Tree Species with Photosynthetic Stems Have Greater Nighttime Sap Flux

Xia Chen 1,2, Jianguo Gao 1, Ping Zhao 1,3*, Heather R. McCarthy 4, Liwei Zhu 1, Guangyan Ni 1 and Lei Ouyang 1

1 Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China, 2 College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China, 3 Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China, 4 Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, United States

An increasing body of evidence has shown that nighttime sap flux occurs in most plants, but the physiological implications and regulatory mechanism are poorly known. The significance of cortical photosynthesis has received much attention during the last decade, however, the knowledge of the relationship between cortical photosynthesis and nocturnal stem sap flow is limited at present. In this study, we divided seven tree species into two groups according to different photosynthetic capabilities: trees of species with (Castanopsis hystrix, Michelia macclurei, Eucalyptus citriodora, and Eucalyptus grandis × urophylla) and without (Castanopsis fissa, Schima superba, and Acacia auriculiformis) photosynthetic stems, and the sap flux (Js) and chlorophyll fluorescence parameters for these species were measured. One-way ANOVA analysis showed that the Fv/Fm (Maximum photochemical quantum yield of PSII) and ΦPSII (effective photochemical quantum yield of PSII) values were lower in non-photosynthetic stem species compared to photosynthetic stem species. The linear regression analysis showed that Js,d (daytime sap flux) and Js,n (nighttime sap flux) of non-photosynthetic stem species was 87.7 and 60.9% of the stem photosynthetic species. Furthermore, for a given daytime transpiration water loss, total nighttime sap flux was higher in species with photosynthetic stems (SlopeSMA = 2.680) than in non-photosynthetic stems species (SlopeSMA = 1.943). These results mean that stem cortical photosynthesis has a possible effect on the nighttime water flow, highlighting the important eco-physiological relationship between nighttime sap flux and cortical photosynthesis.

Keywords: nighttime sap flow, daytime sap flow, stem cortical photosynthesis, oxygen delivery, water-replenishment

INTRODUCTION

The significance of nighttime stem sap flow of plants has been discussed in a number of studies in the past decade (e.g., Mancuso and Marras, 2003; Forster, 2014). However, quantifying nocturnal water use is not easy due to technical limitations. For example, the in situ measurement of nighttime transpiration (E) is difficult and is always overestimated by leaf gas exchange systems (Escalona et al., 2012). The heat dissipation method could simultaneously help us to simply and accurately measure the nighttime sap flow activities and determine the main environmental drivers (Lu et al., 2004; Dawson et al., 2007; Wang et al., 2007; Oishi et al., 2008). Earlier research posited that
non-CAM plants close stomata to reduce water depletion at night, meanwhile reducing the absorption of carbon; however, this view caused an underestimate of whole day sap flow (Forster, 2014). Nighttime E is strongly influenced by daytime physiological processes, which are attributable to the plant growth rate or the environment (Ludwig et al., 2006). The canopy species of the forest community are shade intolerant and have a higher nighttime to daytime sap flux ratio ($J_{s,n}/J_{s,d}$) (Marks and Lechowicz, 2007). Moreover, previous studies revealed that nighttime sap flux is not only a water recharge for stem water deficit caused by intensive daytime transpiration but also a pathway for oxygen delivery for internal sapwood respiration (Gansert, 2003; Daley and Phillips, 2006; Wittmann and Pfanz, 2014). Consistent with these results, Gansert et al. (2001) found that the xylem sap of was filled with dissolved oxygen when radial influx of oxygen into the sapwood in trees of *Betula pendula* Roth at night. However, the relationship between the nighttime sap flux and oxygen delivery and related mechanisms are poorly investigated and understood.

The responses of plant water flux to environmental variables are also regulated by internal biological traits; for instance, recycling of the internal CO$_2$ from the sapwood led to a relative higher whole-tree water use efficiency (WUE) for species that can carry out stem photosynthesis (Nilson et al., 1993; Vick and Young, 2009). Stems with corticular photosynthesis has no or few stomata in the epidermis, which mainly uses internal CO$_2$ for re-assimilation (Wiebe, 1975; Gartner, 1995). And a few carbohydrates produced by corticular photosynthesis are not primarily used for construction purposes but for maintaining and repairing the xylem hydraulic system with the advantage of proximity (Woodruff, 2013; Cernusak and Cheesman, 2015; Bloemen et al., 2016), Schmitz et al. (2012) found that shading the stem of mangrove trees decreased hydraulic conductance and $E$, these results verified the hypotheses that the woody tissue photosynthesis may play a significant role in keeping the balance of the hydraulic transport system.

Based on the perception that there might be a relationship between stem corticular photosynthesis and nighttime water flux, we used TDP (Granier thermal dissipation probes) to measure the nighttime sap flow of seven common tree species with and without photosynthetic tissue in the stem in low subtropical China. The primary goal of this study was to explore whether corticular photosynthesis has an influence on nighttime sap flow. We hypothesized that the nocturnal sap flow of non-photosynthetic stem species would be lower than in photosynthetic stem species, since those species with photosynthetic stems have greater ability to maintain higher hydraulic conductivity and the greater need for oxygen delivery to photosynthetic tissues in the stems.

**MATERIALS AND METHODS**

**Site Description and Examined Tree Species**

The experiment was carried out at three study sites. The first site was located at the Huangmian State Forest Farm, Guangxi Province, China (109°54′ E, 24°46′ N) where *Eucalyptus grandis* × *urophylla* was mainly planted. The research site was 219 m above sea level. Annual precipitation and average temperature were 1750 mm and 19°C, respectively. The *Eucalyptus* plantations mainly contained red and yellow soils. The plantation is routinely managed with phosphorus fertilization. The leaf area index (LAI) of the studied forest during the experiment was 1.92 m$^2$ m$^{-2}$, and the stem density was 1,375 stems ha$^{-1}$. We selected five trees for sap flow measurements from the 1st to the 31st of October 2012.

