Diel patterns of leaf carbohydrate concentrations differ between seedlings and mature trees of two sympatric oak species

Ori Baber[1], Martijn Slot[1,2]*, Gerardo Celis[1], Kaoru Kitajima[1,2,3]

1. Department of Biology, University of Florida, Gainesville, FL 32611, USA

2. Smithsonian Tropical Research Institute, Republic of Panama, Panama

3. Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Corresponding author: Martijn Slot

Email: martijnslot78@gmail.com

Phone: +507 212.8000

Fax: +507 212.8148

*Current address: Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Republic of Panama

Abstract

A fundamental aspect of the carbon cycle is the exchange of carbon between plants and the atmosphere. It is therefore important to understand factors that affect differences in gas exchange and carbon balance within and among species. Concentrations of non-structural carbohydrates are often used as a proxy for carbon balance. We determined diel patterns of leaf carbohydrate concentrations in relation to irradiance (sun vs shade) in seedlings and mature trees of two sympatric oak species (*Quercus virginiana* and *Quercus hemisphaerica*). For seedlings we also measured leaf gas exchange. Higher sun exposure significantly increased photosynthesis and carbohydrate concentrations in both species. Carbohydrate concentrations of seedling leaves showed strong diel fluctuations, whereas concentrations in mature tree leaves did not. This contrast might be attributed to faster carbohydrate export from leaves of mature trees. The difference in sink strength between seedlings and adults may be related to the decreasing ratio of leaf mass to plant mass with ontogeny, increasing the demand for carbohydrates per unit leaf mass. Seedlings and mature trees are clearly functionally different and care must be taken when extrapolating results from seedling experiments to mature trees.

Keywords: Carbohydrate dynamics; Gas exchange; *Quercus virginiana*; *Quercus hemisphaerica*; Plant ontogeny

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Introduction

Non-structural carbohydrates are key components of plant carbon balance (Chapin et al. 1990; Körner 2003), and concentrations of non-structural carbohydrates are often used in ecophysiological studies to compare carbon balance among plant populations (e.g., Bansal and Germino 2008, 2009). Carbon is photo-assimilated into leaves as soluble sugars during the day to meet metabolic demands of these leaves, and excess carbohydrates are either temporarily stored or exported to support carbon sinks away from these source leaves, such as growing shoots and roots. When net photosynthesis exceeds the rate of carbohydrate export, the concentration of non-structural carbohydrates (NSC, the sum of starch and soluble sugars) rises in the leaf, particularly in the form of osmotically inactive starch. During the night, the NSC concentration decreases as starch is broken down and carbohydrates are respired or exported to various sinks (Noguchi and Terashima 1997). The rate of decrease in nighttime NSC concentration differs among species in relation to their growth strategy, with fast-growing, light-demanding species exhibiting more pronounced changes in NSC than slow-growing, shade-tolerant species (Noguchi and Terashima 1997). Hence, while leaf NSC concentration may reflect overall homeostatic control of various metabolic pathways involved in photosynthesis and respiration (Morandini 2009; Sulpice et al. 2009), the concentrations and their diel fluctuations may be species-specific and dependent on the specific growth demands of the plants.

Growth rates and specific developmental requirements affect the source-sink relationships within plants and change with ontogeny (Allen et al. 1998). Ontogeny and plant size also influence leaf traits of long-lived plants (Iida et al. 2014), including gas exchange traits (Steppe et al. 2011). Regardless of ontogeny, NSC concentrations in leaves are affected by environmental factors, since photosynthesis, respiration, and growth are sensitive to

environmental conditions (Farnham et al. 1986; Körner 2003). For example, stimulation of net photosynthetic rates by increasing the irradiance or elevating CO_2 concentration tends to increase NSC accumulation during the day (Allen et al. 1998; Würth et al. 1998, but see Tognetti and Johnson 1999); when the stimulation of photosynthetic production is not matched by an increased demand for export of photosynthates, carbohydrate concentrations increase in leaves.

