Direct and indirect effects of light on plant growth and conduit diameter in Populus seedlings

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August 7, 2023

Abstract

Light can significantly influence plant growth, including stem traits and carbon sources and sinks. These changes in growth require carbon transport, which occurs in the vascular tissue, the phloem, via sieve elements. Light can influence vascular structure in the xylem, but due to methodological constraints there is little data on the degree to which the phloem, and in turn carbon transport, may be affected by the light environment. To examine the role of source/sink activity and plant size on phloem anatomy (i.e., hydraulic conductivity and percent conductive area), we grew Populus tremuloides Michx. seedlings under three different light treatments (high, medium, and low). We measured sieve element diameter and percent conductive area weekly for eight weeks using a newly optimized immunolabeling technique. We compared anatomical data with measurements of plant size, leaf, and root gas exchange. Light treatment altered plant growth and vascular anatomy, especially in the low light seedlings, which had the narrowest conduits (i.e., lowest hydraulic conductivity) and percent phloem conductive area. However, for their stem diameter, the low light seedlings had wider sieve element diameters than expected. Our piecewise SEM for the xylem revealed that stem length was also a driving factor for hydraulic conductivity and percent conductive area. Comparatively, net daily carbon assimilation emerged as an important factor affecting phloem hydraulic conductivity, along with stem diameter, while carbon assimilation was the only significant driver for percent phloem conductive area. Thus, phloem anatomy, and in turn carbon transport, may be more sensitive to environmental factors from both direct effects (i.e., carbon assimilation) and indirect effects (i.e., stem traits) than previously believed.
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INTRODUCTION

Growth requires carbon, nutrients, and signals, which are transported from carbon sources to sinks in the phloem, a vascular tissue, in conduits called sieve elements (Münch, 1930). Differences in plant growth can result from a plant’s light environment, which may impact the size and/or activity of carbon sources (regions in the plant that fix or load carbon) and sinks (areas that metabolize or store carbon) as well as stem length and diameter (Lichtenthaler et al., 1981; Armitage, 1991; Gloser, Scheurwater and Lambers, 1996; Huang, Zhang and Hu, 2014). Vascular anatomy is also impacted by light conditions, but most of this work is focused on the xylem, the vascular tissue that conducts water (Penfound, 1931; Cochrane, Lemoine and Dreyer, 1999; Lemoine, Cochrane and Granier, 2002; Plavcová, Hacke and Sperry, 2011; Gebauer et al., 2012; Noyer et al., 2017) instead of the phloem (but see Segovia and Brown, 1978; Wang and Huang, 2003; Zhang et al., 2016; Stewart, Polutchko, Adams and Demmig-Adams, 2017a). There are many reasons to expect that light will impact phloem anatomy, but because of methodological challenges with identifying sieve elements in cross-sections (Truernit, 2014) relatively little is known about how phloem anatomy changes under different environmental conditions. With the development of a new method for immunolabeling active sieve elements, we can now more quickly measure sieve element diameter and estimate phloem hydraulic conductivity and percent conductive area. This new technique allows us to test critical questions about plant growth and determine whether there is a relationship between light environment and phloem anatomy.

Previous work suggests there might be a relationship between photosynthetic capacity (source activity) and phloem structure because carbon movement in the phloem is driven by pressure differences between source and sink tissue (Adams et al., 2013; Stewart, Polutchko, Adams and Demmig-Adams, 2017a, 2017b). Carbon assimilation often increases under high light, which can increase the fixed carbon in a leaf (Turnbull, Booker and Leyser, 2002). When this occurs, the leaf needs to use, store, or export the fixed carbon, the latter of which requires transport in sieve elements. Studies found increases in foliar phloem volume and cross-sectional area as well as branch phloem cross-sectional area in individuals grown under high light versus low light (Segovia and Brown, 1978; Zhang et al., 2016; Stewart, Polutchko, Adams and Demmig-Adams, 2017a). These findings suggest that changes in the carbon fixation activity of sources may correspond with alterations in phloem cross-sectional area, but we still do not know if sieve element diameter and in turn hydraulic conductivity and percent conductive area are altered.

Light levels also impact carbon sink size and activity. However, the effects of light on sink activity may depend on the species or on the sink organ itself (i.e., fruits, leaves, roots) (Mor and Halevy, 1980; Roopendra, Chandra and Saxena, 2019). When considering fruits, data shows there is a relationship between sink size and phloem cross-sectional area in giant pumpkin fruit (Savage, Haines and Holbrook, 2015) and photosynthate accumulation in Japanese pear was limited by sink strength as opposed to transport capacity (Zhang et al., 2005). Meanwhile, modeling has suggested that increased phloem resistivity in roots, which could occur because of narrower sieve elements, decreases final root length and size (Zhou et al., 2022). Because both sources and sinks are affected by carbon transport, changes in their size and activity that can occur under different light conditions, may lead to alterations in vascular anatomy to maintain transport capacity.