The second site was located at the Heshan National Field Research Station, Chinese Academy of Sciences, Guangdong Province, China (112°54′ E, 22°41′ N). The site elevation was 47 m. This region was dominated by a subtropical monsoon climate, in which annual precipitation and average temperature were 1700 mm and 21.7°C, respectively. This site had reddish soil that was acidic and had low nutrient content (Ren et al., 2007). The forest LAI during the experiment was 2.02 m$^2$ m$^{-2}$, and the stem density was 1,019 stems ha$^{-1}$. We selected 3–5 individual trees of the dominant tree species, *Castanopsis fissa*, *Schima superba*, *Michelia macclurei*, and *Castanopsis hystrix* to measure the sap flow from the 1st to the 31st of October 2012.

The third research site was located in the South China Botanical Garden (113°22′ E, 23°11′ N), Chinese Academy of Sciences, Guangzhou, China, at an elevation of 49 m. Annual precipitation and average temperature were 1696 mm and 21.9°C, respectively. The canopy tree species were mainly composed of *Acacia auriculiformis* and *Eucalyptus citriodora*. The LAI and the stem density of the forest were 1.97 m$^2$ m$^{-2}$ and 804 stems ha$^{-1}$, respectively. The forest soil was acidic and brown. We selected five individual trees for each of *A. auriculiformis* and *E. citriodora*, respectively, the sap flow measurement was also conducted from the 1st to the 31st of October 2013. The characteristics of the studied trees from the three research sites are summarized in Table 1.

All these three sites are located in the low subtropical region of China (at the similar latitude), and characterized by a typical subtropical oceanic monsoon climate. Therefore, the similar climatic and topographic conditions for these three sites, as described above, met the conditions of contrast experiment.

**Environmental Monitoring and Calculation of Vapor Pressure Deficit**

At the Heshan site, we obtained the environmental meteorological data from a meteorological station in the Heshan National Field Research Station, including photosynthetically active radiation (PAR, µmol m$^{-2}$ s$^{-1}$), air temperature (T, °C), air relative humidity (RH, %) and wind speed (m, m s$^{-1}$). At the Huangmian Forest Farm and in South China Botanical Garden, the meteorological data were recorded directly from observation towers (18–23 m) in both forest sites. Photosynthetically active radiation (LI-COR, Lincoln, USA), temperature and humidity (Delta-T Devices Ltd., Cambridge, UK) sensors were mounted on the top of the towers. The vapor pressure deficit (VPD, kPa) was calculated according to the following formula (Campbell and Norman, 1998):
TABLE 1 | Biometric parameters of the sap flow sample trees from the seven studied species in three sites (Site 1: Huangman State Forest Farm; Site 2: Heshan National Field Research Station; Site 3: South China Botanical Garden).

| Sites     | Stem corticular photosynthesis | Tree species                | Code | Family   | n  | DBH (cm)       | Tree height (m) | Sapwood area (cm²) |
|----------|--------------------------------|-----------------------------|------|----------|----|----------------|------------------|-------------------|
| Site 1   | With                           | Eucalyptus grandis × Urophylla | EGU  | Myrtaceae | 5  | 9.96 ± 1.08   | 14.97 ± 6.93    | 69.24 ± 13.57     |
|          |                                |                             |      |           |    |                |                  |                   |
| Site 2   | With                           | Castanopsis hystrix         | CH   | Fagaceae  | 3  | 12.63 ± 0.48  | 11.08 ± 1.52    | 94.05 ± 6.57      |
|          | Without                        | Castanopsis fissa           | CF   | Fagaceae  | 3  | 21.57 ± 0.74  | 10.05 ± 4.08    | 162.88 ± 9.28     |
|          | With                           | Michelia macclurei          | MM   | Magnoliaceae | 5 | 19.54 ± 3.73 | 12.12 ± 4.04    | 239.57 ± 81.71    |
|          | Without                        | Schima superba              | SS   | Theaceae  | 5  | 13.92 ± 5.32  | 7.18 ± 1.81     | 143.43 ± 118.47   |
| Site 3   | Without                        | Acacia auriculiformis       | AA   | Fabaceae  | 5  | 30.84 ± 5.49  | 19.99 ± 1.73    | 174.21 ± 42.96    |
|          | With                           | Eucalyptus citriodora       | EC   | Myrtaceae | 5  | 30.04 ± 4.66  | 26.52 ± 3.54    | 191.34 ± 51.57    |

n is the number of trees selected for sap flux measurement. Data are means ± SD.

\[ VPD = a \times \exp\left(\frac{bT}{T + c}\right)(1 - RH) \]

where \( a, b, \) and \( c \) are fixed parameters, which were 0.611 kPa, 17.502 (unitless), and 240.97°C, respectively.

As described above, because this study was conducted in three different sites, the way that we collected the environmental data was the best approximation that we could attain in consideration of the equipment available.