Evergreen oaks are important in mixed evergreen-deciduous forests in Florida. A high diversity of oak species coexists in Florida, partitioning niches along ecological gradients defined by soil hydrology, nutrients and fire regimes (Cavender-Bares et al. 2004). *Quercus virginiana* (southern live oak) and *Quercus hemisphaerica* (laurel oak) are two of the most abundant canopy oak species in the region, occupying a wide range of soil moisture conditions. These two species often grow side by side, possibly in direct competition for light and other resources (Templeton and Putz 2003; Spector and Putz 2006). Seedlings of the two species exhibit similarly high relative growth rates, the two fastest among nine species compared in a glass house study, even though leaf photosynthetic capacity was higher in *Q. virginiana* than in *Q. hemisphaerica* (Cavender-Bares et al. 2004). High rates of photosynthesis can be expected to result in increased storage of starch in the leaves during the day. On the other hand, fast growing plants have a high rate of sugar export from leaves. This suggests that the diel carbohydrate dynamics in these two species may differ.

Despite an increased interest in plant carbohydrates, e.g., in the context of tree mortality during stress events (e.g., McDowell et al. 2008; Sala et al. 2012), many questions regarding the dynamics of non-structural carbohydrates in woody plants remain unresolved (Dietze et al. 2014). To increase our understanding of carbohydrate dynamics in closely related, but ecologically slightly dissimilar oak species, we analyzed diel dynamics of soluble sugar and

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starch concentrations in the leaves of the two evergreen oak species at seedling and adult stages

under sun and shade conditions, and asked the following questions. 1) Do *Q. virginiana* and *Q.*

hemisphaerica differ in diel patterns of leaf sugar and starch content? 2) What is the effect of

light environment on diel patterns of leaf sugar and starch content? 3) Do seedlings differ from

mature trees in their diel NSC dynamics?

Methods

Study Site and Species

Experiments were conducted on the campus of the University of Florida in Gainesville, FL

(N29°38'22", W82°20'43"). We compared two evergreen oak species, *Quercus virginiana* and

Quercus hemisphaerica. Both species are classified as evergreen, but they exchange the entire

leaf cohort following budbreak and flowering in early spring.

Seedling Greenhouse Experiment

Seeds collected from multiple trees around Gainesville were germinated in sand and grown in

164 ml Ray Leach "Cone-tainers"™ with a 3:1 mixture of sand and vermiculite at a high

irradiance greenhouse (~90% of full sun) for *ca.* 10 months. At this time, the height of *Q.*

hemisphaerica seedlings was 12.1 ± 2.4 cm (mean ± standard deviation) and of *Q. virginiana*

seedlings 19.0 ± 7.4 cm. Both species had an average of 16 leaves per seedling.

A total of 80 seedlings were randomly assigned to a sun (ambient greenhouse

irradiance) or shade treatment. Seedlings were placed in trays (RL98, Stuewe & Sons, Inc.,

Corvallis, OR, USA), keeping an empty cell on all sides of each seedling to prevent neighboring

plants from touching. Plants were frequently rotated within their trays to minimize effects of

spatial heterogeneity within the irradiance treatment. At the start of the experiment all seedlings were provided with ~8.5 mg of slow-release N:P:K fertilizer (Osmocote 19:6:12). Plants were watered in the mornings to field capacity. Shade was provided with neutral density shade cloth supported by a PVC frame, reducing irradiance by 80%, while not significantly changing air temperature. Irradiance (photosynthetic photon flux density; PPFD) and air temperature were monitored with LI-190B PAR sensors (LI-COR, Lincoln, USA), and copper-constantan thermocouples respectively, and 30-minute mean values were logged on a Campbell 21X data logger (Campbell Scientific Inc., Logan, USA).

On 23–24 July 2009, four weeks after initiating the shade treatment, we sampled seedling leaves six times within a 24-hour period, approximately every 4 hours during the day [9:00, 13:00 (≈solar noon), 17:00] and night [20:00 (≈sunset), 24:00, 5:00 (≈sunrise)]. At each sample time, net exchange rates of CO_2 were first measured with an LI-6400 infrared gas analyzer with a standard leaf cuvette (LI-COR), controlled at 30.5 ± 1.0°C, 400 ppm CO_2 and 50–75% relative humidity. Measurements were made on one fully expanded leaf, recently matured under experimental conditions, on each of three randomly selected seedlings per species, in both light environments. Each seedling was measured only once, to avoid potential effects of destructive leaf harvests on the physiology and NSC dynamics during the remainder of the sample day. During daylight sample times, photosynthesis was first measured at ambient light levels ($A_{Ambient}$) of respective conditions (sun or shade) and then light-saturated photosynthesis (A_{Sat}) was determined at 1500 μmol $m^{-2}s^{-1}$. At night only dark respiration (R) was measured (at 29.0 ± 0.5°C). Immediately following the gas-exchange measurements, the leaves were harvested for NSC analyses (see below).

