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Patterns of total root and shoot carbon dioxide fluxes and their impact on daily tree carbon budget in large tropical tree saplings

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Key words

biomass partitioning; carbon budget; *Ceiba pentandra*; coarse-roots; fine-roots; root:shoot ratio; root respiration; root-shoot coupling.

Running head: Daily patterns of total root and shoot CO_2 fluxes

Abstract

A significant amount of the carbon (C) assimilated in photosynthesis by trees is re-emitted to the atmosphere via the respiratory CO_2 flux of roots. Because of technical constraints, we have little understanding of the extent and dynamics of the respiratory CO_2 flux of roots at the root system scale (*RCF*). This study aimed to fill this gap and to quantify the daily C budget of entire trees. We used aeroponics as a novel approach to measure directly and simultaneously *RCF* and the net CO_2 flux of the entire shoot (*SCF*), to estimate their night- and day-time contributions to daily tree CO_2 budget, and to estimate the relative contribution of different root categories to *RCF* in large saplings of the tropical tree species *Ceiba pentandra*. By maintaining root temperature within a narrow range (24-27.5°C), we controlled for its effect on *RCF*, thus allowing to test the potential relationship between *RCF* and *SCF*. The carbon gain of the fast-growing saplings was 0.79±0.10 g C sapling^{-1} d^{-1}, with day-time shoot CO_2 uptake outweighing night-time shoot and day- and night-time root CO_2 losses by a factor of two. Other than a slight rise in the morning hours, *RCF* was relatively stable and not coupled to the daily dynamics of *SCF*. Albeit having lower specific respiration rates compared to fine-roots, the relative contributions of coarse-roots (diameter >2 mm) to *RCF* were substantial because of their large biomass and was estimated to range 43-63% of *RCF* at midday of different days during the growing season. The results of this study suggest that (i) the entire root system needs to be monitored for its impact on the tree CO_2 budget, (ii) *RCF* cannot be derived from *SCF*, and (iii) the importance of coarse-root respiration to *RCF* may be greater than appreciated.

Introduction

About 10-40% of the carbon (C) assimilated by tree canopy photosynthesis is emitted back to the atmosphere as CO_2 through the respiration of tree root systems (Litton et al. 2007). Yet the daily dynamics of the respiratory CO_2 flux of the root system (*RCF*), the partitioning of *RCF* among different root types, and the relationship between the daily patterns of *RCF* to total net shoot CO_2 fluxes (that include net day-time CO_2 uptake and night-time respiration; *SCF*) and the resulting daily CO_2 budget between the two tree parts are not well understood, mainly due to the lack of accessibility to whole tree root systems. Such knowledge is important to better understand tree and forests C dynamics, which control a large proportion of the global C cycle (Luyssaert et al. 2007, Beer et al. 2010).

Tree root systems are composed of different roots types which diverge in their function, structure and longevity (Pregitzer et al. 2002, Marsden et al. 2008, Pérez-Harguindeguy et al. 2013). Fine, non-suberised roots (usually defined by a diameter <2 mm cut-off (Eshel and Grünzweig 2013)) are considered the most active in water and nutrient absorption and transport to supply the daily evaporative demand of the shoot (Makita et al. 2012, McCormack et al. 2015). In contrast, woody coarse-roots store C reserves that buffer asynchronies between supply and demand in the plant (Hartmann and Trumbore 2016). Fine-roots mostly live for a short time and tend to have waves of growth flashes and decay (Pérez-Harguindeguy et al. 2013, Abramoff and Finzi 2015), while coarse-roots live for longer time spans, and develop over the years (Singh and Singh 1993). The difference in the growth dynamics between fine- and coarse-roots dictate different allometric relationships between them for a given ontogenic and phenological phases during a tree lifetime.

The rate of respiration and metabolism per root mass is dictated by the roots' function (Rewald et al. 2014), and tends to decrease with increasing root diameter and density

(Gansert 1994, Pregitzer et al. 1998, Marsden et al. 2008, Makita et al. 2012). Yet the rate of root respiration is not constant over time, and roots exhibit cyclic variations in their respiration rates (Hansen 1977, Wieser and Bahn 2004). Daily cyclic respiration patterns have been observed in fine-roots (Hansen 1977, Drake et al. 2008, Oren et al. 2020), as well as seasonal patterns of respiration rate (Vose and Ryan 2002). In coarse-roots, respiration patterns have been usually demonstrated over time-scales larger than 24 h (Wieser and Bahn 2004, Kuptz et al. 2011, Brændholt et al. 2018), due to their function as a non-structural carbohydrates pool (Furze et al. 2019), used to bridge asynchronies between C supply and demand over seasonal to annual time scales (Sala et al. 2012, Hartmann and Trumbore 2016).

Root functionality is achieved through the energy released in cellular respiration, which metabolises sugars and produces CO_2 as a by-product (Amthor 2000). It is well known that respiration, being a biochemical process, is regulated by temperature (Atkin and Tjoelker 2003). Rarely, studies found a very weak relationship between temperature and soil respiration (e.g. Tang et al. 2005), while others isolated a temperature independent component of soil respiration that was related to photosynthetically active radiation, used as a proxy for photosynthesis (Liu et al. 2006). This suggests that the daily C dynamics of the root system is coupled to some degree to that of the shoot. Recently we showed that respiration of fine-roots is coupled to leaf C dynamics in tropical tree saplings on a daily time scale, with a maximal coupling under high-light conditions (Oren et al. 2020). Our findings were based on measurements of fine-roots only, as direct respiration measurements on excised coarse-roots would mean damaging large portions of the root system, which might affect the root system sink size and potential coupling dynamics. Here we present the results of further CO_2 flux measurements, done and analysed separately on a larger scale to encompass the entire root system and the shoot. By doing so, we tested

76 whether a similar relationship exists between the CO_2 fluxes of the entire root system and
77 the entire shoot in trees.

78 Carbon assimilated during photosynthesis is either utilised by the leaves or is loaded
79 to the phloem, and is then translocated to the different sink organs along the phloem
80 pathway from the shoot to the roots (Mannerheim et al. 2020). When sugars are un-loaded
81 from the phloem in the roots, the C that they contain is incorporated into new structural
82 biomass, into C reserves, mostly in coarse-roots, or is emitted out of the tree as CO_2 after
83 being consumed by respiration to maintain the root metabolism and functioning
84 (Hartmann et al. 2018). The balance between the sizes of the source and sink organs
85 determines the magnitude of the entire tree C budget and whether there is a net C gain or
86 loss, and thus it depends on the organs' activity patterns, specific rates of metabolic
87 processes, and their total biomass. Respiratory losses in mature tropical forests have been
88 estimated to range ~55-75% out of the annual gross primary production (Luyssaert et al.
89 2007, Malhi et al. 2017), with pioneer species having lower respiratory losses due to
90 proportionally smaller root system (Veneklaas and Poorter 1998).

91 Here we used the soil-free environment, achieved through growing trees in
92 aeroponics, to directly measure root respiration and shoot CO_2 fluxes over different scales,
93 ranging from separated organs, such as fine- and coarse-roots, to the entire root system
94 and shoot. By doing so, we aimed to achieve the following goals: (a) to quantify the relative
95 contributions of day- and night-time *SCF* and *RCF* to the daily whole tree CO_2 budget in
96 large *Ceiba pentandra* saplings, (b) to estimate the relative contributions of fine- and
97 coarse-roots to *RCF*, and (c) to determine whether *RCF* is related to *SCF* during a daily
98 timescale when light is ample and root temperature is maintained stable. We hypothesized
99 that (a) root:shoot biomass ratio (R:S) that is in favour of the shoot, combined with specific
100 CO_2 flux rates of the shoot organs (i.e. leaves, branches, stem), lead to larger CO_2 fluxes of
101 the shoot compared to the root system CO_2 emissions, and to a domination of the daily tree

CO_2 budget by the shoot CO_2 fluxes. Moreover, we hypothesized that (b) the daily pattern of *RCF* is coupled to the temporal variability in *SCF*, mainly due to fine-roots respiration, which - albeit their small biomass fraction – is (c) hypothesized to be the major component of *RCF* due to a higher specific respiration rate compared to coarse-roots. Our approach was to measure CO_2 fluxes of the total root system and the shoot, as well as respiration rates of different root diameter classes, ranging from diameter <2 mm to >10 mm, and to quantify the biomass partitioning of large saplings (up to 7.1±0.1 m long shoot-root axis) of the tropical tree species *Ceiba pentandra*.

Materials and Methods

To achieve the goals of this study, several steps and different measurements were taken over the years and in different facilities. Figure S1 (available as Supplementary Data at *Tree Physiology* Online) presents a roadmap to guide the reader through the various activities and their contributions to the data presented in this study.

Tree species, seed germination and growing conditions

We used saplings of the tropical tree species *Ceiba pentandra* (L.) Gaertn. (kapok), native to Western Africa, South and Central America (termed by the genus name *Ceiba* from here on). This tree species is ecologically important as it is a pioneer species, yet persistent in climax forests, reaching a height of up to 60 m at maturity. Hence it dominates the tropical forest canopy, and when flowering, provides nectar to a large number of animal species, including insects, birds, and mammals (Murawski and Hamrick 1992, Zotz and Winter 1994).

Seeds of *Ceiba* were collected in July of every year (2014-2015) from a mother tree in the Dead Sea Ein Gedi Botanic Garden, Israel. The mother tree itself was germinated

from a seed batch collected in Kenya. We kept the seeds in a refrigerator and germinated them every year in the autumn in a vermiculite bed that was irrigated every second day in a temperature-controlled room (19/26°C, night/day temperature). Two-and-a-half months after sowing, we transplanted the seedlings into 16 cm diameter pots in a controlled temperature regime (22/28°C) greenhouse. Two months later, at the end of the winter (usually during March), we moved the pots to a greenhouse with minimal temperature control to acclimatize the seedlings to a greater climatic variability. Five-and-a-half months after seed sowing, we either transferred the seedlings to the aeroponics facility or replanted them into 50 l containers containing sandy soil (see *Study facilities* section below; Figure S1 available as Supplementary Data at *Tree Physiology* Online).

Study facilities

i. Aeroponics

Saplings were grown in the Sarah Racine Root Research Laboratory aeroponics facility in Tel Aviv University, Tel Aviv, Israel. The aeroponics facility is a large temperature-controlled greenhouse, divided by a concrete floor into shoot compartments of 2.8-4.5 m height of an arched ceiling, and root compartments of 6 m depth in which full accessibility to root system is possible (Waisel 2002). In aeroponics, plants grow without any substrate, which enables direct access to the root system without damaging fine roots while measuring them (Waisel 2002). The root compartment in the aeropinics facility contained a moist environment, in which nutrients were added through an intermittent mist irrigation (Eshel and Grünzweig 2013). The nutrient solution contained 0.6 g l^{-1} compound fertilizer 20:20:20 (containing 20% N compounds, 20% P_2O_5, 20% K_2O, and microelements; Haifa Fertilizers, Israel), 0.11 g l^{-1} $MgSO_4 \cdot 7H_2O$ (Merck, Germany), 0.09 g l^{-1} $CaCl_2 \cdot 2H_2O$ (Merck, Germany) and 0.025% liquid fertilizer containing Fe-EDTA, B and other microelements (Fertilizers & Chemicals, Israel).

153 The main growing seasons reported here are spring to autumn 2015 and 2016
154 (Figure S1 available as Supplementary Data at *Tree Physiology* Online). Growing conditions
155 in the aeroponics facility were similar in 2015 and 2016, though maximum daily PPFD was
156 slightly higher and maximal daily vapour pressure deficit (VPD) was slightly lower in
157 summer 2015 than in summer 2016 (see Oren et al. 2020). Mean daily temperatures of
158 the root and shoot compartments were similar in both years (see *Oren* et al. 2020).

