



1 Comparison of CO₂ and O₂ fluxes demonstrate retention of 2 respired CO₂ in tree stems from a range of tree species

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22 **Abstract.** The ratio of CO₂ efflux to O₂ influx (ARQ, apparent respiratory quotient) in tree stems is expected to
23 be 1.0 for carbohydrates, the main substrate supporting stem respiration. In previous studies of stem fluxes, ARQ
24 values below 1.0 were observed and hypothesized to indicate retention of respired carbon within the stem. Here,
25 we demonstrate that stem ARQ <1.0 values are common across 85 tropical, temperate, and Mediterranean forest
26 trees from 9 different species. Mean ARQ values per species per site ranged from 0.39 to 0.78, with an overall
27 mean of 0.59. Assuming that O₂ uptake provides a measure of *in situ* stem respiration (due to the low solubility
28 of O₂), the overall mean indicates that on average 41% of CO₂ respired in stems is not emitted from the local stem
29 surface. The instantaneous ARQ did not vary with sap flow. ARQ values of incubated stem cores were similar to
30 those measured in stem chambers on intact trees. We therefore conclude that dissolution of CO₂ in the xylem sap
31 and transport away from the site of respiration cannot explain the low ARQ values. We suggest to examine
32 refixation of respired CO₂ in biosynthesis reactions as possible mechanism for low ARQ values.

33 1 Introduction

34 The global annual CO₂ efflux from tree stems to the atmosphere is estimated at 6.7 ±1.1 Pg C yr⁻¹ (Yang et al.,
35 2016), but the drivers of stem CO₂ efflux are not well understood (Trumbore et al., 2013). CO₂ in tree stems
36 originates primarily from aerobic respiration, which consumes oxygen (O₂). Respiratory quotient (RQ) is defined
37 as the ratio between produced CO₂ and consumed O₂, and its value is derived from the metabolized substrate.
38 Carbohydrates are believed to be the main respiratory substrate in tree stems (Hoch et al., 2003; Plaxton and
39 Podestá, 2006), and their metabolism results in an RQ of ~1.0. Metabolism that relies entirely on lipids yields an



40 RQ value of ~0.7, but significant storage of lipids in stems is uncommon and limited to several tree genera called
41 'fat-trees' (Sinnott, 1918). RQ values greater than 1.0 are associated with organic acid catabolism.
42 Initial measurements of the ratio of CO₂ efflux to O₂ influx from the stem surface for six tree species found values
43 mostly below 1.0 (the expected value for RQ from carbohydrate metabolism) (Angert and Sherer, 2011; Angert
44 et al., 2012). The flux ratio is referred to in those studies, and here, as the “apparent” RQ (ARQ), because it
45 potentially includes processes that incorporate CO₂ and/or O₂ in the stem in addition to the respiration taking place
46 in tissue beneath a chamber placed on the stem surface. Processes that can potentially reduce the emission of CO₂
47 and thereby decrease ARQ below 1.0 include dissolution and transport of CO₂ in the xylem sap (Teskey et al.,
48 2008), and carboxylating reactions during biosynthesis of compounds more oxidized than carbohydrates
49 (Lambers et al., 2008). Alternatively, it may be hypothesized that ARQ below 1.0 is the result of non-respiratory
50 O₂ uptake, e.g. by oxidases and hydroxylases that are O₂ consuming enzymes.
51 Carbon dioxide is ~30 times more soluble in water than O₂, and dissolved CO₂ reacts with water to form
52 bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions, further increasing the amount of dissolved inorganic carbon
53 (DIC). The rate of O₂ uptake is thus assumed to provide a better measure of stem respiration than CO₂ efflux,
54 which can be complicated by dissolution and transport within the xylem sap (Teskey et al., 2008), potentially
55 contributing to low ARQ values. If transport of CO₂ within the stem is important, ARQ measured at the stem
56 surface is expected to be inversely related to sap velocity. As the difference in solubility between CO₂ and O₂
57 decreases with increasing temperature (Gevantman, 2018), ARQ might be expected to increase with temperature
58 if all other factors remain constant. In addition, variations of ARQ with stem height are to be expected. A model
59 of CO₂ diffusion and advection in the xylem sap by Hölttä and Kolari (2009) predicted that the accumulation of
60 dissolved CO₂ in the ascending xylem sap, together with a reduction in stem diameter with height, induces faster
61 CO₂ diffusive loss to the atmosphere in the upper parts of the stem. Thus, an increase in ARQ (higher CO₂ loss
62 per mole of O₂ uptake) with stem height is expected. However, there is evidence from studies with an isotopically
63 labeled stem CO₂ pool that a significant portion of C is transported as DIC to photosynthetic tissues where it might
64 be refixed to organic C (Bloemen et al., 2013; McGuire et al., 2009; Powers and Marshall, 2011). To date, studies
65 of these processes in large trees are scarce, and it is not clear which process are responsible for low ARQ. If lower
66 than unity ARQ values are prevalent and result from processes that retain CO₂ in the stem, estimates of tree stem
67 respiration based on CO₂ efflux measurements must be reconsidered. Thus, the first objective of this work is to
68 determine whether ARQ values lower than 1.0 is observed in a variety of trees from different biomes and across
69 seasons. A secondary objective of this study was to test whether ARQ varies with xylem stream characteristics or
70 with tree height.

71 **2 Materials and Methods**

72 **2.1 Methods for evaluating ARQ**

73 We report tree stem ARQ results based on measurement methods described in (Hilman and Angert, 2016). These
74 methods overcome the difficulty of measuring small changes in O₂ against the high atmospheric background by
75 using a static stem chamber, in which the O₂ changes are considerably larger than in an open flow chamber.

76 We used three different approaches to measuring ARQ: two are based on discrete gas samples of headspace air,
77 and one based on direct measurement of instantaneous fluxes using gas sensors in the first hour after chamber



78 sealing. Discrete gas samples are either taken within 30 minutes to few hours after chamber sealing
79 (“instantaneous” sampling) or after the chamber has been sealed to the stem for more than 24 hours, once steady
80 state conditions have been achieved (“steady state”). These timings were confirmed by continuous measurements
81 with sensors (Hilman and Angert, 2016).

82 2.1.1 ARQ measurement from discrete samples

83 The evaluation of ARQ from discrete gas measurements is based on a one-box model that describes gas dynamics
84 in the headspace of a static chamber sealed to the surface of a tree stem (Angert and Sherer, 2011; Angert et al.,
85 2012; Hilman and Angert, 2016). In the model, the gas in the chamber headspace has initial mean atmospheric
86 values (20.95% O₂, 0.04% CO₂), ensured by flushing the chamber with ambient air before measurement. Once
87 the chamber is closed and the headspace above the stem surface is isolated, metabolic reactions in the stem control
88 the chamber's air composition. For the first few hours, headspace concentrations of CO₂ increase and O₂ decrease
89 at rates that are roughly linear with time (“instantaneous” incubation, Fig. 1,S1). During this linear stage, ARQ is
90 calculated by:

$$91 \text{ARQ} = \frac{\text{CO}_2 \text{ efflux}}{\text{O}_2 \text{ influx}} = \frac{\Delta\text{CO}_2}{\Delta\text{O}_2} \quad (1)$$

92 where ΔCO_2 and ΔO_2 are the changes in [CO₂] and [O₂] during the initial period after the chamber was sealed,
93 and for discrete samples can also be determined from the difference in concentrations between the chamber air
94 sampled at a specific time and the initial atmosphere. “Instantaneous” fluxes of CO₂ and O₂ reported here are
95 obtained either by monitoring concentration change during the first hour following chamber closure with sensors
96 directly in the field or by sampling headspace air with glass flasks within 30 minutes to a few hours of closing the
97 chamber. The flasks were transported to the laboratory for measurement of CO₂ and O₂.