Mature Tree Field Study

Four pairs of mature trees were selected. The trees in each pair (one *Q. hemisphaerica*, one *Q. virginiana*) were growing in close proximity to each other (6–12 m apart), were similar in size, and were assumed to experience similar ambient conditions. The trees were sampled on 14–15 July 2009. Sampling consisted of seven harvests 4 hours apart within a 24-hour period, including approximate times of sunrise, solar noon, and sunset. For each tree, two twigs were harvested for NSC analyses (see below) at each time from approximately the same height within the tree; one from the outer canopy exposed to full sunlight and another from the shaded interior part of the canopy. The temporal pattern of above-canopy irradiance for the 24-hour period was obtained from the Florida Automated Weather Network station in Alachua *ca*. 20 km from Gainesville, and for shade branches, we estimated the irradiance pattern by dividing the above-canopy irradiance by 2 based on spot measurements of PPFD with a handheld LI-190B sensor (LI-COR).

Total Non-Structural Carbohydrate Analysis

Leaves and twigs were stored on ice during transport. Upon return to the laboratory, five leaves were selected from each twig sampled from mature trees. For all leaves, area was measured with an LI-3050 leaf area meter (LI-COR), and leaves were dried in individual paper envelopes at 60°C for 72 hours. Although minor enzymatic degradation of NSC was possible during oven drying, it is unlikely to have influenced the patterns of NSC reported, because all leaves were treated equally. Leaves were ground with a Whiley mill to pass through a 40-mesh screen (Thomas Scientific, Swedesboro, USA). The leaves collected from the mature trees were ground together to make two pooled samples per individual; one of five sun leaves and one of five shade

leaves. Seedling replicates were analyzed as individual samples. Concentrations of carbohydrates were determined following Dubois et al. (1956) with modifications. Briefly, soluble simple sugars were extracted in 80% (v/v) ethanol from 10–15 mg sample by shaking overnight at 27°C followed by two, 2-hour incubations. The supernatant from each sample was collected in a volumetric flask and brought up to 10 ml with distilled water. This long extraction period at relatively low temperature more successfully extracts soluble sugars than shorter hot-ethanol extraction (Kitajima, unpublished data). Starch in the pellet was digested to glucose by amyloglucosidase in a sodium acetate buffer. Glucose concentrations were determined colorimetrically at 487 nm via the phenol- sulfuric acid method. Starch concentrations were determined as glucose equivalents.

Statistical Analysis

To account for the factor time in the analyses of the variance in carbohydrate concentrations and rates of net CO_2 exchange rates measured at different times of the day, we used a variance-covariance structure similar to that of repeated-measures ANOVA. In the seedling study different individuals were measured at each sampling time, but as all seedlings within a treatment by species combination were maintained under very uniform conditions within a single tray, plants measured at e.g., t = 1 were treated as correlated replicates of the plants of the same species and treatment that were measured at t = 0. This method enabled us to assess the effects of species identity, treatment, and ontogenetic phase on the response variables while accounting for temporal changes in the repeatedly measured subjects (individual trees, or seedlings within uniform treatment groups). At the same time, the significance of the within-subject variation

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could be determined, and assigned to the time of sampling/measurement. All statistical analyses were performed using JMP 8.0.1 Software (SAS, Cary, USA).

Results

Diel Non-Structural Carbohydrate Dynamics

Carbohydrate concentrations in seedling leaves fluctuated considerably during the 24 hours of observation, particularly concentrations of starch (Fig. 1). During the day, starch concentrations increased, reaching peak values between solar noon and 17:00. Starch concentrations then decreased until midnight, after which they were maintained at approximately steady levels. Concentrations of soluble simple sugars followed similar, but less pronounced patterns. Treatment (sun vs shade) significantly influenced the concentrations (but not the diel pattern) of sugar, starch, and NSC (Table 1), and time was a significant factor explaining within-subject variance. In contrast, sugar and starch concentrations in leaves of mature trees showed minimal diel variation (Fig. 2). These patterns did not differ between the species (Table 1). A significant time×treatment interaction effect on starch and NSC reflects the greater peak of starch concentration in sun than in shade leaves, associated with higher PPFD in the sun (Fig. 1).

