Comparison of CO_2 and O_2 fluxes demonstrate retention of respired CO_2 in tree stems from a range of tree species

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Abstract. The ratio of CO_2 efflux to O_2 influx (ARQ, apparent respiratory quotient) in tree stems is expected to be 1.0 for carbohydrates, the main substrate supporting stem respiration. In previous studies of stem fluxes, ARQ values below 1.0 were observed and hypothesized to indicate retention of respired carbon within the stem. Here, we demonstrate that stem ARQ <1.0 values are common across 85 tropical, temperate, and Mediterranean forest trees from 9 different species. Mean ARQ values per species per site ranged from 0.39 to 0.78, with an overall mean of 0.59. Assuming that O_2 uptake provides a measure of *in situ* stem respiration (due to the low solubility of O_2), the overall mean indicates that on average 41% of CO_2 respired in stems is not emitted from the local stem surface. The instantaneous ARQ did not vary with sap flow. ARQ values of incubated stem cores were similar to those measured in stem chambers on intact trees. We therefore conclude that dissolution of CO_2 in the xylem sap and transport away from the site of respiration cannot explain the low ARQ values. We suggest refixation of respired CO_2 in biosynthesis reactions as possible mechanism for low ARQ values.

1 Introduction

The global annual CO_2 efflux from tree stems to the atmosphere is estimated at 6.7 ±1.1 Pg C yr^{-1} (Yang et al., 2016), but the drivers of stem CO_2 efflux are not well understood (Trumbore et al., 2013). CO_2 in tree stems originates primarily from aerobic respiration, which consumes oxygen (O_2). The respiratory quotient (RQ) is defined as the ratio between CO_2 produced and O_2 consumed, and its value is derived from the stoichiometry of the metabolized substrate. Carbohydrates are believed to be the main respiratory substrate in tree stems (Hoch et al., 2003; Plaxton and Podestá, 2006), and their metabolism results in an RQ of ~1.0. Respiration that relies

entirely on lipids predicts RQ values of ~0.7, but it is not clear to what extent lipids are stored and used in trees as they are rarely measured (Hartmann and Trumbore, 2016). Current understanding suggests that significant storage of lipids in stems is uncommon and limited to several tree genera, the so-called 'fat-trees' (Sinnott, 1918). RQ values greater than 1.0 are associated with organic acids catabolism, due to the greater O content of the molecules being oxidized. For these reasons, we expect principally carbohydrate metabolism in tree stems and an RQ of approximately 1.0.

Initial measurements of the ratio of CO_2 efflux to O_2 influx from the stem surface for six tree species found values mostly below 1.0 (Angert and Sherer, 2011; Angert et al., 2012). The flux ratio is referred to in those studies, and here, as the "apparent" RQ (ARQ), because it potentially includes additional sources or sinks of CO_2 and/or O_2 in the stem in addition to the respiration taking place in tissue beneath a chamber placed on the stem surface. Processes that can potentially reduce the emission of CO_2 and thereby decrease ARQ below 1.0 include: (1) dissolution and transport of CO_2 in the xylem sap (Teskey et al., 2008), and (2) carboxylating reactions during biosynthesis of compounds more oxidized than carbohydrates that involve refixation of CO_2 by the enzyme phosphoenolpyruvate carboxylase (PEPC) (Lambers et al., 2008). Alternatively, it may be hypothesized that ARQ below 1.0 is the result of non-respiratory O_2 uptake, e.g. by oxidases and hydroxylases that are O_2 consuming enzymes.

Carbon dioxide is ~30 times more soluble in water than O_2, and dissolved CO_2 reacts with water to form bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions, further increasing the amount of dissolved inorganic carbon (DIC). The rate of O_2 uptake is thus assumed to provide a better measure of stem respiration than CO_2 efflux, which can be complicated by dissolution and transport within the xylem sap (Teskey et al., 2008), potentially contributing to low ARQ values. There is evidence from studies with an isotopically labeled stem CO_2 pool that a significant portion of C is transported as DIC to photosynthetic tissues where it might be refixed to organic C (Bloemen et al., 2013; McGuire et al., 2009; Powers and Marshall, 2011). If transport of CO_2 within the stem is important, ARQ measured at the stem surface is expected to be inversely related to sap velocity. As the difference in solubility between CO_2 and O_2 decreases with increasing temperature (Gevantman, 2018), ARQ also might be expected to increase with temperature if all other factors remain constant. In addition, variations of ARQ with stem height are to be expected. A model of CO_2 diffusion and advection in the xylem sap by Hölttä and Kolari (2009) predicted that the accumulation of dissolved CO_2 in the ascending xylem sap, together with a reduction in stem diameter with height, induces faster CO_2 diffusive loss to the atmosphere in the upper parts of the stem. Thus, we expect an increase in ARQ (higher CO_2 loss per mole of O_2 uptake) with stem height if dissolution and transport of CO_2 in the xylem sap is important.

The second possible explanation for low ARQ is local dark refixation in the stem by PEPC (Angert et al., 2012). PEPC is present in tree stems (Berveiller and Damesin, 2008; Höll, 1974; Ivanov et al., 2005), and its activity was suggested to be sufficient to have a measureable impact on respired CO_2 in *Ricinus communis* (Gessler et al., 2009). Stem ARQ values would remain below unity as long as the products of PEPC fixation (e.g. malate and citrate) are not inhibiting further fixation. To date, studies of these processes in large trees are scarce, and it is not clear which processes are responsible for low ARQ. If ARQ values lower than unity are prevalent and result from processes that retain CO_2 in the stem, estimates of tree stem respiration based on CO_2 efflux measurements must be reconsidered. Thus, the first objective of this work is to determine whether ARQ values

lower than 1.0 are observed in a variety of trees from different biomes and across seasons. A secondary objective of this study is to test whether ARQ varies with xylem stream characteristics or with tree height.

2 Materials and Methods

2.1 Methods for evaluating ARQ

We report tree stem ARQ results based on measurement methods described in Hilman and Angert (2016a). These methods overcome the difficulty of measuring small changes in O_2 against the high atmospheric background by using a static stem chamber, in which the O_2 changes are considerably larger than in an open flow chamber.

We used three different approaches to measuring ARQ: two are based on discrete gas samples of headspace air, and one is based on direct measurement of the headspace air using gas sensors ("continuous" sampling). Discrete gas samples are either taken within 30 minutes to several hours after chamber sealing ("instantaneous" sampling) or after the chamber has been sealed to the stem for more than 24 hours, once steady state conditions have been achieved ("steady state"). These methods and the time required for achieving steady state were confirmed by comparing with "continuous" measurements (Hilman and Angert, 2016a). For each site and experiment described in section 2.2, we identify the method used to estimate ARQ as "instantaneous", "steady state" (for flask samples) or "continuous" (Table 1).