98 After the first hours, the initially linear rates of change in headspace gas concentration with time decline, and
99 concentrations eventually remain constant (Fig. 1,S1). In this phase the gases in the chamber and the outer part of
100 the stem, where most of the metabolism takes place, are assumed to be in equilibrium. This “steady state” occurs
101 when the rates of addition of CO₂ and loss of O₂ from the stem to the chamber headspace are balanced by diffusive
102 (assuming no strong wind) exchange of headspace air with outside air through porous portions of the outer stem.
103 For “steady state” samples, the chamber is sealed to the surface of the stem and left for a period longer than 24
104 hours, after which the headspace air is sampled using glass flasks. The CO₂ and O₂ concentrations must be
105 corrected for differences in diffusivity between CO₂ and O₂, as detailed in (Angert and Sherer, 2011; Angert et
106 al., 2012; Hilman and Angert, 2016) in order to estimate the ratio of the gas fluxes from the concentrations in the
107 static chamber:

$$108 \text{ARQ} = \frac{g_{\text{CO}_2} \times \Delta\text{CO}_2}{g_{\text{O}_2} \times \Delta\text{O}_2} \quad (2)$$

109 where g_{CO_2} and g_{O_2} are the CO₂ and O₂ conductance values in the outer layer of the stem between the chamber
110 and the atmosphere. The structure of the path along which diffusion occurs is the same for CO₂ and O₂ and hence
111 the conductance ratio $g_{\text{CO}_2}/g_{\text{O}_2}$ depends solely on the ratio of diffusivities of the gases in air, which is 0.76
112 (Massman, 1998). As a result, at steady state:

$$113 \text{ARQ} = 0.76 \times \frac{\Delta\text{CO}_2}{\Delta\text{O}_2} \quad (3)$$

114 Hilman and Angert (2016) demonstrated excellent agreement for direct comparisons of the “instantaneous” and
115 “steady state” measurement methods, and the results are further compared here.



116 The data we report here were collected in different sites and over different years, and chamber designs and
117 methods applied varied from site to site, as described in Sect. 2.2 and in Table 1. In all cases, a chamber is attached
118 to the surface of the stem with an air-tight seal (using a sealant in most cases – see Table 1 for details). Ports (to
119 which sampling flasks can later be attached) or a separate lid with ports allow the chamber to remain open to the
120 atmosphere when not in use; openings are covered with screen to prevent insect damage inside the chambers. For
121 a measurement, the chamber is first flushed with ambient air using a syringe, then all openings are closed, and
122 CO₂ is allowed to accumulate (and O₂ to be consumed) in the headspace trapped within the chamber. The
123 chambers contain sampling ports to which glass flasks equipped with O-ring valves (LouwersHanique, Hapert,
124 The Netherlands) are attached. Initially the valves are open. Air from the chambers is sampled passively by closing
125 the valves. For “steady state” field measurements, two glass flasks are connected to a stem chamber and closed
126 after at least one day of incubation. For “instantaneous” ARQ, the valves are closed after shorter incubation
127 periods (30 minutes to a few hours).

128 The flasks were analyzed in the laboratory at the Hebrew University in Jerusalem in a closed system [The
129 *Hampadah* (Hilman and Angert, 2016)]. Two analyzers are included in the *Hampadah* system; an infra-red gas
130 analyzer (IRGA) for CO₂ measurement (LI 840A LI-COR; Lincoln, NE, USA) and a fuel-cell based analyzer
131 (FC-10; Sable Systems International, Las Vegas, NV, USA) for measuring O₂. The principle of operation of the
132 *Hampadah* is measurement of the change in CO₂ and O₂ concentrations in the system's air after flask opening,
133 and calculation of the concentration in the flask that would yield such change.

134 2.1.2 Continuous ARQ measurements

135 Sensitive detection of small changes in O₂ is difficult in the field, which is why we used the flask samples and
136 long chamber closure times (“steady state”) in most field sites. However, to measure diurnal changes in stem ARQ
137 values of *Malus domestica*, we were able to make continuous measurements with a small IRGA CO₂ sensor
138 (COZIR Wide Range 0-20% CO₂ Sensor, CO2Meter, Inc.) and a quenching based optode (Fibox 3, PreSens-
139 Precision Sensing) for O₂ measurement (Hilman and Angert, 2016). The sensors' reading was extracted every 30
140 seconds. A temperature sensor was placed next to the optode sensor for temperature and water vapor corrections.
141 The inlet of a small diaphragm pump (KNF micro-pump) and a non-return valve (SMC AKH 12mm, RS, UK)
142 were connected to the chamber headspace, for periodic automatic venting of the chamber every 4 hours. The CO₂
143 efflux and the O₂ influx were calculated using a linear fit over ~120 gas concentration measurements during the
144 first hour of incubation, the chamber volume, and the stem surface area under the chamber. We used the data from
145 this experiment to examine the sensitivity of ARQ to temperature, which affects the gas solubility constants. The
146 strongest effects are expected during the night, when daytime influences on stem fluxes associated with sap flow
147 and low turgor pressure (Salomón et al., 2018) are minimized.

148 For each site and experiment described below, we identify the method used to estimate ARQ as
149 “instantaneous”, “steady state” (for flask samples) or “continuous”.

150 2.2 Study sites and experimental design

151 For addressing our first goal of determining the variation in stem ARQ values across a range of tree species and
152 environments, we measured ARQ in trees located in tropical forests in the Republic of Panama and in Brazil, in
153 temperate forests in the northeast US (Bartlett and Harvard forests), in Mediterranean savanna in Spain, and in



154 Israel where we sampled various species on the Hebrew University campus in Jerusalem and the adjacent
155 Botanical Gardens, and in natural Mediterranean shrubland that is located on the Carmel Ridge (Table 1, Fig. 3).
156 Seasonal measurements were performed in Jerusalem, US, and Brazil sites. In Jerusalem, five individual trees
157 from five different species (first five species in Table 1) were measured every 2-3 months between December-
158 February 2011 and July 2014, except for the *M. domestica*, which was measured at monthly intervals between
159 July 2011 and July 2013 (“steady state”). Phenology of the deciduous trees (all except *Quercus calliprinos*) was
160 classified into four groups (Fig. 4). In addition, in the same site, we sampled four *Quercus ilex* trees in July 2016
161 (“steady state”). Five individuals of *Acer rubrum* were measured at each of the sites in the US in September 2012
162 (“steady state”). Trees at the northern site (Bartlett Experimental Forest) had fall color development, while leaves
163 at Harvard Forest were still green. We questioned if ARQ would vary with the phenological differences. After
164 analysis we excluded results from three trees because of suspected air leakage from the chamber ($O_2 > 20\%$ after
165 six days of stem incubation). In Brazil six *Scleronema micranthum* trees were measured in five campaigns
166 between March 2012 and March 2014. In the two first campaigns “instantaneous” ARQ was measured, while
167 “steady state” ARQ was measured in the three later campaigns. After analysis we excluded results from four
168 measurements because of a weak signal ($O_2 > 20.7\%$ and $SD > 0.1$ after 3 h of incubation). In Panama we sampled
169 42 *Tetragastris panamensis* trees (“steady state”) in three campaigns: September 2012, September-October 2013,
170 March-April 2014. Some individuals were sampled more than once. The trees grew in plots that were part of a
171 fertilization and litter manipulation projects (Wright et al., 2011; Sayer and Tanner, 2010). No treatment effects
172 were found (Fig. S2). In Spain we sampled 16 *Q. ilex* trees during May 2015 (“steady state”). On Carmel Ridge
173 we sampled ARQ of four *Q. calliprinos* trees (“steady state”) during April 2012, September 2012, and January
174 2013.

175 For our second objective, to explore the potential for low ARQ values to reflect dissolution and transport
176 of CO_2 in the xylem sap, we measured instantaneous ARQ at varying sap flow velocities and in different times of
177 a day. Transport of CO_2 was previously reported to be correlated with sap flow (McGuire and Teskey, 2004;
178 Bowman et al., 2005; McGuire et al., 2007). Thus, anti-correlation of ARQ with sap flux, expressed in maximal
179 values during night in diel course, would provide evidence to support the transport of locally respired C as DIC.
180 We also measured vertical transects of ARQ including in-stem measurements, using chambers and probes placed
181 at different heights on a single stem, and in incubations from stem cores. We performed a number of experiments:
182 1) ARQ (“instantaneous”) was measured simultaneously with sap flux density measurements in nine *Q. ilex* trees
183 with similar diameter (0.35 to 0.49 m at breast height) in the site in Spain.
184 2) At sites where we could not measure sap flux, we measured day-night variation in ARQ. ARQ
185 (“instantaneous”) was measured during daytime, at pre-dawn when the transpiration stream should reach its
186 minimum, and again during the next day. We conducted two day-night campaigns on the trees at the site in
187 Jerusalem, during July 2012 and April 2013. Additionally, during 24-28 April 2013, ARQ values were measured
188 every 4 h from the *M. domestica* tree in Jerusalem (“continuous”).
189 3) ARQ (“steady state”) was measured over spring, summer and winter in the *Q. calliprinos* trees on Carmel Ridge
190 site, simultaneously with pre-dawn shoot water potential (Ψ_{pd}). Ψ_{pd} is a measure for available soil water and
191 therefore is also a rough proxy for seasonal differences in transpiration rates (Aranda et al., 2005; Bucci et al.,
192 2005).



