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ORIGINAL ARTICLE

Importance of stem photosynthesis in plant carbon allocation of *Clusia minor*

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Abstract

Key message **Restricted access of light for stems reduced carbon acquisition there and limited the biomass growth of the roots.**

Abstract Light access can affect the microatmosphere within stems, creating favourable conditions for photosynthesis. We tested the hypothesis that stem photosynthesis modifies carbon allocation within plants and also can affect root growth. To verify this hypothesis, parts of *Clusia minor* L. stems were covered with dark material for 8 months to block light access to stems, and then, we compared morphological traits, biomass increment, photosynthetic activity and carbon isotopic signature ($\delta^{13}C$) in plants with dark- and light-exposed stems. *Clusia minor* stems were characterized by chlorophyll presence from pith to cortex, active photosystem II and 79% re-assimilation of respired CO_2. We also revealed 24-h changes in the $\delta^{13}C$ of carbohydrates exported from leaves. Keeping stems in darkness led to a significant lowering in root biomass and shoot-to-root weight index (I_w). Moreover, reductions in stem CO_2 efflux and the $\delta^{13}C$ in the roots and stems were also observed. Our results indicate that the lack of stem photosynthesis affects photosynthate flux to heterotrophic organs, such as roots, stems and probably expanding leaves.

Keywords Roots · Biomass · ^{13}C discrimination · Carbon allocation · Stem photosynthesis · Stems

Introduction

The tree stem is thought to have several major functions: support, transport and storage (Givnish 1995). In addition to these well-known functions, photosynthesis seems to be important at least in trees with green (non-lignified) stems. Many living tissues in stems are equipped with chloroplasts, demonstrating that their photochemistry may affect carbon and energy balance (Yiotis and Manetas 2010); and recently, the participation of stem photosynthesis in drought stress tolerance was indicated (Cernusak and Cheesman 2015; Vandegehuchte and Bloemen 2015; Ávila-Lovera et al. 2017; Ávila-Lovera and Tezara 2018).

Stem photosynthesis is often an underestimated process especially in trees where green cells are deeply hidden by the cork layer (Yiotis et al. 2009). When it is transmitted through the cork, light energy in the photosynthetically active radiation (PAR) range can be utilized in stems. Often its intensity is weak and spectrally different in comparison with incident irradiation as a result of the cork reflectance and absorption (Pfanz 1999; Pilarski et al. 2008; Wittmann and Pfanz 2016).

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The limited permeability of stem tissue for water vapour and other gases is at least partially responsible for high CO_2 concentration and lower O_2 abundance when compared with the surrounding atmosphere (Maier and Clinton 2006; Teskey et al. 2008; Kocurek and Pilarski 2012). CO_2 refixation measured as percent reduction of CO_2 efflux in light is considered a main parameter describing the photosynthetic activity of stems. However, this parameter does not take into account the entire complexity of processes related to photosynthesis and occurring in the veins of midribs, petioles and stems (Yiotis and Manetas 2010; Kuźniak et al. 2016; Miszalski et al. 2017). Photosynthesis in the stems' cells can be a source of energy (ATP) for these cells or carbohydrate transportation over long distances. Another benefit of this phenomenon is that it provides the amount of oxygen in the stem, necessary for respiration. It is known that the respiration decreases with lowering O_2 concentration (Spicer and Holbrook 2005) and stem photosynthesis enables higher respiration rates (Wittmann and Pfanz 2018). In addition, CO_2 released during dark respiration can be re-assimilated by stem photosynthesis.

The benefits from stem photosynthesis are not necessarily limited to the anatomical part in which they appear. Saveyn et al. (2010) revealed that light exclusion from stems in three woody species resulted in a reduction in chlorophyll concentration and radial growth. They also revealed that in defoliated plants, darkening of trunks caused a reduction in bud biomass (Saveyn et al. 2010); thus, the photosynthetic activity of stems influences the growth of other organs, likely by affecting carbohydrate allocation.

The mechanism of carbon distribution and carbohydrate transport can be tracked by changes in $\delta^{13}C$ in organic matter (OM) and in respiratory CO_2 (Moore et al. 2008). The first step of carbon discrimination in plants includes fractionation during CO_2 diffusion through the leaf boundary layer and stomata, and discrimination by RubisCO and PEPC. Although carbon isotope discrimination during photosynthetic CO_2 fixation is a rather well described and understood phenomenon (Farquhar et al. 1982; Fung et al. 1997; Borland and Dodd 2002), much less is known about the isotopic fractionation associated with the post-photosynthetic processes following carboxylation in leaf tissues (Badeck et al. 2005; Ghashghaie and Badeck 2014; Miszalski et al. 2016). It is proposed that post-photosynthetic carbon fractionation is clearly displayed in whole plant ^{13}C distribution. According to Cernusak et al. (2009), non-photosynthetic/heterotrophic tissues (e.g. stems, fruits and roots) in C_3 plants tend to be enriched in ^{13}C compared with the leaves that supply these tissues with photosynthate. Also, young emerging leaves of C_3 plants for which growth may be mostly heterotrophic, tend to be ^{13}C enriched (Damesin and Lelarge 2003) and later during the development of the photosynthetic apparatus, $\delta^{13}C$ is lowered.

Cernusak et al. (2009) described six hypotheses explaining post-photosynthetic isotope fractionation. One of the hypotheses (Tcherkez et al. 2004) stipulates that post-photosynthetic carbon fractionation is initiated by aldolase, which condenses two trioses to form fructose 1–6 bisphosphate, discriminates carbon isotopes in favour of ^{13}C and enriches hexose molecules (Rossmann et al. 1991; Gleixner and Schmidt 1997; Gilbert et al. 2011, 2012; Ghalagashie and Badeck 2014) and subsequently transitory starch in ^{13}C. The remaining ^{13}C-depleted trioses are transported to the cytosol, forming sucrose that is expected to be ^{13}C depleted compared with sucrose derived from the degradation of transitory starch at night. The proportion between night and day sucrose affects $\delta^{13}C$ sucrose levels loaded into the phloem sap. Thus, light period-derived sucrose and other carbohydrates that migrate in the phloem sap are typically ^{13}C depleted compared with those loaded and transported during the night (Gessler et al. 2007, 2008).