159 ii. Greenhouse

160 The main growing season reported here for the greenhouse is 2015 (no trees were grown
161 in the greenhouse in 2016). Saplings were grown in a 4.5 m tall greenhouse with limited
162 temperature control in the Robert H. Smith Faculty of Agriculture, Food and Environment,
163 Hebrew University of Jerusalem, Rehovot, Israel. Seedlings were planted in 50 l containers
164 filled with sandy soil and were drip-irrigated five times a day with a compound nutrient
165 solution containing 100 g l^{-1} of 5:3:8+B (containing 5% N compounds, 3% P_2O_5, 8% K_2O,
166 72 ppm B and other microelements; Haifa Fertilizers, Israel). Saplings in the greenhouse
167 were only used to quantify biomass partitioning and R:S (see *Biomass partitioning and*
168 *allometric relationships* below).
169

170 *Total root system respiration and shoot carbon dioxide fluxes*

171 Day- and night-time *RCF* and *SCF* were measured simultaneously, using two teams
172 working in parallel, by enclosing the root system and the shoot in custom-made cuvettes.
173 Measuring campaigns took place four times between early June and late July 2015 (9 June
174 at 12:20-16:50, 22 June at 08:10-16:20, 20 July at 04:00-18:50 (dawn at 5:00, dusk at
175 18:30) and 30 July 2015 at 00:40-09:40 (dawn at 05:10)). The same three saplings were
176 measured repeatedly, one after the other, at each of the measuring days. On each

177 measuring day, the time window in which measurements were done differed yet
178 overlapped, to some extent, with measurements taken on the other days.

179 The large cuvettes used to measure *RCF* and *SCF* consisted of an aluminium frame
180 and a clear polyethylene sheet (150 μm, 92% transmission of PPFD, Ginegar Plastic
181 Products, Israel; Figure S2 available as Supplementary Data at *Tree Physiology* Online). The
182 top part of the shoot cuvette was cylindrical (height of 1 m, inner diameter of 0.8 m); its
183 bottom part was conical (height of 0.3 m, maximal inner diameter of 0.8 m), starting out
184 at the bottom of the cylindrical part and reaching down to the trunk base at floor level, to
185 which it was sealed (Figure S2a available as Supplementary Data at *Tree Physiology*
186 Online). The total volume of the shoot cuvette was 550 l, and it was connected to a LI-8100
187 infrared gas analyser (IRGA) (LI-COR, Lincoln, Nebraska, USA).

188 The root cuvette was applied upside down compared to the shoot cuvette, that is,
189 its bottom part was cylindrical (height of 2 m, inner diameter of 0.6 m); its top part was
190 conical (height of 0.5 m, maximal inner diameter of 0.6 m), starting out at the top of the
191 cylindrical part and reaching up to the trunk base below the point where the shoot cuvette
192 was attached (Figure S2b available as Supplementary Data at *Tree Physiology* Online).
193 Since the actual trunk base was located below the floor level to allow the initial shallow
194 roots to be surrounded well in the root compartment atmosphere, a short trunk segment
195 of *c*. 35 cm was enclosed by the root cuvette. The total volume of the root cuvette was 610
196 l, and it was connected to a LI-7500 IRGA (LI-COR) in closed mode (calibration tube
197 installed to create closed loop system).

198 Installation of the cuvettes was done with extreme care so that minimal change in
199 organ position was forced onto the saplings. Ice packs were attached to the aluminium
200 frame of the shoot cuvette to minimize heating. Temperature inside the shoot cuvette
201 around mid-day generally did not exceed the temperature outside the cuvette (*c*. 31°C) by

more than 6°C. Temperature inside the root cuvette generally did not exceed the temperature outside the cuvette (*c*. 27°C) by more than 1.5°C. Fans were installed in both root and shoot cuvettes to maintain a gentle air mixing throughout the measurements. Both the root and the shoot gas exchange systems were built in a closed loop design and CO_2 fluxes were calculated according to LI-COR Inc. (2008):

$$Flux\ (\mu mol\ kg^{-1}\ s^{-1}) = \frac{P*V*(1000-W)}{R*(T+273.16)*DW} * \frac{\Delta[CO_2]_{dry}}{\Delta t} \qquad \text{(eq. 1)}$$

where P is air pressure (kPa), V is the system volume (m^3), W is water vapor concentration, R is the universal gas constant (m^3 kPa $mmol^{-1}$ $°K^{-1}$), T is temperature (°C), DW is the dry weight of a sampled root segment (kg), $[CO_2]_{dry}$ is a dilution-corrected CO_2 concentration (µmol mol^{-1}), and t is time (s).

Flux measurements were conducted in parallel for root and shoot, and lasted 2 min. After allowing air mixing for 20 seconds, instantaneous fluxes were calculated based on the next 100 seconds of measurement. The cuvettes were virtually air-tight, with the CO_2 concentration declining during a leak test by 0.2% and 0.45% per minute in the root and shoot cuvettes, respectively. We tested for leaks by increasing CO_2 concentration within empty cuvettes up to threefold that of ambient air and monitoring its decrease over 2 min. Both root and shoot cuvettes were opened and aired using a large fan between measurements.

Specific rates and scaled-up respiration of roots of different diameter classes

Specific respiration rates (µmol kg^{-1} s^{-1}) of roots in different diameter classes (diameter <2 mm, 2-5 mm, 5-10 mm, and >10 mm) were measured during destructive harvests of saplings grown in aeroponics. The CO_2 flux system was built as a closed loop where air circulated between the LI-7500 IRGA (calibration tube installed) and a small cuvette. Each flux measurement (two replicates per sapling and root diameter class) took 2 min, starting after a root segment was put inside the cuvette immediately after its

excision, and the cuvette was closed and put in the dark at ambient temperature. The total system volume was 472 cm^3 and the CO_2 flux was calculated according to LI-COR Inc. (2008) (see *Total root system respiration and shoot carbon dioxide fluxes* above).

Specific respiration rates were measured in October 2015 (n=3 saplings), April 2016 (n=2 saplings) and in October 2016 (n=3 saplings; Figure S1 available as Supplementary Data at *Tree Physiology* Online). Measurements were conducted between 8:00 to 14:00 (Israel Standard Time) in both 2015 and 2016. Root respiration was measured under ambient temperature and in the dark. Scaling up *specific* respiration to *total* root diameter class respiration per sapling was done using eq. 2:

$$R_{total} = R_{specific} * B \quad \text{(eq. 2)}$$

where R_{total} is the scaled-up respiration of a given root diameter class for a sapling (μmol $sapling^{-1}$ s^{-1}), $R_{specific}$ is the measured *specific* respiration rate (μmol kg^{-1} s^{-1}), and B is the corresponding harvested or estimated biomass per sapling of a root diameter class for a given date (kg $sapling^{-1}$) (see *Biomass partitioning and allometric relationships* below).

Biomass partitioning and allometric relationships

The saplings used for specific respiration rates measurements (see *Specific rates and scaled-up respiration of roots of different diameter classes* above) were destructively harvested, and their root system was separated into the different root diameter classes to determine their biomass during three harvests: in October 2015, April 2016 and October 2016. The <2 mm root diameter class included some non-suberized fine-roots that their diameter was slightly larger than 2 mm (in this case, the emphasis was put on potential root functionality rather than on their diameter). In addition to the root system, each sapling shoot was divided at harvest into leaf blades, leaf rachises, branches and trunk.

To compare biomass partitioning and R:S ratio between aeroponics- and container-grown saplings, we also harvested five *Ceiba* saplings in October 2015 in the greenhouse. For saplings grown in the greenhouse, soil was gently but thoroughly washed from the root system, without separating roots to diameter classes. The harvested tree organs in both aeroponics and greenhouse were dried at 60°C until constant weight was achieved to determine their dry weight (DW).

Allometric relationships between all tree organs (i.e. leaf rachises, branches, trunk and roots of different diameter classes) were established by carrying out destructive harvests of additional saplings. These saplings were grown in the aeroponics facility under similar growing conditions in consecutive years from 2014 to 2016, and were harvested at different ages and sizes during these years. In total 20 saplings were used to establish the allometric relationships. Trunk base diameter and shoot height were recorded prior to harvesting the saplings.

Trunk base diameter was found to predict the biomass and surface area of the different tree organs rather well (i.e. leaf blades, petioles, trunk and branches, and roots of the following diameters: <2 mm, 2-5 mm, 5-10 mm and >10 mm; Table S1 available as Supplementary Data at *Tree Physiology* Online). Thus, it was used to fit the following power function for each tree organ:

$$Y = aD^b \qquad \text{(eq. 3)}$$

where Y is biomass (g), a and b are parameters, and D is the trunk base diameter (cm). Organ biomass was estimated for any given date by applying eq. 3 to the base diameter, recorded throughout the seasons (Figure S3c available as Supplementary Data at *Tree Physiology* Online). By measuring trunk base diameter during the 2015 growing season, we could quantify the biomass or surface area of any organ for any given date.

Daily tree carbon budget

Using the CO_2 fluxes and biomass data recorded on 9 June, 22 June, 20 July, and 30 July 2015, we estimated and compared a daily C budget in two different ways:

1. Carbon dioxide flux budget

The daily budget is the integrated sum of root system and shoot CO_2 exchange processes (i.e. respiration or net CO_2 uptake) over a day, and it determines whether the saplings were taking up or losing C and at what rate. We regard the fluxes in a tree-centric point of view - values of net CO_2 uptake are defined as positive and those of respiration as negative. The daily CO_2 budget was calculated by integrating and summing the fluxes between two successive measuring points of instantaneous *RCF* and *SCF* (µmol sapling^{-1} s^{-1}).

A daily tree CO_2 budget (24 h) was calculated for 25 July 2015, based on the CO_2 measuring campaigns described above in *Carbon dioxide fluxes of the total root system and the shoot of Ceiba*. This day (25 July 2015) was chosen as the average date between two CO_2 flux measurement campaigns of the total root system and the shoot, conducted on 20 and 30 July, on the same three *Ceiba* saplings. *RCF* and *SCF*, measured on similar times of day but on different dates, did not differ statistically (Table S2 available as Supplementary Data at *Tree Physiology* Online). Additionally, microclimate conditions were similar on both measuring days (Figure S4 available as Supplementary Data at *Tree Physiology* Online). Shoot height increased between 20 and 30 July 2015 from 1.9 to 2 m, root system length was stable at 1.7 m, canopy surface area increased from 1.1 to 1.3 m^2, root system surface area from 0.65 to 0.85 m^2, root system biomass from 58 to 75 g, and shoot biomass from 164 to 205 g.

Therefore, we combined the *c*. 18 h of flux data into one dataset (ranging 00:40-18:50) and regarded this combined dataset as one average day (25 July 2015). For the *c*. 6 h of the night in which no measurements were taken (18:50-00:40), we averaged *RCF* and *SCF*

304 rates of the pre-dawn and early evening measurements (*RCF* predawn -0.75±0.03; *RCF*
305 early evening -0.32±0.08 µmol sapling^{-1} s^{-1}; *SCF* predawn -0.44±0.07 µmol sapling^{-1} s^{-1}; *SCF*
306 early evening -0.52±0.22 µmol sapling^{-1} s^{-1}), assuming a stable change in respiration rate
307 during this time span, as was found in other studies (Liu et al. 2006, Saveyn et al. 2007,
308 Makita et al. 2014, Salomón et al. 2018).

309 2. Carbon biomass budget

310 Another approach to estimate the daily C budget was calculating the biomass
311 increment for 25 July 2015 after fitting the following exponential curve to root and shoot
312 biomass for the period from 9 June to 30 July 2015, as follows:

313 $Biomass = a * b + \exp(c * date)$ (eq. 4)

314

315 where *a*, *b* and *c* are parameters, and *date* is the day difference from 9 June 2015. Root and
316 shoot biomass on 9 June, 22 June, 20 July, and 30 July 2015 were calculated by applying
317 eq. 3 (see *Biomass partitioning and allometric relationships* section) to the documented
318 trunk base diameter on these dates. Daily biomass increment (g sapling^{-1} d^{-1}) is the first
319 derivative of the exponential fit. The daily C biomass increment was calculated by
320 multiplying the daily biomass change by the mean %C in biomass (0.43) in *Ceiba* organs,
321 determined in an earlier examination (data not shown).

