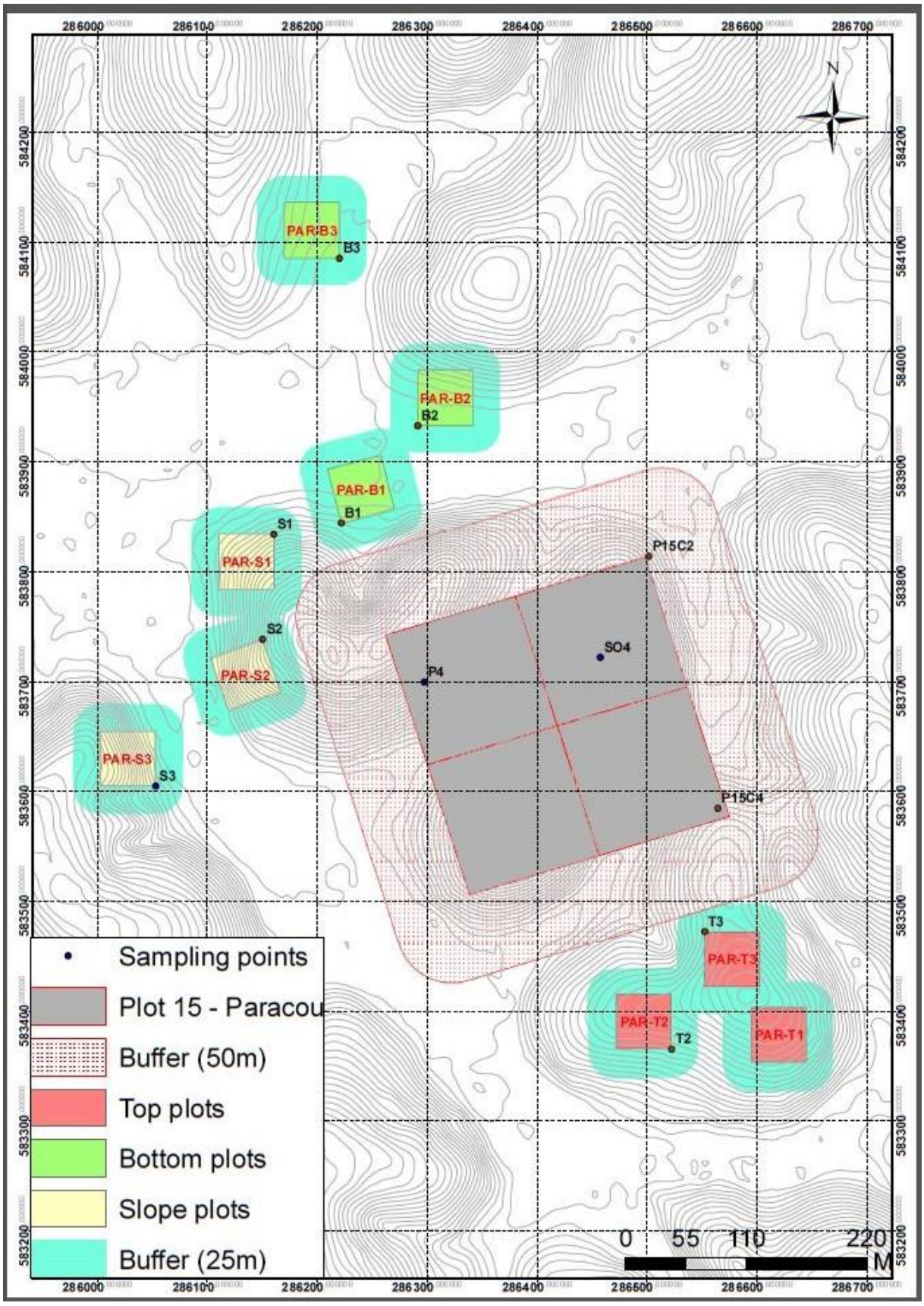


Figure A3. Plots of Paracou. Plots T4, B4 and S4 are indicated as control plots, but this is not the case for this research.