To date, most of the investigations have been focused on the benefits stems gain based on their photosynthetic activity (Pfanz 1999; Eyles et al. 2009; Miszalski et al. 2017; Ávila-Lovera and Tezara 2018; Wittmann and Pfanz 2018). To the best of our knowledge, the only attempt of Saveyn et al. (2010) to show the effect of photoassimilates produced in stems on other organs was observed in buds.

Our hypothesis assumes that limited stem photosynthesis will affect root biomass. For research, we have chosen the tropical tree *Clusia minor* L. of the *Clusiaceae* family. The previous results considering *Clusia multiflora* and *C. rosea* proved that their stems are characterized by high permeability for water vapour which is an adaptation to low water availability. Also, cross sections of *Clusia* stems showed well-developed water–storage cortex (Lüttge 2008; Kocurek et al. 2015; Miszalski et al. 2017). This tissue develops a photosynthetically active cell layer, several times thicker than the one in temperate trees (Dima et al. 2006; Berveiller et al. 2007). Here, we report the $\delta^{13}C$ values in leaves, stems and roots of *C. minor* cultivated with full access to light and partially darkened stems. In our experiments, heterogeneity of carbohydrates and its distribution in the diurnal cycle was also tested. Moreover, we discuss other possible explanations concerning $\delta^{13}C$ heterogeneity and carbon allocation in woody C_3 plants.

Materials and methods

Plant material

The experiments were performed on 8-month-old cuttings from 2-year-old C*lusia minor* L. plants. Two-leaf pair cuttings were obtained from mother plants. The cuttings were rooted for 4 weeks in tap water. After roots appeared, the

cuttings were transferred to individual pots with 500 g soil. This soil was composed of universal substrate (Substral Natural, Substral Evergreen Garden Care Poland, Warsaw, Poland) containing NPK 9:5:10 (pH 6.0–6.5) mixed in a 1:1 ratio with quartz sand. Plants were divided in two groups (5 plants per each). Stems of one group were darkened with black cotton tape; only approximately 10% of whole stem area above the black tape of shoots was not covered with the tape. However, after 8 months of growth (January–September), the portion of the stem above the darkened part accounted for approximately 40% of the whole stem area. Plants were grown under a natural photoperiod in a 12 m^2 growth room located in Kraków, Poland (50°08 N, 19°84 E). The experimental plants covered around 20% of the maximal capacity of the well-ventilated growth room, in order to reduce fluctuations in isotope composition of source CO_2. However, the exact fluctuation was not measured.

Clusia minor is categorized as a C_3-CAM intermediate plant (Borland et al. 1998) that develops CAM when exposed to stress; thus, extensive watering is crucial to sustain C_3 metabolism. After an 8-month growth period, plants developed 6–10 new leaves. The plants were thoroughly irrigated every third day using 50 ml of tap water. All measurements except growth analyses were performed after 8 months of experiments, and then phloem sap and fresh material were obtained for $\delta^{13}C$ and carbohydrates content determination.

Chlorophyll localization in stems

The distribution of chlorophyll in stems was determined based on the autofluorescence of chl *a*. Hand-cut cross-sections were obtained approximately 2 cm below the upper part of black tape and approximately 1 cm above it (Fig. 1). Cross-sections were observed in distilled water using a Nikon ECLIPSE Ni light and epifluorescence microscope (Nikon, Japan) equipped with a Digital Sight series DS-Fi1c microscope camera and NIS Imaging software (Nikon version 4.11). Red and green colours correspond to autofluorescence of chlorophyll and cell walls, respectively.

Fluorescence measurements

Chlorophyll fluorescence imaging was performed using a pulse-amplitude modulated system (Imaging PAM, Walz, Effeltrich, Germany) equipped with a bank of blue ($\lambda = 450$ nm) LEDs and a CCD camera capturing fluorescence images. Measurements were performed between 7:00 and 11:00 h on dark-adapted (30 min) plants. The completely dark-adapted parts of *C. minor* stems were cut and moved to a container filled with distilled water to avoid tissue desiccation. Then, the stems were split with a razor blade and placed into a transparent polyethylene (9 cm × 13 cm) sealable bag completely filled with water. Such packed samples of stems were given a saturating (ca. 3500 µmol m^{-2} s^{-1}) pulse to assess the maximum PSII photochemical efficiency as $F_v/F_m = (F_m - F_0)/F_m$, where F_0 and F_m are the fluorescence yields with open and closed PSII reaction centres, respectively.

Phloem sap samples

To obtain phloem sap, leaves were detached at the base of the petiole. Then, each leaf was inserted with the petiole into a vial containing 1.5 ml of 15 mM sodium hexametaphosphate (Sigma-Aldrich, St Louis, MO, USA). Exudates from the first hour were discarded, and the vials replaced by new vials containing the same amount of solution. Sap collections were stopped after 5 h, and samples were boiled for 1 min to stop enzyme activities (Wild et al. 2010). In the obtained exudates, $\delta^{13}C$ and carbohydrate content were determined.

Carbon isotope analysis in organic samples

Frozen samples of leaves, barks and roots were oven-dried for 24 h at 105 °C before being ground to a fine powder for isotope analysis. The samples of phloem sap were lyophilized for 24 h before $\delta^{13}C$ determination. Isotope ratio measurements of ^{13}C were performed on a DELTA^{plus} Mass Spectrometer (Finnigan, Germany) coupled with a Flash1112 NC Series Elemental Analyzer (ThermoFinnigan, Italy) in a continuous flow mode. Laboratory isotope standards (sorghum flour $\delta^{13}C = -13.68‰$ and protein $-26.98‰$, IVA Analysentechnik, Germany) were used in measurements to calculate the final $\delta^{13}C$ results as follows (Malec-Czechowska and Wierzchnicki 2013):

$$\delta^{13}C \equiv \delta(^{13}C/^{12}C) = \left(\frac{R - R_{stnd}}{R_{stnd}}\right) \times 1000 \quad [‰], \tag{1}$$

where R is the isotope ratio $^{13}C/^{12}C$ in the sample, and R_{stnd} is the isotope ratio $^{13}C/^{12}C$ in international standard PDB (Pee Dee Belemnite).