2.1.1 ARQ measurement from discrete samples

The evaluation of ARQ from discrete gas measurements is based on a one-box model that describes gas dynamics in the headspace of a static chamber sealed to the surface of a tree stem (Angert and Sherer, 2011; Angert et al., 2012; Hilman and Angert, 2016a). In the model, the gas in the chamber headspace has initial mean atmospheric values (20.95% O_2, 0.04% CO_2), ensured by flushing the chamber with ambient air before measurement. Once the chamber is closed and the headspace above the stem surface is isolated, metabolic reactions in the stem control the chamber's air composition. For the first few hours, headspace concentrations of CO_2 increase and O_2 decrease at rates that are roughly linear with time ("instantaneous" incubation, Fig. 1, S1). During this linear stage, ARQ is calculated by:

$$\mathrm{ARQ} = \frac{\mathrm{CO_2\ efflux}}{\mathrm{O_2\ influx}} = \frac{\Delta \mathrm{CO_2}}{\Delta \mathrm{O_2}} \quad (1)$$

where ΔCO_2 and ΔO_2 are the changes in $[CO_2]$ and $[O_2]$ during the initial period after the chamber was sealed, and for discrete samples can also be determined from the difference in concentrations between the chamber air sampled at a specific time and the initial atmosphere. "Instantaneous" fluxes of CO_2 and O_2 reported here are obtained either by monitoring concentration change during the first hour following chamber closure with sensors directly in the field or by sampling headspace air with glass flasks within 30 minutes to a few hours of closing the chamber. The flasks were transported to the laboratory for measurement of CO_2 and O_2.

After the first hours, the initially linear rates of change in headspace gas concentration with time decline, and concentrations eventually remain constant (Fig. 1, S1). In this phase the gases in the chamber and the outer part of the stem, where most of the metabolism takes place, are assumed to be in equilibrium. This "steady state" occurs when the rates of addition of CO_2 and loss of O_2 from the stem to the chamber headspace are balanced by

diffusive (assuming no strong wind) exchange of headspace air with outside air through porous portions of the outer stem. For "steady state" samples, the chamber is sealed to the surface of the stem and left for a period longer than 24 hours, after which the headspace air is sampled using glass flasks. The CO_2 and O_2 concentrations must be corrected for differences in diffusivity between CO_2 and O_2, as detailed in (Angert and Sherer, 2011; Angert et al., 2012; Hilman and Angert, 2016b) in order to estimate the ratio of the gas fluxes from the concentrations in the static chamber:

$$\mathrm{ARQ} = \frac{gCO_2 \times \Delta CO_2}{gO_2 \times \Delta O_2} \quad (2)$$

where gCO_2 and gO_2 are the CO_2 and O_2 conductance values in the outer layer of the stem between the chamber and the atmosphere. The structure of the path along which diffusion occurs is the same for CO_2 and O_2 and hence the conductance ratio gCO_2/gO_2 depends solely on the ratio of diffusivities of the gases in air, which is 0.76 (Massman, 1998). As a result, at steady state:

$$\mathrm{ARQ} = 0.76 \times \frac{\Delta CO_2}{\Delta O_2} \quad (3)$$

Assuming constant CO_2/O_2 fluxes over time, samples taken either by "instantaneous" or "steady state" methods will yield the same ARQ values. Indeed, Hilman and Angert (2016a) demonstrated excellent agreement for direct comparisons of the "instantaneous" and "steady state" measurement methods, and the results are further compared here.

2.1.2 Stem chambers and gas measurements

All data reported here was collected by using chambers attached to the stem surface to create a gas-tight incubation headspace. Chamber designs and sampling details differed between sites (see section 2.2 and Table 1), but generally all chambers were equipped with sampling ports for attaching glass flasks equipped with O-ring valves (LouwersHanique, Hapert, The Netherlands). Outside incubations, permanently installed chambers were protected against insect infestation using screens. Incubations were always started at ambient concentration, and flasks were allowed to equilibrate with the headspace by opening the flasks' valves during incubation. Incubation time varied from between 30 minutes to a few hours for "instantaneous" ARQ samples to more than 24h for "steady state" samples. At the end of the incubation period, the flask valve was closed and the gas sample was shipped to the laboratory for analysis. Each reported ARQ measurement is the average of duplicate flasks taken from the stem chamber, and the error is the standard deviation.

The CO_2/O_2 ratios in the flasks were analyzed in the laboratory at the Hebrew University in Jerusalem in a closed system (The *Hampadah* (Hilman and Angert, 2016b)). Two analyzers are included in the *Hampadah* system; an infra-red gas analyzer (IRGA) for CO_2 measurement (LI 840A LI-COR; Lincoln, NE, USA) and a fuel-cell based analyzer (FC-10; Sable Systems International, Las Vegas, NV, USA) for measuring O_2. The principle of operation of the *Hampadah* is measurement of the change in CO_2 and O_2 concentrations in the system's air after the addition of the air from a given sample flask of known volume, and calculation of the concentration in the flask that would yield that overall concentration change (Hilman and Angert, 2016b).

2.1.3 Continuous ARQ measurements

Sensitive detection of small changes in O_2 is difficult in the field, which is why we used the flask samples and long chamber closure times ("steady state") in most field sites. However, to measure diurnal changes in stem

ARQ values of *Malus domestica*, we were able to make continuous measurements with a small IRGA CO_2 sensor (COZIR Wide Range 0-20% CO_2 Sensor, CO2Meter, Inc., Ormond Beach FL, USA) and a quenching based optode (Fibox 3, PreSens-Precision Sensing, Regensburg, Germany) for O_2 measurement (Hilman and Angert, 2016a). The sensors' reading was extracted every 30 seconds. A temperature sensor was placed next to the optode sensor for temperature and water vapor corrections. The inlet of a small diaphragm pump (KNF micro-pump) and a non-return valve (SMC AKH 12mm, RS, UK) were connected to the chamber headspace and used to automatically vent the chamber headspace every 4 hours. The CO_2 efflux and the O_2 influx were calculated using a linear fit over ~120 gas concentration measurements during the first hour of incubation, the chamber volume, and the stem surface area under the chamber. We used the data from this experiment to examine the sensitivity of ARQ to temperature, which affects the gas solubility constants. The strongest effects are expected during the night, when daytime influences on stem fluxes associated with sap flow and low turgor pressure (Salomón et al., 2018) are minimized.