322 Over a day, C is also lost via root exudation, hence the daily root system exudation
323 rate was estimated to account for the loss of C via this pathway. Root exudation was
324 measured according to the guidelines of Vranova et al. (2013). Three fine-root tips per
325 sapling were excised in October 2015 between 09:30 and 10:00 am, and were immediately
326 inserted into vials containing the same nutrient solution used for irrigation and
327 fertilization. We made sure that the cut ends of the roots (i.e. the distal side from the root
328 cap) were above the nutrient solution in the vial to avoid potential leakage from the

wounded tissue into the solution. After 15 min, the roots were removed from the vials and dried for DW determination. Vials with nutrient solution only served as blanks. After roots were removed, the vials were stored on ice and later frozen at -20°C. Total organic C was determined using a Total Organic Carbon Analyser (TOC-VCSH, Shimadzu, Kyoto, Japan). Root exudation rate was calculated as follows:

$$E = \frac{\mathrm{TOC} * V}{\mathrm{DW} * t} \qquad \text{(eq. 5)}$$

where E is exudation rate (mg C g^{-1} h^{-1}), TOC is the total organic C concentration (mg C l^{-1}), V is the sample volume (l), DW is the excised root biomass (g), t is the time of exudation measurement (h).

Measured instantaneous exudation rates were then scaled up to a daily rate (g C $sapling^{-1}$ d^{-1}) by multiplying the measured rate by the tree fine-root biomass, assuming a constant flux over 24 h. The daily C budget was calculated as the sum of daily C biomass increment and daily C loss through root exudation.

Statistical analyses

We used Mixed Model regressions to determine the relationships between *RCF* to the explanatory variables root compartment temperature and *SCF*; and between *SCF* to PPFD and shoot compartment temperature, with sapling and date as crossed random effects, so that the dependency between repeated measurements within and between dates of the same sapling was accounted for. The same three saplings were measured repeatedly over the day during the flux measurement campaigns, with one flux per sapling used as the replicate unit for analysis to avoid pseudo-replication. We used one-way ANOVA to test the differences in total harvested biomass between years and facilities; between biomass partitioning in aeroponics- and container-grown saplings; and between the DW and respiration rates of roots of different diameter classes. We used one-way Repeated

Measures ANOVA to test the differences between different data-points of *RCF* or *SCF* measured on different time of day between 9 June to 30 July 2015, with a combined date-cycle variable as the main fixed effect, to account for the effect of both date and time of day (i.e. cycle) on the CO_2 flux, and sapling as a random effect. Means were compared with Tukey's HSD test. All statistical analyses were performed using JMP 14.0.0 software (SAS Institute, Inc., Cary, NC, USA).

Results

Growth and biomass partitioning of aeroponics- and container-grown saplings

Ceiba saplings grew in height from <0.5 to 4.2 m and from <0.5 to 4.0 m over 6-7 months in 2015 and 2016, respectively (Figure S3a available as Supplementary Data at *Tree Physiology* Online). The roots reached maximal length of 3.4 m by the end of the growing seasons in both years (Figure S3b available as Supplementary Data at *Tree Physiology* Online).

In very small saplings harvested in April 2016, fine-roots (diameter <2 mm) had the largest biomass fraction of the total root system biomass, while in large saplings harvested at the end of the growing season (i.e. in both October 2015 and 2016), most of the root system biomass consisted of coarse-roots, mainly in roots with a diameter >10 mm (Table 1). As saplings in October 2015 tended to have more biomass compared to saplings harvested in October 2016, we found significant differences in the allometric relationships between the fine-root to total biomass fraction: fine-root fraction was significantly higher in October 2015 (*c*. 9%) compared to October 2016 (*c*. 4%) (P<0.001; Table 1).

Total harvested biomass did not differ between the aeroponics- and container-grown saplings in 2015 (P=0.773), nor did the biomass data from aeroponics between

2015 and 2016 (P=0.077; Table 1). In addition, we found that saplings grown in aeroponics and in the 50 l containers in the greenhouse in 2015 did not differ in their biomass partitioning to the root system, shoot, leaf blades, petioles and trunk (Table S3 available as Supplementary Data at *Tree Physiology* Online). Notably, the root system comprised 30-32% and the shoot 68-70% of total tree biomass in both study facilities and both years (Table 1). These values translate into R:S ratios of 0.43±0.03 in both aeroponics- and container-grown saplings in 2015. There was a linear relationship (P<0.001) between root and shoot biomass (Figure 1).

Rates and daily patterns of total root system respiration and shoot carbon dioxide fluxes

Microclimatic conditions during the measuring campaigns of *RCF* and *SCF* followed a similar pattern on each of the measuring days in June and July 2015. PPFD, VPD and shoot compartment temperature showed a typical daily pattern, while root compartment temperature remained rather stable throughout the day, with an average daily amplitude of 2.6°C during June and July 2015 (Figure S4 available as Supplementary Data at *Tree Physiology* Online).

SCF ranged between maximal CO_2 uptake of 4.83±0.90 μmol sapling^{-1} s^{-1} around 10:00 and night-time respiration of -0.70±0.18 μmol sapling^{-1} s^{-1} (Figure 2, Table S2 available as Supplementary Data at *Tree Physiology* Online). *RCF* rate was smaller and less variable compared to day-time *SCF* on all four measuring days. During night-time, when both the shoot and the root system were emitting CO_2 via respiration, *RCF* rate was larger than *SCF*. *SCF* per leaf area and per biomass decreased with sapling size and date, while *RCF* per root surface area and biomass was rather stable during June and July 2015 (Figure S5 available as Supplementary Data at *Tree Physiology* Online).

SCF per leaf area was well related to PPFD and shoot compartment temperature on three out of four measuring days (Table S4 available as Supplementary Data at *Tree Physiology* Online), and a model combining PPFD and shoot compartment temperature explained 87% of the variability in *SCF* per leaf area (Figure S6 and Table S5 available as Supplementary Data at *Tree Physiology* Online) ($P<0.001$). The variation in mass-based *RCF* could not be separately explained by root compartment temperature nor *SCF* separately *per date* (Figure 3). When all dates were included in a Mixed Model regression that used *SCF* and root compartment temperature as fixed effects, and sapling and date as crossed random effects, showed that only root compartment temperature had a marginally significant effect on mass-based *RCF* ($R^2=0.11$, $P=0.024$) (Table S5 available as Supplementary Data at *Tree Physiology* Online), yet a model that included only root compartment temperature as a fixed main effect, explained only a very small fraction of the variation in mass-based *RCF* (*RCF*=1.79**Root compartment temperature*-55.5, $R^2=0.11$, $P=0.006$) (Figure 3).

Respiration rates of different root diameter classes and their contribution to total root system respiration

Specific respiration rates (μmol kg^{-1} s^{-1}), determined during harvests in October 2015, April 2016 and October 2016, were significantly higher in fine-roots compared to coarser roots (Figure S7a, b, and c available as Supplementary Data at *Tree Physiology* Online). When scaled up to total biomass of each root diameter class (μmol $sapling^{-1}$ s^{-1}), fine-root respiration was significantly higher than respiration of other root diameter classes in large saplings in October 2015 and in small saplings in April 2016, but not in large saplings in October 2016 (Figure S7d, e, and f available as Supplementary Data at *Tree Physiology* Online). Coarse-roots (i.e. all roots with a diameter >2 mm) contributed 24, 16 and 68%

to *RCF* on these dates, respectively. Coarse-root biomass contained 87-94% of the total root system biomass between 9 June to 30 July 2015 (Figure 4a, c, e, and g), and scaling up respiration rates to total biomass on these days, based on specific respiration rates measured in October 2015 with the same three saplings used to measure RCF and SCF with the large cuvettes between 9 June to 30 July 2015, showed that 43-63% of *RCF* originated from coarse-root respiration (Figure 4b, d, f, and h).

Daily carbon dioxide and carbon biomass budgets

Integrating *RCF* and *SCF* over 25 July 2015 yielded a relatively large day-time CO_2 uptake by the shoot and smaller CO_2 losses from *RCF* and night-time shoot respiration (Table 2). The pattern of net tree CO_2 flux, i.e. the sum of instantaneous *RCF* and *SCF* resembled that of the shoot (Figure S8 available as Supplementary Data at *Tree Physiology* Online). The daily CO_2 budget for *Ceiba* saplings amounted to 0.79±0.10 g C sapling^{-1} d^{-1}, which was 50% of the net day-time shoot CO_2 uptake (i.e. 1.58±0.18 g C sapling^{-1} d^{-1}).

Allometry-derived root and shoot C biomass increased over the course of 25 July 2015 by 0.88±0.17 and 2.13±0.44 g C sapling^{-1} d^{-1}, respectively, and the daily loss of C via root exudation was 0.33±0.01 g C sapling^{-1} d^{-1}. Hence the total daily C budget for 25 July 2015 was 3.35±0.61 g C sapling^{-1} d^{-1}.

Discussion

In this study we measured total root and shoot CO_2 fluxes by enclosing the root system and the shoot in large cuvettes and quantified the relative day- and night-time contributions of *RCF* and *SCF* to the daily CO_2 budget in large *Ceiba* saplings. We also estimated the relative contribution of the different root diameter classes, coarsely divided into fine- and coarse-roots, within a root system to *RCF* at midday under high-light

conditions. Lastly, we tested the relationship between *RCF* and *SCF* under high-light conditions, while maintaining root temperature within a narrow range. We found that the daily CO_2 budget was dominated by the shoot, and that day-time CO_2 uptake by the shoot outweighed night-time shoot and day- and night-time root CO_2 losses by a factor of two, leading to a positive daily C budget and a high growth rate. We found no relationship between the relatively low and stable *RCF* to the high and variable rates of *SCF*. While the specific respiration rate of fine-roots was much higher than that of coarse-roots, the latter contributed significantly to *RCF* due to their large biomass proportion. This, in turn, may have affected the daily pattern of *RCF* and whole tree C dynamics.

Root:shoot biomass ratio and carbon budget

More biomass was allocated to the shoot than to the roots, as observed in almost all tree species globally (Mokany et al. 2006). The R:S ratio did not change with sapling size, and was within the range reported for tropical forests (0.21-0.56) (Jackson et al. 1996, Mokany et al. 2006). However, the biomass proportions between root diameter classes did change with tree size, leading to different estimations of coarse-roots contribution to *RCF*. The striking similarity in R:S ratio, practically an identity, between aeroponics- and container-grown saplings supports the conclusion by Eshel and Grünzweig (2012) that aeroponics is a valid tool to study tree root systems, and provides evidence of a tightly maintained quantitative relationship between root and shoot biomass in trees.

The low CO_2 concentration in the belowground component of aeroponics (*c*. 400 μmol mol^{-1}, data not shown) may be conductive to increased *RCF* rates, compared with the high CO_2 existing in soils (Qi et al. 1994). Hence, one could have expected that by the end of the growing season in 2015, the aeroponics-grown saplings would grow less compared to the container-grown saplings in the greenhouse due to potential increased respiratory losses

478 of C. However, we found no difference in total sapling biomass or in the R:S ratio between
479 the saplings in the two facilities. Hence, the root respiration rates reported here likely
480 represent typical rates in *Ceiba* trees growing in soils. Yet we acknowledge that aeroponics
481 is an artificial environment, in which potential different anatomical features in aeroponics-
482 grown roots, the lack of mechanical impedance, root hairs, and mycorrhizal symbionts
483 might have affected respiration rates to some degree.

484 The high rates of CO_2 uptake by the shoot during day-time, compared to night-time
485 shoot respiration and daily *RCF*, led to a positive daily CO_2 budget and a high growth rate,
486 thus confirming our first hypothesis that the positive daily C budget is dominated by the
487 shoot due to higher shoot biomass and specific assimilative CO_2 flux rates. Our estimate of
488 the daily CO_2 budget yielded respiratory losses of 50% out of the net day-time shoot CO_2
489 uptake. This is comparable to the findings of Schäfer et al. (2003) and Wieser et al. (2005)
490 for mature temperate pine trees, but lower than the percentage observed in mature
491 Mediterranean trees (Klein and Hoch 2015) and tropical forests (Luyssaert et al. 2007,
492 Malhi et al. 2017), perhaps due to the fact that *Ceiba* is recognised as a pioneer species,
493 that are associated with high growth rates and low respiratory losses (Veneklaas and
494 Poorter 1998). We admit that our CO_2 budget may present a slight over- or
495 underestimation due to the potential elimination of daily fluctuations caused by the
496 narrow range of root temperature in our experimental set-up. However, given the small
497 proportion of *RCF* compared to the *SCF*, it is unlikely that the conditions in our
498 experimental set-up affected the entire tree daily CO_2 budget dramatically.