Gas exchange measurements

The measurements of gas exchange were conducted with a Portable Photosynthesis System LI-6400XT (LI-COR Inc., Lincoln, NE, USA) using leaves and stems under greenhouse conditions. Gas (CO_2 and H_2O) efflux rates were measured using a standard chamber for leaves and a conifer chamber 6400-05 for stems. Measurements of gas exchange of the leaves were performed continuously (every 15 min) under greenhouse conditions under sun exposure (30–37 °C, 28–48% relative humidity (RH), 400–900 µmol mol^{-1} CO_2, and 0–1250 µmol PAR m^{-2} s^{-1}). Refixation of stems was

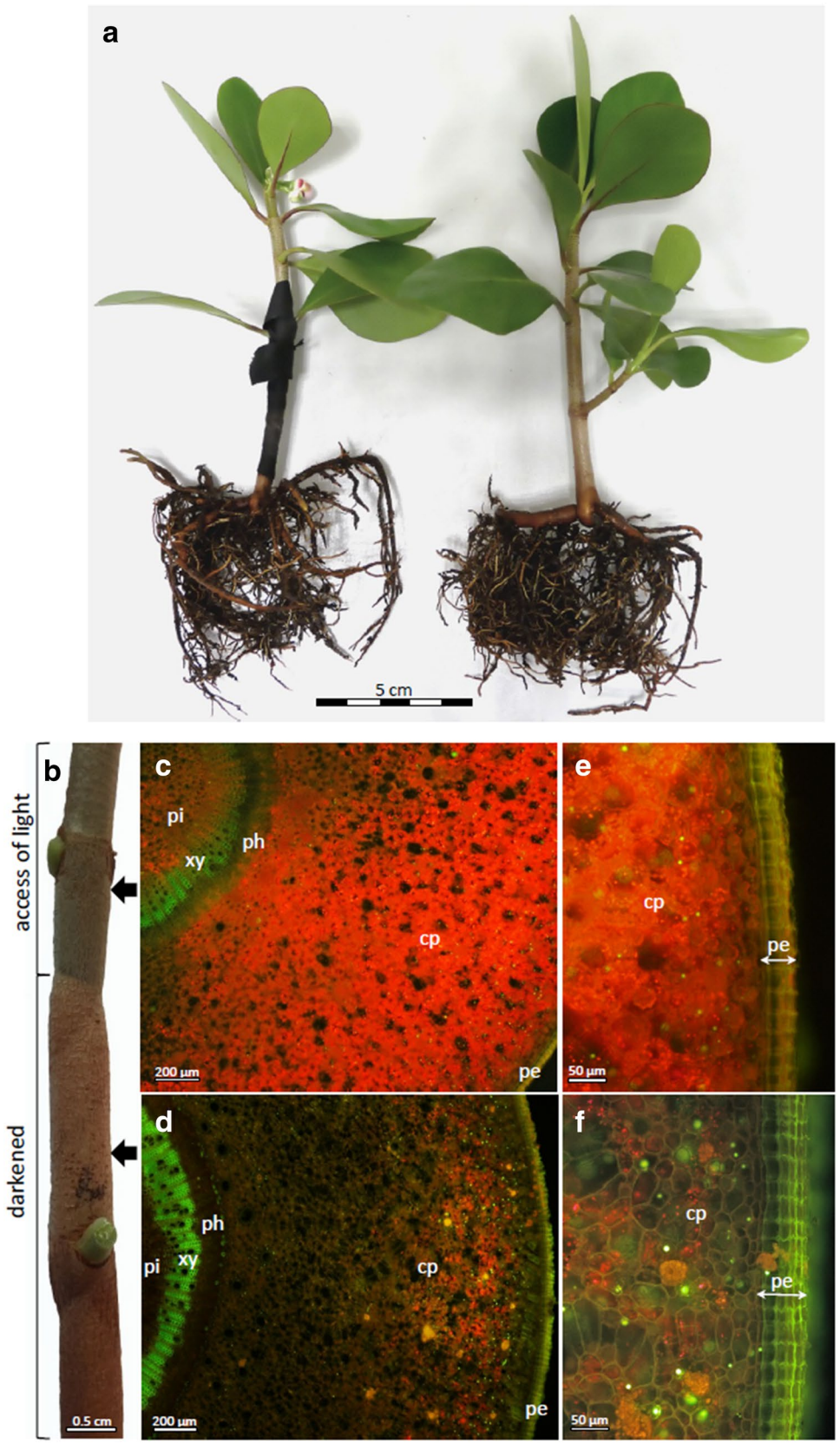

Fig. 1 Eight-month-old cuttings of *C. minor* partly darkened with black tape (left) and with access to light (right) (**a**); partly darkened stem (**b**); cross-sections of the portion of the stem with access to light (**c**, **e**); cross-sections of portion of stem subject to dark for 8 months (**d**, **f**) observed by epifluorescence microscopy; *pi* pith, *cp* cortex parenchyma, *ph* phloem, *xy* xylem, *pe* peridermis. Red and green colours correspond to autofluorescence of chlorophyll and cell walls, respectively. Black arrows indicate locations where cross-sections were obtained

estimated from CO_2 efflux under laboratory conditions (30 °C, 30% RH, CO_2 concentration 400 µmol mol^{-1}, in darkness or 1000 µmol PAR m^{-2} s^{-1}). Refixation by stems was estimated according to Cernusak and Marshall (2000) and obtained from CO_2 efflux in the dark (*R*d) and under illumination (*R*lt) as follows:

$$\text{refixation} = \left(\frac{Rd - Rlt}{Rd}\right) \times 100 \quad [\%]. \tag{2}$$