193 4) ARQ was measured at different heights on the same tree stem from the stem surface using stem chambers, and
194 also from inside the stem. In the ARQ seasonal measurements in Jerusalem, the *Q. calliprinos* and the *Platanus*
195 *occidentalis* trees were measured at their stem base, in addition to the breast height measurement ("steady state").
196 In Brazil, we measured "instantaneous" ARQ from stem chambers installed up to 11 m above the ground on a
197 single *S. micranthum* tree. To evaluate the influence of the internal ARQ on the surface ARQ, we measured in the
198 same tree in-stem gas concentrations and ARQ.

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

203 2.3 Sap flux density

204 Sap flux density was monitored in 9 trees at the site in Majadas de Tietar (Spain) using heat ratio method (HRM)
205 sensors (SFM1 Sap Flow Meter, ICT International). A description of the installation and measurement is presented
206 in Methods S1. The detailed procedures for sap flux corrections and calculations are described in (Perez-Priego
207 et al., 2017). We tested, whether the daily maximum sap flux density (i.e. average of measurements between 10:00
208 and 17:00 during the day of the ARQ measurement), which correlated with CO₂ dissolution fluxes (Bowman et
209 al., 2005), would explain variability in "instantaneous" ARQ.

210 2.4 Shoot water potential

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

215 2.5 In-stem measurements

216 For sampling gas from inside the stem, stainless-steel tubes (1.3 cm diameter) were installed 4, 8, and 12 cm deep
217 into the stem, in various stem heights on the same *S. micranthum* tree in Brazil where the vertical ARQ transects
218 were measured. Installation procedure was according to Muhr et al. (2013) and tubes were sealed between
219 sampling dates. Using rubber tubing we connected the sampling flasks to the tubes for incubation of 4 days. The
220 flasks were then analyzed for CO₂ and O₂ in the *Hampadah*. Assuming steady state, ARQ was calculated using
221 Eq (3) (Angert et al., 2012).

222 2.6 Measuring ARQ of incubated tissues

223 Stem cores were extracted in Panama, Spain, and Jerusalem using 1.2 cm diameter cork borer, right after the
224 chamber incubation experiment. The outer bark and green tissues, as well as sapwood sieves (with paler color
225 than the phloem tissues), were removed from the cores. The cores were cut to fit into the incubation system,
226 wrapped with moist gauze cloth to avoid desiccation, and inserted into gas-tight set of flasks (two or three)
227 connected by Swagelok Ultra-Torr fittings (Swagelok, Solon, OH, USA, Fig. S3). At the end of the incubation



228 period, the flasks were closed and analyzed in the *Hampadah*. Since the incubations took place in a closed system,
229 the change with time in $[\text{CO}_2]$ and $[\text{O}_2]$ are assumed to be linear, and ARQ can be calculated using Eq (1).

230 In Panama and Spain the incubations started immediately upon core extraction, at ambient temperature, and lasted
231 8 h and 3 h, respectively. In Israel, the *Q. ilex* cores were kept on moist gauze cloth for 2 h before being sealed in
232 the incubation system that were kept at 25°C in environmental chamber. Repeated incubations were performed in
233 series, with the incubation systems flushed in between with ambient air. Simultaneously, from each tree, four
234 leaves from an understory branch were cut and inserted into the same incubation systems, for the same incubation
235 durations. The O_2 uptake rate ($\text{nmol O}_2 \text{ g.FW}^{-1} \text{ s}^{-1}$) was calculated as follows [adopted from Pruyt et al. (2002a)]:

$$236 \text{ O}_2 \text{ uptake rate} = \frac{\Delta\text{O}_2}{100} \times \frac{V_H}{T \times M_{\text{FW}} \times V_m} \times 10^9 \quad (4)$$

237 where ΔO_2 is the decrease in $[\text{O}_2]$ during the incubation, V_H is volume of headspace (ml), T is incubation period
238 (s), M_{FW} is fresh weight (g), V_m is the molar volume, and 10^9 converts units to nmol.

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

244 2.7 Statistical analysis

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

252 3 Results

253 The ARQ estimated from “instantaneous” and “steady state” measurements were in good agreement over a large
254 range of ARQ (Fig. 2). The average “steady state” ARQ value across all species and sites, including results from
255 (Angert et al., 2012), was 0.59 ($n = 229$) and the average ARQ of species in the different sites ranged between
256 0.39 and 0.78 (Fig. 3). For individual measurements, a minimum ARQ value of 0.27 was recorded for *Q. ilex* in
257 Spain and for *T. panamensis*. The highest value was 0.99 for *M. domestica* and *Populus deltoids*.

258 Phenology or seasonality had some effect on ARQ (Figure 4). In Brazil, ARQ varied between 0.41 ± 0.15 in the
259 wetter season and 0.82 ± 0.12 in the drier season. In Jerusalem, the ARQ of *Q. calliprinos* and *Pistacia atlantica*
260 had lowest values during spring and highest values in fall and winter (Fig. 4). The average ARQ of the *A. rubrum*
261 trees at Harvard Forest, where all leaves were green, was significantly greater than the average ARQ of the trees
262 at Bartlett Experimental Forest, where the leaves had autumn color development (0.69 vs. 0.57 , $P < 0.05$ in a
263 Student's t test).



264 3.1 ARQ values under varying xylem stream flow and temperature

265 Instantaneous ARQ values of nine *Q. ilex* trees were invariable (mean \pm SD of 0.42 ± 0.04) in comparison with the
266 larger variation in maximum daily sap flux density among these trees ($0.15 \pm 0.05 \text{ m}^3 \text{ H}_2\text{O m}^{-2} \text{ h}^{-1}$), and no
267 correlation was found between the ARQ and sap flux density ($r^2=0$, $P=0.9891$).

268 Mean ARQ \pm SD values ("steady state") of the oaks at the Carmel Ridge site were 0.62 ± 0.06 , 0.68 ± 0.07 and 0.69
269 ± 0.08 for spring, summer and winter, respectively. Repeated-measures analysis of variance found no significant
270 difference between seasons ($F_{2,2}=2.52$, $P=0.28$), while Ψ_{pd} varied significantly with seasons ($F_{2,2}=207.85$, P
271 $=0.0048$). During summer, Ψ_{pd} was -2.65 MPa, much lower than the spring and winter values (-0.64 and -0.86
272 MPa, respectively).

273 In the day-night campaigns done at Hebrew University and the adjacent arboretum, ARQ
274 ("instantaneous") values ranged between 0.52 and 1.05, across all trees, seasons, and sample times (Fig.5). Pre-
275 dawn ARQ values higher than daylight values (beyond the duplicates error) were observed during the summer in
276 *M. domestica* and in the upper chamber on *Q. calliprinos*. No significant diurnal effect was found in repeated-
277 measures analysis of variance of the breast height chambers, neither when results of all the trees was grouped by
278 season, nor when results were grouped by stem chamber. In "continuous" measurement of *M. domestica*, ARQ
279 value obtained every 4 hours. ARQ during night was not significantly higher than the day time ARQ value [P
280 >0.76 in a student's t test, 0.70 ($n=12$) vs 0.71 ($n=11$) respectively, Fig. 6]. The variations among the nighttime
281 values were best fitted with temperatures measured 235 minutes before measurement ($r^2=0.84$, $P=0.0001$, ARQ
282 $=0.01 \times \text{Temperature (C}^\circ) + 0.54$). With the same time lag, the coefficient of determination for the daytime values
283 is $r^2=0.44$ ($P=0.0266$).

284

285 3.2 Stem surface and in-stem ARQ vertical transects

286 In *Q. calliprinos*, measured over three years, ARQ did not differ significantly ($P>0.33$ in student's t test) between
287 breast height and stem base (ARQ of 0.56 vs. 0.59 respectively, $n=14$, Fig. 4). For *P. occidentalis* measured for
288 the same period the ARQ measured at breast height was significantly higher than ARQ measured at the stem base
289 (0.74 vs. 0.64 respectively, $n=12$, $P=0.003$ in student's t test, Fig. 4). For a single *S. micranthum* tree in Brazil,
290 ARQ values measured at heights of 6.5 m and 11 m above the ground were similar to ARQ measured at breast
291 height (Fig. 7), but also show differences with the stem base. In this tree, ARQ measured in March (0.46 ± 0.11)
292 was lower than in October (0.89 ± 0.16). The in-stem ARQ values ranged between 0.25 and 0.56, with average
293 \pm SD of 0.46 ± 0.07 in both seasons and at all stem positions and depths. The in-stem ARQ, as well as $[\text{CO}_2]$ values,
294 had no clear vertical trend (Fig. 7,S4).