### Sap Flux Measurements

Self-made Granier thermal dissipation probes (TDP) were used to measure sap flow of trees in this study (Granier, 1987). These sensors were consisted of a pair of 20 mm long, 2 mm diameter stainless steel probes, which were vertically inserted into the hydroactive xylem \( \sim 10 \) cm apart. The upper probe was heated by a direct current of 120 mA with a constant power of 0.2 W, whereas the lower probe remained unheated. The Delta-T logger (DL2e, UK) transformed the instantaneous temperature difference of the two probes into a voltage value and collected every 30 s, averaged 10 min (Zhao et al., 2005). Then the sap flow density (g H₂O m⁻² s⁻¹) was calculated according to the following formula:

\[ J_s = 119 \times \left(\frac{\Delta T_m - \Delta T}{\Delta T}\right)^{1.231} \]

where \( \Delta T_m \) is the temperature difference obtained under zero flow conditions, which was determined separately for each tree over 7–10 days to avoid the underestimation of nocturnal sap flow (Lu et al., 2004). \( \Delta T \) is the instantaneous temperature difference.

### Tree Morphological Attributes

Considering the possible influences of tree structural factors on nighttime water flux, Pearson’s correlation analysis between \( J_{sn} \) and \( J_{sn} / J_{sd} \) and biometric parameters (DBH, tree height, sapwood area) (Table 1) was performed. We chose 14–31 trees of each species in different diameter classes around the plot and used an increment borer to drill out wood core from the stems at breast height. The coloration changes between the heartwood and sapwood helped us to measure Sapwood width.

We determined the wood density and wood water content, as well as the saturated wood water content by following Borchert’s method (Borchert, 1994):

Wood density = dry mass/fresh volume

Woody water content = 100 × (fresh mass–dry mass)/fresh mass

Saturated woody water content = 100 × (saturated mass–dry mass)/dry mass.

### Measurement of Chlorophyll Fluorescence

The stem chlorophyll fluorescence of the seven tree species was determined by a pulse-modulated fluorometer (PAM-2100; Walz, Effeltrich, Germany) to compare the photosynthetic performance among them. We chose three trees from each species to measure chlorophyll fluorescence between 9:00 and 11:00 in the morning. Before measurement, the stems were covered with aluminum foil for 2 h of dark adaptation. Initial fluorescence (F₀) was measured under a weak modulated radiation (0.5 \( \mu \)mol m⁻² s⁻¹), and the dark maximum fluorescence (Fₘ) was induced by a 0.8-s pulse of saturating light (2,700 \( \mu \)mol m⁻² s⁻¹). The maximal fluorescence in the light (Fₘ) was recorded after a second saturating pulse, which is normally lowered with respect to Fₘ by non-photochemical quenching. Then the minimum fluorescence of the light-adapted (F₀) can be measured after a weak far-red light. The variable fluorescence (Fᵥ) was calculated as \( Fₔ = (Fₘ - F₀) \). The maximum photochemical quantum yield of PSII (Fᵥ/Fₘ) for dark adapted stems was calculated according to the following equation:

\[ Fᵥ/Fₘ = (Fₔ/Fₘ - F₀)/Fₘ \] (Maxwell and Johnson, 2000), and effective photochemical quantum yield of PSII (Φₚₛₛᵦ) was calculated by using the formula as follows: \( Φ₊ᵦ = (Fₔ/Fₘ - F₀)/Fₘ \) (Genty et al., 1989). Besides, the non-photochemical quenching (NPQ) was determined according to the equation:

\[ NPQ = (Fₘ - Fₔ)/Fₘ \] and the photochemical quenching coefficient (qP) was calculated as \( qP = (Fₔ/Fₘ - F₀) \) (Maxwell and Johnson, 2000).

### Statistical Analysis

A one-way ANOVA followed by a Duncan’s test was conducted to test for significant differences (\( P < 0.05 \)) in the chlorophyll...
fluorescence parameters ($F_s/F_m$, $\Phi_{PSII}$, NPQ, $qP$) among the seven tree species. Pearson correlation analysis was performed to test for significant differences ($P < 0.05$) between nighttime sap flux ($J_{s,n}$) and the ratio of nighttime to daytime sap flux ($J_{s,n}/J_{s,d}$) with tree biometric parameters (DBH, tree height, sapwood area) and soil chemical property (soil pH, soil organic matter, total nitrogen, available phosphorous). Independent-sample $t$-test was performed to test for significant differences ($P < 0.05$) in sap flux of photosynthetic stem species and non-photosynthetic stem species for daytime and nighttime. Statistical analyses were performed in SAS 9.2 (SAS Institute, Cary, NC, USA). Figures were plotted using Origin 8.6 (OriginLab Corp., USA). Partial analyses of variations and ANCOVA were evaluated using the Predictive Analytics Software (PASW, IBM, USA). After Ln-transforming variables, the bivariate relationships between daytime and nighttime sap flux were analyzed using standardized major axis (SMA) regression, and the equation parameters was calculated by using SMATR Version 2.0 (Warton et al., 2006).

RESULTS

Relationship of $J_{s,n}$ with Environmental Factors and Biometric Parameters

The daily sap flow pattern of the seven studied trees is shown in (Figure 1). Pearson’s correlation analysis between $J_{s,n}$ and $J_{s,n}/J_{s,d}$ and biometric parameters (DBH, tree height, sapwood area) showed that the correlation coefficients were very low and non-significant (Table 2), suggesting that canopy position and tree morphological features had little effect on nighttime water flux.

Partial correlation analysis showed a high correlation between $J_{s,n}$ and VPD for E. grandis × urophylla ($P < 0.000$), but there were low correlation coefficients for the other six species, indicating that $J_{s,n}$ was mainly for E in E. grandis × urophylla, while it was for water recharge in the other species (Table 3).