Carbohydrate contents

The contents of soluble sugars were determined according to Black et al. (1996) with some modifications according to Janeczko et al. (2010). Approximately 5 mg of the lyophilized samples (LABCONCO, USA) were homogenized with 80% (v:v) ethanol at 80 °C for 40 min and then centrifuged at 13 000 × *g* for 10 min. After dissolving in 100 ml of ultra-pure water, samples were filtered through a 0.22-µm filter (Costar Spin-x, Coring, NY, USA). Samples (1–2 ml) were injected onto a Hamilton RCX-10 (250 mm × 4.1 mm) column (Hamilton, Reno, NV, USA) and separated by high-performance liquid chromatography (HPLC, Beckman, Fullerton, CA, USA) using the Beckman System Gold 125 NM Solvent Module equipped with an ESA Coulochem II Analytical Cell 5040 and gold electrode (ESA Chelmsford, MA, USA).

Statistics

Statistical analyses of the data were performed using Statistica 12.0 (Statsoft, Tulsa, OK, USA). Morphological parameters, $\delta^{13}C$ values, gas exchange and carbohydrate levels were evaluated by statistical analysis of variance (ANOVA). Images from chlorophyll *a* fluorescence and epifluorescence microscopy represent typical examples of at least five repetitions. Detailed information about statistic tests and the number of replicates is indicated in the description of tables and figures.

Results

Stems were strongly affected by the 8-month period of darkening treatment (Fig. 1). The darkened parts of the stem became brown because of the cork layer, whereas the light exposed parts exhibited a visibly green colour. The changes also included the interior of stems and concerned chlorophyll amount, which was clearly visible on the cross-sections as a red signal. Initially, chlorophyll presence was observed in all tissues of the stem and subsequently disappeared from the pith, pith rays and phloem. Ultimately, only small amounts of chlorophyll remained in the upper cortex parenchyma.

The maximum PSII photochemical efficiency (F_v/F_m) measured at the cross-section of stems exposed to light (Fig. 2) was observed in all tissues based on the presence of chlorophyll as noted above on the epifluorescence pictures (Fig. 1). F_v/F_m values varied from 0.84 in cortex parenchyma to 0.83 in the piths, whereas areas containing no chlorophyll, such as wood, appeared black, which is similar to the background signal.

The gas exchange values of plants with lightened and darkened stems are shown in Table 1. The net photosynthesis of illuminated stems was slightly negative at 1000 µmol m^{-2} s^{-1} PAR, whereas dark respiration of those stems reached 0.9 µmol m^{-2} s^{-1}. Plants with darkened stems were characterized by reduced dark respiration. Refixation calculated from respiration and net photosynthesis was possible only in the case of illuminated stems. Conductance of water vapour was significantly increased in lightened stems when compared with darkened ones.

The $\delta^{13}C$ values are presented in Fig. 3. The lowest $\delta^{13}C$ was noted in leaves of plants with light-exposed stems. These values were significantly higher in the barks of stems and roots. In plants with darkened stems, $\delta^{13}C$ values for leaves, barks and roots were reduced by approximately

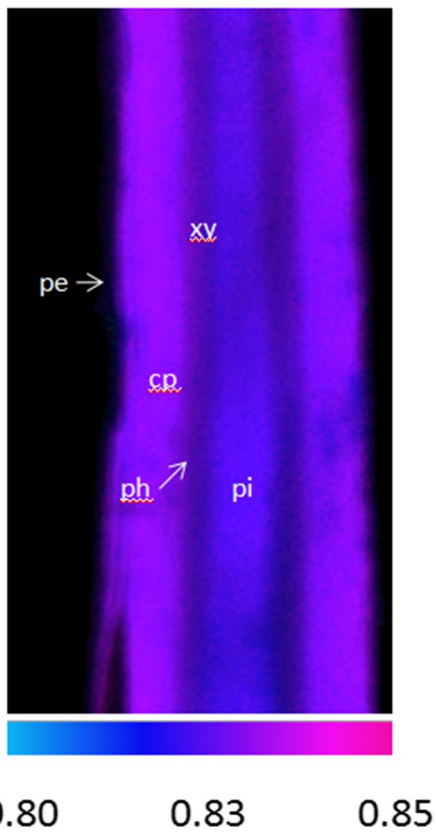

Fig. 2 Chl *a* fluorescence imaging of F_v/F_m. All images are normalised to the false-colour bar provided. The pixel value display is based on a false-colour scale ranging from blue to purple (0.80–0.85)

Table 1 Stem gas exchange in plants of *C. minor* growing in light and dark conditions (means ± SD)

Parameters	Growth conditions	
	Light	Dark
Net photosynthesis [μmol m^{-2} s^{-1}]	− 0.190 ± 0.042	–
Dark respiration [μmol m^{-2} s^{-1}]	− 0.905 ± 0.039a	− 0.198 ± 0.066b
Conductance [mmol m^{-2} s^{-1}]	0.824 ± 0.110a	0.569 ± 0.124b
Refixation [%]	79.0 ± 4.7	–

The differences between mean values marked with different letters in rows are statistically significant according to the Duncan's test ($P \leq 0.05$, $n = 10$)

1.0, 2.5 and 2.7‰, respectively. Moreover, in plants with darkened stems, clear differences in isotopic ratio between organs were not observed.