499 The C budget derived from the biomass increment method was higher than the value
500 derived from the CO_2 fluxes method. We evaluated the sensitivity of the methods used to
501 estimate daily tree C budget by changing each component in the daily budget calculations
502 by ±10%, as performed in other studies (Schäfer et al. 2003). This resulted in ±18% (0.65-
503 0.93 g C sapling^{-1} d^{-1}) for the CO_2 fluxes method and in changes of ±9% (3.05-3.65 g C

504 sapling^{-1} d^{-1}) for the biomass increment method, relative to the original estimates. Such a
505 discrepancy between the methods suggests unaccounted C fluxes or pools, which differ for
506 each method (Schäfer et al. 2003).

507

508 *Respiration partitioning between root diameter classes*

509 The functions of fine- and coarse-roots dictate different allocation (Mannerheim et al.
510 2020), metabolic and respiration rates (Pregitzer et al. 1998, Chen et al. 2010), so that the
511 thinner a root is, the higher is its respiration rate. Our findings confirm this trend in all the
512 measurements of respiration of different root diameter classes, regardless of tree size and
513 year. The rates reported here are of the same order of magnitude as were found for fine-
514 root respiration in other studies on tropical tree species (Marsden et al. 2008, Makita et
515 al. 2012).

516 The allometric relationships between organs change in trees with ontogeny and
517 phenological phase (Hartmann et al. 2018). Indeed, we found that the allometric
518 relationships between the biomass of different root diameter classes differed between
519 trees of different size. Such changes in biomass relationships may also affect the different
520 relative contribution of each root diameter classes to *RCF*. When we scaled up specific
521 respiration rates of different root diameter classes to their corresponding biomass, we
522 found that the relative contribution of total fine- and coarse-root respiration to *RCF*
523 differed appreciably between saplings of different sizes. These findings only partially
524 confirm our third hypothesis that the major component of *RCF* derives from fine-root
525 respiration. The proportion of coarse-root respiration to *RCF* found here, when the shoot
526 was exposed to high-light conditions, is larger (43-63%) than the annual proportion found
527 in the past in temperate trees (8-29%) (Ryan et al. 1996, Hamilton et al. 2002, Drake et al.
528 2011). Hence, the data suggest that coarse-roots may play a role, greater than thought

529 before, in shaping the daily pattern of *RCF* in tropical trees. We note, however, that the
530 proportion of coarse-root respiration to *RCF* is allometry-dependent, and hence may
531 change with size and may differ in mature *Ceiba* trees. Nonetheless, these findings stress
532 that to accurately predict net tree CO_2 dynamics, it is important to have detailed
533 information about the respiration magnitude of all root classes within a root system over
534 time. This is especially true for assessments of seasonal and annual CO_2 fluxes and C
535 budgets, as within such time scales the allometric relationships within tree root systems
536 will change (Hartmann et al. 2018). These changes can be triggered partly by quick
537 responses of fine-root production but also by mortality due to environmental drivers
538 (Steinaker and Wilson 2008, Cordeiro et al. 2020).

539

540 *Contribution and potential impact of coarse-root respiration on total root system respiration*
541 *pattern*

542 Relationships between respiration and temperature have been well documented in
543 previous studies, with respiration increasing with the increase in temperature (Atkin and
544 Tjoelker 2003, Griffin and Prager 2017). At the same time, relationships between soil
545 respiration and photosynthesis have been documented as well (Tang et al. 2005, Kayler et
546 al. 2017), and the coupling between an autotrophic component of soil respiration and
547 photosynthetically active radiation was identified, without any further relation to soil
548 temperature (Liu et al. 2006). These findings suggest that *RCF*, or at least a portion of *RCF*,
549 should be coupled to the shoot CO_2 dynamics.

550 Direct coupling between the daily pattern of fine-root respiration and leaf and
551 canopy photosynthesis have been identified in previous studies (Tang et al. 2005, Liu et
552 al. 2006, Drake et al. 2008, Blessing et al. 2015, Kayler et al. 2017, Oren et al. 2020). Here,
553 we aimed at testing at a broader scale the potential coupling between the entire root

554 system and shoot CO_2 fluxes, hypothesizing to find similar daily relationships as those
555 found in the past between fine-roots and leaves CO_2 dynamics. To control for temperature,
556 we maintained the temperature in the root compartment within a narrow range
557 throughout the day, which led to a very weak relationship between *RCF* and temperature.
558 Measuring *RCF* and *SCF* under high-light conditions should have allowed reaching
559 maximal potential coupling (Oren et al. 2020). Surprisingly, and contrary to our second
560 hypothesis, we found that the patterns of *RCF* were not related to *SCF*, neither immediately
561 nor with a time-lag on all four measuring days, spanning almost two months.

562 A possible explanation to the lack of coupling between *RCF* to *SCF* may rely on the
563 different scales of observation, i.e. due to the fact that a root system is composed of
564 different root types that are probably regulated by different internal and external factors
565 (Gansert 1994, Kuzyakov and Gavrichkova 2010). We estimated that a large proportion of
566 *RCF* at midday (43-63%, depending on the date) had derived from respiration of woody
567 coarse-roots, including the massive root crown, which is seldom sampled (Mokany et al.
568 2006). Hence, the lack of coupling between *RCF* and *SCF* suggests that coarse-root
569 respiration is not related to the daily pattern of *SCF*. Moreover, considering past reports
570 on daily variability of fine-root respiration (Hansen 1977, Drake et al. 2008, Oren et al.
571 2020), it follows that the integrated daily pattern of *RCF* in this study, which tended to be
572 relatively stable, resulted possibly from a cancelling out of fine- and coarse-root
573 respiration patterns to a certain degree. Although we could not isolate the daily pattern of
574 coarse-root respiration and determine the factors regulating it, we nonetheless
575 demonstrated the quantitative importance of coarse-root respiration to *RCF*.

576

577

578

Conclusions

Shoot CO_2 fluxes dominated the daily tree CO_2 budget. The daily pattern of *RCF* did not resemble that of *SCF* and was not related to it, even though this study was conducted under high-light conditions, which were supposed to promote such a relationship through increased phloem loading in the canopy. Total coarse-roots respiration contributed appreciably to *RCF*, and was even estimated to dominate *RCF* during some of our measurements. The proportions between fine- and coarse-root respiration were allometry-dependent. As such, the stable daily *RCF* and the significant proportions of coarse-root respiration suggest that coarse-root respiration is not regulated by the shoot activity. We stress here the need to quantify total coarse-root respiration to explain daily *RCF* patterns, as it can contribute significantly to *RCF* and may vary appreciably over different tree sizes and phenological phases. Monitoring *RCF* for its impact on net tree CO_2 fluxes is important to improve the quality of tree CO_2 budgets.

Supplementary Data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

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609

610 **Author contribution**

611 J.M.G., A.F. and N.B. planned and designed the study; I.O. and N.M. conducted
612 measurements in the aeroponics facility; and I.O. and J.M.G. analyzed the data and wrote
613 the first drafts of the manuscript. All authors contributed to the final draft of the
614 manuscript.

615

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794 lowland forest in Panama. Tree Physiol 14:1291–1301.

795

796 **List of figures**

797 Figure 1 – Root to shoot biomass relationships in *Ceiba pentandra* (*Ceiba*) saplings,
798 harvested between 2014 and 2016. Data points are individually harvested *Ceiba* saplings
799 grown in aeroponics (n=20) (open symbols) or in 50 l containers in the greenhouse (n=5)
800 (closed symbols). The fitted line for *Ceiba* in aeroponics only is *Root biomass*=0.47**Shoot*
801 *biomass*-13.52, R^2=0.93, P<0.001. Dotted-dashed line represents a 1:1 line.

802 Figure 2 – Net CO_2 flux of the shoot (*SCF*; closed symbols) and root (*RCF*; open symbols) of
803 *Ceiba pentandra* saplings measured during night- and/or day-time on four days during
804 summer 2015. Positive values indicate CO_2 taken up by the saplings, and negative values
805 indicate CO_2 lost from the saplings. Data are means±SE, n=3 saplings. Grey areas indicate
806 night-time on 25 July 2015.

807 Figure 3 – Relationship between total root system respiration (*RCF*) (a) to root
808 compartment temperature or (b) to net shoot CO_2 flux (*SCF*) of *Ceiba pentandra* saplings.
809 The relationships were tested using Mixed Model regressions per date (fitted dashed
810 lines) with sapling as a random effect. *RCF* was not related to *SCF* or to root compartment
811 temperature on any measuring day (Table S4 available as Supplementary Data at *Tree*
812 *Physiology* Online). Mixed Model regression that included both root compartment
813 temperature and *SCF* as main fixed effects, and sapling and date as crossed random effects

814 revealed that *RCF* was only related to root compartment temperature with the following
815 inverse relationship: *RCF*=1.79**Root compartment temperature*-55.5 (R^2=0.11, *P*=0.006)
816 (fitted continuous black line in (a)). Data were recorded during June and July 2015. *n*=65
817 single measured CO_2 fluxes.

818 Figure 4 – Biomass (a, c, e, and g) and respiration (b, d, f, and h) of different root diameter
819 classes of *Ceiba pentandra* saplings, calculated for different days during the growing season
820 in June and July 2015. Respiration of root diameter class (µmol sapling^{-1} s^{-1}) in (b), (d), (f),
821 and (h) was derived from scaling up specific respiration rates (µmol kg^{-1} s^{-1}) by the
822 corresponding total root diameter class biomass in (a), (c), (e), and (g). Specific respiration
823 rates were measured in October 2015 at harvest (Figure S7a available as Supplementary
824 Data at *Tree Physiology* Online). Bars with non-identical letters are significantly different
825 at *P*≤0.05 (Tukey's HSD test). Data are means±SE, *n*=3 saplings.