Chlorophyll Fluorescence of Tree Stems

Light is the energy source driving photosynthesis and the signal that regulates plant growth and development. The stems of tree species with chlorophyll can carry out stem photosynthesis (Supplementary Figure 1). Two Eucalyptus species had cortical green tissue with a thickness of ca. 2 mm, and C. hystrix and M. macclurei also had green bark, but its thickness was ca. 0.5 mm. In contrast, C. fissa, S. superb, and A. auriculiformis had no distinct green tissue on the trunk. To evaluate the different photosynthetic capacities of the stems, chlorophyll fluorescence parameters were measured in the sample trees of the seven species. The results showed that $F_s/F_m$ (Maximum photochemical quantum yield of PSII) and the $\Phi_{PSII}$ (effective photochemical quantum yield of PSII) values were lower in non-stem photosynthetic species (C. fissa, S. superb, and A. auriculiformis) compared to stem photosynthetic species (C. hystrix, M. macclurei, E. citriodora, and E. grandis × urophylla; Figure 2).

![FIGURE 1](Image)

Diurnal patterns of sap flux density in seven studied trees. CH, Castanopsis hystrix; MM, Michelia macclurei; EC, Eucalyptus citriodora; EGU, Eucalyptus grandis × urophylla; CF, Castanopsis fissa; SS, Schima superba; AA, Acacia auriculiformis. Data point is the average of all days across the study period; bars indicating SD are omitted for clarity.

| Variables                           | $J_{s,n}$  | $J_{s,n}/J_{s,d}$ |
|-------------------------------------|------------|-------------------|
| $r$   | $P$       | $r$   | $P$       |
| DBH (cm)  | 0.010    | 0.959 | 0.270 | 0.142 |
| Tree height (m) | 0.024    | 0.899 | 0.155 | 0.405 |
| Sapwood area (cm²) | −0.060   | 0.748 | 0.102 | 0.586 |
| Soil pH          | 0.161    | 0.386 | 0.237 | 0.199 |
| Soil organic matter (g kg⁻¹) | −0.069   | 0.712 | −0.341 | 0.060 |
| Soil total nitrogen (g kg⁻¹) | 0.056   | 0.764 | −0.181 | 0.329 |
| Available phosphorous (mg kg⁻¹) | −0.141   | 0.448 | −0.058 | 0.756 |

The quantification of soil nutrient factors is referenced from Lu et al. (2007). Soil chemical properties are for the 0–20 cm soil layer.

![TABLE 2](Image)

| Tree species | VPD |
|--------------|-----|
|              | $r$ | $P$ |
| Photosynthetic stem | CH | −0.143 | 0.549 |
|                  | EC | −0.094 | 0.676 |
|                  | EGU | 0.814 | 0.000 |
|                  | MM | −0.028 | 0.898 |
| Non-photosynthetic stem | AA | 0.408 | 0.067 |
|                  | CF | −0.169 | 0.431 |
|                  | SS | 0.187 | 0.417 |

Seven tree species were divided into two groups according to the species had corticular photosynthesis or not. Significant effects ($P < 0.05$) are indicated in bold.
As shown in Table 4, there was a significant difference in the \( F_v/F_m \) value and the \( \Phi_{PSII} \) value in the sampled trees. However, no significant difference in \( qP \) and \( NPQ \) among the sampled trees was observed (Table 4).

**Pattern of Nighttime Sap Flow and Daytime Sap Flow for Species with and without Photosynthetic Stems**

We plotted \( J_{s,n} \) as a function of \( J_{s,d} \) for the two groups of species, namely those with photosynthetic stems (\( C. hystrix, M. macclurei, E. citriodora, \) and \( E. grandis \times urophylla \)) and without photosynthetic stem (\( C. fissa, S. superb, \) and \( A. auriculiformis \)). As shown in Figure 3, the standardized major axis slope of the photosynthetic stem group (\( Slopes_{SMA} = 2.680 \)) was higher than that of the non-photosynthesis stem group (\( Slopes_{SMA} = 1.943; P = 0.008 \)), indicating that the \( J_{s,n} \) of the trees with stem cortical photosynthesis was more active. After removing the data of \( E. grandis \times urophylla \), where \( J_{s,n} \) was mainly for \( E \) as demonstrated by the correlation analysis in Table 3, the slope of the other three photosynthetic stem species (\( Slopes_{SMA} = 3.244, r^2 = 0.543 \)) was even higher than that of the non-photosynthetic stem trees (\( P = 0.001 \)). If the data were not Ln-transformed and plotted by OLS regression, the slope of species with stem cortical photosynthesis (\( Slopes_{OLS} = 0.167, r^2 = 0.364, P < 0.0001 \)) would still be significantly higher than that of non-photosynthetic stem species (\( Slopes_{OLS} = 0.071, r^2 = 0.198, P < 0.0001; P < 0.001 \)). We have also conducted ANCOVA analyses and found that the group effect (stem cortical photosynthetic capability) was significant if \( J_{s,d} \) is incorporated (Table 5).

As shown in Figure 5, there was a significant difference in \( J_{s,d} (P = 0.013) \) and \( J_{s,n} (P < 0.000) \) between the two groups of tree species, both \( J_{s,d} \) and \( J_{s,n} \) were higher in stem photosynthetic species than in non-stem photosynthetic species.