2.2 Study sites and experimental design

For addressing the variation in stem ARQ values across a range of tree species and environments, our study included trees located in tropical forests (Panama and Brazil), in temperate forests (Bartlett and Harvard, USA), and in a Mediterranean savanna (Spain) and a Mediterranean shrubland (Carmel Ridge, Israel). We also included five trees located on the Hebrew University campus in Jerusalem (Israel) and in the adjacent Botanical Gardens. The trees in Panama were part of a fertilization and litter manipulation projects (Wright et al., 2011; Sayer and Tanner, 2010). No treatment effects were found (Fig. S2, this topic is not in the scope of this paper). Details about the sites, tree species, stem chambers, stem dimensions, and experiments conducted in each of the sites are presented in Table 1 and Figure 3. The list below summarizes what data was available from the different sites and what questions in particular we addressed with this data (the numbering of the experiments matches Table 1):

A. Seasonal and/or phenological measurements of stem ARQ were performed in Jerusalem, US, and Brazil sites. In Jerusalem, five individual trees from five different species (first five species in Table 1) were measured. The phenological state of deciduous trees (all except *Quercus calliprinos*) was separated into four classes (Fig. 4). In the US sites, trees measured at the northern site (Bartlett Experimental Forest) had fall color development, while leaves at Harvard Forest (southern site) were still green. After analysis of flasks, we excluded results from three trees because of suspected air leakage from the chamber (O_2 >20% after six days of stem incubation). In Brazil, six trees were measured. After analysis we excluded results from four out of twelve “instantaneous” measurements because of a weak signal (O_2 >20.7% and SD >0.1 after 3 h of incubation).

For our second objective, to explore the potential for low ARQ values to reflect dissolution and transport of CO_2 in the xylem sap, we measured instantaneous ARQ at varying sap flow velocities and at different times of a day. Transport of CO_2 was previously reported to be correlated with sap flow (McGuire and Teskey, 2004; Bowman et al., 2005; McGuire et al., 2007). Thus, anti-correlation of ARQ with sap flux, expressed via maximal ARQ values during the night when transport is at a minimum, would provide evidence that low ARQ can be explained by export of locally respired CO_2 (as DIC) out of the stem region being measured (experiments B, C, and E, below). If transport of dissolved CO_2 is the main driver of low ARQ values, we would also expect that: (D)

higher ARQ values will be observed at higher temperatures (due to differential temperature dependences of CO_2/O_2 solubility coefficients); (F) ARQ values will increase with stem height due to DIC accumulation and stem tapering that induce stronger CO_2 diffusive loss; (F) ARQ values will decrease with depth in the stem (due to the greater proximity to the water conducting vessel elements); and (G) ARQ values in incubated stem cores will be higher than measured values at the stem surface (due to the detachment from the transport system).We performed a number of experiments to test each of these predictions (additional details in Table 1):

B. ARQ ("instantaneous") was measured simultaneously with sap flux density measurements in nine *Quercus ilex* trees with similar diameter (0.35 to 0.49 m at breast height) at the site in Spain.

C. ARQ ("instantaneous") was measured during daytime, at pre-dawn when the transpiration stream should reach its minimum, and again during the next day. We conducted two day-night campaigns on the trees at the site in Jerusalem. Additionally, during 4 days, ARQ ("continuous") values were measured every 4 h from the *M. domestica* tree in Jerusalem.

D. Nighttime results of the “continuous” ARQ measurements on the *M. domestica* enabled us to examine the relationship between temperature and ARQ. During the night, when sap flux is minimal, the temperature effect on the gases solubility should have its maximum effect on ARQ values.

E. ARQ ("steady state") was measured over spring, summer and winter for *Quercus calliprinos* trees on Carmel Ridge site, simultaneously with pre-dawn shoot water potential (Ψ_{pd}). Ψ_{pd} is a measure for available soil water and therefore is also a rough proxy for seasonal differences in transpiration rates (Aranda et al., 2005; Bucci et al., 2005).

F. ARQ was measured at different heights on the same tree stems, while simultaneously ARQ was determined from air sampled inside the stem. During the seasonal measurements in Jerusalem, ARQ (“steady state”) was measured at the stem base of the *Q. calliprinos* and the *Platanus occidentalis* trees as well as at breast height. In Brazil, we measured ARQ ("instantaneous") from stem chambers and in-stem probes to sample in-stem gases from the tree base up to 11 m above the ground on a single *Scleronema micranthum* tree on two separate days.

G. ARQ ("steady state") measured from stem chambers was compared with ARQ measurements through incubation of stem cores. Measurement of stem tissues should provide better estimation for the stem outer layers’ RQ by excluding dissolution and advection in the xylem stream. Incubations were performed on cores taken from four species in four different sites (Table 1). In Jerusalem, we compared repeated stem incubation ARQ with that of leaf incubation.

2.3 Sap flux density

Sap flux density was monitored in 9 trees at the site in Spain using heat ratio method (HRM) sensors (SFM1 Sap Flow Meter, ICT International). A description of the installation and measurement is presented in Methods S1. The detailed procedures for sap flux corrections and calculations are described in (Perez-Priego et al., 2017). We tested whether the daily maximum sap flux density (i.e. average of measurements between 10:00 and 17:00 during the day of the ARQ measurement), which correlated with CO_2 dissolution fluxes (Bowman et al., 2005) could explain variability in ARQ ("instantaneous").

2.4 Shoot water potential

Pre-dawn shoot water potential (Ψ_{pd}) on Carmel Ridge was measured using a pressure chamber (PMS Instrument Company, Corvallis, Oregon, USA). At each sampling time, we sampled 2-3 terminal twigs containing 5-10 leaves from each tree. The samples were wrapped in plastic, placed on ice and measured within an hour of sampling using the pressure chamber technique (Scholander et al., 1965).

2.5 In-stem measurements

For sampling gas from inside the stem, stainless-steel tubes (1.3 cm diameter) were installed 4, 8, and 12 cm deep into the stem, in various stem heights on the same tree in Brazil where the vertical ARQ transects were measured. Installation procedure was according to Muhr et al. (2013) and tubes were sealed between sampling dates. Using rubber tubing we connected the sampling flasks to the tubes for incubation of 4 days. The flasks were then analyzed for CO_2 and O_2 in the *Hampadah*. Assuming steady state, ARQ was calculated using Eq (3) (Angert et al., 2012).

2.6 Measuring ARQ of incubated tissues

Stem cores were extracted immediately after the chamber incubation experiment in Panama, Spain, and Jerusalem using a 1.2 cm diameter cork borer. The outer bark and green tissues, as well as sapwood sieves (with paler color than the phloem tissues), were removed from the cores. The cores were cut into longitudinal halves, wrapped with moist gauze cloth to avoid desiccation and placed in an air-tight incubation system to which glass flasks (two or three) were connected by Swagelok Ultra-Torr fittings (Swagelok, Solon, OH, USA, Fig. S4). At the end of the incubation period, the flasks were closed and analyzed in the *Hampadah*. Since the incubations took place in a closed system (no diffusive exchange with outside air), the change with time in $[CO_2]$ and $[O_2]$ are assumed to be linear, and ARQ can be calculated using equation (1).