The $\delta^{13}C$ values were also measured in the phloem sap obtained from petioles of the plants with lightened stems (Fig. 4). The values were rather stable during the light period of the day and were significantly higher between 8.00 and 13.00 h compared with evening and night (18.00–23.00 h). Plants were maintained in the mode of C_3 photosynthesis by regular watering, so the daily net photosynthesis plot in leaves showed positive values only during the day with two depressions around midday and 15.30 h. Leaves performed

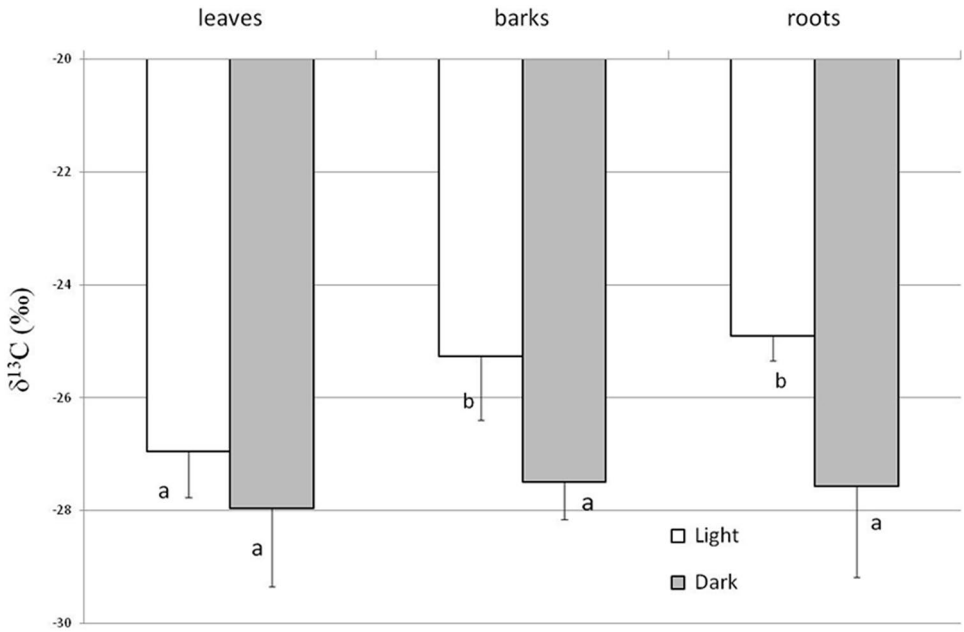

Fig. 3 Values of $\delta^{13}C$ in leaves, barks and roots of plants growing in standard conditions and plants with stems darkened for 8 months (means ± SD). The mean values marked with the same letters are not statistically significant according to the Duncan's test ($P \leq 0.05$, $n = 5$)

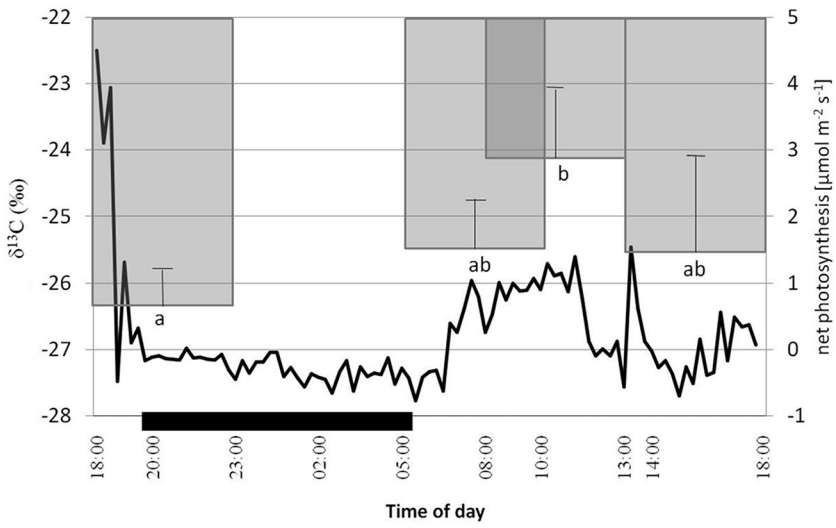

Fig. 4 Diurnal changes in $\delta^{13}C$ carbohydrate levels in phloem exudates (shaded bars) and net photosynthesis (black curve) in leaves of plants with lightened stems. Exudation lasted for 5 h. The solid bar on the *x*-axis indicates the dark period. Data on $\delta^{13}C$ are the means ± SD of five replicates. The mean values marked with the same letters are not statistically significant according to the Duncan's test ($P \leq 0.05$, $n = 5$). Net photosynthesis curve is representative of three replicates ($n = 3$)

dark respiration at levels not exceeding 0.85 µmol m^{-2} s^{-1} and mostly at the level of 0.2–0.5 µmol m^{-2} s^{-1}. The daily trend graph also reveals sugar levels in phloem sap (Fig. 5). The highest sugar concentration was recorded at the beginning of the day between 5.00 and 10.00 h and gradually decreased thereafter. Fluctuations in sugar content were low (2.4–3.9 mM) and significantly different only between morning (5.00–10.00 h) and late evening hours (18.00–23.00 h).

Plant biomass of cuttings (shoots + roots) with darkened stems was characterized by approximately 23% reduced weight compared with illuminated stems. In the case of roots, this value was more pronounced and reached 34% (Table 2). This result indicates that darkening affected shoots and roots differently. This divergence between organs was also evident in the shoot/root weight index (l_w), which was increased in darkened (1.39) compared with lightened plants (1.02). Consequently, the shoot length of plants with lightened stems (15 cm) was approximately twofold higher when compared with darkened stems. However, darkening did not significantly affect leaf development. During the experiment, the leaf area increased by approximately 200% in plants with darkened stems and 230% in light-exposed stems (however not statistically significant).