**DISCUSSION**

We investigated the possible influences of tree morphological attributes (DBH, tree height, sapwood area), meteorological environmental factors and soil environmental factors on the nighttime sap flow in this study. Wood density may exert an influence on sap flow due to its high correlation with hydraulic conductance and carbon assimilation (Santiago et al., 2004; Pratt et al., 2007). For example, De Dios et al. (2013) reported that nighttime stomatal conductance (\( g_s \)) was negatively correlated with wood density in \( E. tereticornis \). In our study, there were no significant correlations between \( J_{s,n} \) or \( J_{s,n}/J_{s,d} \) and wood density, wood water content, or saturated wood water content (Figure 4), indicating that nighttime water flux was not influenced by wood type among these seven tree species. Furthermore, the effects of soil chemical properties on \( J_{s,n} \) were also quantified, and the results showed that neither \( J_{s,n} \) nor \( J_{s,n}/J_{s,d} \) were affected by soil acid alkalinity, soil organic matter or available phosphorous (Table 2). Consistent with these results, the research on \( Arabidopsis thaliana \) also indicated that nighttime water flux had no relationship with soil nutrient conditions (Christman et al., 2009). Interestingly, nearly every soil nutrient indicator was negatively correlated with \( J_{s,n} \) (Table 2), suggesting that nighttime \( J_s \) may be indirectly used for nutrient uptake, which was consistent with the observations by Scholz et al. (2007). However, the decreased nighttime \( J_s \) could not be attributed to soil nutrient status due to a negative relationship between wood density and \( J_{s,d} \) that was observed simultaneously (\( r = -0.709, P = 0.074 \), data not shown). All results demonstrated that tree form features and soil factors

### Table 4 | Variance analysis of the chlorophyll fluorescence parameters \( F_v/F_m \) (Maximum photochemical quantum yield of PSII), \( \Phi_{PSII} \) (effective photochemical quantum yield of PSII), \( qP \) (photochemical quenching), and \( qN \) (Non-photochemical quenching) of stem among seven studied species.

| Variable | Source | Sum of squares | df | Mean square | F-value | P-value |
|----------|--------|----------------|----|-------------|---------|---------|
| \( F_v/F_m \) | Model | 1.469 | 6 | 0.245 | 7.510 | 0.001 |
| Error | 0.457 | 14 | 0.033 | | | |
| Corrected total | 1.926 | 20 | | | | |
| \( \Phi_{PSII} \) | Model | 0.761 | 6 | 0.127 | 5.970 | 0.003 |
| Error | 0.297 | 14 | 0.021 | | | |
| Corrected total | 1.058 | 20 | | | | |
| \( qP \) | Model | 0.449 | 6 | 0.075 | 0.600 | 0.725 |
| Error | 1.738 | 14 | 0.124 | | | |
| Corrected total | 2.187 | 20 | | | | |
| \( qN \) | Model | 0.396 | 6 | 0.066 | 0.58 | 0.744 |
| Error | 1.606 | 14 | 0.115 | | | |
| Corrected total | 2.002 | 20 | | | |
TABLE 5 | ANCOVA results of the effects of group, \( J_{\text{s,d}} \) (daytime sap flux) or the interaction of group and \( J_{\text{s,d}} \) on \( J_{\text{s,n}} \) (nighttime sap flux).

| Source          | Type III Sum of squares | df | Mean squares | \( F \) | Significance |
|-----------------|-------------------------|----|--------------|-------|-------------|
| Corrected model | 18.925                  | 3  | 6.308        | 44.849| 0.000       |
| Intercept       | 6.547                   | 1  | 6.547        | 46.548| 0.000       |
| Group           | 1.597                   | 1  | 1.597        | 11.356| 0.001       |
| \( J_{\text{s,d}} \) | 13.707                 | 1  | 13.707       | 97.450| 0.000       |
| Group \( \times \) \( J_{\text{s,d}} \) | 1.704                 | 1  | 1.704        | 12.113| 0.001       |
| Error           | 24.052                  | 171| 0.141        |       |             |
| Total           | 734.485                 | 175|              |       |             |
| Corrected total | 42.977                  | 174|              |       |             |

Seven tree species were divided into two groups according to the species had corticular photosynthesis or not.

Chen et al. Photosynthetic Stems and Sap Flow

FIGURE 3 | Nighttime sap flux as a function of daytime sap flux in trees with or without stem photosynthesis. The sap flux values reported for each species are the means of 3–5 trees. The solid gray line represents the standardized major axis regression across the four species with stem photosynthesis: \( y = 2.680x - 10.261 \left( r^2 = 0.485, P < 0.0001 \right) \); the solid black line represents the standardized major axis regression across the three species without stem photosynthesis: \( y = 1.943x - 6.936 \left( r^2 = 0.211, P < 0.0001 \right) \).

TABLE 2 | Seven tree species were divided into two groups according to the species had corticular photosynthesis or not.

| Species | Type III Sum of squares | df | Mean squares | \( F \) | Significance |
|---------|-------------------------|----|--------------|-------|-------------|
| Corrected model | 18.925                  | 3  | 6.308        | 44.849| 0.000       |
| Intercept | 6.547                   | 1  | 6.547        | 46.548| 0.000       |
| Group | 1.597                   | 1  | 1.597        | 11.356| 0.001       |
| \( J_{\text{s,d}} \) | 13.707                 | 1  | 13.707       | 97.450| 0.000       |
| Group \( \times \) \( J_{\text{s,d}} \) | 1.704                 | 1  | 1.704        | 12.113| 0.001       |
| Error | 24.052                  | 171| 0.141        |       |             |
| Total | 734.485                 | 175|              |       |             |
| Corrected total | 42.977                  | 174|              |       |             |

Seven tree species were divided into two groups according to the species had corticular photosynthesis or not.

did not contribute to variation in nighttime sap flow in this study (Table 2). Although the possible effects of soil condition and meteorological factors on sap flow were discussed as above, it is noteworthy that the imbalance of the selection of seven tree species in three sites cannot be ignored, which caused the precondition of detecting sites effects is not available in this study.