295 3.3 Tissue incubations

296 The average ARQ values of the stem core incubations were similar to the stem chamber ARQ for the four
297 sites/trees where these comparisons were made (Fig. 8). In the time series incubations of *Q. ilex* stem cores and
298 leaves, significant effects of time, tissue (leaves, stem cores), and their interactions (time \times tissue) on ARQ and
299 O_2 uptake rates were observed. ARQ of the stem cores increased from 0.44 ± 0.08 (mean \pm SD, $n=4$) after 3 h to
300 0.94 ± 0.03 at the end of the experiment (32 h; Fig. 9). The ARQ of incubated leaves of the same trees showed
301 higher initial ARQ of 0.80 ± 0.02 , with an increase over time to 0.92 ± 0.02 .



302 4 Discussion

303 4.1 ARQ is lower than 1.0 for a wide range of tree species

304 The ARQ measured in stem chambers installed on 85 individual trees of 9 species including tropical, temperate
305 and Mediterranean forest trees was considerably and almost universally lower than 1.0. ARQ values as low as 0.7
306 could indicate that lipids were used exclusively as substrates for respiration. Lipids respiration is often associated
307 with environmental stresses, for example, initial RQ of ~1 measured in branches of *Pinus sylvestris* L. declined in
308 response to 11 days of shading and drought treatments to values of 0.77-0.75, reflecting mixture of substrates (Hanf
309 et al., 2015). However, many ARQ values are below 0.7, so substrate use alone cannot explain them. Additionally,
310 as ARQ values above 1.0 are expected when lipids are produced (De Vries et al., 1974), ARQ <1.0 resulting from
311 lipid metabolism must be mirrored with ARQ >1.0 at a different time (assuming the lipids are produced locally).
312 However, ARQ almost never exceeded 1.0. The results demonstrate that O₂ influx to the stems usually exceeded
313 the CO₂ efflux, regardless of tree species, site, season, and time of day. Assuming O₂ uptake provides a measure
314 of *in situ* respiration (due to the low solubility of O₂), values of ARQ averaging 0.59 indicate that on average 41%
315 of the CO₂ produced by respiration was not locally emitted to the atmosphere, but apparently retained in the stem.
316 For sites where we have time series data for the same individuals, considerable variation in ARQ values was
317 observed over two years in Brazil and over three years in Israel. A decrease in ARQ values was often observed
318 during entrance to dormancy for the deciduous trees in Jerusalem, and an apparent minimum in ARQ for *P.*
319 *atlantica* and *Q. calliprinos* in spring (Fig. 4). This seems to be in agreement with the findings of significantly
320 lower ARQ for Bartlett forest, where leaves were beginning to senesce, compared to the more southerly Harvard
321 forest, where leaves were still green.

322 The possibility of measurement artifacts as the source for the low ARQ values seems unlikely, as Hilman
323 and Angert (2016) demonstrated the validity of the measurement methods and the box-model approach. Further
324 support comes from the slope (1.006) of the linear regression of "instantaneous" ARQ vs. "steady state" ARQ
325 measured for the same tree, which is extremely close to 1 (Fig. 2). The considerable scattering in the regression
326 may be attributed to temporal differences in the time integrated by the two types of measurement: the
327 "instantaneous" sampling was typically conducted few days before the "steady state" sampling on the same tree,
328 and to lower precision in "instantaneous" samples due to smaller changes in O₂ over the shorter time periods
329 (Hilman and Angert, 2016). We also found strong similarities between the ARQ measured for intact stems with
330 chambers and by incubating cores (Fig. 8), which provides another, indirect, confirmation that the low ARQ
331 values obtained with the stem chamber measurement approaches are measuring something that is occurring in the
332 stem tissues.

333 4.2 Dissolution and transport of respired CO₂ in xylem stream cannot explain the low ARQ values

334 Given the low solubility of O₂, stem flux ARQ values <1.0 (or potentially 0.7 for 'fat' trees) are either result of
335 respired CO₂ that is exported from the site of respiration before it can be emitted to the atmosphere or refixed in
336 biosynthesis processes. As noted earlier, a second possibility is non-respiratory O₂ uptake, e.g. by oxidases and
337 hydroxylases that are O₂ consuming enzymes, most notably used in lignin biosynthesis. However, stoichiometric
338 analysis of this pathway shows that the CO₂ produced from the sucrose that is the lignin's substrate usually exceeds
339 the O₂ consumption, so that the net effect of lignin biosynthesis should be a local increase in ARQ (Amthor,



340 2003). To the best of our knowledge, there are no other significant O₂ consuming processes in tree stems that
341 might affect the ARQ value.

342 We conclude that the low ARQ much be the result of CO₂ being locally fixed or transported away from
343 the site of respiration. If CO₂ dissolution and DIC transport is the main export mechanism, we would expect ARQ
344 to be related to temperature (i.e. according to solubility changes with temperature), anti-correlated with sap flow
345 (McGuire and Teskey, 2004; McGuire et al., 2007; Bowman et al., 2005), and further that ARQ should increase
346 with height in the stem (Hölttä and Kolari, 2009). Three observations support the idea that this export mechanism
347 controls some of the variability in ARQ. First, in the diurnal measurements of the *M. domestica*, the nighttime
348 ARQ results were indeed correlated with temperature, an expected trend given the greater temperature sensitivity
349 of the CO₂ solubility in comparison with O₂ (Gevantman, 2018). Second, the *P. occidentalis* had higher ARQ
350 values in the upper stem position, especially during the growing seasons (Fig. 4). Third, relatively high ARQ
351 values were observed at 0.2 m above the ground in the *S. micranthum* tree (Fig. 7), which may reflect a burst of
352 in-stem CO₂ that originated from belowground respiration (McGuire and Teskey, 2004; Levy et al., 1999).
353 However, in most of our observations ARQ did not vary as expected if CO₂ dissolution and transport were the
354 main CO₂ export mechanism.

355 When sap flux density was measured directly, it did not explain the variation in ARQ among *Q. ilex* trees
356 in Spain. Mean ARQ values were fairly stable over spring, summer and winter (0.62-0.69) for *Q. calliprinos* in
357 the Carmel Ridge site, while the transpiration stream probably varied greatly between seasons if related to Ψ_{pd} .
358 Additionally, during dormancy when no leaves were in place to force the transpiration stream, we found ARQ
359 values <1.0 in four deciduous trees (black markers in Fig. 4). Transpiration streams are also assumed to decline
360 during the night, but ARQ values <1.0 during nighttime were measured in five species, and in most cases no
361 nocturnal increase of ARQ in comparison to daytime values was observed (Fig. 5,6). Thus, the temperature
362 dependency observed for the *M. domestica* tree during the night, which explained variability in ARQ values
363 between 0.65-0.75, must be a second order control on ARQ variability and cannot explain the big deviation from
364 unity (according to the linear fit, an ARQ of 1.0 is expected at the unreasonable temperature of 63°C). Also, the
365 vertical transects of ARQ for *Q. calliprinos* and *S. micranthum*, including in-stem ARQ for the later (Fig. 4,7,S4),
366 showed no consistent pattern of ARQ increasing with stem height, unlike the ARQ increase with height measured
367 in the *P. occidentalis* (Fig. 4). Likewise, no trend of in-stem [CO₂] increase with stem height was observed,
368 suggesting no CO₂ accumulation in the ascending xylem sap (Fig. S4).