Discussion

In cross-sections of *C. minor* stems, using the epifluorescence microscopy technique, we can observe particular layers of tissues emitting a red signal originating from chlorophyll molecules. Using this technique, Dima et al. (2006) also detected chloroplasts in the cortex of 20 examined woody species, and these chloroplasts were also observed in perimedullar rays and piths of 19 and 16 species, respectively. In our study, chloroplasts were present in the cortex and pith. Moreover, fluorescence images indicate that chlorophyll-rich tissues showed a highly efficient PSII apparatus (F_v/F_m above 0.82). Similar observations have been made by Yotis et al. (2009) who revealed a continuous decrease in the F_v/F_m value (from 0.80 to 0.65) along the depth of stems of *Eleaganus angustifolia* L. A CO_2-rich environment in stems impedes photochemical activity possibly through acidification of the cytoplasm (Pfanz 1999; Manetas 2004), and an active PSII will produce O_2 that can be used for respiration and can support photorespiration. Thus, it seems that the stem photochemical activity will provide energy (ATP) and photosynthate and counteracts O_2 deficiency (Kuźniak et al. 2016; Wittmann and Pfanz 2018).

Stems responsible for transport of water and photoassimilates often are very tight, impeding gas exchange with the surrounding atmosphere. As shown in our experiments on *Clusia* species (Kocurek et al. 2015), stems are well protected from water evaporation. In young stems of *C. minor*, H_2O conductance was approximately 0.8 mmol m^{-2} s^{-1}, while this value reached 30 mmol m^{-2} s^{-1} in leaves (data not shown). Other examined trees also showed similar conductance, e.g. *ca.* 1.0 mmol m^{-2} s^{-1} in 4-year old stems of *Pinus monticola* Dougl. ex D. Don (Cernusak and Marshall 2000) or 1.1 mmol m^{-2} s^{-1} in young stems of *Betula pendula* Roth (Wittmann et al. 2006). The presence of cork increases diffusion resistance values that typically cause an even more drastic reduction in the stems' conductance, e.g. 0.15–0.20 mmol m^{-2} s^{-1} in 8- to 10-year-old stems of *Clusia multiflora* Kunth and *Clusia rosea* Jacq. (Kocurek et al. 2015). The limited conductance of the cork favours accumulation of CO_2 produced via respiration or/and transported from roots in xylem sap (Teskey et al. 2008). Finally, stem CO_2 efflux is modulated by actual efficiency

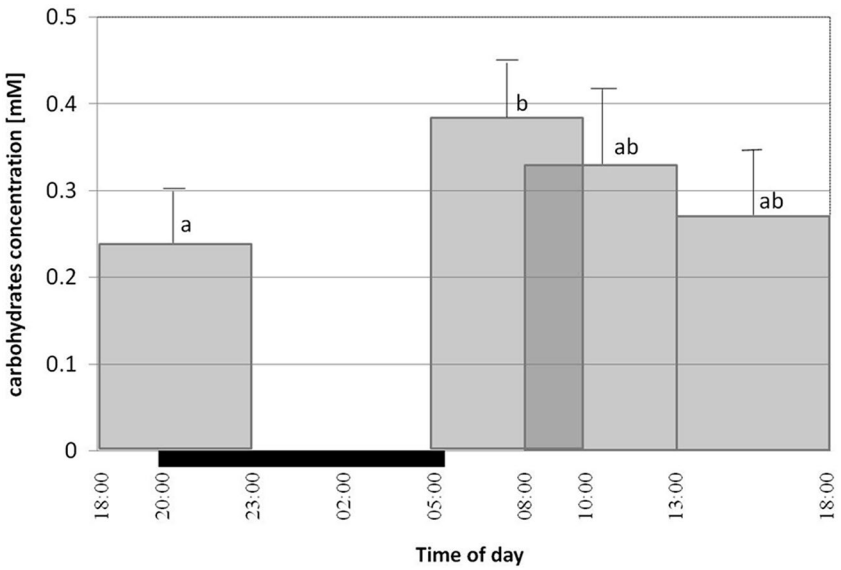

Fig. 5 Diurnal changes in carbohydrate concentrations in phloem exudates (shaded bars) in *C. minor* with lightened stems. Exudation lasted for 5 h. The solid bar on the *x*-axis indicates the dark period. Data are the means ± SD of five replicates. The mean values marked with the same letters are not statistically significant, according to the Duncan's test ($P \leq 0.05$, $n = 5$)