Seedling Diel Gas Exchange

The time course of seedling photosynthesis at ambient irradiance produced similar patterns across species and treatments (Fig. 3). Photosynthesis increased in the morning and peak rates were observed at approximately solar noon (13:00) when ambient PPFD was highest. Photosynthesis declined in the afternoon toward sunset at which time it was zero (sun treatment)

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or negative (shade treatment). After 20:00, respiration resulted in a net loss of CO_2, peaking around midnight in all species by treatment combinations (Fig. 3).

Photosynthetic rates at ambient PPFD were marginally higher in the sun than in the shade ($P = 0.05$) (Table 2). Time significantly affected $A_{Ambient}$ without significant differences between species (Table 2). A_{Sat} did not differ significantly between species either (Table 3), but in contrast to $A_{Ambient}$, A_{Sat} was not affected by treatment and time of measurement. Similarly, average nighttime respiration rates were not significantly different between species and treatments (Table 3). Diel fluctuations of NSC concentrations paralleled the patterns of net photosynthetic rates (Fig. 3), resulting in a positive correlation between the rate of photosynthesis and NSC concentration across species and treatments ($R^2 = 0.58$; $P<0.01$).

Discussion

No Species Differences in Diel Patterns of Carbohydrates

Quercus virginiana and *Q. hemisphaerica* are morphologically, and architecturally dissimilar (Templeton and Putz 2003), yet they exhibited similar diel patterns of carbohydrate accumulation and gas exchange. We found photosynthetic capacity and ambient photosynthesis rates to be similar in *Q. virginiana* and *Q. hemisphaerica*. As a result, only the shade treatment and time of day had significant effects on $A_{Ambient}$, factors that also had a significant effect on leaf carbohydrate concentrations.

Diel Carbohydrate Dynamics Similar in Sun and Shade Leaves
Diel carbohydrate dynamics followed similar patterns in sun and shade leaves, but with lower overall concentrations in shade leaves. This difference in overall concentrations is likely

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explained by lower net photosynthesis rates in the shade. Diel patterns in gas exchange of seedling leaves roughly followed changes in PPFD, and leaf carbohydrate concentrations correlated with gas exchange rates. In mature trees, sun leaves exhibited overall higher NSC concentrations (Fig. 2), but in contrast to seedling leaves, carbohydrates in leaves of mature trees showed little diurnal fluctuation despite large diurnal changes in PPFD and likely variation in photosynthetic rates during the day. Compared to seedlings, adult tree leaves had overall lower and more stable concentration of starch (30–70 mg g^{-1} (daily minimum and maximum) compared to 70–250 mg g^{-1}), with smaller differences between sun-exposed and shaded leaves.

In both light environments the concentrations of simple sugars were more stable than the concentration of starch. Maintenance of sugar homeostasis in leaves is important because sugars can modulate expression of genes important in photosynthesis and respiration (Koch 1996). Starch concentrations of seedling leaves on the other hand, fluctuated 3–5 fold over a 24-hour period. Leaf starch concentration is commonly more variable than soluble sugar concentrations (e.g., Fondy and Geiger 1982; Morin et al. 2011). However, in leaves of mature trees, neither sugar nor starch concentrations changed significantly during 24 hours.

Strong Effect of Ontogeny on Diel Carbohydrate Fluctuations
Leaf carbohydrate concentrations fluctuated considerably in seedlings, but diel fluctuations in their mature counterparts were minimal. Low diel variation in carbohydrate concentrations has previously been reported for conifer seedlings (Bansal and Germino 2009) and saplings (Yang et al. 2002). Bansal and Germino (2009) argued that perhaps slow-growing conifers show less dynamic patterns of carbohydrate concentrations than fast-growing herbs, because they accumulate more reserves in their evergreen leaves to tolerate stressful conditions. This

argument is unlikely to apply only to the mature trees of the oak species in the current study and not to the seedlings. Instead, the relatively stable NSC concentration throughout the day may be explained by high sink strength in mature trees, resulting in immediate export of synthesized carbohydrates, rather than buildup during the day. Leaf mass as a proportion of total plant mass decreases with ontogeny in woody plants (Poorter et al., 2012a), as larger plants need to invest more in structural support. The proportionally smaller mass of photosynthetic material in big trees may contribute to a larger demand for photosynthates per unit leaf mass than in seedlings, in which a larger fraction of the biomass contributes to photosynthetic production.