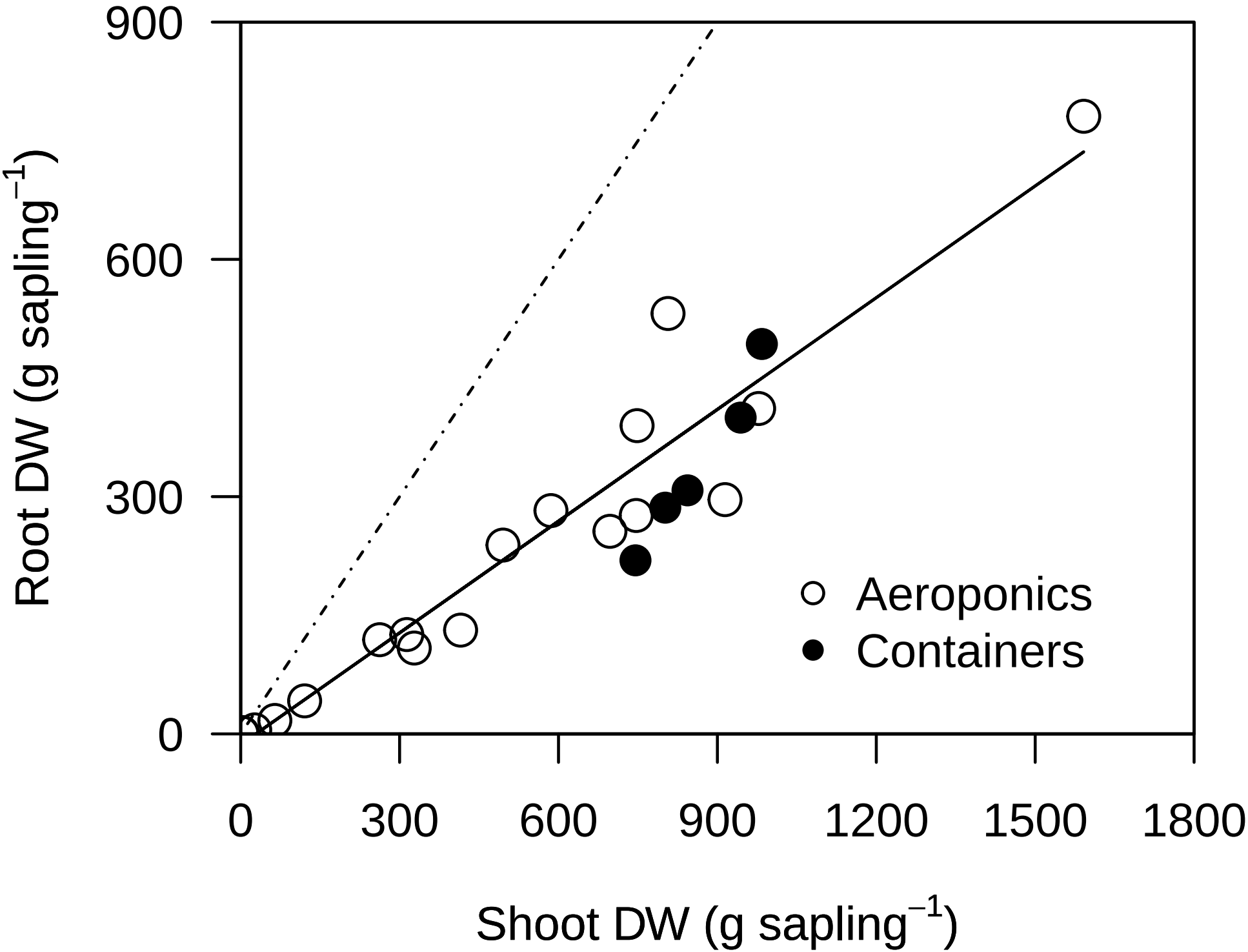
826

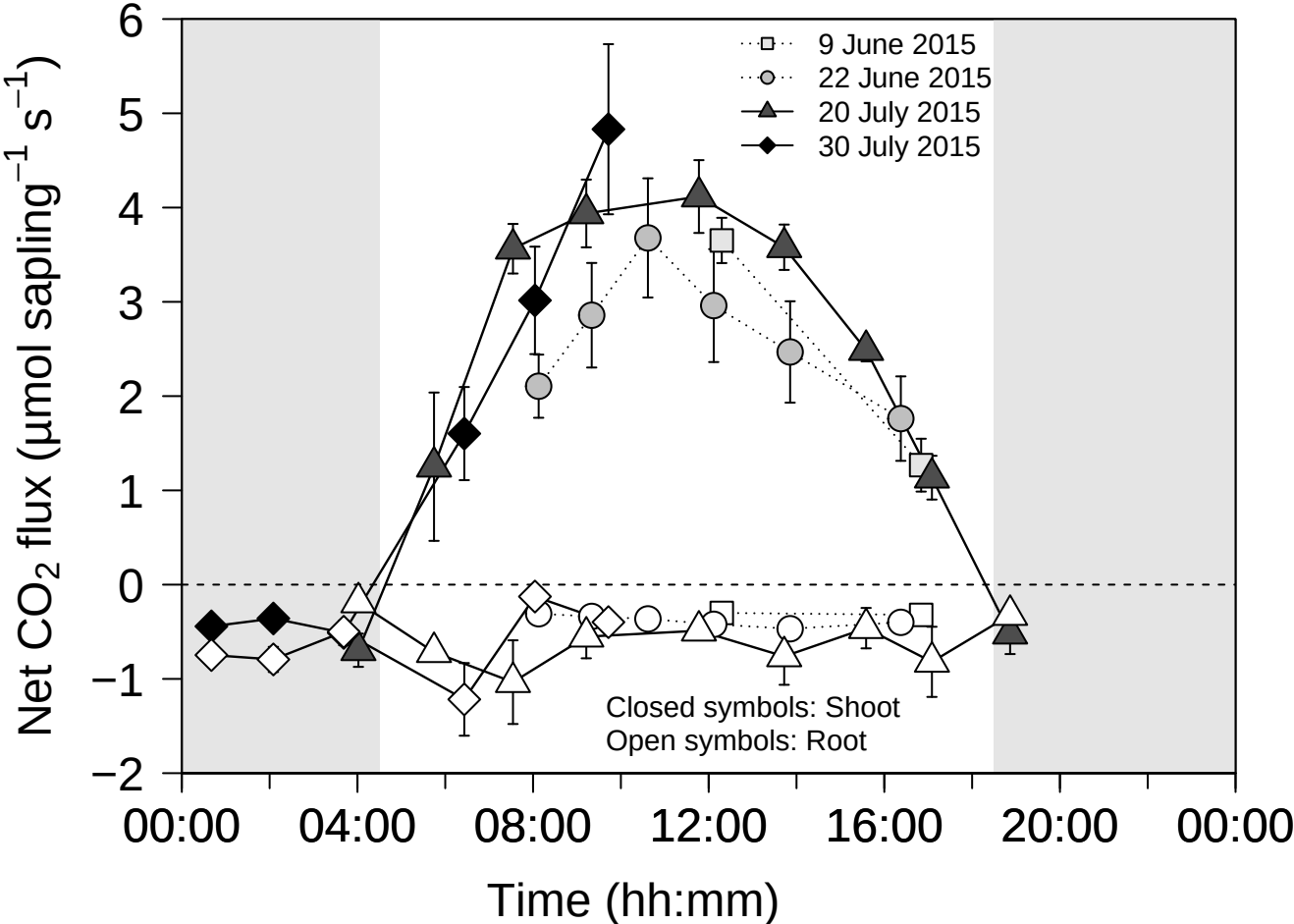
Table 1 – Biomass partitioning of *Ceiba pentandra* to different organs at harvests carried in 2015 and 2016. Data are means±SE. *n*=5 aeroponics- and container-grown saplings in 2015, *n*=2 aeroponics-grown saplings in April 2016, and *n*=6 aeroponics-grown saplings in October 2016.

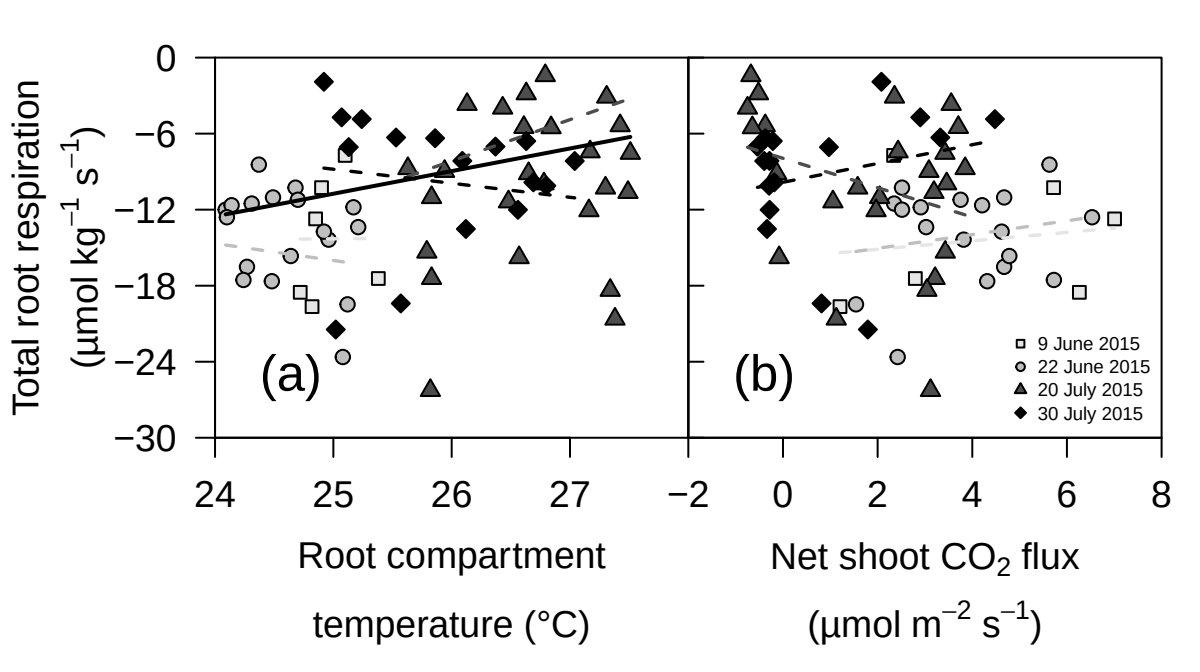
Tree part	Plant organ	Aeroponics						Container	
		October 2015		April 2016		October 2016		October 2015	
		Biomass (g)	Fraction of total biomass (%)	Biomass (g)	Fraction of total biomass (%)	Biomass (g)	Fraction of total biomass (%)	Biomass (g)	Fraction of total biomass (%)
Sapling	*Total*	*1375.2±260.1*	*100±0*	*4.4±0.2*	*100±0*	*856.1±146.4*	*100±0*	*1242.8±96.8*	*100±0*
Shoot	*Total*	*952.3±167.1*	*69.9±1.5*	*3.0±0.02*	*67.7±2.1*	*588.5±94.5*	*69.7±2.4*	*863.6±44.3*	*70.1±1.9*
	Leaf blade	195.5±36.9	14.2±0.4	1.3±0.1	29.0±2.9	189.4±37.2	22.2±1.6	169.6±11.8	13.8±0.9
	Leaf rachis	39.6±6.5	2.9±0.1	0.2±0.01	5.1±0.3	41.3±6.9	5.0±0.6	33.4±2.5	2.7±0.1
	Trunk+branches	717.2±123.9	52.7±1.2	1.6±0.1	35.6±0.8	357.8±56.8	42.5±3.0	659.6±36.1	53.5±1.5
Root	*Total*	*422.9±94.5*	*30.1±1.5*	*1.4±0.1*	*32.3±2.1*	*267.6±60.6*	*30.3±2.4*	*379.2±53.0*	*29.9±1.9*
	<2 mm	128.9±31.7	9.1±0.5	1.0±0.1	23.3±1.9	30.6±8.8	3.7±0.7		
	2-5 mm	30.0±9.1	2.2±0.7	0.2±0.04	3.6±0.8	79.6±21.9	8.7±1.1		
	5-10 mm	16.6±6.0	1.1±0.3	0.4±0.04	8.7±1.1	7.0±2.3	0.7±0.1		
	>10 mm	247.4±53.5	17.7±0.9			150.4±37.4	17.1±2.1		

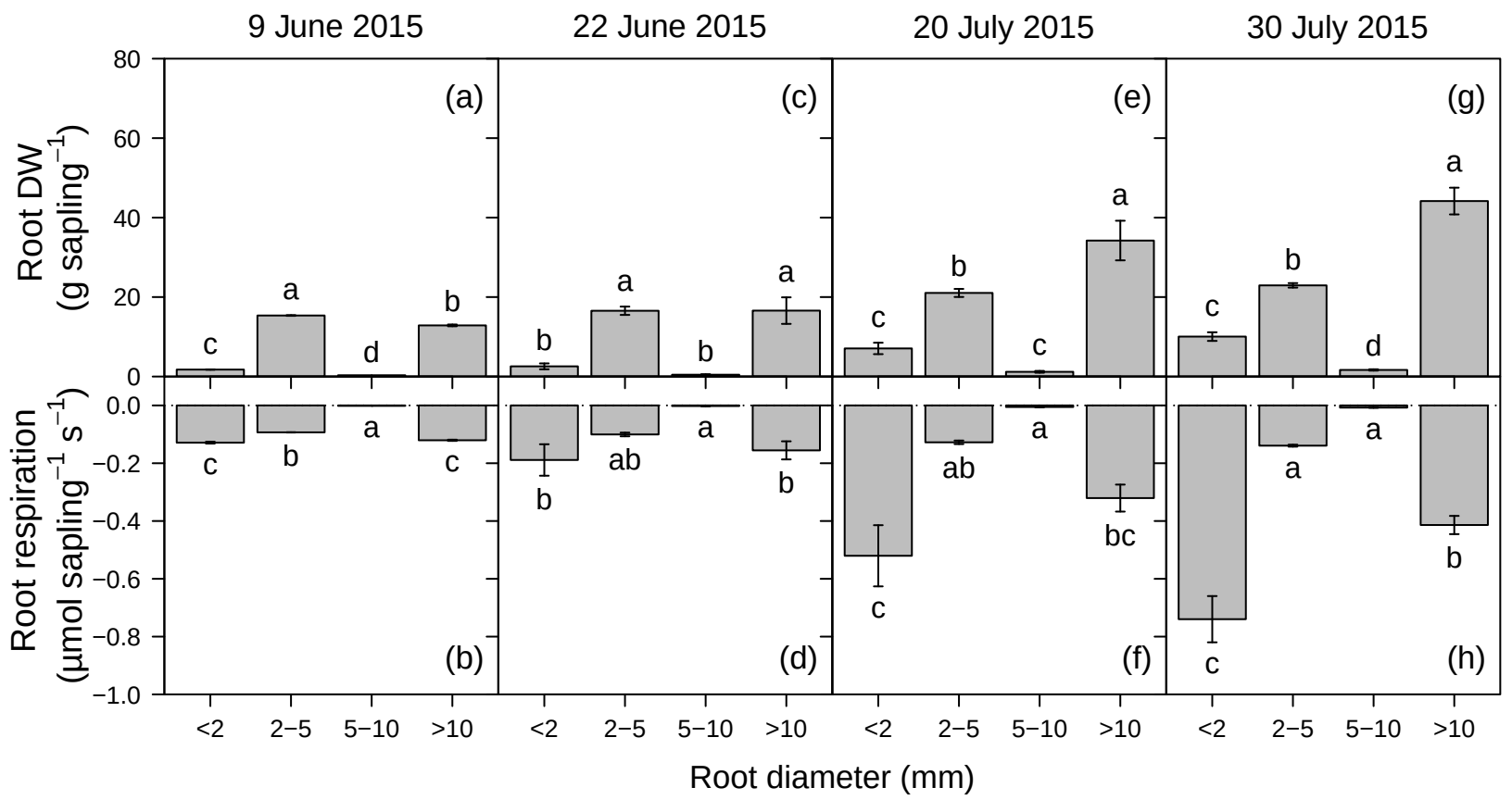
Table 2 – Daily carbon budget of *Ceiba pentandra* saplings, derived from integration of total root system respiration (*RCF*) and shoot CO_2 flux (*SCF*) measurements during day-time (13.5 h), night-time (10.5 h), and combined (24 h) on 25 July 2015. Positive or negative values represent CO_2 taken-up or emitted by the saplings, respectively. Data are means±SE of three saplings.