In Panama and Spain the incubations were started immediately upon core extraction, at ambient temperature, and lasted 8 h and 3 h, respectively. In Jerusalem the cores were kept on moist gauze cloth for 2 h before being sealed in the incubation system and kept at 25^{o}C in an environmental chamber. Repeated incubations were performed in series, with the incubation systems flushed in between with ambient air. Simultaneously, from each tree, four leaves from an understory branch were cut and inserted into the same incubation systems, for the same incubation durations. The O_2 uptake rate (nmol O_2 g.FW^{-1} s^{-1}) was calculated as follows (adopted from Pruyn et al. (2002)):

$$O_2 \text{ uptake rate } = \frac{\Delta O_2}{100} \times \frac{V_H}{T \times M_{FW} \times V_m} \times 10^9 \quad (4)$$

where ΔO_2 is the decrease in $[O_2]$ during the incubation, V_H is volume of headspace (ml), T is incubation period (s), M_{FW} is fresh weight (g), V_m is the molar volume, and 10^9 converts units to nmol. We dried the samples in an oven at 60^{o}C for two days for the dry weight.

In Brazil, stem cores were extracted by using a 5.15 mm diameter increment corer. After bark was removed the cores were cut to a length of 6 cm each and then allowed to equilibrate with the atmosphere for 6-8 hours, while continually being kept moist. After equilibration, each core was transferred to an incubation chamber equipped with flasks. Prior to starting the incubation, a few ml of water were added to keep the core tissue moist. In this case, incubations were left at room temperature (~25^{o}C) for 24 h before flasks were closed and removed.

2.7 Statistical analysis

All statistical analysis was done using JMP (JMP®, JMP Pro 13, SAS Institute Inc., Cary, NC, USA). Repeated measures analysis of variance was used to evaluate how the interaction of tissue (stem core/leaves) with ARQ and O_2 uptake varies with time in the repeated incubations of the tissues from the trees in Jerusalem. Mauchly's test indicated violation of sphericity in the ARQ response in the repeated incubations experiment (χ^2 =18.132, *P* =0.021), therefore the Greenhouse-Geisser adjusted F test was chosen. One-way analysis of variance (ANOVA) followed by Tukey-Kramer HSD was used to perform comparisons among time points in every tissue. Student's t-test was used for comparisons between stem cores and leaves at each time point.

3 Results

The ARQ estimated from "instantaneous" and "steady state" measurements were in good agreement over a large range of ARQ (Fig. 2). The mean difference between the two assessments is 0.02, and RMSD is 0.15. The average ARQ ("steady state") value across all species and sites, including results from (Angert et al., 2012), was 0.59 (n =229) and the average ARQ of species in the different sites ranged between 0.39 and 0.78 (Fig. 3). For individual measurements, a minimum ARQ value of 0.27 was recorded for *Q. ilex* in Spain and for *Tetragastris panamensis* in Panama. The highest value was 0.99 for *M. domestica* and *Populus deltoids* in Jerusalem.

Phenology or seasonality had some effect on ARQ. In Jerusalem, the ARQ of *Q. calliprinos* and *Pistacia atlantica* was lower during spring and higher in fall and winter (Fig. 4). In Brazil, ARQ varied between 0.41 ±0.15 in the wet season (March) and 0.82 ±0.12 in the dry season (October, Fig. S3). The average ARQ of the *Acer rubrum* trees at Harvard Forest, where all leaves were green, was significantly higher than the average ARQ of the trees at Bartlett Experimental Forest, where the leaves had autumn color development (0.69 vs. 0.57, *P* <0.05 in a Student's t test).

3.1 ARQ values under varying xylem stream flow and temperature

ARQ ("Instantaneous") values of nine *Q. ilex* trees in Spain were invariable (mean ±SD of 0.42 ±0.04) in comparison with the larger variation in maximum daily sap flux density among these trees (0.15 ±0.05 m^3 H_2O m^{-2} h^{-1}), and no correlation was found between the ARQ and sap flux density (r^2 =0, *P* =0.9891).

Mean ARQ ±SD values ("steady state") of the trees at the Carmel Ridge site were 0.62 ±0.06, 0.68 ±0.07 and 0.69 ±0.08 for spring, summer and winter, respectively. Repeated-measures analysis of variance found no significant difference between seasons ($F_{2,2}$ =2.52, *P* =0.28), while Ψ_{pd} varied significantly with seasons ($F_{2,2}$ =207.85, *P* =0.0048). During summer, Ψ_{pd} was -2.65 MPa, much lower than the spring and winter values (-0.64 and -0.86 MPa, respectively).

In the Jerusalem day-night campaigns, ARQ ("instantaneous") values ranged between 0.52 and 1.05, across all trees, seasons, and sampling times (Fig. 5). Pre-dawn ARQ values exceeding than daylight values (by amounts larger than the differences between duplicates) were observed during the summer in *M. domestica* and in the upper chamber on *Q. calliprinos*. No significant diurnal effect was found in repeated-measures analysis of variance of the breast height chambers, neither when results of all the trees was grouped by season, nor when results were grouped by stem chamber. In "continuous" measurements of *M. domestica*, with ARQ values obtained every 4 hours, ARQ during the night (0.70; n=12) was not significantly (*P* >0.76 in a student's *t* test)

304 greater than in the day (0.71; n =11; Fig. 6). The variations among the nighttime values were best explained
305 using temperatures measured 235 minutes before the ARQ measurement (r^2 =0.84, *P* =0.0001, ARQ =0.01 ×
306 Temperature (C^o) + 0.54). With the same time lag, the coefficient of determination for the daytime values is r^2
307 =0.44 (*P* =0.0266).
308

309 **3.2 Stem surface and in-stem ARQ vertical transects**

310 In *Q. calliprinos*, measured over three years in Jerusalem, ARQ did not differ significantly (*P* >0.33 in student's
311 *t* test) between breast height and stem base (ARQ of 0.56 vs. 0.59 respectively, n =14, Fig. 4). For *P.*
312 *occidentalis* measured for the same period the ARQ measured at breast height was significantly higher than
313 ARQ measured at the stem base (0.74 vs. 0.64 respectively, n =12, *P* =0.003 in student's *t* test, Fig. 4). For a
314 single *S. micranthum* tree in Brazil, ARQ values measured at heights of 6.5 m and 11 m above the ground were
315 similar to ARQ measured at breast height (Fig. 7), but also show differences with the stem base. In this tree,
316 ARQ measured in March (0.46 ±0.11; wet season) was lower than in October (0.89 ±0.16; dry season). The in-
317 stem ARQ values ranged between 0.25 and 0.56, with average ±SD of 0.46 ±0.07 in both seasons and at all stem
318 positions and depths. The in-stem ARQ, as well as [CO_2] values, had no clear vertical trend (Fig. 7; S5).