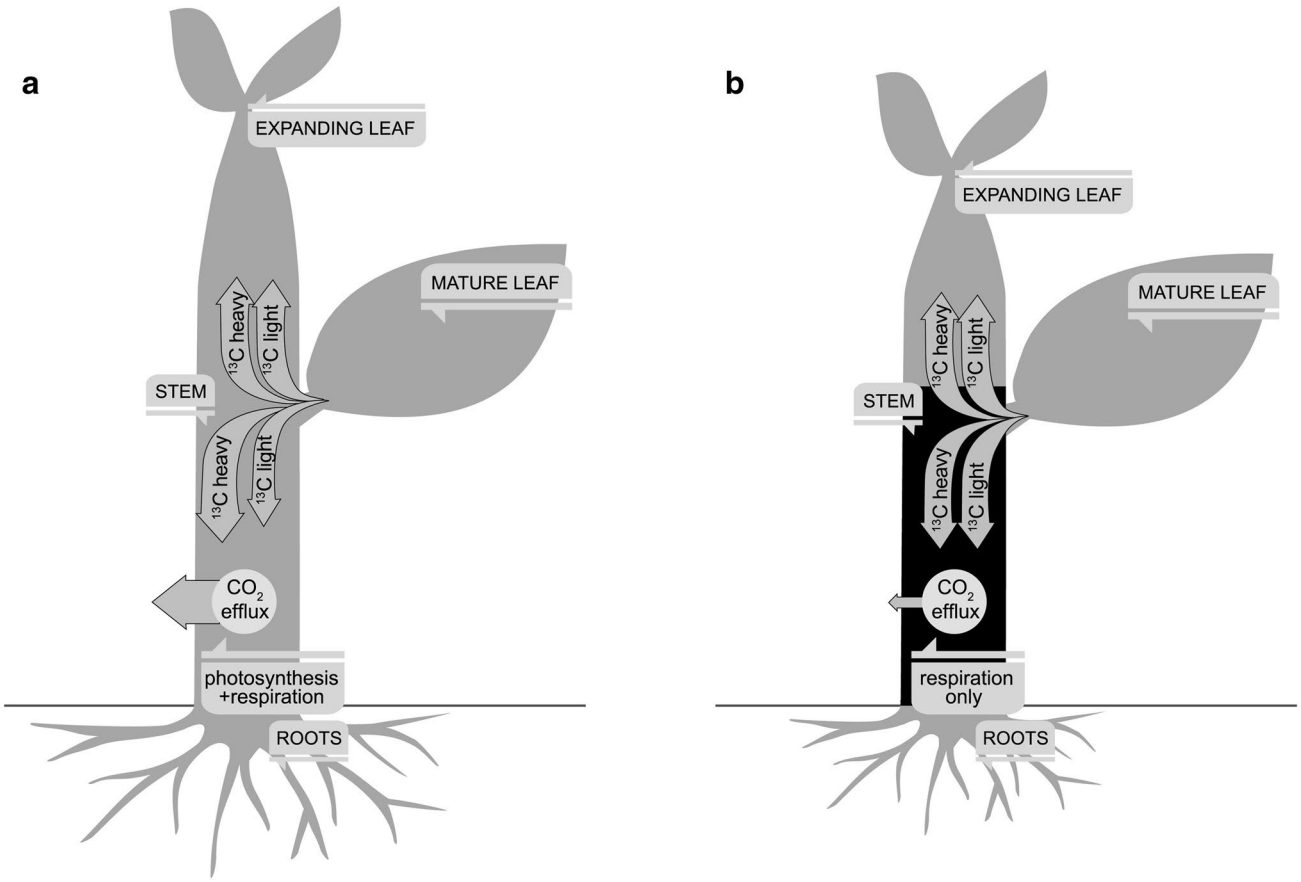

Fig. 6 Schematic characteristics of carbon allocation and its consequence on $\delta^{13}C$ distribution among organs of *C. minor* plants with lightened (**a**) and darkened (**b**) stems. The widths of arrows correspond to proposed ^{13}C heavy or light photosynthate streams and CO_2 efflux

Table 2 Morphological parameters of plants of *C. minor* growing in light and dark conditions

Growth conditions	Weight [g]			I_w	Increase of	
	Shoots	Roots	Shoots + roots		Shoot length [cm]	Leaves area [%]
Light	16.2 ± 4.9a	15.7 ± 3.5a	31.9 ± 8.4a	1.02 ± 0.08a	15.0 ± 4.6a	231 ± 70a
Dark	14.2 ± 2.8a	10.3 ± 1.0b	24.5 ± 2.9a	1.39 ± 0.29b	7.8 ± 3.5b	204 ± 46a

I_w—index of weight = shoot weight/root weight

The mean values marked with the same letters in columns are not statistically significant according to the Duncan's test ($P \leq 0.05$, $n = 5$)

of photosynthesis. Based on the CO_2 efflux calculations (in light and dark conditions), efficient refixation of respiratory CO_2 is considered a photosynthetic activity of plant stems (Pfanz and Aschan 2001; Pfanz et al. 2002; Cerasoli et al. 2009).

In our experiments, the relatively high conductance of 2-year-old stems was manifested by a high CO_2 efflux (0.9 µmol m^{-2} s^{-1}), and approximately 79% of CO_2 was refixed by stem tissues. Our calculations of CO_2 refixation in strong light (1000 µmol m^{-2} s^{-1} PAR) in *C. minor* yield high levels compared with those (7–123%) reported for other species (Cernusak and Marshall 2000; Teskey et al. 2008; Cerasoli et al. 2009; Ávila et al. 2014).

The physiological role of stem photosynthesis is clearly observed when stems are kept for a long time in darkness. Restricted access of light led to the disappearance of chlorophyll as observed in cross-sections of *C. minor* stems. Such treatment also limited the biomass of the whole plant by approximately 20%. This result is attributed to reduced carbon acquisition in the darkened stem area that was estimated at approximately 60% of the whole stem and limited transport of carbohydrates due to ATP deficiency, as suggested above. A significant photosynthesis rate in the entire plant

area (stem + leaves) allows estimation of the stem's contribution to total plant photosynthetic production (Aschan and Pfanz 2003). Cernusak and Hutley (2011) estimated that 11% of wood in the branches of *Eucalyptus miniata* A. Cunn. ex Schauer originates from corticular photosynthate. Kharouk et al. (1995) also reported 30–50% re-assimilation of respiratory CO_2 by the bark of *Populus tremuloides* Michx., which constitutes a 10–15% contribution to the CO_2 acquisition during the summer months.

Interestingly, stem darkening affected root biomass even more than shoots. Such treatment lowers shoot weight by approximately 10% and root weight by 34%. To our knowledge, this is the first time that the effect of stem photosynthesis on root development has been quantified. Earlier, Saveyn et al. (2010) observed reduced bud development on darkened stems and revealed that buds are supplied photosynthate derived from stem photosynthesis.

Darkened stems become a more heterotrophic organ likely because photosynthates are transported from other organs via the phloem. We expect that changes in carbon distribution between auto- and heterotrophic organs can be predicted based on $\delta^{13}C$.