The large carbohydrate fluctuations in seedling leaves in contrast, suggest weak sink strength. Decreased sink strength reduces the rate of translocation of sugar out of the leaf and under such conditions sugars accumulate in source leaves (Würth et al., 1998; Ainsworth and Bush 2011). Weak sink strength could be caused by restricted root growth in pot-bound plants or nutrient limitation, reducing overall plant growth rates (Townend and Dickinson 1995; Whitfield et al. 1996). Plants in most greenhouse studies exceed the biomass threshold beyond which pot volume negatively affects growth (plant dry mass > 1 gram per liter pot volume. Poorter et al. 2012b). Although the seedlings used in the current experiment were fertilized and well-watered, it is possible that growth restriction by small pots reduced sink demands in seedlings compared to the ground-rooting, irrigated mature trees. Tognetti and Johnson (1999) examined the influence of nutrient addition and elevated CO_2 concentration on photosynthetic capacity and NSC concentrations in *Q. virginiana* seedlings, and found no effects of nutrients on foliar carbohydrates. They observed somewhat higher A_{Sat} (13–15 µmol CO_2 m^{-2} s^{-1}, compared to 10–11 µmol CO_2 m^{-2} s^{-1} in the current study), but much lower leaf starch concentrations. The time of the day in which they sampled leaves for NSC analysis is unknown, but our results suggest that

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diel dynamics of photosynthate accumulation can easily swamp the treatment effects in plants with weak sink strength.

Concluding Remarks

In both species, we observed greater diel dynamics of photosynthates in seedlings than in adult trees. We suspect these differences to be driven by differences in sink strength either due to inherent ontogenetic size difference or environmental factors, such as pot restriction, that restricted seedling growth. Our results illustrate that seedlings are not functionally similar to mature trees, and care will thus have to be taken when extrapolating results from seedling pot experiments to trees of larger size, especially if those are not pot grown. Thus, it is important for future studies to use ground-growing plants to address how resource limitations may influence coupling of diel dynamics of photosynthetic activity and carbohydrate accumulation.

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Table captions

Table 1.Summary of repeated measures ANOVAs for the effects of treatment (sun vs shade), species (*Quercus hemisphaerica* vs *Q. virginiana*), and ontogeny (seedlings vs mature trees) on leaf carbohydrate concentrations (Simple sugars, Starch, and total non-structural carbohydrates (NSC)).

Table 2. Results of a repeated measures ANOVA for the effect of treatment (sun vs shade), species (*Quercus hemisphaerica* vs *Q. virginiana*), and time of day on rates of CO_2 exchange in seedling leaves at ambient conditions. Significant effects are indicated in bold.

Table 3. Light-saturated photosynthesis rate (A_{Sat}), and photosynthesis at ambient irradiance ($A_{Ambient}$) measured at solar noon, and dark respiration (R) average over two nighttime measurements, for sun-exposed and shaded seedlings of *Quercus hemisphaerica* and *Q. virginiana* (means ± s.d.). Daily minimum and maximum concentrations of carbohydrates (Simple sugars, Starch, and total non-structural carbohydrates (NSC)) and the time of day (TOD) at which these occurred are also shown.

Table 1.

Source of variance	Simple sugars	Starch	NSC
Between Subjects			
Species	*ns*	*ns*	*ns*
Ontogeny	*ns*	***	***
Treatment	***	***	***
Species×Ontogeny	*ns*	*ns*	*ns*
Species×Treatment	*ns*	*ns*	*ns*
Within Subjects			
Time	***	***	***
Time×Species	*ns*	*ns*	**
Time×Ontogeny	***	***	***
Time×Treatment	*ns*	***	***

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Table 2

Source of variance	F	DF	*P*
Between Subjects			
All Between	1.2	3	0.09
Species	0.4	1	0.10
Treatment	0.7	1	**0.05**
Species×Treatment	0.1	1	0.30
Within Subjects			
Time	61.4	5	**0.001**
Time×Species	1.7	5	0.40
Time×Treatment	2.9	5	0.20