Appendix B

Table B1. Species list of the measured trees in Nouragues

Plot	Family	Tree species
NOU-B1	Fabaceae	*Vouacapoua americana*
	Fabaceae	*Eperua falcata*
	Lecythidaceae	*Lecythis sp*
	Lecythidaceae	*Eschweilera coriacea*
	Myristicaceae	*Osteophleum platyspermum*
NOU-B2	Lecythidaceae	*Eschweilera sp*
	Sapotaceae	*Sapotaceae sp*
	Chrysobalanaceae	*Licania alba*
NOU-B3	Fabaceae	*Dicorynia guianensis*
	Moraceae	*Brosimum guianense*
	Fabaceae	*Alexa sp*
NOU-B4	Fabaceae	*Eperua falcata*
	Moraceae	*Helicostylis pedunculata*
	Burseraceae	*Protium opacum*
	Fabaceae	*Bocoa prouacensis*
	Sapotaceae	*Pradosia ptychandra*
NOU-S1	Annonaceae	*Unonopsis flavescens*
	Elaeocarpaceae	*Sloanea sp*
	Myristicaceae	*Virola michelii*
NOU-S2	Lauraceae	*NA*
	Fabaceae	*Inga sp*
	Fabaceae	*Vouacapoua americana*
	Sapotaceae	*Micropholis melinoniana*
	Lecythidaceae	*Lecythis sp*
NOU-S3	Burseraceae	*Tetragastris sp*
	Fabaceae	*Elizabetha princeps*
	Sapotaceae	*Pouteria gongrijpii*
	Sapotaceae	*NA*
	Nyctaginaceae	*Neea sp*
NOU-S4	Sapotaceae	*Chrysophyllum sanguinolentum*
	Rubiaceae	*Capirona decorticans*
	Chrysobalanaceae	*Couepia caryophylloides*
	Chrysobalanaceae	*Licania alba*
	Fabaceae	*Dicorynia guianensis*
NOU-T1	Sapotaceae	*NA*
	Fabaceae	*Hymanea courbaril*
	Fabaceae	*Dicorynia guianensis*
	Lecythidaceae	*Eschweilera coriacea*
	Fabaceae	*Vouacapoua americana*
NOU-T2	Lecythidaceae	*Lecythis poiteaui*
	Fabaceae	*Dicorynia guianensis*
	Chrysobalanaceae	*Licania alba*
	Rubiaceae	*Chimarrhis turbinata*
	Opiliaceae	*Agonandra silvatica*
NOU-T3	Caryocaraceae	*Caryocar glabrum*
	Chrysobalanaceae	*Licania sp*
	Chrysobalanaceae	*Licania alba*
	Lauraceae	*Sextonia rubra*
	Vochysiaceae	*NA*
NOU-T4	Chrysobalanaceae	*Licania canescens*
	Chrysobalanaceae	*Licania alba*
	Sapindaceae	*Talisia praealta*
	Sapotaceae	*Pouteria eugeniifolia*
	Lecythidaceae	*Lecythis zabucajo*

Table B2. Species list of the measured trees in Paracou

Plot	Family	Tree species
PAR-B1	Putranjivaceae	*Drypetes variabilis*
	Clusiaceae	*Tovomita sp*
	Malvaceae	*Sterculia pruriens*
	Fabaceae	*Eperua falcata*
	Meliaceae	*Carapa surinamensis*
PAR-B2	Elaeocarpaceae	*Sloanea sp*
	Lecythidaceae	*Gustavia hexapetala*
	Fabaceae	*Eperua falcata*
	Malvaceae	*Catostemma fragrans*
	Fabaceae	*Dipteryx odorata*
PAR-B3	Fabaceae	*Eperua falcata*
	Fabaceae	*Dipteryx odorata*
	Lecythidaceae	*Eschweilera sp*
	Clusiaceae	*Tovomita sp*
	Chrysobalanaceae	*Licania alba*
PAR-B4	Fabaceae	*Eperua falcata*
	Chrysobalanaceae	*Licania alba*
	Clusiaceae	*Symphoniaglobulifera*
PAR-T1	Annonaceae	*Oxandra asbeckii*
	Fabaceae	*Eperua grandiflora*
	Lecythidaceae	*Eschweilera sp*
	Clusiaceae	*Moronobea coccinea*
PAR-T2	Lecythidaceae	*Eschweilera sp*
	Malvaceae	*Sterculia sp*
	Fabaceae	*Eperua grandiflora*
PAR-T3	Fabaceae	*Bocoa prouacensis*
	Sapotaceae	/
	Fabaceae	*Vouacapoua americana*
PAR-T4	Dichapetalaceae	*Tapura capitulifera*
	Malvaceae	*Lueheopsis rugosa*
	Euphorbiaceae	*Chaetocarpus schomburgkianus*
	Sapotaceae	*Pouteria eugeniifolia*