Even though the trunk surface has low radiation transmittance, stems containing green tissue can use the limited energy source of radiation for effective carbon assimilation (Saveyn et al., 2010; Wittmann and Planz, 2016). Due to morphological features (for example, the absence of stoma or similar organelles) and weak photosynthesis, it is difficult to apply a gas exchange method that is based on the infrared CO₂-absorption principle for the in situ measurement of stem photosynthesis (Teskey et al., 2008; Zhu et al., 2012). Therefore, as an efficient and non-destructive method, the in vivo measurement of chlorophyll fluorescence has been widely used in plants and is considered an appropriate way to evaluate stem photosynthesis (Wittmann and Planz, 2016). An increase in \( F_{v}/F_{m} \) and \( \Phi_{\text{PSII}} \) reflects higher light use efficiency in plants (Baker and Rosenvist, 2004; Baker, 2008). The results of our study showed that there were lower values of \( F_{v}/F_{m} \) and \( \Phi_{\text{PSII}} \) in the species without stem green tissue compared to those with stem green tissue (Figure 2), demonstrating that stem photosynthetic species had a higher utilization of the weak light energy.

In our study, we found that species with chlorophyll in the stem have a more active \( J_{\text{s,n}} \) (Figure 3). On the one hand, species that have photosynthetic stems can use the weak light to produce carbohydrates during daytime. The accumulation of photosynthates helps the stem maintain a high metabolic rate at night, which depends on nocturnal sap flux to deliver oxygen. Similar results have also been reported by Del Hierro et al. (2002), which indicated that the higher \( J_{\text{s,n}} \) in the species have stem photosynthetic capacity is probably favorable for oxygen transport to xylem or sapwood parenchyma. In addition, the research on bark chlorophyll content and photosynthesis reported that photosynthesis and respiration were higher in the tree species with more chlorophyll in the stem (Ren et al., 2010; Wittmann and Planz, 2016).
And highly positive correlation was found between branch photosynthesis and respiration in nine tree species (Berveiller et al., 2007). Tree species with stem photosynthesis can use internal stem CO₂ to produce extra O₂ during the day (Pfanz et al., 2002; Borisjuk and Rolletschek, 2009). Nevertheless, the stem green tissue is not “oxygen source” but “oxygen sink” due to that the light is unavailable at night. Despite the respiration during the night is not as intensive as that during the daytime because of the lower nocturnal temperature, the respiration of green tissue is always higher than that of xylem living cells, which probably leads to hypoxia (Butler and Landsberg, 1981; Pallardy, 2008). Thus, the O₂ conveyed by nighttime sap flux can alleviate hypoxia stress to some extent (Eklund, 2000; Gansert, 2003; Mancuso and Marras, 2003; Sorz and Hietz, 2006, 2008). On the other hand, the carbohydrates produced by stem corticular photosynthesis may have a role in maintaining and repairing the xylem hydraulic system (Steppe et al., 2015; Bloemen et al., 2016).

Then, a high transpiration rate during the day can lead to an intensive stem water deficit, thus requiring more water recharge during the following night (Fuentes et al., 2013). We found that \( J_{s,d} \) and \( J_{s,n} \) of non-photosynthetic stem species were lower \((P = 0.013\) and \(P < 0.000\), respectively; Figure 5) than photosynthetic stem species. Similarly, Schmitz et al. (2012) found that the hydraulic conductivity was lower in the non-photosynthetic stem, demonstrating that the stem corticular photosynthesis has a significant role in regulating water transport. Based on the above analysis, our study provided observation data explaining that...
corticular photosynthesis has an effect on the nighttime water flow.

**CONCLUSION**

Nocturnal water flux plays a role in delivering oxygen to the internal parenchymatous tissues of the stem xylem wood and in replenishing water consumed through transpiration during day. In our study, we found that species with stem corticular photosynthesis are likely to have higher $F_s$, which is largely consistent with our hypothesis that the nocturnal sap flow of non-photosynthetic stem species would be lower than in photosynthetic stem species. These prospective result will be verified by the improvement of experimental scheme, and we suggest that it is time to rethink the relationship between corticular photosynthesis and nocturnal water flux. However, due to the existence of species-specific effects, additional studies should be done to fully assess the influence of stem photosynthesis on nighttime water flux, one of the issues that is worth paying attention to is the possible chemical composition variation in the cortex below the bark and the dilution effect from sap flow. In addition, the quantification of oxygen concentration in the sapwood needs to be investigated in the future studies.

**REFERENCES**

Baker, N. R. (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* 59, 89–113. doi: 10.1146/annurev.arplant.59.032607.092759

Baker, N. R., and Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J. Exp. Bot.* 55, 1607–1621. doi: 10.1093/jxb/erh196

Berveiller, D., Kierzkowski, D., and Damesin, C. (2007). Interspecific variability of stem photosynthesis among tree species. *Tree Physiol.* 27, 53–61. doi: 10.1093/treephys/27.1.53

Blenken, J., Vergeynst, L., Orlaat-Michels, L., and Steppe, K. (2016). How important is woody tissue photosynthesis in poplar during drought stress? *Trees* 30, 63–72. doi: 10.1007/s00468-014-1132-9

Borchert, R. (1994). Soil and water storage determine phenology and distribution of tropical dry forest trees. *Ecology* 75, 1437–1449. doi: 10.2307/1937467

Borisjuk, L., and Rolletschek, H. (2009). The oxygen status of the developing seed. *New Phytol.* 182, 17–30. doi: 10.1111/j.1469-8137.2008.02752.x

Butler, D. R., and Landsberg, J. J. (1981). Respiration rates of apple trees, estimated by CO$_2$-efflux measurements. *Plant Cell Environ.* 4, 153–159. doi: 10.1111/j.1365-3040.1981.tb01037.x

Campbell, G. S., and Norman, J. M. (1998). *An Introduction to Environmental Biophysics*, 2nd Edn. New York, NY: Springer.