319 **3.3 Tissue incubations**

320 The average ARQ values of the stem core incubations were similar to the stem chamber ARQ for the four
321 sites/trees where these comparisons were made (Fig. 8). When incubations were repeated over time for *Q. ilex*
322 stem cores and leaves, significant effects of time, tissue (leaves, stem cores), and their interactions (time ×
323 tissue) on ARQ and O_2 uptake rates were observed. ARQ of the stem cores increased from 0.44 ±0.08 (mean
324 ±SD, n =4) after 3 h to 0.94 ±0.03 at the end of the experiment (32 h; Fig. 9). The ARQ of incubated leaves of
325 the same trees showed higher initial ARQ of 0.80 ±0.02, with an increase over time to 0.92 ±0.02.

326 **4 Discussion**

327 **4.1 ARQ is lower than 1.0 for a wide range of tree species**

328 The ARQ measured in stem chambers installed on 85 individual trees of 9 species including tropical, temperate
329 and Mediterranean forest trees was considerably and almost universally lower than 1.0. ARQ values as low as
330 0.7 could indicate that lipids were used exclusively as substrates for respiration, but current understanding
331 suggests this scenario is implausible. However, this understanding relies on low and constant lipid
332 concentrations over seasonal sampling (Hoch et al., 2003); daily changes in lipid concentrations and RQ were
333 measured in response to shading and drought treatments, indicating this substrate might be more important than
334 commonly thought (Fischer et al., 2015; Hanf et al., 2015). Nevertheless, many of the measured ARQ values
335 were below 0.7, so substrate use alone cannot explain them. Additionally, as ARQ values above 1.0 are
336 expected when lipids are produced (De Vries et al., 1974), ARQ <1.0 resulting from lipid metabolism must be
337 mirrored with ARQ >1.0 at a different time (assuming the lipids are produced locally). However, ARQ almost
338 never exceeded 1.0. The results demonstrate that O_2 influx to the stems usually exceeded the CO_2 efflux,
339 regardless of tree species, site, season, and time of day. Assuming O_2 uptake provides a measure of *in situ*

respiration (due to the low solubility of O_2) and carbohydrates are the main substrate, values of ARQ averaging 0.59 indicate that on average 41% of the CO_2 produced by respiration was not locally emitted to the atmosphere, but apparently retained in the stem.

For sites where we have time series data for the same individuals, considerable variations in ARQ values were observed over two years in Brazil (Fig. S3) and over three years in Jerusalem (Fig. 4). A decrease in ARQ values was often observed during entrance to dormancy for the deciduous trees in Jerusalem, and an apparent minimum in ARQ for *P. atlantica* and *Q. calliprinos* in spring (Fig. 4). The autumn decrease seems to be in agreement with the finding of significantly lower ARQ for Bartlett Experimental forest, where leaves were beginning to senesce, compared to the more southerly Harvard forest, where leaves were still green.

The possibility of measurement artifacts as the source for the low ARQ values seems unlikely, as Hilman and Angert (2016a) previously demonstrated the validity of the measurement methods and the box-model approach. Further support comes from the small mean difference (0.02) between the "instantaneous" and "steady state" ARQ measured for the same tree, which reflects overall agreement between the measures. The considerable scatter around perfect agreement (Fig. 2), expressed also in RMSD of 0.15 could be taken as an indication that the measurement methods differ significantly. However, since the model assumes constant ARQ with time, and temporal changes in ARQ are obviously present as shown in Fig. 5 and 6, the scatter could also be attributed to temporal differences in the time integrated by the two types of measurement: the "instantaneous" sampling was typically conducted few days before the "steady state" sampling on the same tree. Additionally, the precision for "instantaneous" ARQ was lower than for “steady state” values, due to smaller changes in O_2 over the shorter time periods. This may also contribute to the scatter in Fig. 2 (Hilman and Angert, 2016a). We also found strong similarities between ARQ measured on intact stems using chambers and ARQ determined by incubating stem cores (Fig. 8). This provides another, indirect, confirmation that the low ARQ values obtained with the stem chamber measurement approaches are measuring something that is occurring in the stem tissues.

4.2 Dissolution and transport of respired CO_2 in xylem stream cannot explain the low ARQ values

Given the low solubility of O_2, stem flux ARQ values <1.0 (or potentially <0.7 for ‘fat’ trees) are the result of respired CO_2 either being exported from the site of respiration before it can be emitted to the atmosphere or being refixed during biosynthesis processes within the stem. As noted earlier, a second possibility is non-respiratory O_2 uptake, e.g. by oxidases and hydroxylases that are O_2 consuming enzymes, most notably used in lignin biosynthesis. However, stoichiometric analysis of this pathway shows that the CO_2 produced from the sucrose that is the lignin's substrate usually exceeds the O_2 consumption, so that the net effect of lignin biosynthesis should be a local increase in ARQ (Amthor, 2003). To the best of our knowledge, there are no other significant O_2 consuming processes in tree stems that might affect the ARQ value.

We conclude that the low stem ARQ must be the result of CO_2 being locally fixed or transported away from the site of respiration. If CO_2 dissolution and DIC transport is the main export mechanism, we would expect ARQ to increase with temperature (i.e. according to solubility changes with temperature), be anti-correlated with sap flow (McGuire and Teskey, 2004; McGuire et al., 2007; Bowman et al., 2005), and to increase with height in the stem (Hölttä and Kolari, 2009). Three observations support the idea that this export mechanism controls some of the variability in ARQ. First, nighttime ARQ in *M. domestica* was indeed correlated with temperature, an expected trend given the greater temperature sensitivity of the CO_2 solubility in comparison with O_2

(Gevantman, 2018). Second, the *P. occidentalis* had higher ARQ values in the upper stem position, especially during the growing season (Fig. 4). Third, relatively high ARQ values were observed at 0.2 m above the ground in the *S. micranthum* tree (Fig. 7), which may reflect a burst of in-stem CO_2 that originated from belowground respiration (McGuire and Teskey, 2004; Levy et al., 1999). However, in most of our observations ARQ did not vary as expected if CO_2 dissolution and transport were the main CO_2 export mechanism.