Daily carbohydrate concentrations in phloem depend on the species. In general, a higher concentration is noted during the day in conditions favourable for photosynthesis (Sharkey and Pate 1976; Wild et al. 2010; Kallarackal et al. 2012). Transport of carbohydrates in phloem is regulated by source and sink organs and the circadian rhythm of growth (Paul and Foyer 2001; Borland and Dodd 2002; Ceusters et al. 2009a, b). Leaves grow during the day and use "in situ" produced photosynthate (Walter and Schurr 2005). In contrast, woody stem growth occurs mostly at night (Steppe et al. 2005; Saveyn et al. 2007), whereas roots do not exhibit a diurnal cycle (Walter and Schurr 2005). In our experiments in *C. minor,* phloem sap showed diurnal variations in $\delta^{13}C$ of up to 2.2‰, and we also observed higher carbohydrates concentration during morning (significant) and midday (not significant) compared to late evening hours. We expect that carbohydrate distribution can change the ^{13}C discrimination pattern between organs. The lack of photosynthates produced by the darkened stem likely led to a change in the distribution of sugars reaching the new leaves, stems and roots. The $\delta^{13}C$ heterogeneity in *C. minor* organs revealed that leaves of plants with light-exposed stems export ^{13}C-enriched sugars mainly derived from transitory starch to stems and roots (Fig. 6). Darkening of stems disturbed this process because heterotrophic stems need more photosynthate. Thus, more sugars with reduced ^{13}C levels are directed to the stems and roots and subsequently results in no differences in $\delta^{13}C$ between organs. The changes in $\delta^{13}C$ distribution after darkening of the stems correspond with biomass limitations: lower in shoots and higher in roots. It seems that stems kept in the dark and unable to drive photosynthesis consume a portion of sugars transferred to roots. Thus, root growth suffers more than other organs.

Carbohydrates produced in photosynthetically active tissues and exported to heterotrophic organs are used in respiration (Borland and Dodd 2002). Thus, another explanation of the $\delta^{13}C$ distribution in *C. minor* organs after darkening is the possibility of ^{13}C fractionation during respiration. The respiratory CO_2 efflux from stem of trees and shrubs was widely observed in the range of 0.2–4 μmol m^{-2} s^{-1} (Wittmann et al. 2006; Saveyn et al. 2007; Cerasoli et al. 2009, Yiotis and Manetas 2010). In *C. minor*, respiration evaluated as CO_2 efflux in dark- and in light-exposed stems reached 0.9 μmol m^{-2} s^{-1}, whereas the maximum respiration of leaves is 0.85 μmol m^{-2} s^{-1}. These results indicate intensive stem metabolism.

Trunk and branch respiration in some species may represent 26% of the total carbon assimilated by leaves in a beech forest (Damesin et al. 2002). Thus, it seems that disturbances in respiration levels may substantially modify $\delta^{13}C$ in stem tissues. The CO_2 respired in the dark by C_3 leaves is generally ^{13}C enriched compared with leaf organic matter (or leaf sugar pools), and the latter is subsequently depleted in ^{13}C (Ghashghaie et al. 2003; Badeck et al. 2005; Werner et al. 2012). On the other hand, respiring roots generally release ^{13}C-depleted CO_2 compared with root organic matter. However, roots of lignified plants show ^{13}C enrichment in respired CO_2. Conversely, in stems and leaves of woody plants (herbaceous plants were not investigated), effluxing CO_2 is ^{13}C enriched (Damesin et al. 2002; Saveyn et al. 2010), and the remaining organic matter of leaves is ^{13}C depleted. Moreover, the intensity of stem photosynthesis is reflected in the $\delta^{13}C$ value of CO_2 released from stems. Cernusak et al. (2001) revealed strong stem photosynthesis modulation in $\delta^{13}C$ of CO_2 released from stems of two woody plants. As a result of darkening, an increased $\delta^{13}C$ in organic matter of stems was observed. Similar results were also published by Saveyn et al. (2010) and Cernusak and Hutley (2011). These results (Cernusak et al. 2001; Saveyn et al. 2010; Cernusak and Hutley 2011) are clearly in contrast to the $\delta^{13}C$ levels observed in organic matter in stems of *C. minor* kept in the dark. However, without detailed analyses of the $\delta^{13}C$ level of CO_2 released from *C. minor* stems, we can only hypothesize that respiration in darkened stems fractionates carbon isotopes in an opposite manner as reported for other investigated plants (release of increased ^{13}C levels of CO_2 than organic matter). Hypothetical changes in respiratory metabolism consistent with the observed $\delta^{13}C$ levels in stems and roots of *C. minor* should involve a high contribution of the pyruvate dehydrogenase reaction (PDH) pathway (Ghashghaie and Badeck 2014).

Furthermore, the observed patterns could at least partially result from the anaplerotic fixation of HCO_3^- by PEPC into ^{13}C-enriched malate. PEPC activity in stems and roots

of C_3 plants is significantly higher than that in the leaves (Gao et al. 1996; Berveiler and Damesin 2008; Kocurek and Pilarski 2011). According to the hypothesis formulated by Hibberd and Quick (2002) and Brown et al. (2010), the roots are to some extent the source of malate transported in xylem sap and then decarboxylated in the vascular bundles of stems, petioles and leaves where CO_2 is re-assimilated. We hypothesize that an enhanced anaplerotic pathway in heterotrophic roots and stems after darkening increased malate production. In addition, ^{13}C-enriched malate is transported to leaves, and the carbon remaining in organic matter is ^{13}C depleted, which may explain the more negative $\delta^{13}C$ signal due to stem darkening.

Conclusion

A portion of energy needed for transport, storage and development of stems is provided from photochemical activity itself. The darkening of stems reduces this important energy source and significantly reduces root weight. Additionally, darkening of stems reduces the level of CO_2 efflux, which indicates a reduction in the level of metabolism. Changes in $\delta^{13}C$ levels in *Clusia* organs with darkened stems reveal the impact on the flow of carbohydrates produced by the leaf.

In lightened stems, carbohydrates with high $\delta^{13}C$ levels derived from leaves flow to the roots and stems. Darkening disturbs this allocation, and carbohydrates with lower $\delta^{13}C$ levels are transferred to stems and roots. We do not know which particular carbohydrates are involved in this mechanism, and whether this phenomenon equally occurs in trees or plants from other families.

We conclude that the role of stem photosynthesis in shaping root growth should require further, more extensive research.

Author contribution statement ZM, MK: conceived and designed experiments; MK UL and ZM: wrote the manuscript; MK: performed most experimental analyses; RW: performed isotopic analysis; AK: made microscopic plant anatomy observations.

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