369 The in-stem ARQ measured in the *S. micranthum* ranged between 0.25 and 0.56. Even lower values,
370 with typical ARQ of 0.13-0.18, have been reported before (Angert et al., 2012). These low values are consistent
371 with in-stem measurements of small CO₂ increases and large O₂ reductions in comparison to atmospheric
372 concentrations (Pruyn et al., 2002b; Eklund, 1990; Eklund, 1993). Thus, one can speculate that the low ARQ
373 values at the trunk surface are the reflection of the low ARQ in the sapwood itself. However, there are
374 contradicting assessments about the influence of in-stem CO₂ on the CO₂ efflux from the stem surface; while
375 some studies interpreted tight covariations of in-stem CO₂ and surface efflux as strong in-stem influence (Teskey
376 and McGuire, 2007; Steppe et al., 2007; Teskey and McGuire, 2002), other studies inferred only marginal
377 influence of in-stem processes on surface efflux (Ubierna et al., 2009; Maier and Clinton, 2006). Unlike
378 covariation observations, which do not necessarily represent cause-and-effect relationships (Maier and Clinton,
379 2006), Muhr et al. (2013) utilized the difference in ¹⁴C signature of in-stem CO₂ (5 cm deep) and surface efflux



380 to estimate that <20% of total emitted CO₂ originates from the inner stem. To assess the potential influence on
381 ARQ measured at the surface, we used a two pool model for sources of the surface CO₂ efflux: (1) an in-stem
382 CO₂ pool that is affected by sap flow transport, and (2) CO₂ produced locally by stem tissues (mostly close to the
383 stem surface) fully released to the atmosphere. Using the results of the *S. micranthum*, we can evaluate the
384 contribution of the in-stem ARQ to the stem surface ARQ. The mean ARQ values for the 4 cm deep probes and
385 for the surface were ~0.5 and 0.67, respectively. Assuming 20% of the CO₂ efflux comes from the in-stem due
386 the diffusive gradient between the sapwood and the atmosphere, the other 80% comes from metabolism in the
387 stem tissues close to the stem surface. With an in-stem ARQ of 0.5, which means that the O₂ influx induced by
388 concentration gradient is twice the CO₂ flux after correcting for relative diffusivity, the O₂ influx between the
389 atmosphere and the inner-stem would be equivalent to 40% of the CO₂ efflux. To explain the overall ARQ
390 (measured at the stem surface) of 0.67, the O₂ influx to the stem tissues must therefore be the equivalent of 110%
391 of the CO₂ efflux, i.e. the total ARQ representing the sum of fluxes from diffusion and metabolism would be
392 $(20+80)/(40+110) = 0.67$. The ARQ of the fluxes stem tissue metabolism alone would be $80/110 = 0.73$, which is
393 still lower than unity.

394 ARQ values <1.0 observed in stem core incubations, where tissues are isolated from the influence of
395 transport (Fig. 8,9) further support our conclusion that ARQ resulting from local metabolism of the stem tissue
396 near the surface is <1.0. In light of that, the apparent decoupling between ARQ, sap flux density, and Ψ_{pd} presented
397 above might be derived from strong diffusion barriers that restrict gas exchange between the sapwood and the
398 external cambium, phloem, and bark tissues (Ubierna et al., 2009). A major contribution from respiratory activity
399 concentrated in the outer stem tissues to overall stem respiration would further reduce sap flow effects on surface
400 fluxes (Hölttä and Koları, 2009; Maier and Clinton, 2006; Ubierna et al., 2009).

401 Overall, our results suggest that CO₂ dissolution and removal in the xylem stream are not the main cause of the
402 low ARQ values that are common to the trees we measured. At the same time, observed ARQ values may be
403 influenced by the cumulative effects of some dissolution and transport, partial lipid metabolism, and non-
404 respiratory O₂ consumption. One potential explanation for low ARQ values could be biosynthesis with
405 engagement of the enzyme phosphoenolpyruvate carboxylase (PEPC), which is able to fix respired CO₂. Indirect
406 evidence for PEPC activity can be found in the increase of the ARQ values with time in our repeated incubations,
407 while cellular activity was retained as reflected in O₂ uptake rates (Fig. 9). Such a pattern may reflect a biochemical
408 process, e.g. C fixation by the enzyme PEPC, that decreases with time due to self-inhibition by the accumulation
409 of the products (Kai et al., 1999; Huber and Edwards, 1975). Based on mass balance calculation for the stem cores
410 incubations and published PEPC fixation rates in tree stems (Table 2), PEPC fixation rates can easily explain the
411 retained CO₂. The fixation products, organic and amino acids, may be exported via the xylem stream and/or via
412 the phloem (Hoffland et al., 1992; Schill et al., 1996). Further investigation into the potential role of PEPC,
413 including direct measurement of PEPC activity, would be needed to assess whether PEPC plays a role in lowering
414 ARQ values to the levels observed. To complete stem carbon balance, additional evaluation of the relations
415 between the in-stem and the stem surface fluxes are also needed, as well as analysis of how organic and amino
416 acids vary in the stem.

417 **4.3 Implications of low ARQ**

418 From a whole ecosystem perspective, if respired CO₂ in the stem returns to the atmosphere elsewhere (e.g. in the
419 soil, canopy), the overall ecosystem-atmosphere carbon fluxes will not be affected, and high ARQ associated with
420 the release of the transported CO₂ will balance the low ARQ in the stem. However, such internal transport can
421 cause a discrepancy between the measured above-ground and below-ground CO₂ effluxes and the locations where
422 respiration is actually occurring (Aubrey and Teskey, 2009), and lead to false attribution of respiration responses
423 to environmental conditions. Moreover, the different long-term temperature sensitivity of CO₂ efflux and O₂ influx
424 is of interest, and might explain part of the gap between modeled and observed Q₁₀ values of tree respiration
425 (Griffin and Prager, 2017). For example, decrease in ARQ with rising temperature (due to higher PEPC activity
426 for example) might result in a slow increase in CO₂ efflux, whereas the respiration rate (O₂ uptake) is actually
427 increasing sharply, together with the internal carbon flux. Future studies should determine how temperature and
428 nutrients control long term changes in ARQ, and aim to identify the biochemical process that control the low ARQ
429 reported by the current study.

430

431 *Author Contribution.* B.H and A.A planned and designed the research. B.H performed the ARQ analysis and led
432 the writing of the manuscript. J.M and S.T carried out the field work in Brazil, and M.S.C carried out the field
433 work in USA. P.Y measured shoot water potential. S.J.W designed the long term experiment in the Republic of
434 Panama. G.M, O.P, M.M, and A.C contributed to the campaign in Spain. O.P measured the sap flux density.
435 J.M.G and Y.O contributed to the campaigns in the Carmel Ridge. T.W contributed to the campaigns in Spain and
436 in Givat Ram campus. 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.

437

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

439

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

441

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594 **Table 1** Study sites, tree species sampled at each site, stem chambers and stems dimensions.

Site and coordinates	Species	Chamber type, sealant	Diameter at the base of the chamber (cm) ^a			Measurements made in the site	ARQ measurement method
			Mean ± SD	minimum	maximum		
Givat Ram campus, Jerusalem, Israel (31.77°N, 35.20°E)	<i>Populus deltoids</i>	Perspex [®] , hot glue	60.2			ARQ seasonal comparison	Steady state, Instantaneous,
	Bartr. Ex Marsh		43.4				
	<i>Platanus occidentalis</i> L.		21.2			Day-night ARQ variation	Continuous
	<i>Pistacia atlantica</i> Desf.		24.3				
	<i>Quercus calliprinos</i> Webb.	Perspex [®] , vacuum grease	20 ± 8	12	29	ARQ vs. temperature ARQ vertical transects	ARQ of incubated stem cores and leaves
	<i>Malus domestica</i> Borkh.						
	<i>Quercus ilex</i> L.						
Ramat Hanadiv Nature Park Carmel Ridge, Israel (32.55°N, 34.94°E)	<i>Quercus calliprinos</i> Webb.	Perspex [®] , hot glue	11.2 ± 1.2	9.8	12.7	Simultaneous measurements of ARQ and shoot water potential	Steady state
Bartlett Experimental forest, NH, USA (44.06°N, 71.29°W)	<i>Acer rubrum</i> L.	Polypropylene caulking	20 ± 10	10	34	ARQ seasonal comparison	Steady state
Harvard forest, MA, USA (42.53°N, 72.17°W)	<i>Acer rubrum</i> L.	Polypropylene, caulking	18 ± 9	9	26	ARQ seasonal comparison	Steady state
Majadas de Tiétar, Caceres, Spain (39°56'25" N, 5°46'28" W)	<i>Quercus ilex</i> L.	Perspex [®] , vacuum grease	45 ± 7	35	64	ARQ survey of 16 trees	Steady state, Instantaneous
Gigante peninsula, Barro Colorado Nature Monument, Republic of Panama (9°06'31" N, 79°50'37" W)	<i>Tetragastris panamensis</i> (Engl.) Kuntze	Perspex [®] , vacuum grease	30.0 ± 12.5	12.6	66.8	ARQ survey of 42 trees	Steady state, Instantaneous
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")	<i>Scleronema micranthum</i> (Ducke) Ducke	Polypropylene ^d	41.2 ± 13.3	27.0	57.8	Seasonal comparison ARQ vertical transects	Steady state, Instantaneous
						In-stem ARQ and gases concentrations	
						ARQ of incubated stem cores	

595

596 ^aAll chambers were installed at ~1.3 m above the ground, except for the *Q. calliprinos* on Carmel Ridge that were
597 placed near to the ground due to the shrubby canopy, the low branching of the trunk and the constraint of the size
598 of the chamber.