When sap flux density was measured directly, it did not explain the variation in ARQ among *Q. ilex* trees in Spain. Mean ARQ values were fairly stable over spring, summer and winter (0.62-0.69) for *Q. calliprinos* in the Carmel Ridge site, while the transpiration stream probably varied greatly between seasons if related to Ψ_{pd}. Additionally, during dormancy when no leaves were in place to force the transpiration stream, we found ARQ values <1.0 in four deciduous trees (black markers in Fig. 4). Sap flow rates are assumed to decline during the night, but ARQ values <1.0 during nighttime were measured in five species, and in most cases no nocturnal increase of ARQ in comparison to daytime values was observed (Fig. 5, 6). Thus, the temperature dependency observed for the *M. domestica* tree during the night, which explained variability in ARQ values between 0.65-0.75, must be a second order control on ARQ variability and cannot explain the big deviation from unity (according to the linear fit, an ARQ of 1.0 is expected at the unreasonable temperature of 63^{o}C). Also, the vertical transects of ARQ for *Q. calliprinos* and *S. micranthum*, including in-stem ARQ for the later (Fig. 4, 7, S5), showed no consistent pattern of ARQ increasing with stem height, unlike the ARQ increase with height measured in the *P. occidentalis* (Fig. 4).

ARQ values measured in the stem core incubations, where tissues are isolated from the influence of transport in the xylem stream, were well below 1.0 and similar to the chambers' values (Fig. 8, 9). The in-stem ARQ measured in the *S. micranthum* was likewise <1.0, but although the proximity to the xylem was greater, the values were not necessarily lower than the surface ARQ (Fig. 7). It is likely that in-stem ARQ values are influenced by dissolution in the xylem water, but the question is what is the contribution of in-stem CO_2 to the CO_2 efflux from the stem surface? There are contradicting assessments, and the influence likely is related to wood-anatomy. For example, studies of ring- and diffuse-porous species observed tight covariations of in-stem CO_2 and surface efflux and have interpreted this as evidence of strong influence of in-stem CO_2 concentrations (Teskey and McGuire, 2007; Steppe et al., 2007; Teskey and McGuire, 2002), while other studies conducted on conifers with tracheid anatomy inferred only marginal influence of in-stem processes on surface efflux (Ubierna et al., 2009; Maier and Clinton, 2006). Nevertheless, observations of covariation in in-stem [CO_2] and CO_2 efflux do not necessarily represent cause-and-effect relationships (Maier and Clinton, 2006). Muhr et al. (2013) utilized the difference in ^{14}C signature of in-stem CO_2 (5 cm deep) and surface efflux, to estimate that <20% of total emitted CO_2 originates from the inner stem in three tropical non-coniferous tree species. Small contribution of in-stem CO_2 to the surface efflux can be easily explained by the slow diffusion through wood of all three anatomical groups (Sorz and Hietz, 2006). The woody diffusional barrier can explain the apparent decoupling between ARQ, sap flux density, and Ψ_{pd} presented above. A major contribution from respiratory activity concentrated in the outer stem tissues to overall stem respiration would further reduce sap flow effects on surface fluxes (Hölttä and Kolari, 2009; Maier and Clinton, 2006; Ubierna et al., 2009).

An alternative explanation for low ARQ values could be the fixation of CO_2 by biosynthesis with engagement of the enzyme PEPC, which is able to fix respired CO_2. Indirect evidence for PEPC activity can be found in the increase of the ARQ values with time in our repeated incubations, while cellular activity was retained as

reflected in O_2 uptake rates (Fig. 9). Such a pattern may reflect a biochemical process, e.g. CO_2 fixation by the enzyme PEPC, which decreases with time due to self-inhibition by the accumulation of the products (Kai et al., 1999; Huber and Edwards, 1975). PEPC fixation rates can easily explain the retained CO_2, according to mass balance calculation for the stem cores incubations and published PEPC fixation rates in young tree stems (Table 2). Assuming refixation is important, the fact that ARQ measured from intact stems is almost always lower than unity indicates that the fixation products, organic acids like malate and citrate or amino acids, are not inhibiting the fixation or being oxidized locally, and are further metabolized or allocated elsewhere in the stem. The malate can be transported in the xylem stream as indicated by an upwards concentration increase in *Acer platanoides* stems (Schill et al., 1996). A possible fate of the malate might be similar to 'C4-like photosynthesis' observed in tobacco, where xylem-transported malate contributes C to photosynthesis in leaves (Hibberd and Quick, 2002). Alternatively, the fixation products might be exported via the phloem. One possible sink is excretion of organic acids to the rhizosphere as root exudates, which can account for ample fraction of overall GPP in forests (Abramoff and Finzi, 2016; Finzi et al., 2015). Indications for the transport of organic acids from upper parts of the plant to the roots have already been reported (Hoffland et al., 1992; Shane et al., 2004).

Overall, our results suggest that CO_2 dissolution and removal in the xylem stream are not the main cause of the low ARQ values that are common to the trees we measured. We speculate the observed ARQ values resulted by PEPC refixation, with possible cumulative effects of some dissolution and transport, partial lipid metabolism, and some non-respiratory O_2 consumption. Corticular photosynthesis may theoretically influence ARQ, but in complex manner; with assumed O_2/CO_2 exchange ratio of 1.0 and given all other processes yield ARQ <1.0, the photosynthesis will reduce the CO_2 and O_2 concentration gradients between stem-atmosphere in the same absolute numbers, which will cause to further ARQ decrease. However, in our measurements photosynthesis was prevented by shading the measured stem surface. Additionally, most evidence for significant corticualr photosynthesis come from twigs and young stems (Pfanz et al., 2002; Ávila et al., 2014), while stems in current study were mature. Wood anatomy may further impact ARQ by modifying the contribution of internal stem processes on surface fluxes. The numerous mechanisms potentially responsible to ARQ probably varied with the broad range of species and wood anatomies we investigated. Further research to pursue the potential role of PEPC, including direct measurement of PEPC activity, would be needed to assess whether PEPC plays a role in lowering ARQ values to the levels observed. To complete the stem C balance, additional evaluation of the relations between the in-stem and the stem surface fluxes are also needed, as well as analysis of how organic and amino acids vary in the stem.