Appendix C

Here we describe the equations used by the LI-6400XT to calculate transpiration and photosynthesis. Other equations used by the LI-6400XT to calculate e.g. relative humidity are documented in Chapter 14 of the manual (L. I. C. O. R., 2005).

Transpiration

The mass balance of water vapor in an open system is given by

$$sE = u_o w_o - u_i w_i \quad \text{(Eq. 1)}$$

S is leaf area (m^{-2}), E is transpiration rate (mol m^{-2} s^{-1}), u_i and u_o are incoming and outgoing flow rates (mol s^{-1}) from the chamber, and w_i and w_o are incoming and outgoing water mole fractions (mol H_2O mol air^{-1}).

Since

$$u_o = u_i + sE \quad \text{(Eq. 2)}$$

we can write

$$sE = (u_i + sE)w_o - u_i w_i \quad \text{(Eq. 3)}$$

which rearranges to

$$E = \frac{u_i(w_o - w_i)}{s(1 - w_o)} \quad \text{(Eq. 4)}$$

The relationships between the terms in (eq. 1-4) and what the LI-6400XT measures are

$$u_i = F/10^6$$

$$w_i = W_r/10^3$$

$$w_o = W_S/10^3$$

$$s = S/10^4 \quad \text{(Eq. 5)}$$

where F is flow rate (µmol s^{-1}), W_s and W_r are sample and reference water mole fractions (mmol H_2O mol air^{-1}), and S is leaf area (cm^{-2}). The equation that the LI-6400 uses for transpiration is thus

$$E = \frac{F(W_S - W_r)}{100S(1000 - W_S)}$$

Total Conductance to Water Vapor

The total (includes stomatal and boundary layer) conductance of the leaf g_{tw} (mol H_2O m^{-2} s^{-1}) is given by

$$g_{tw} = \frac{E(1000 - \frac{W_l + W_S}{2}}{W_l - W_S}$$

where W_l is the molar concentration of water vapor within the leaf (mmol H_2O mol air^{-1}), which is computed from the leaf temperature T_l (C) and the total atmospheric pressure P (kPa)

$$W_l = \frac{e(T_l)}{P}.1000$$

The function *e*(T) is saturation vapor pressure (kPa) at temperature T (°C). The formula used by the LI-6400 is

$$e(T) = 0.061365e^{\frac{17.502T}{240.97+T}}$$

Stomatal Conductance to Water Vapor

The stomatal conductance g_{sw} to water vapor (mol H_2O m^{-2} s^{-1}) is obtained from the total conductance by removing the contribution from the boundary layer.

$$g_{sw} = \frac{1}{\frac{1}{g_{tw}} - \frac{k_f}{g_{bw}}}$$

where k_f is a factor based on the estimate K of the fraction of stomatal conductance of one side of the leaf to the other,

$$k_f = \frac{K^2 + 1}{(K+1)^2}$$

and g_{bw} is the boundary layer conductance to water vapor (mol H_2O m^{-2} s^{-1}) from one side of the leaf. The boundary layer conductance correction thus depends on whether the leaf has stomata on one or both sides of the leaf.