599 ^b Chambers were made of 10 cm × 12 cm Perspex[®] plate with four connectors to allow attachment of sampling
600 flasks. The chamber on the *M. domestica* was slightly larger, 12 cm × 19 cm, with six flasks connectors. Chambers
601 were placed on top of a closed cell foam frame that allowed an air-tight seal between the rigid chamber and the



602 uneven surface of the tree stem. We used nylon straps to compress the foam, while the sealant was applied between
603 the foam and stem for ensuring the seal (Hilman and Angert, 2016). Sealants were silicone based vacuum grease
604 (Silicaid®1010 manufactured by Aidchim Ltd., Raanana, Israel) or hot glue applied by a hot-glue gun.
605 ^c The chambers are described in (Muhr et al., 2013; Carbone et al., 2013). Briefly, the chambers were made from
606 an opaque plastic polypropylene pipe T-fitting with fittings for sampling flasks. Sealants were caulking (Nautiflex;
607 OASE GmbH, Oerel- Barchel, Germany) or hot glue applied by a hot-glue gun.
608 ^d Chambers were built from a 15 cm long piece of polypropylene (PP) tubing (6.5 cm OD) that was welded shut
609 on both sides with a PP disc (6.7 cm diameter). By cutting off a segment (height 2 cm) the tube was turned into
610 an incubation chamber. Opposite the chamber opening, three fittings (Sprint ESKV 20, Wiska, Germany) were
611 installed and sealed around the edges with liquid rubber (Dichtfix, Bindulin, Fürth, Germany). For sampling,
612 chambers were attached to the trees with 4 lashing straps. To achieve a gas tight seal, a frame (25 mm thick) made
613 from closed-porous cellular rubber (EPDM-quality, REIFF Technische Produkte GmbH, Reutlingen, Germany)
614 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 rate.

	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 C g.DW ⁻¹ s ⁻¹)	PEPC fixation rate ^d (nmol C g.DW ⁻¹ s ⁻¹)
<i>Quercus ilex</i> (n=4)	0.44 ± 0.08 ^c	3.84 ± 0.30	<u>2.15</u>	
<i>Tetragastris panamensis</i> (n=11)	0.33 ± 0.07	1.40 ± 0.69	<u>0.93</u>	
<i>Fagus sylvatica</i> L.				<u>12.6</u>

^a Values are mean ± SD

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

^c Calculated as O₂ uptake × (1-ARQ), which is an estimation of the flux of respired CO₂ 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

10 The chosen PEPC activity was the lowest among seasonal measurements.

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Figure captions

Figure 1: Modeled changes in a tree stem chamber of the concentrations of CO₂, O₂, and the ratio between Δ CO₂ and Δ O₂, 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
5 arbitrary fluxes and ARQ = 0.5. The two time frames in which ARQ, the ratio of CO₂ efflux/O₂ influx, can be measured from the ratio Δ CO₂/ Δ O₂ are indicated in the figure.

Figure 2: Relation between “instantaneous” ARQ (ratio of CO₂ efflux/O₂ 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). The regression forced to go through 0. The P value is the
10 significance of the slope estimation.

Figure 3: Summary of “steady state” apparent respiratory quotient (ARQ) measurements (ratio of CO₂ efflux/O₂ 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
15 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” apparent respiratory quotient (ARQ, the ratio of CO₂ efflux/O₂ 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
20 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). Markers connected with dashed lines represent measurements with chambers positioned at the stem base. The trees grew on Hebrew University campus in Jerusalem, Israel.

25 Figure 5: Instantaneous apparent respiratory quotient (ARQ, ratio CO₂ efflux/O₂ influx of a stem, \pm SD of duplicates) values measured over a day-night-day transition in Jerusalem, Israel in July 2012 (a) and April 2013 (b) from different trees growing on Hebrew University campus in Jerusalem, Israel. *Q. 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).

30 Figure 6: Diurnal patterns of (a) O₂ influx to the stem and CO₂ efflux from the stem, (b) chamber temperature, and (c) instantaneous apparent respiratory quotient (ARQ, ratio CO₂ efflux/O₂ influx for tree stems). Shaded areas



ndicate 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 apparent respiratory quotient (ARQ, ratio of CO₂ efflux/O₂ influx for tree stems) measured from stem chambers installed at different heights above the ground on a *S. micranthum* tree in Brazil.

5 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 apparent respiratory quotient (ARQ, ratio CO₂ efflux/O₂ influx for tree stems, “steady state”) to ARQ measured from incubations of stem cores (ratio CO₂ increase/O₂ decrease), by species (n individuals) in different sites. Values are means ±SD.

10 Figure 9: (a) O₂ uptake rate (nmol g.FW⁻¹ s⁻¹) and (b) apparent respiratory quotient (ARQ, ratio CO₂ increase/O₂ 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.

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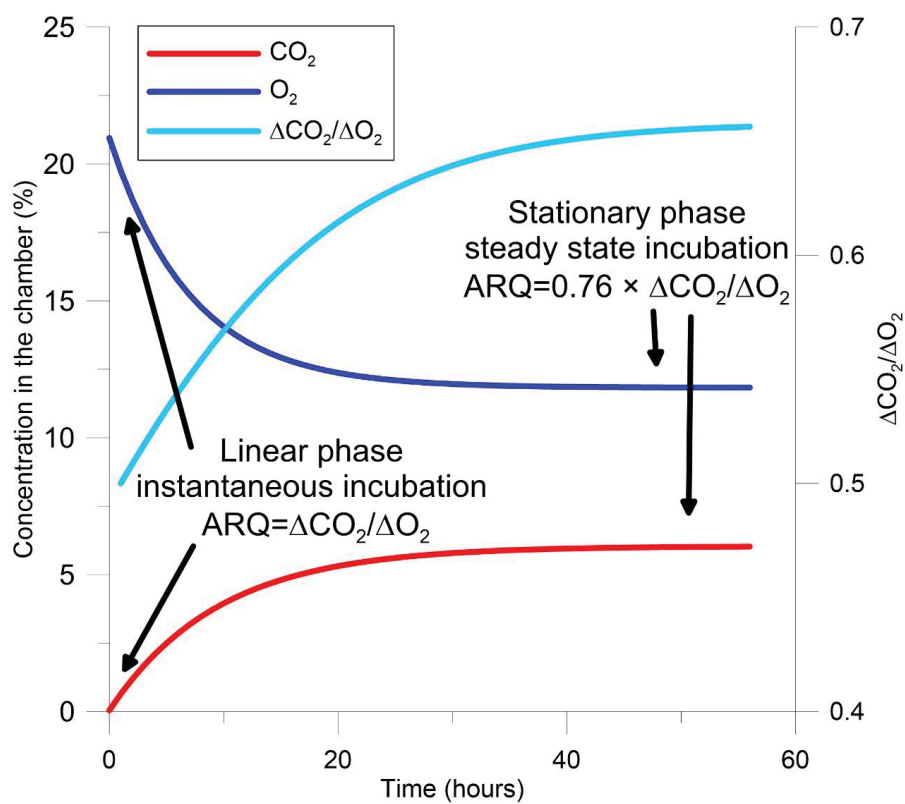
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10 Figure 1