4.3 Implications of low ARQ

From a whole ecosystem perspective, if respired CO_2 in the stem returns to the atmosphere elsewhere (e.g. in the soil, canopy), the overall ecosystem-atmosphere C fluxes will not be affected, and high ARQ associated with the release of the transported CO_2 will balance the low ARQ in the stem. Such ARQ >1.0 values are expected in the rhizosphere where organic acids are decomposed. In the canopy, greater refixation of internal C is expected to increase the photosynthetically oxidative ratio (O_2 produced/CO_2 consumed), as the internally transported C replaces the atmospheric CO_2 when assimilation is measured. Additionally, such internal transport can cause a discrepancy between the measured above-ground and below-ground CO_2 effluxes and the locations where respiration is actually occurring (Aubrey and Teskey, 2009), and lead to false attribution of respiration

responses to environmental conditions. Moreover, the different long-term temperature sensitivity of CO_2 efflux and O_2 influx is of interest, and might explain part of the gap between modeled and observed Q_{10} values of tree respiration (Griffin and Prager, 2017). For example, decrease in ARQ with rising temperature (due to higher PEPC activity for example) might result in a slow increase in CO_2 efflux, whereas the respiration rate (O_2 uptake) is actually increasing sharply, together with the internal C flux. Future studies should determine how temperature and nutrients control long term changes in ARQ, and aim to identify the biochemical process that control the low ARQ reported by the current study.

Author Contribution. B.H and A.A planned and designed the research. B.H performed most of the ARQ sampling and analysis, and led the writing of the manuscript. J.M, N.K and S.T carried out the field work in Brazil, and M.S.C carried out the field work in USA. P.Y measured shoot water potential. S.J.W designed the long term experiment in the Republic of Panama. G.M, O.P, M.M, and A.C contributed to the campaign in Spain. O.P measured the sap flux density. J.M.G and Y.O contributed to the campaigns in the Carmel Ridge. T.W contributed to the campaigns in Spain and Jerusalem. J.M, S.T, S.J.W, G.M, O.P, M.M, J.M.G and A.A contributed to the discussion and writing.

Data availability. Data used in this study can be found in figures, tables and in the Supplement.

Competing interests. The authors declare that they have no conflict of interest.

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653 **Table 1** Study sites, tree species sampled at each site, stem chambers, stems diameters, and experiments done in the site (A-

654 G list in section 2.2)**.**

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Site and coordinates	Species	Chamber type, sealant	Stem diameter (cm, mean ± SD (n))[a]	Experiments in the site (as listed in section 2.2) – dates of samplings (stem chamber measurement method: "steady state" (s), "instantaneous" (i), "continuous" (c))
Givat Ram campus, Jerusalem, Israel (31.77°N, 35.20°E)	*Populus deltoids* Bartr. Ex Marsh *Platanus occidentalis* L. *Pistacia atlantica* Desf. *Quercus calliprinos* Webb. *Malus domestica* Borkh. *Quercus ilex* L.	Perspex ®[b], hot glue Perspex ®, vacuum grease	60.2 (1) 43.4 (1) 21.2 (1) 24.3 (1) 16.3 (1) 20 ± 8 (4)	Seasonal and phenological measurements (A) and vertical transects for *P. occidentalis* and *Q. calliprinos* (F) - every 1-3 months between July 2011 and July 2014 (s, i). Day-night variation (C) - July 2012 and April 2013 (i). Day-night variation (C) and temperature effect on ARQ for the *M. domestica* (D) - April 2013 (c). Comparison between stem chambers and incubated stem cores ARQ, and repeated incubations of stem cores and leaves (G) - July 2016 (s, i).
Ramat Hanadiv Nature Park, Carmel Ridge, Israel (32.55°N, 34.94°E)	*Quercus calliprinos* Webb.	Perspex ®, hot glue	11.2 ± 1.2 (4)	Simultaneous measurements of ARQ and pre-dawn shoot water potential (E) - April 2012, September 2012, and January 2013 (s).
Bartlett Experimental forest, NH, USA (44.06°N, 71.29°W)	*Acer rubrum* L.	Polypropylene [c], caulking	20 ± 10 (4)	Comparison to Harvard forest based on different phenology (A) - September 2012.
Harvard forest, MA, USA (42.53°N, 72.17°W)	*Acer rubrum* L.	Polypropylene, caulking	18 ± 9 (3)	Comparison to Bartlett Experimental forest based on different phenology (A) - September 2012.
Majadas de Tiétar, Caceres, Spain (39°56'25" N, 5°46'28" W)	*Quercus ilex* L.	Perspex ®, vacuum grease	45 ± 7 (16)	Simultaneous measurements of ARQ and sap flux density (B) and comparison between stem chambers and incubated stem cores ARQ (G) - May 2015 (s, i).
Gigante peninsula, Barro Colorado Nature Monument, Republic of Panama (9°06'31" N, 79°50'37" W)	*Tetragastris panamensis* (Engl.) Kuntze	Perspex ®, vacuum grease	30.0 ± 12.5 (42)	Comparison between stem chambers and incubated stem cores ARQ (G) – September-October 2013 (s). Additional stem chamber ARQ measurements - September 2012, September-October 2013, March-April 2014 (s, i).
A station of the Brazilian National Institute for Research in the Amazon (INPA), north west of Manaus, Brazil (2°38'23" S, 60°09'51")	*Scleronema micranthum* (Ducke) Ducke	Polypropylene [d]	41.2 ± 13.3 (7)	Seasonal variability (A) - five campaigns between March 2012 and March 2014 (6 trees) (i in the two first campaigns, s in the three later campaigns). Vertical transects including in-stem measurements (F) - March and October 2012 (i). Ccomparison between stem chambers and incubated stem cores ARQ (G) - March 2014 (s).

656 [a]All chambers were installed at ~1.3 m above the ground, except for the *Q. calliprinos* on Carmel Ridge that

657 were placed near to the ground due to the shrubby canopy, the low branching of the trunk and the constraint of

658 the size of the chamber.

659 [b]Chambers were made of 10 cm × 12 cm Perspex® plate with four connectors to allow attachment of sampling

660 flasks. The chamber on the *M. domestica* was slightly larger, 12 cm × 19 cm, with six flasks connectors.

661 Chambers were placed on top of a closed cell foam frame that allowed an air-tight seal between the rigid

chamber and the uneven surface of the tree stem. We used nylon straps to compress the foam, while the sealant was applied between the foam and stem for ensuring the seal (Hilman and Angert, 2016a). Sealants were silicone based vacuum grease (Silicaid®1010 manufactured by Aidchim ltd., Raanana, Israel) or hot glue applied by a hot-glue gun.

[c]The chambers are described in (Muhr et al., 2013; Carbone et al., 2013). Briefly, the chambers were made from an opaque plastic polypropylene pipe T-fitting with fittings for sampling flasks. Sealants were caulking (Nautiflex; OASE GmbH, Oerel- Barchel, Germany) or hot glue applied by a hot-glue gun.