Table 3

Trait	Units		*Quercus hemisphaerica*				*Quercus virginiana*			
			Sun	TOD	Shade	TOD	Sun	TOD	Shade	TOD
A_{Sat}	µmol m^{-2} s^{-1}		5.1 ± 2.1	noon	6.6 ± 2.4	noon	11.1 ± 3.3	noon	9.6 ± 1.3	noon
$A_{Ambient}$	µmol m^{-2} s^{-1}		4.1 ± 1.5	noon	3.5 ± 1.0	noon	7.4 ± 2.6	noon	5.5 ± 0.3	noon
R	µmol m^{-2} s^{-1}		1.5 ± 0.7	night	1.3 ± 0.4	night	1.6 ± 0.7	night	1.5 ± 0.9	night
Simple sugars	mg g^{-1}	Min*	85 ± 8	5:00	68 ± 7	5:00	75 ± 7	5:00	67 ± 20	24:00
	mg g^{-1}	Max	145 ± 16	17:00	107 ± 25	17:00	160 ± 88	13:00	117 ± 15	9:00
Starch	mg g^{-1}	Min	69 ± 22	24:00	45 ± 13	5:00	42 ± 6	5:00	35 ± 7	24:00
	mg g^{-1}	Max	254 ± 30	17:00	166 ± 11	13:00	229 ± 35	13:00	157 ± 27	13:00
NSC	mg g^{-1}	Min**	164 ± 15	5:00	114 ± 20	5:00	117 ± 8	5:00	102 ± 16	24:00
	mg g^{-1}	Max	399 ± 40	17:00	270 ± 70	13:00	390 ± 86	13:00	295 ± 70	13:00

* Significant treatment effect with *P* <0.05

** Significant treatment effect with *P* <0.01

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Figure legends

Figure 1. Time course of leaf sugar and starch concentrations (left axis) and photosynthetic photon flux density at plant height (right axis) for seedlings of *Quercus hemisphaerica* grown in sun (A) and shade (B) , and *Q. virginiana* grown in sun (C) and shade (D), measured on 23–24 July, 2009. Error bars indicate one s.d. (n = 3).

Figure 2. Time course of sugar and starch concentrations (left axis) and photosynthetic photon flux density at a nearby meteorological station at 2 m height (right axis) for mature tree leaves of *Quercus hemisphaerica* exposed to sun(A) and shade (B), and *Q. virginiana* exposed to sun (C) and shade (D), measured on 14–15 July 2009. For shade leaves photon flux density is shown as 50% that of full sun. Error bars indicate one s.d. (n = 4).