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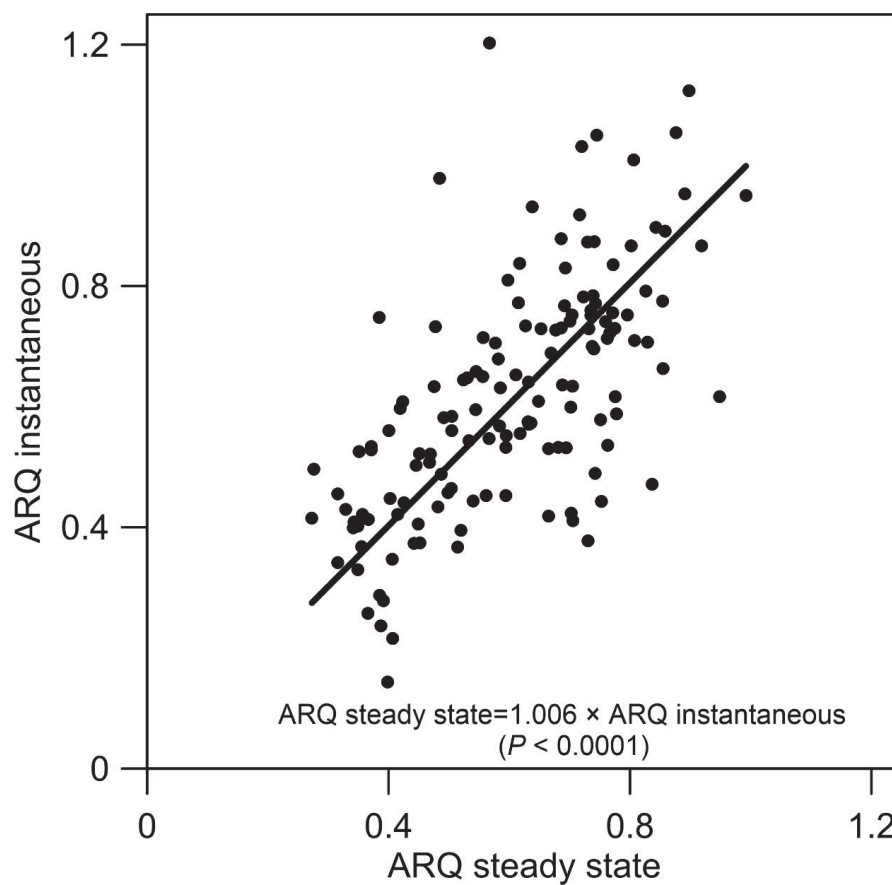
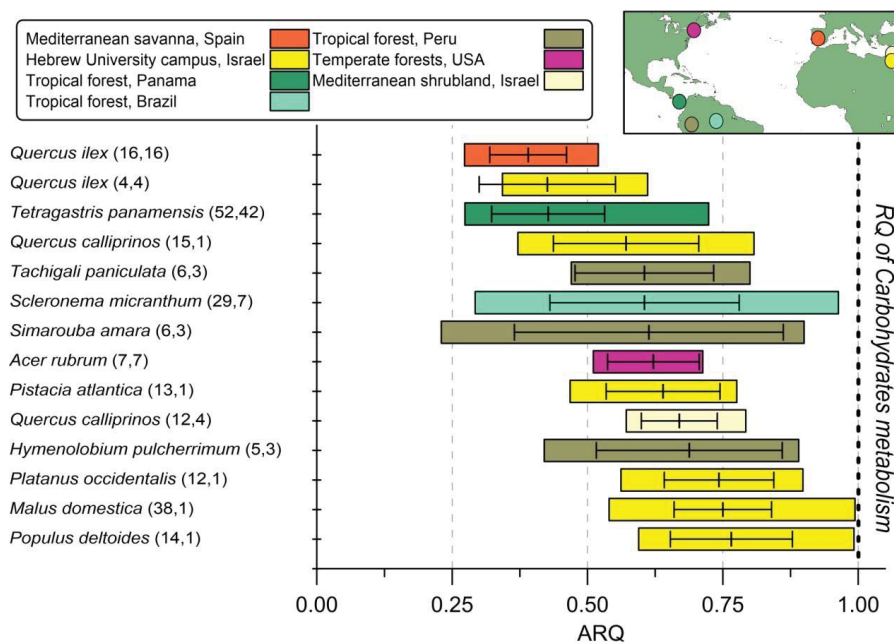


Figure2



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Figure 3

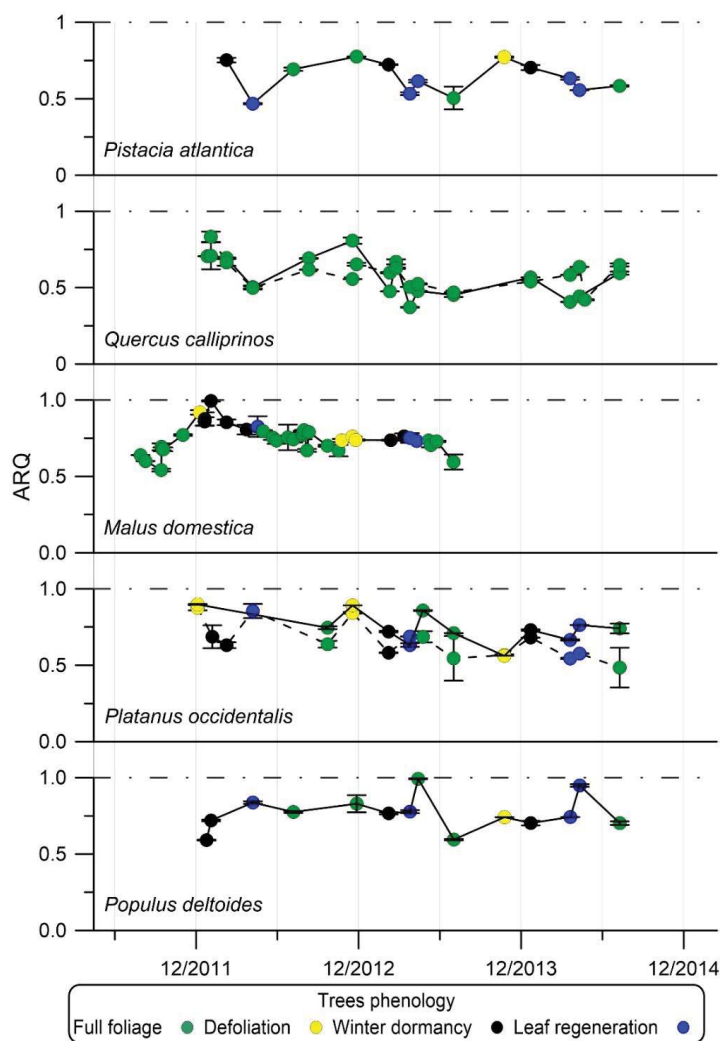


Figure 4

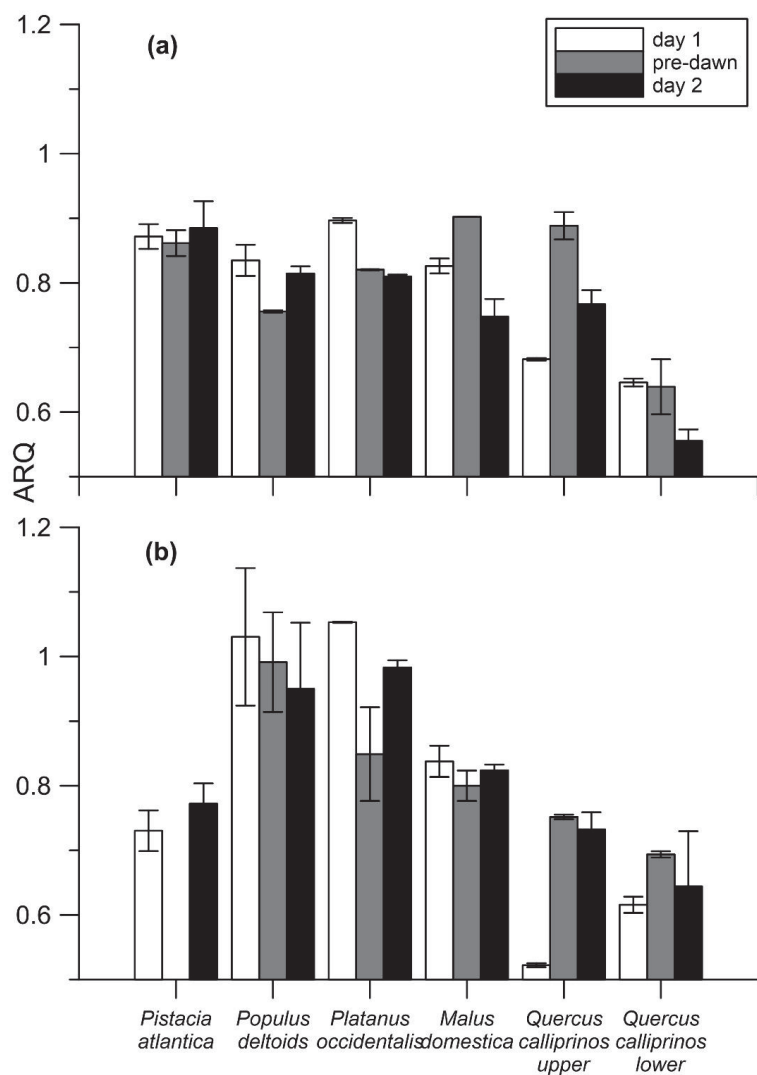
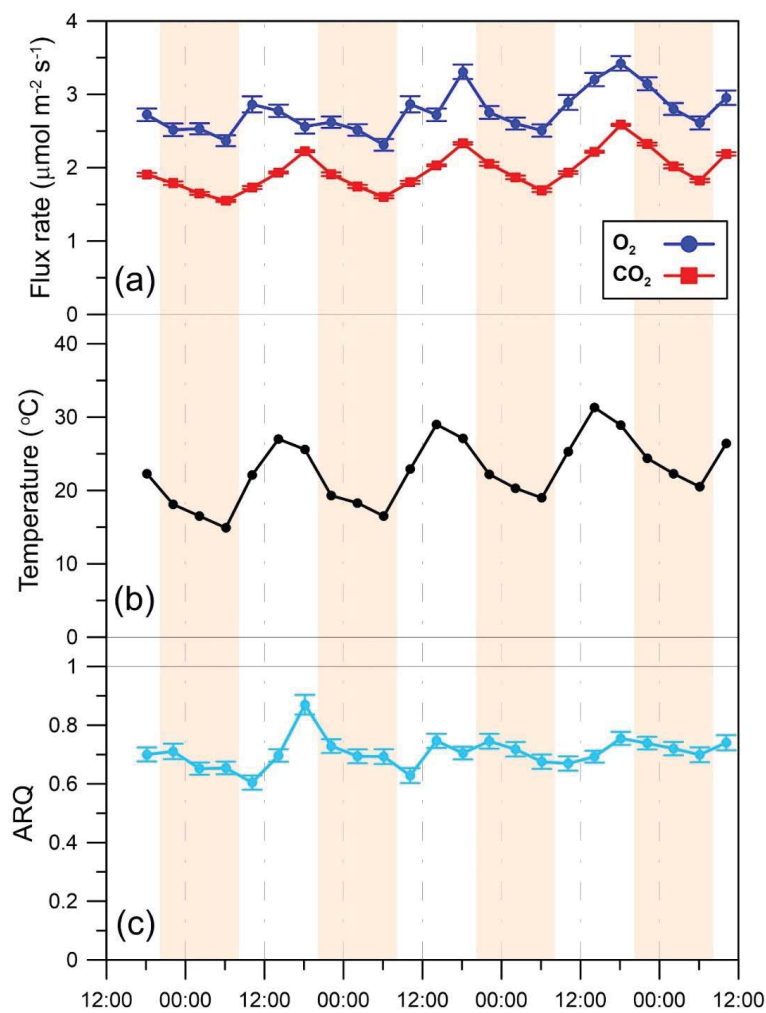