Time of day	Tree part	Net CO_2 flux (g C sapling^{-1} d^{-1})
Day-time	Shoot	1.58±0.18
	Root	-0.33±0.06
	Total sapling	1.24±0.12
Night-time	Shoot	-0.21±0.04
	Root	-0.25±0.01
	Total sapling	-0.46±0.04
Daily	Shoot	1.37±0.14
	Root	-0.58±0.06
	Total sapling	0.79±0.10


900
600
300
0
Root DW (g sapling^{-1})
0
300
600
900
1200
1500
1800
Shoot DW (g sapling^{-1})
Aeroponics
Containers


9 June 2015
22 June 2015
20 July 2015
30 July 2015
Closed symbols: Shoot
Open symbols: Root
Net CO_2 flux (µmol sapling^{-1} s^{-1})
Time (hh:mm)
6
5
4
3
2
1
0
−1
−2
00:00
04:00
08:00
12:00
16:00
20:00
00:00


Total root respiration
(µmol kg^{-1} s^{-1})
0
−6
−12
−18
−24
−30
(a)
(b)
24
25
26
27
−2
0
2
4
6
8
Root compartment
temperature (°C)
Net shoot CO_2 flux
(µmol m^{-2} s^{-1})
9 June 2015
22 June 2015
20 July 2015
30 July 2015


9 June 2015
22 June 2015
20 July 2015
30 July 2015
Root DW (g sapling^{-1})
Root respiration (µmol sapling^{-1} s^{-1})
Root diameter (mm)
<2
2–5
5–10
>10
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

Supplementary Data

Table S1 – Allometric equations describing organ biomass and surface area of *Ceiba pentandra* and their goodness of fit (R^2). The following power function was fitted: $Y=aD^b$, where a and b are parameters and *D* is base diameter (cm).

Biomass (g)	Parameter *a*	Parameter *b*	R^2
Shoot	27.2	2.31	0.96
Leaf blade	13.47	1.84	0.85
Leaf rachis	4.58	1.50	0.82
Trunk+branches	12.94	2.56	0.94
Root system	7.29	2.66	0.95
Root <2 mm	0.22	4.02	0.87
Root 2-5 mm	8.80	1.24	0.31
Root 5-10 mm	0.08	3.45	0.74
Root >10 mm	4.03	2.69	0.91
Total	33.59	2.42	0.97
Surface area (m^2)			
Leaf blade	0.28	1.80	0.79
Fine root	91.43	4.03	0.87

Table S2 – Total shoot CO_2 fluxes (*SCF*; µmol m^{-2} s^{-1}) and root system respiration (*RCF*; µmol kg^{-1} s^{-1}), measured during the 9 June, 22 June, 20 July and 30 July 2015 campaigns. In each campaign, the same saplings were repeatedly sampled for their CO_2 fluxes. Measured fluxes were grouped according to similar time windows. Means were compared within a column using Tukey's HSD test following one-way Repeated Measures ANOVAs with a combined date-cycle variable as the main fixed effect, to account for the effect of both date and time of day (i.e. cycle) on the CO_2 flux, and sapling as a random effect (ANOVA results for *SCF*: F ratio=15.4, $P<0.001$; for *RCF*: F ratio=2.77, $P=0.007$). Means with non-identical letters are significantly different at $P\leq0.05$. No differences were found among fluxes measured within the same time window. Data are means±SE, n=1-3 saplings per a measured CO_2 flux.

Time window	Date	*SCF*	*RCF*
04:15-05:30	20 July 2015	-0.70±0.18 ^{e}	-0.19±0.04 ^{a}
	30 July 2015	-0.51±0.13 ^{e}	-0.50±0.03 ^{ab}
06:15-08:00	20 July 2015	1.25±0.79 ^{de}	-0.72±0.05 ^{ab}
	30 July 2015	1.60±0.49 ^{cd}	-1.22±0.39 ^{b}
08:00-09:30	22 June 2015	2.10±0.33 ^{bcd}	-0.31±0.07 ^{ab}
	20 July 2015	3.56±0.26 ^{abc}	-1.03±0.44 ^{ab}
	30 July 2015	3.02±0.57 ^{abcd}	-0.13 ^{ab}
09:45-11:00	22 June 2015	2.85±0.56 ^{abcd}	-0.34±0.07 ^{ab}
	20 July 2015	3.94±0.36 ^{ab}	-0.55±0.23 ^{ab}
	30 July 2015	4.83±0.90 ^{a}	-0.40±0.04 ^{ab}
11:45-13:45	9 June 2015	3.66±0.24 ^{abc}	-0.30±0.06 ^{ab}
	22 June 2015	2.96±0.60 ^{abcd}	-0.42±0.11 ^{ab}
	20 July 2015	4.12±0.39 ^{ab}	-0.49±0.09 ^{ab}
14:15-15:30	22 June 2015	2.47±0.54 ^{bcd}	-0.47±0.09 ^{ab}
	20 July 2015	3.58±0.24 ^{abc}	-0.76±0.30 ^{ab}
17:00-18:30	9 June 2015	1.27±0.28 ^{de}	-0.32±0.08 ^{ab}
	22 June 2015	1.72±0.46 ^{cd}	-0.40±0.06 ^{ab}
	20 July 2015	1.13±0.23 ^{de}	-0.82±0.37 ^{ab}

Table S3 - Results of one-way ANOVAs testing the differences of biomass partitioning to tree organs (% of biomass of total harvested biomass partitioned to an organ) between aeroponics- and container-grown saplings, harvested in October 2015. There were no significant differences ($P \leq 0.05$) in biomass partitioning between facilities for all tree organs. *F* means F ratio and *P* means p-values.

	Organ									
	Shoot		Leaf blade		Leaf rachis		Trunk and branches		Root system	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Facility	0.006	0.937	0.17	0.693	2.12	0.183	0.16	0.703	0.006	0.937

Table S4 – Results of Mixed Model regressions testing the relationship between net shoot CO_2 flux (*SCF*; μmol m^{-2} s^{-1}) to photosynthetically photon flux density (PPFD; μmol m^{-2} s^{-1}) or shoot compartment temperature (°C), and between root system respiration (*RCF*; μmol kg^{-1} s^{-1}) to root compartment temperature (°C) or *SCF* (μmol m^{-2} s^{-1}), by date, with sapling as a random effect. Significant values ($P \leq 0.05$) are shown in bold.

CO_2 flux	Variables							
SCF								
	PPFD				Shoot compartment temperature			
Date	Model	R^2	*P*	*n*	Model	R^2	*P*	*n*
9 June 2015	log(*SCF*)=0.45* log(*PPFD*)-0.39	0.80	**0.016**	6	log(*SCF*)=8.20* log(*Shoot compartment temperature*)-10.97	0.99	**0.005**	6
22 June 2015	log(*SCF*)=0.13* log(*PPFD*)+0.41	0.14	0.124	18	log(*SCF*)=0.72* log(*Shoot compartment temperature*)-0.26	0.04	0.442	27
20 July 2015	log(*SCF*)=0.31* log(*PPFD*)-0.16	0.86	**<0.001**	27	log(*SCF*)=3.27* log(*Shoot compartment temperature*)-4.15	0.43	**<0.001**	18
30 July 2015	log(*SCF*)=0.26* log(*PPFD*)+0.01	0.99	**<0.001**	18	log(*SCF*)=6.24* log(*Shoot compartment temperature*)-8.26	0.81	**<0.001**	18
RCF								
	Root compartment temperature				*SCF*			
Date	Model	R^2	*P*	*n*	Model	R^2	*P*	*n*
9 June 2015	*RCF*=-0.08**Root compartment temperature*+16.19	0.22	0.993	6	*RCF*=-0.33**SCF*+15.80	0.15	0.778	6
22 June 2015	*RCF*=1.34**Root compartment temperature*-17.46	0.32	0.424	18	*RCF*=-0.55**SCF*+16.14	0.31	0.382	18
20 July 2015	*RCF*=-3.26**Root compartment temperature*+93.02	0.08	0.181	25	*RCF*=1.16**SCF*+7.95	0.10	0.127	25
30 July 2015	*RCF*=1.11**Root compartment temperature*-18.93	0.03	0.544	16	*RCF*=-0.74**SCF*+9.84	0.05	0.398	16

Table S5 - Results of Mixed Model regressions testing the relationship between net shoot CO_2 flux (*SCF*; μmol m^{-2} s^{-1}) to photosynthetically photon flux density (PPFD; μmol m^{-2} s^{-1}) and shoot compartment temperature (°C), and between root system respiration (*RCF*; μmol kg^{-1} s^{-1}) to root compartment temperature (°C) and *SCF* (μmol m^{-2} s^{-1}) with sapling and date as crossed random effects. *n*=69 and 65 measured CO_2 fluxes for *SCF* and *RCF*, respectively, measured on 9 June 2015, 22 June 2015, 20 July 2015, and 30 July 2015. Significant values (*P*≤0.05) are shown in bold, marginally significant values (0.05<*P*≤0.10) are indicated with a plus sign.