Net Photosynthesis

The mass balance of CO_2 in an open system is given by

$$sa = u_i c_i - u_o c_o$$

where a is assimilation rate (mol CO_2 m-2 s-1), c_i and c_o are incoming and outgoing mole fractions (mol CO_2 mol air-1) of carbon dioxide. Using (2), we can write

$$sa = u_i c_i - (u_i + sE)c_o$$

which rearranges to

$$a = \frac{u_i(c_i - c_o)}{s} - Ec_o$$

To write this equation in terms of what the LI-6400 measures, we use (5) and

$$c_i = C_r/10^6$$

$$c_o = C_S/10^6$$

$$a = A/10^6$$

where C_r and C_s are sample and reference CO_2 concentrations (µmol CO_2 mol air^{-1}), and A is net assimilation rate of CO_2 by the leaf (µmol CO_2 m^{-2} s^{-1}). Substitution yields

$$A = \frac{F(C_r - C_S)}{100S} - C_S E$$

Intercellular CO2

The intercellular CO_2 concentration C_i (µmol CO_2 mol air^{-1}) is given by

$$C_i = \frac{\left(g_{tc} - \frac{E}{2}\right) C_S - A}{g_{tc} + \frac{E}{2}}$$

where g_{tc} is the total conductance to CO_2, and is given by

$$g_{tc} = \frac{1}{\frac{1.6}{g_{sw}} + \frac{1.37k_f}{g_{bw}}}$$

1.6 is the ratio of the diffusivities of CO_2 and water in air, and 1.37 is the same ratio in the boundary layer.

Appendix D

Table D1. Parameters used in our model, taken from De Pury and Farquaher (1997).

Parameter	Value	Definition
α	0.24 radians	Angle of beam irradiance to the leaf normal
K_c25	272.38 kPa	Michaelis constant for carboxylation at 25 °C
ΔH_aK_c	23.72 J mol^{-1}	Energy of activation K_c
K_o25	165.82 kPa	Michaelis constant for oxygenation at 25 °C
ΔH_aK_o	80.99 J mol^{-1}	Energy of activation K_o
Γ*25	37.43 kPa	Photosynthetic CO_2 compensation point at 25 °C
ΔH_a Γ*	24.46 J mol^{-1}	Energy of activation Γ*
O	210 kPa	Oxygen partial pressure
θ	0.7	Curvature factor of response of canopy photosynthesis to irradiance

Table D2. Equations (1-9) of the Farquhar et al. (1980) leaf photosynthesis model. Equation 10 is taken from Von Caemmerer (2000).

Equation	Definition	No.
$A = \min\{A_j, A_v\} - R_1$	Net rate of leaf photosynthesis	(1)
$A_v = V_1 \frac{p_i - \Gamma*}{p_i + K'}$, providing $p_i > \Gamma*$	Rubisco-limited photosynthesis	(2)
$K' = K_c\,(1 + O/K_O)$	Effective Michaelis-Menten constant	(3)
$A_j = J \frac{p_i - \Gamma*}{4(p_i + 2\Gamma*)}$, providing $p_i > \Gamma*$	Electron-transport limited rate of photosynthesis	(4)
$\theta_1 J^2 - (I_{le} + J_m)J + I_{le}J_m = 0$	Irradiance dependence of electron transport	(5)
$I_{le} = I_1(1 - f)/2$	PAR effectively absorbed by PSII	(6)
$k_T = k_{25}\exp(E_a(T - 25)/[298\,R(T + 273)]$	Arrhenius function	(7)
$\Gamma* = 3 \cdot 69 + 0.188(\mathrm{T} - 25) + 0 \cdot 0036(\mathrm{T} - 25)^2$	Temperature dependence of Γ*	(8)
$J_{m_r} = J_{m_{25}} \cdot \exp[\frac{(T_K - 298)E_a}{RT_K \cdot 298}] \frac{[1 + \exp(\frac{S \cdot 298 - H}{R \cdot 298})]}{[1 + \exp(\frac{ST_K - H}{RT_K})]}$	Temperature (K) dependence of J_{max}	(9)
0.361 * PAR	Absorptance of light	(10)

The model was built for a temperature of 25 °C. Here, leaf temperature was controlled to be around 30 °C, during the measurements small differences were possible. This deviation from the model was corrected by using an Arrhenius equation:

$$V_{cmax}(T_k) = V_{cmax}(25)\exp[\frac{E_a(T_k - 25)}{298RT_k}]$$