Cernusak, L. A., and Cheesman, A. W. (2015). The benefits of recycling: how photosynthetic bark can increase drought tolerance. *New Phytol.* 208, 993–997. doi: 10.1111/nph.13723

Christman, M. A., Donovan, L. A., and Richards, J. H. (2009). Magnitude of nighttime transpiration does not affect plant growth or nutrition in well-watered Arabidopsis. *Physiol. Plant.* 136, 264–273. doi: 10.1111/j.1399-3054.2009.01216.x

Daley, M. J., and Phillips, N. G. (2006). Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiol.* 26, 411–419. doi: 10.1093/treephys/26.4.411

Dawson, T. E., Burgess, S., Tu, K. P., Oliveira, R. S., Santiago, L. S., Fisher, J. B., et al. (2007). Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiol.* 27, 561–575. doi: 10.1093/treephys/27.4.561

De Dios, V. R., Turnbull, M. H., Barbour, M. M., Ontedhu, J., Ghannoum, O., and Tissue, D. T. (2013). Soil phosphorous and endogenous rhythms exert a larger impact than CO$_2$ or temperature on nocturnal stomatal conductance in Eucalyptus tereticornis. *Tree Physiol.* 33, 1206–1215. doi: 10.1093/treephys/tpo091

Del Hierro, A. M., Kromberger, W., Hietz, P., and Offenthaler, I. (2002). A new method to determine the oxygen concentration inside the sapwood of trees. *J. Exp. Bot.* 53, 559–563. doi: 10.1093/jxb/53.3.559

Ekuland, L. (2000). Internal oxygen levels decrease during the growing season and with increasing stem height. *Trees* 14, 177–180. doi: 10.1007/PL00009761

Escalona, J. M., Fuentes, S., Tomás, M., Martorell, S., Flexas, J., and Medrano, H. (2012). Responses of leaf night respiration and transpiration to water stress in *Vitis vinifera* L. *Agri. Water Manage.* 118, 50–58. doi: 10.1016/j.agwat.2012.11.018

Forster, M. A. (2014). How significant is nocturnal sap flow? *Tree Physiol.* 34, 757–765. doi: 10.1093/treephys/tpu051

Fuentes, S., Mahadevan, M., Bonada, M., Skewes, M. A., and Cox, J. W. (2013). Night-time sap flow is parabolically linked to midday water potential for field-grown almond trees. *Irrig. Sci.* 31, 1265–1276. doi: 10.1007/s00271-013-0403-3

Gansert, D. (2003). Xylem sap flow as a major pathway for oxygen supply to the sapwood of birch (*Betula pubescens* Ehr.). *Plant Cell Environ.* 26, 1803–1814. doi: 10.1046/j.1365-3040.2003.01097.x

Gansert, D., Burgdorf, M., and Lösch, R. (2001). A novel approach to the in situ measurement of oxygen concentrations in the sapwood of woody species. *Plant Cell Environ.* 24, 1053–1064. doi: 10.1046/j.1365-3040.2001.00751.x

Garten, B. L. (1995). *Plant Stems: Physiology and Functional Morphology*. City, UT: Academic Press.

Genty, B., Briantais, J. M., and Baker, N. R. (1989). The relationship between corticular photosynthesis and nocturnal water flow. *Physiol. Plant.* 75, 777–785. doi: 10.1111/j.0031-1320.1989.tb04310.x

Granier, A. (1987). Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree Physiol.* 3, 309–320. doi: 10.1093/treephys/3.4.309

**AUTHOR CONTRIBUTIONS**

XC and JG: Conducted the experiments, analyzed the experimental data, and wrote the manuscript; JG and PZ: Designed the experiment. PZ, HM, LZ, GN and LO: Contributed to revising manuscript.

**ACKNOWLEDGMENTS**

This study was financially supported by the National Natural Science Foundation of China (Grant No.: 31670410, 41030638) and National key Research and Development Program (Grant No.: 2016YFC0500106-02).

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.00030/full#supplementary-material

**Supplementary Figure 1** | The seven studied tree species with stem photosynthesis: *Castanopsis hystrix* (a), *Michelia macclurei* (b), *Eucalyptus citroidora* (c), *Eucalyptus grandis × urrophylla* (d), and without stem photosynthesis: *Castanopsis fissa* (e), *Schima superba* (f), and *Acacia auriculiformis* (g).
Lu, P., Urban, L., and Zhao, P. (2004). Granier’s thermal dissipation probe (TDP) method for measuring sap flow in trees: theory and practice. *Acta Bot. Sin.*, 46, 631–646. doi: 10.3321/j.issn:1672-9072.2004.06.001

Lu, Y., Feng, H., and Gan, H. H. (2007). Soil fertility characteristics and enzyme activity for urban parks in Guangzhou City. *J. Soil Water Conserv.* 21, 106–163. doi: 10.13870/j.cnki.sjswch.2007.01.039

Ludwig, F., Jewitt, R. A., and Donovan, L. A. (2006). Nutrient and water addition effects on day- and night-time conductance and transpiration in a C3 desert annual. *Oecologia* 148, 219–225. doi: 10.1007/s00442-006-0367-6

Marks, C. O., and Lechowicz, M. J. (2007). The ecological and functional correlates of nocturnal transpiration. *Tree Physiol.* 27, 577–584. doi: 10.1093/treephys/27.4.577

Mancuso, S., and Marras, A. M. (2003). Different pathways of the oxygen supply in the sapwood of young Olea europaea trees. *Plantia* 216, 1028–1033. doi: 10.1007/s00425-002-0956-5