CO_2 flux			Variables	
SCF				
			PPFD	Shoot compartment temperature
	Model	R^2	*P*	*P*
	log(*SCF*)=0.35*log(*PPFD*)-1.58*log(*Shoot compartment temperature*)+2.05	0.87	**<0.001**	**0.0002**
RCF				
			Root compartment temperature	*SCF*
	Model	R^2	*P*	*P*
	RCF=1.68**Root compartment temperature*-0.07**SCF*-52.8	0.11	0.093+	0.889

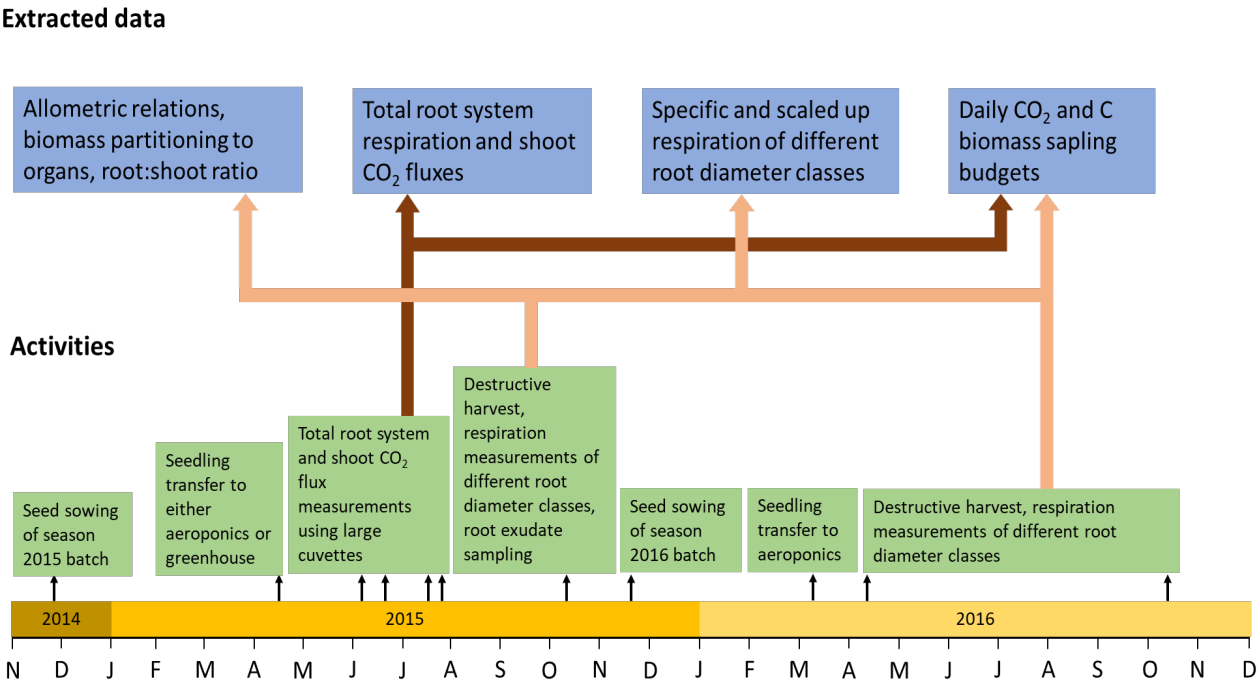


Figure S1 – Schematic representation of the different activities (green squares) done at certain time points (black vertical arrows) over the years 2014-2016, and their contributions to the different topics (blue squares) addressed in this study. Note that some activities were repeated several times in one year (with one seed batch), or several times in different years (with two seed batches, separately).

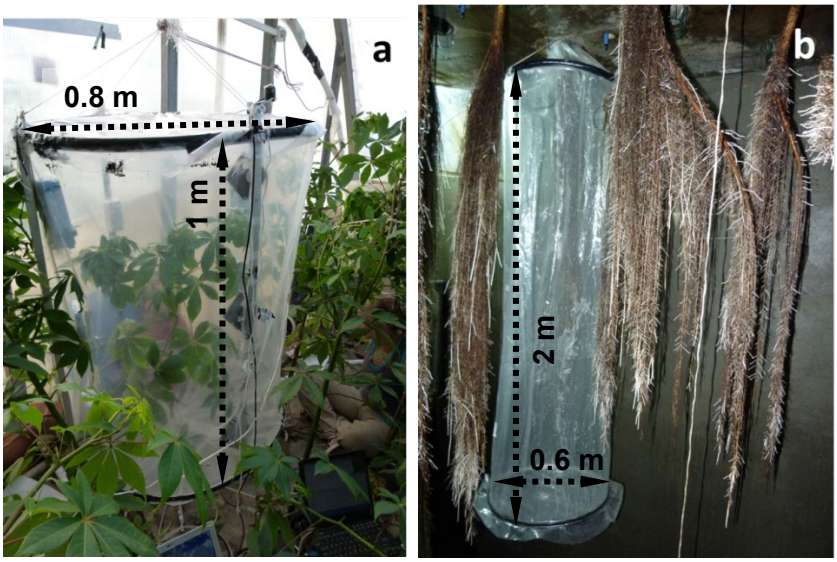


Figure S2 – Cuvettes used to measure (a) entire shoot CO_2 fluxes and (b) total root system respiration.

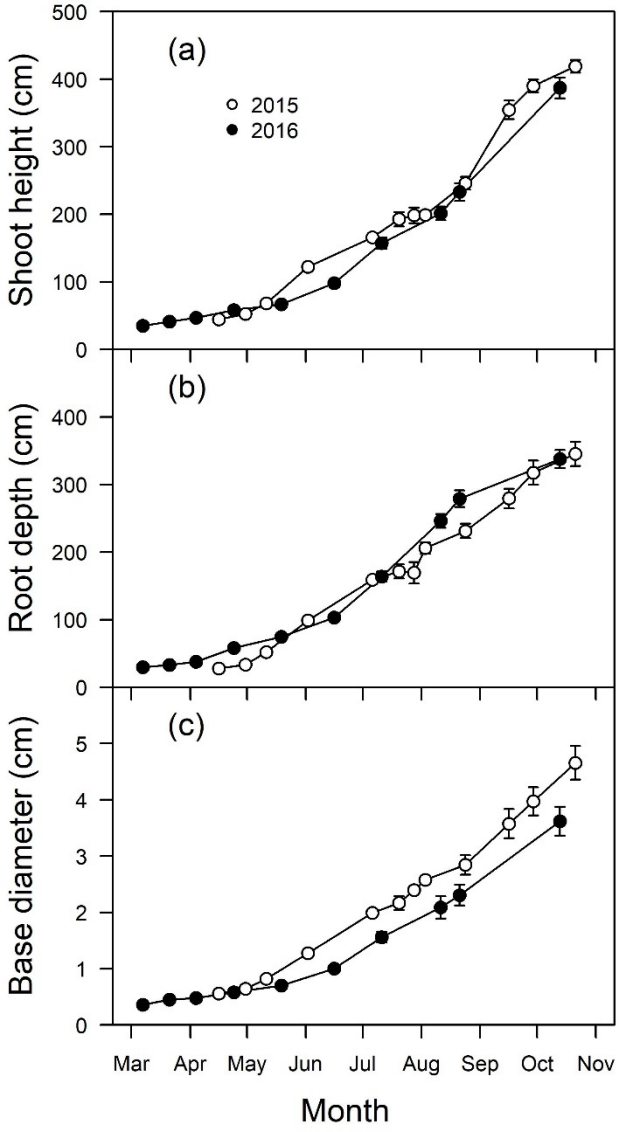


Figure S3 – (a) Shoot height, (b) root depth , and (c) base diameter of *Ceiba pentandra* saplings, measured over the growing seasons 2015 and 2016. Scaling-up of biomass was based on the data presented here. Data are means±SE. *n*=3-14 saplings.

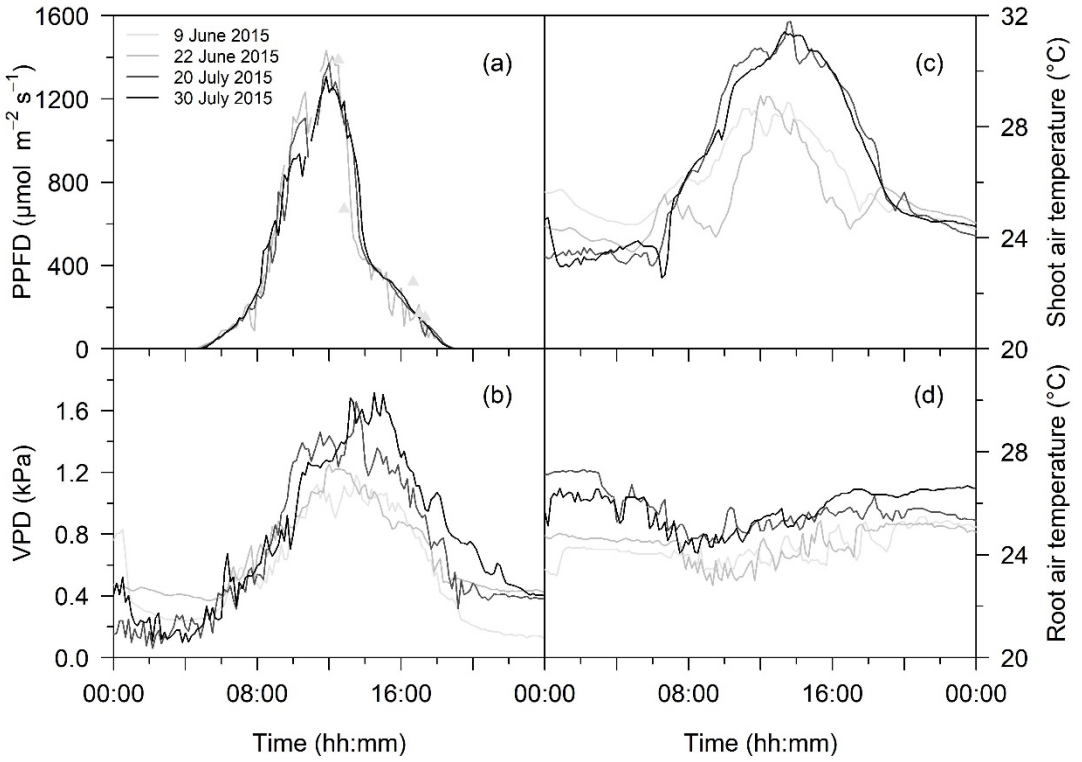


Figure S4 – Microclimate data during the total root system respiration and entire shoot CO_2 flux measurements of *Ceiba pentandra* saplings, measured on 9 June, 22 June, 20 July and 30 July 2015: (a) Photosynthetic photon flux density (PPFD), (b) vapour pressure deficit (VPD), (c) shoot air temperature and (d) root air temperature. 9 June PPFD data (grey triangles) originates from a PAR sensor fixed on top of the measuring cuvette as continuous data from the fixed sensor in the aeroponics facility were not available.

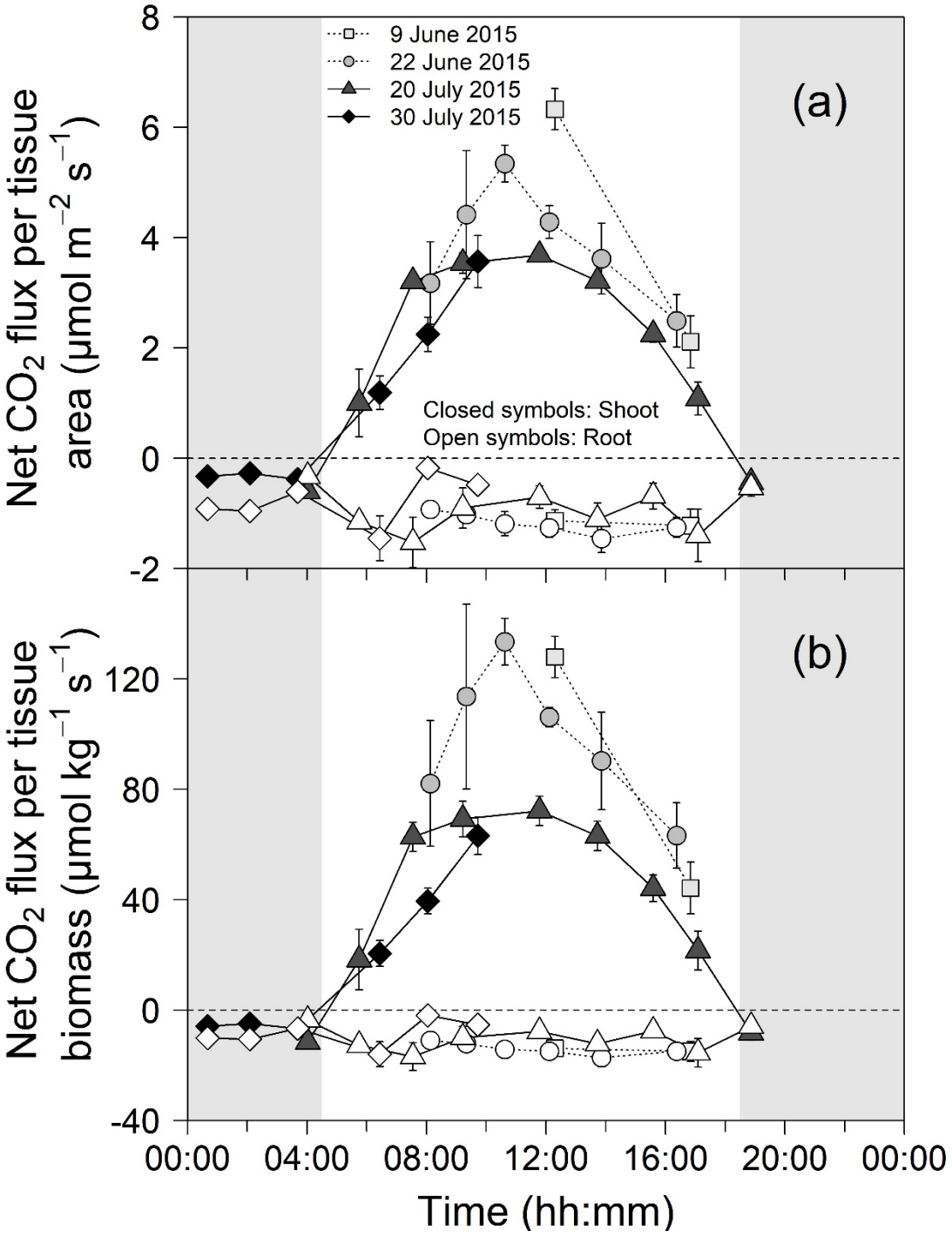


Figure S5 - Total shoot CO_2 flux (closed symbols) and root system respiration (open symbols) (a) per leaf and root surface area and (b) per leaf and root biomass of *Ceiba pentandra* saplings measured during night- and/or day-time on four days during summer 2015. Positive and negative values indicate CO_2 uptake by and CO_2 efflux from the saplings, respectively. Data points are means±SE, *n*=3 saplings. Grey areas indicate night-time on 25 July 2015.