[d]Chambers were built from a 15 cm long piece of polypropylene (PP) tubing (6.5 cm OD) that was welded shut on both sides with a PP disc (6.7 cm diameter). By cutting off a segment (height 2 cm) the tube was turned into an incubation chamber. Opposite the chamber opening, three fittings (Sprint ESKV 20, Wiska, Germany) were installed and sealed around the edges with liquid rubber (Dichtfix, Bindulin, Fürth, Germany). For sampling, chambers were attached to the trees with 4 lashing straps. To achieve a gas tight seal, a frame (25 mm thick) made from closed-porous cellular rubber (EPDM-quality, REIFF Technische Produkte GmbH, Reutlingen, Germany) was placed between the chamber and the stem.

Table 2 Comparison between the calculated PEPC fixation rates required to explain measured ARQ in stem cores incubations and reported PEPC fixation rates for young stems.

	ARQ[a] (CO_2 efflux/O_2 uptake)	O_2 uptake[b] (nmol g.DW^{-1} s^{-1})	PEPC fixation rate required to explain the observed ARQ[c] (nmol CO_2 g.DW^{-1} s^{-1})	PEPC fixation rate[d] (nmol C g.DW^{-1} s^{-}1)
Quercus ilex (n =4)	0.44 ±0.08[c]	3.84 ±0.30	2.15	
Tetragastris panamensis (n =11)	0.33 ±0.07	1.40 ±0.69	0.93	
Fagus sylvatica L.				12.6
Pinus sylvestrys L.				16.74

[a]Values are mean ±SD

[b]Dry weight (DW) was determined after drying in an oven at 60°C for two days.

[c]Calculated as O_2 uptake × (1-ARQ), which is an estimation of the flux of respired CO_2 that didn't diffused out from the core. Based on the assumption that carbohydrates with ARQ =1 are the respiratory substrate.

[d]We calculated PEPC fixation rate of *Fagus sylvatica* L. with data from Berveiller and Damesin (2008) as follow:

PEPC activity (nmol C mg^{-1} chl s^{-1}) × total chl (mg g.DW^{-1}) = ~30 × 0.42 = 12.6 nmol C g.DW^{-1} s^{-1}

The chosen PEPC activity was the lowest among seasonal measurements.

We calculated PEPC fixation rate of *Pinus sylvestrys* L. with data from Ivanov et al. (2005) as follow:

PEPC activity (μmol C mg^{-1} chl min^{-1}) × total chl (μg g.FW^{-1}) × g.FW/g.DW (using assumed water content of 0.5) × conversion to seconds = 1.04 × 483.02 × 2 × 1/60 = 16.74 nmol C g.DW^{-1} s^{-1}

PEPC activity was measured during winter

Figure captions

Figure 1: Modelled changes in a tree stem chamber of the concentrations of CO_2, O_2, and the ratio between ΔCO_2 and ΔO_2, which are the changes in the gases concentrations from their initial values, and are also the difference in concentrations between the chamber and the atmosphere. The gas dynamics are based on a one-box model with arbitrary fluxes and ARQ (ratio of CO2 efflux/O2 influx for tree stems) = 0.5. The two time frames in which ARQ can be measured from the ratio $\Delta CO_2/\Delta O_2$ are indicated in the figure.

Figure 2: Scatter plot of "instantaneous" ARQ (ratio of CO_2 efflux/O_2 influx for tree stems) measured in stem chambers after incubation of 30 minutes to a few hours and "steady state" ARQ measured in the same experiment with typically two days of incubation (n = 139).

Figure 3: Summary of "steady state" ARQ (ratio of CO_2 efflux/O_2 influx for tree stems) for 12 species (n measurements, n individuals). Gases were sampled from chambers at breast height (~1.3 m above soil surface), except for the *Q. calliprinos* in the Mediterranean shrubland, in which chambers were placed near the stem base due to branching stems. Vertical lines are mean values, error bars represent one standard deviation, and colored bars represent the range of measured ARQ values. The Peru data is after Angert et al. (2012). The horizontal bars were ordered according to increasing mean ARQ.

Figure 4: Seasonal dynamics of "steady state" ARQ (ratio of CO_2 efflux/O_2 influx for tree stems) of five individual trees from five different species. Phenology stage index determined according to: "Defoliation"- from beginning of autumn color development to the end of the fall, "Winter dormancy"- when the tree was bare from leaves, "Leaf regeneration"- from bud burst to early leaf development stage. The *Q. calliprinos* is evergreen. Markers are mean values and error bars are SD of duplicate samples from the same stem chamber. Markers connected with solid lines represent measurements with chambers at breast height (~1.3 m above soil surface). Smaller markers connected with dashed lines represent measurements with chambers positioned at the stem base. The trees grew on Hebrew University campus in Jerusalem, Israel.

Figure 5: Instantaneous ARQ (ratio CO_2 efflux/O_2 influx of a stem, ± SD of duplicates) values measured over a day-night-day transition during July 2012 (a) and April 2013 (b) from different trees growing on Hebrew University campus in Jerusalem, Israel. *Quercus calliprinos* was measured at two different heights on the stem. First sampling was taken during daylight (day 1), next sampling before dawn (pre-dawn) and last sampling during daylight of the successive day (day 2).

Figure 6: Diurnal patterns of (a) O_2 influx to the stem and CO_2 efflux from the stem, (b) chamber temperature, and (c) instantaneous ARQ (ratio CO_2 efflux/O_2 influx for tree stems). Shaded areas indicate night periods.

Error bars are 95% confidence bounds. All data were obtained from a single *M. domestica* tree during 24-28 April 2013 on Hebrew University campus in Jerusalem, Israel.

Figure 7: Instantaneous ARQ (ratio of CO_2 efflux/O_2 influx for tree stems) measured from stem chambers installed at different heights above the ground on a *S. micranthum* tree in Brazil. At the same heights ARQ was measured from 4 cm in-stem probes. The measurements were conducted during 30 March and 18 October 2012. Error bars represents SD of duplicate samples from the same stem chamber.

Figure 8: Comparisons of stem chamber steady state ARQ (ratio CO_2 efflux/O_2 influx for tree stems) to ARQ measured from incubations of stem cores (ratio CO_2 increase/O_2 decrease), by species (n individuals) in different sites. Values are means ±SD.

Figure 9: (a) O_2 uptake rate (nmol $g.FW^{-1}$ s^{-1}) and (b) ARQ (ratio CO_2 increase/O_2 decrease) of *Q. ilex* leaves and stem cores incubated in a closed system (n =4). Values are means ± SD. Asterisks indicate significant difference between tissues at each time step (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$ in Student's t-test). Different letters indicate significant difference in Tukey-Cramer HSD analysis that followed one-way analysis of variance (ANOVA) within tissue type, between time steps.