Figure 3. Time course of photosynthetic rates in the ambient condition (left axis) and total non-structural carbohydrate (NSC) per gram of dry leaf mass (right axis) for seedlings of *Quercus hemisphaerica* grown in sun(A) and shade (B), and *Q. virginiana* grown in sun (C) and shade (D). A secondary right axis shows the photosynthetic photon flux density at plant height in the different treatments (gray shading). Error bars (one s.d.) are shown for photosynthesis (n = 3). For NSC, variation among individuals in sugars and starch is shown separately in Fig. 1.
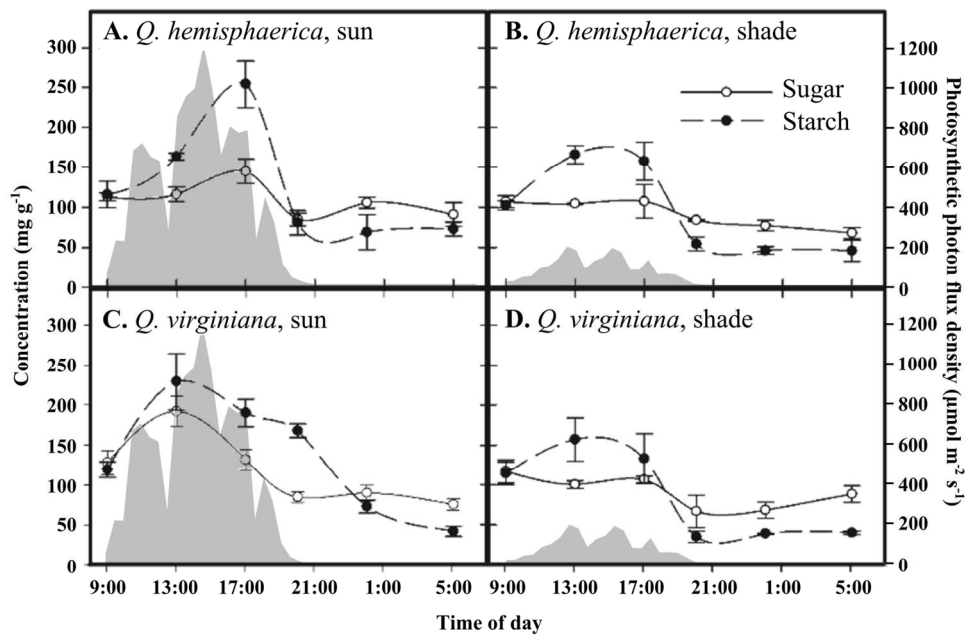
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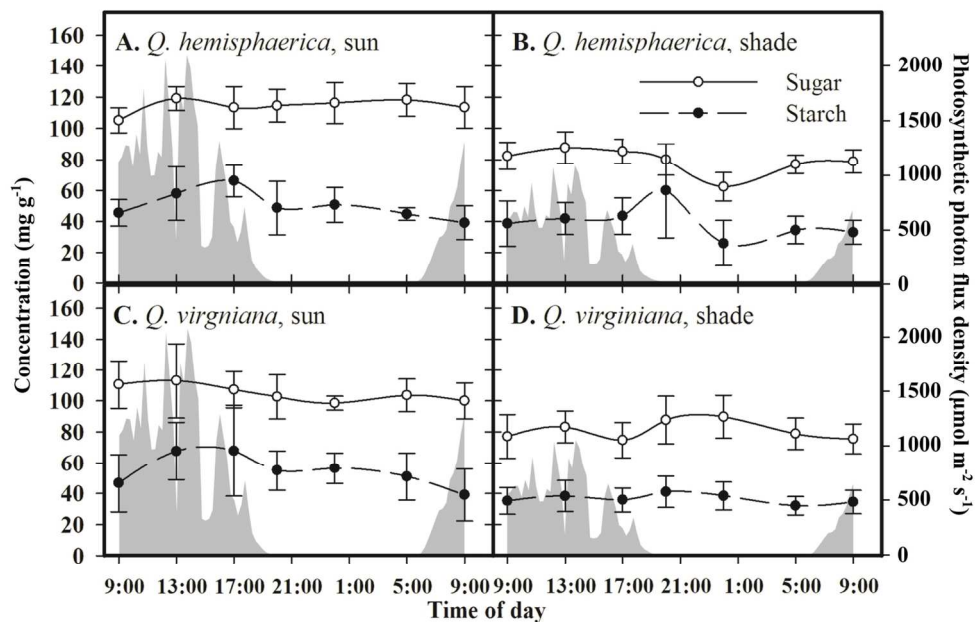144x101mm (300 x 300 DPI)

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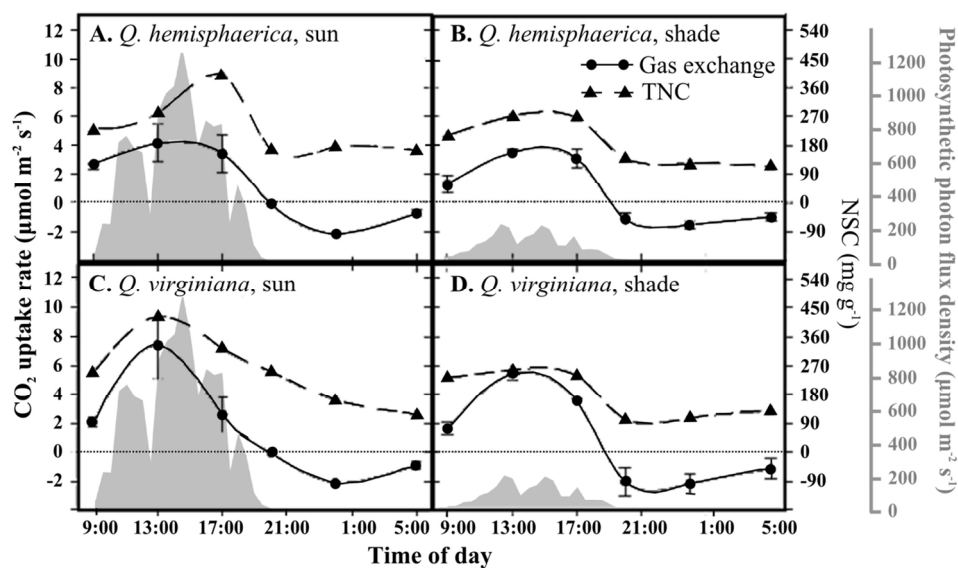143x100mm (300 x 300 DPI)

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129x82mm (300 x 300 DPI)