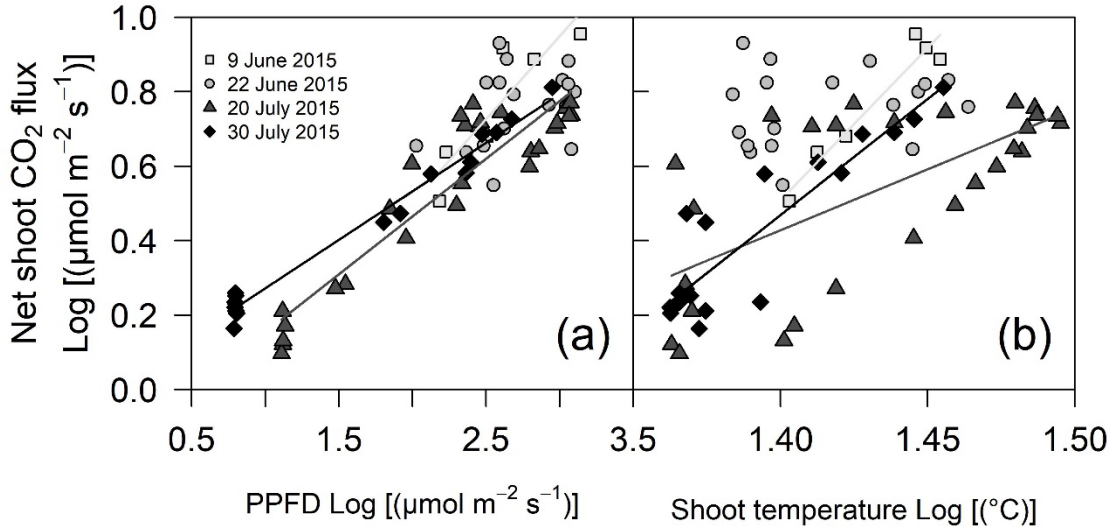


Figure S6 – Relationship between net shoot CO_2 flux (*SCF*) per leaf area to (a) photosynthetic photon flux density (PPFD), and (b) shoot compartment temperature of *Ceiba pentandra*, tested using Mixed Model regressions per date with sapling as a random effect. *SCF* was significantly related to PPFD and shoot temperature (continuous lines, see fitted lines' details in Table S4 above) on all measuring days except 22 June 2015. Modelling *SCF* for all dates using PPFD and shoot compartment temperature as fixed effects and sapling and date as crossed random effects, yielded the following relationship: log(*SCF*)=0.35*log(PPFD)-1.58*log(*Shoot compartment temperature*)+2.05 (R^2=0.87, P<0.001). Data were recorded during June and July 2015. n=69.

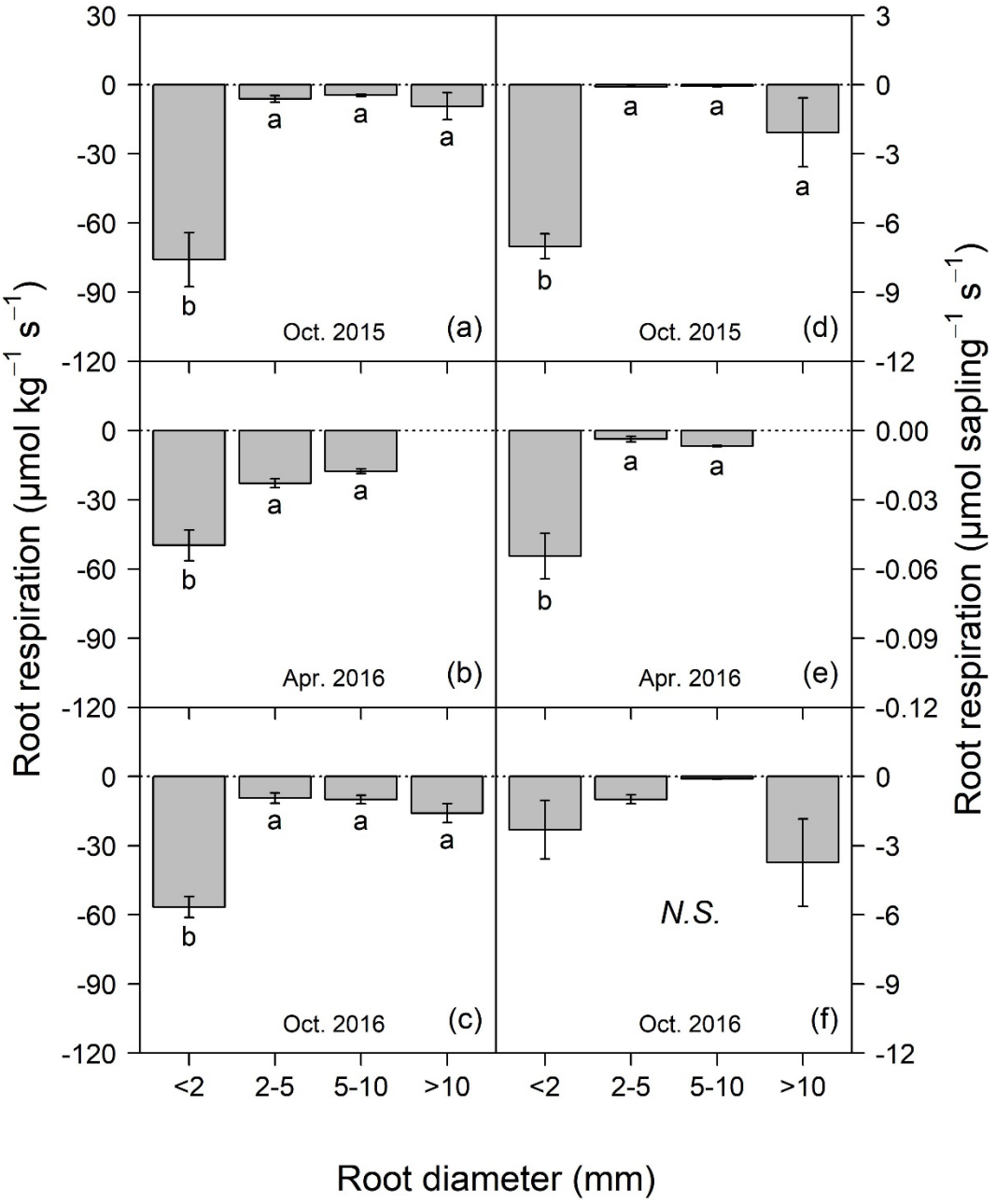


Figure S7 – Specific root respiration (µmol kg^{-1} s^{-1}) (a, b, c) and total organ root respiration (µmol sapling^{-1} s^{-1}) (d, e, f) of *Ceiba pentandra* grown in 2015 (a, d) and 2016 (b, c, e, f), measured in April (b, e) or October (a, c, d, f) of the specific years. Roots were classified according to their diameter classes, i.e., fine-roots (<2 mm diameter) and coarse-roots (2-5, 5-10 or >10 mm diameter). In April 2016 the saplings were too small to have roots with diameter >10 mm. Data are means±SE, *n*=2-3 saplings. Bars with non-identical letters within a panel are significantly different at $P \leq 0.05$ (Tukey's HSD test).

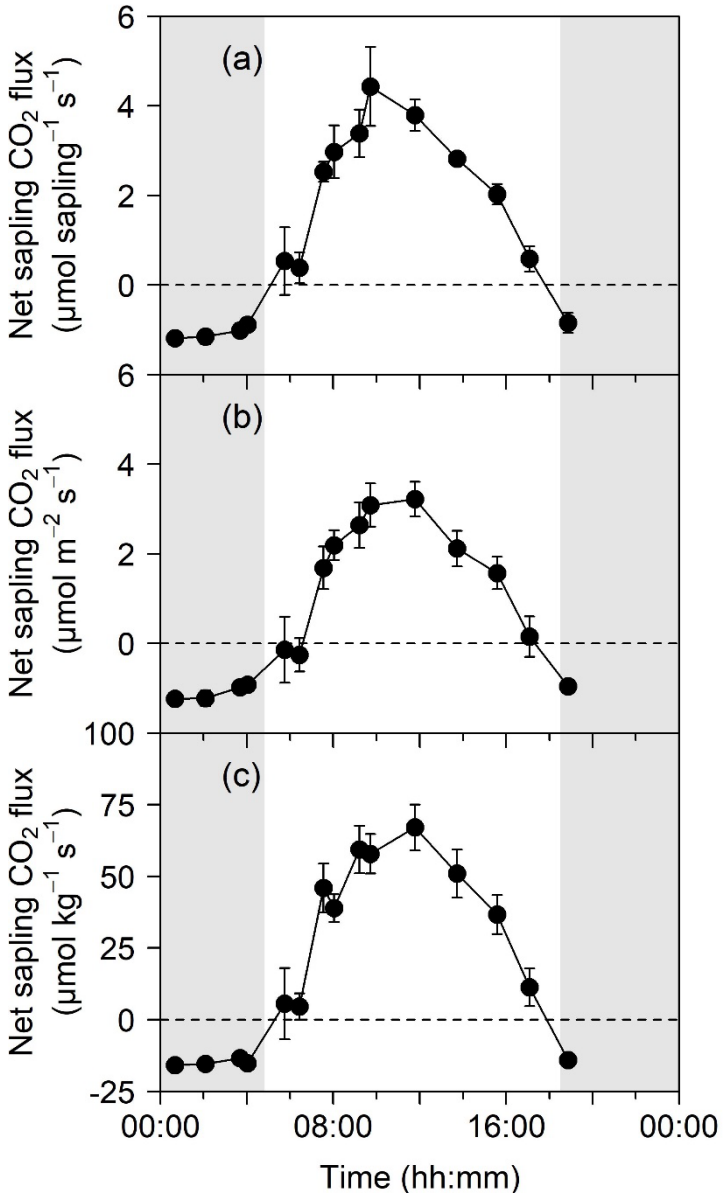


Figure S8 – Net sapling CO_2 fluxes (a) per sapling, (b) per leaf and root surface area, and (c) per leaf and root biomass of *Ceiba pentandra* saplings. Net shoot CO_2 fluxes and total root system respiration were measured during night- and/or day-time on 20 and 30 July 2015 and were integrated into net tree CO_2 fluxes over one average day, 25 July 2015. Positive and negative values indicate CO_2 uptake by and CO_2 efflux from the saplings, respectively. Data are means±SE, *n*=3 saplings. Grey areas indicate night-time on 25 July 2015.