Maxwell, K., and Johnson, G. N. (2000). Chlorophyll fluorescence: a practical guide. *Elsevier Inc.*

Mott, G. T. (1985). Spacing and density effects on day- and night-time conductance and transpiration in a C3 desert annual. *Tree Physiol.* 14, 103–114. doi: 10.1093/treephys/14.2.103

Nilsen, E. T., Karpa, D., Mooney, H. A., and Field, C. (1993). Patterns of stem photosynthesis in two invasive legumes (*Spartium junceum, Cytisus scoparius*) of the California coastal region. *Am. J. Bot.* 80, 1126–1136. doi: 10.2307/2445540

Oishi, A. C., Oren, R., and Stoy, P. C. (2008). Estimating components of forest evapotranspiration: a footprint approach for scaling sap flux measurements. *Agric. For. Meteorol.* 148, 719–732. doi: 10.1016/j.agrformet.2008.06.013

Pallardy, S. G. (2008). *Tree Physiology of Woody Plants*, 3rd Edn. Salt Lake City, UT: Elsevier Inc.

Pfanz, H., Aschan, G., Langenfeld-HEYser, R., Wittmann, C., and Loose, M. (2002). Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. *Naturwissenschaften* 89, 147–162. doi: 10.1007/s00114-002-0309-z

Pratt, R. B., Jacobsen, A. L., Ewers, F. W., and Davis, S. D. (2007). Relationships among xylem transport, biomechanics and storage in stems and roots of nine Rhamnaceae species of the California chaparral. *New Phytol.* 174, 787–798. doi: 10.1111/j.1469-8137.2007.02061.x

Ren, F. F., Sun, G. Y., Hu, Y. B., Fan, C. H., and Cai, S. Y. (2009). A preliminary study on photosynthetic characteristics of *Corylonema* in several tree barks. *Plant Physiol. J.* 45, 249–252. doi: 10.13592/j.cnki.pjjp.2009.03.021

Ren, H., Du, W. B., Wang, J., Yu, Z. Y., and Guo, Q. F. (2007). Natural restoration of degraded rangeland ecosystem in Heshan hilly land. *Environ. Exp. Bot.* 59, 353–3600. doi: 10.1016/S1872-2032(07)60076-6

Santiago, L. S., Goldstein, G., Meinzer, F. C., Fisher, J. B., Machado, K., Woodruff, D., et al. (2004). Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140, 543–550. doi: 10.1007/s00442-004-1624-1

Scholz, F. G., Bucci, S. J., Goldstein, G., Meinzer, F. C., Franco, A. C., and Miralles-Wilhelm, F. (2007). Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree Physiol.* 27, 551–559. doi: 10.1093/treephys/27.4.551

Soro, J., and Hietz, P. (2006). Gas diffusion through wood: implications for oxygen supply. *Trees* 20, 34–41. doi: 10.1007/s00468-005-0010-x

Soro, J., and Hietz, P. (2008). Is oxygen involved in beech (*Fagus sylvatica*) red heartwood formation? *Trees* 22, 175–185. doi: 10.1007/s00468-007-0187-2

Steppe, K., Sterck, F., and Deslauriers, A. (2015). Diel growth dynamics in tree stems: linking anatomy and ecophysiology. *Trends Plant Sci.* 20, 335–343. doi: 10.1016/j.tplants.2015.03.013

Teskey, R. O., Saveyn, A., Steppe, K., and McGuire, M. A. (2008). Origin, fate and significance of CO2 in tree stems. *New Phytol.* 177, 17–32. doi: 10.1111/j.1399-3054.2008.01861.x

Vick, J. K., and Young, D. R. (2009). Corticular photosynthesis: a mechanism to enhance shrub expansion in coastal environments. *Photosynthetica* 47, 26–32. doi: 10.1007/s11099-009-0006-7

Vang, H., Zhao, P., Cai, X. A., Wang, M. A., L., Rao, X. Q., et al. (2007). Partitioning of night sap flow of *Acacia mangium* and its implication for estimating whole-tree transpiration. *Chin. J. Plant Ecol.* 31, 777–786. doi: 10.17521/cjpe.2007.0099

Warton, D. I., Wright, I. J., Falster, D. S., and Westoby, M. (2006). Bivariate line-fitting methods for allometry. *Biol. Rev.* 81, 259–291. doi: 10.1017/S1464793106007007

Wiebe, H. (1975). Photosynthesis in wood. *Physiol. Plant.* 33, 245–246. doi: 10.1111/j.1399-3054.1975.tb03162.x

Wittmann, C., and Pfanz, H. (2014). Bark and woody tissue photosynthesis means to avoid hypoxia or anoxia in developing stem tissues. *Funct. Plant Biol.* 41, 940–953. doi: 10.1071/FP14046

Wittmann, C., and Pfanz, H. (2016). The optical, absorptive and chlorophyll fluorescence properties of young stems of five woody species. *Environ. Exp. Bot.* 121, 83–93. doi: 10.1016/j.envexpbot.2015.05.007

Woodruff, D. R. (2013). The impacts of water stress on phloem transport in Douglas fir trees. *Tree Physiol.* 34, 5–14. doi: 10.1093/treephys/tpt106

Zhu, L. W., Zhao, P., Cai, X. A., Zeng, X. P., Ni, G. Y., Zhang, J. Y., et al. (2012). Effects of sap velocity on the daytime increase of stem CO2 efflux from stems of *Schima superba* trees. *Trees* 26, 535–542. doi: 10.1007/s00468-011-0615-1

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Chen, Gao, Zhao, McCarthy, Zhu, Ni and Ouyang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.