Figure 5



5 Figure 6

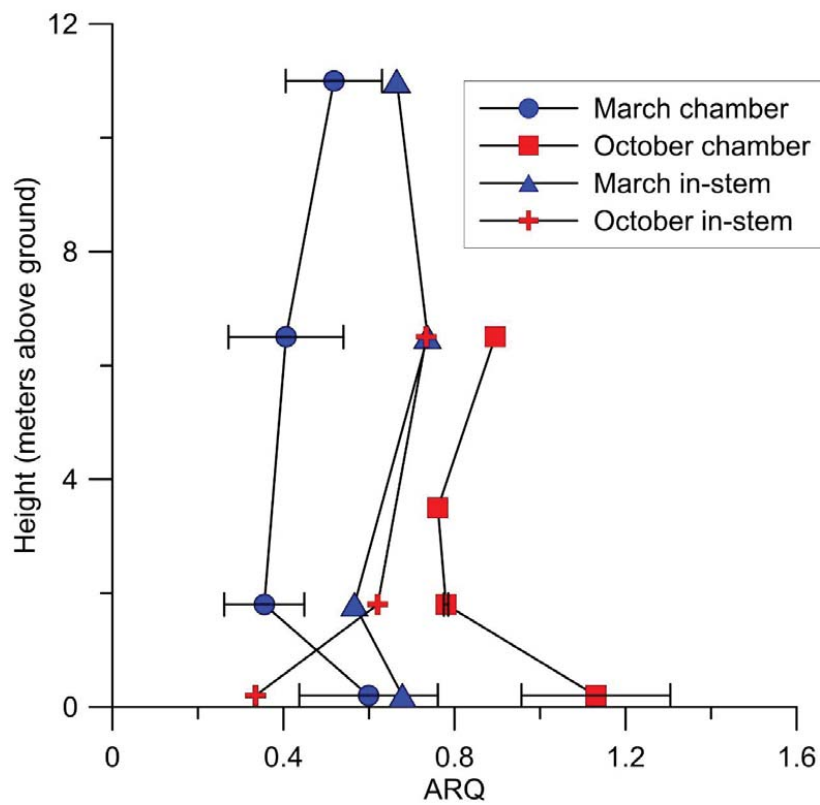
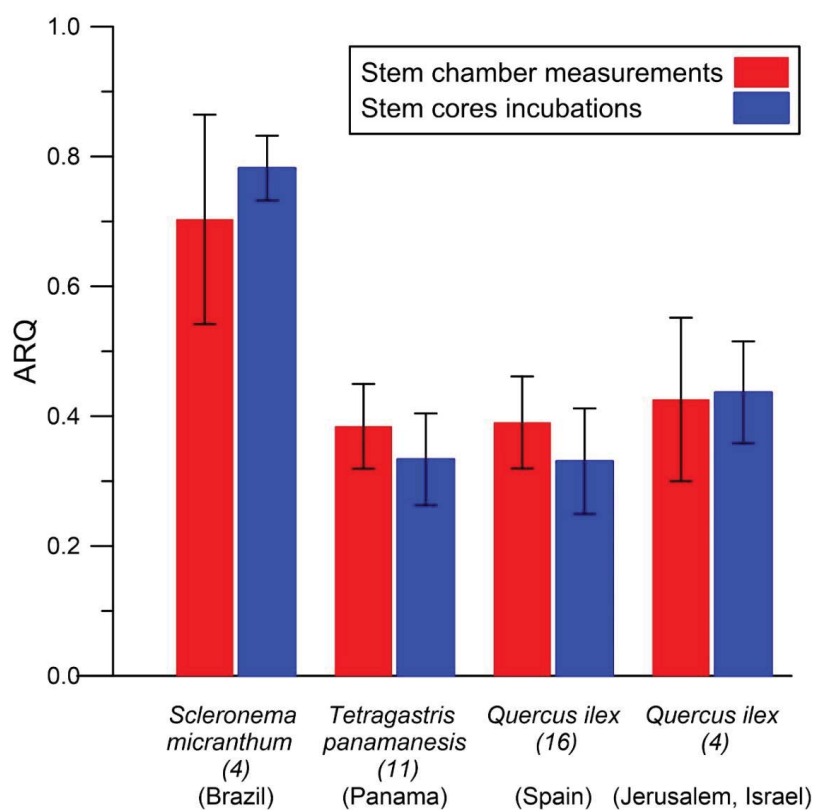


Figure 7



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Figure 8

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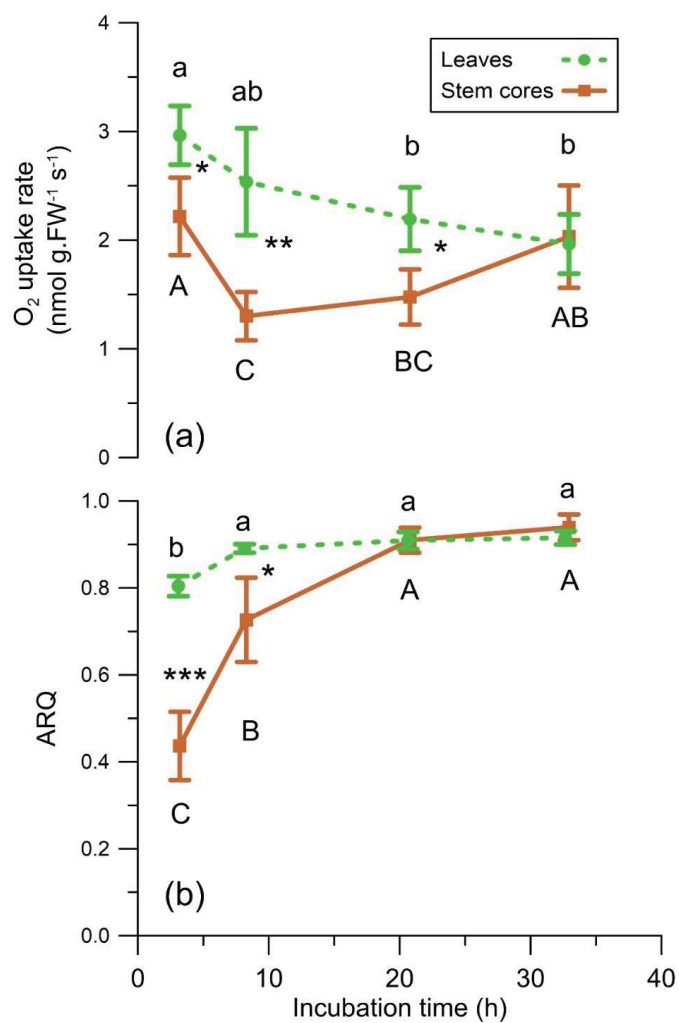


Figure 9