Variations in source and/or sink activity can also lead to changes in stem length and diameter, and thus indirectly impact conduit diameter in the phloem and xylem (Aslani et al., 2020). As stems elongate, the resistance to vascular transport should increase, but wider conduits at the stem base reduce this resistance, which allows plants to maintain similar carbon and water flow rates as they get taller (McCulloh and
Sperry, 2005; Mencuccini et al., 2007; Anfodillo, Petit and Crivellaro, 2013; Olson et al., 2014; Petit and Crivellaro, 2014; Savage et al., 2017). Conduit diameter also scales with stem diameter likely due to allometric relationships between stem length and diameter (Niklas, 1993; Niklas and Spatz, 2004; Anfodillo et al., 2006; Savage et al., 2010; Olson and Rosell, 2013; Ziaco et al., 2014; Jyske and Hölttä, 2015; Pertiwi et al., 2018; Tanget et al., 2022). Even seedlings, which should have minimal resistance to transport, have wider xylem conduits at their base (Zhong et al., 2019). Thus, differences in stem length and/or diameter caused by light may indirectly contribute to changes in conduit diameter.

Because the phloem and xylem are spatially close and hydraulically and developmentally linked since the xylem supplies the water in phloem sap (Sevanto, Hölttä and Holbrook, 2011), it seems likely that any changes in phloem anatomy would be mirrored in the xylem. Isometric relationships between phloem and xylem cross-sectional areas have been found in at least twelve species under controlled conditions (Carvalho et al., 2017; Ray and Jones, 2018). However, studies have found that phloem cross-sectional area may be less sensitive to the environment than xylem cross-sectional area, which suggests the phloem may be less plastic to environmental changes (Gričar et al., 2014; Swidrak, Gruber and Oberhuber, 2014; Miller et al., 2020).

The close spatial arrangement, hydraulic linkage, and isometric relationships between the phloem and xylem suggests that any structural or functional changes in one tissue may be seen in the other but this remains to be extensively tested.

To determine how source leaf activity, sink root activity, and stem length and diameter alter phloem hydraulic conductivity and percent conductive area, we manipulated growth of Populus tremuloides (Michx.) seedlings by placing them under three different light conditions for eight weeks. Using a new technique that allows for better visualization of sieve elements, we did an in-depth analysis of phloem anatomy (Torode et al., 2018; Ray and Savage, 2020). Furthermore, we measured the growth, net daily carbon assimilation and daily root respiration of these seedlings. In doing so, we sought to answer the following questions: (1) Under different light conditions, how do *P. tremuloides* seedlings alter their growth and vascular anatomy? (2) Does source activity, sink activity, or stem length and diameter impact phloem hydraulic conductivity and percent phloem conductive area? (3) Are the allometric relationships between the phloem and xylem different under different light conditions? and (4) Are the predicted stem and conduit scaling relationships the same for seedlings as for mature plants even under different light conditions?

**MATERIALS AND METHODS**

**Planting and growth conditions**

Quaking aspen (*P. tremuloides*) seeds were collected in Duluth, Minnesota, USA (46.7867° N, 92.1005° W) from May 24th to May 28th, 2021. The seeds were cleaned using seed screens going from high grade to progressively finer grade. After cleaning, the first cohort (140 seedlings) of seeds was planted in 3.8 x 19.5 cm Ray Leach cone-tainers (Stuewe and Sons, Oregon, USA) filled with a 50:50 mixture of sand and sieved peat moss mixed with slow-release fertilizer pellets at a nutrient level of 3.5 g/L of soil (Osmocote 14/14/14) on June 14th, 2021. The remaining seeds were stored at 3°C until the second cohort (140 seedlings) was planted on August 24th, 2021. We measured leaf gas exchange and anatomical traits on the first cohort, and biomass and root gas exchange on the second cohort. We grew the seedlings from each cohort for eight weeks in a growth chamber (Percival Advanced Intellus, Iowa, USA) with LED lights set to mimic the day length and climate of Minnesota in June (15-hour light cycle, 21.5°C day and 17°C night temperature). Each seedling’s germination date was recorded and marked after a hypocotyl appeared. To ensure sufficient germination, we planted multiple seeds per cone-tainer, and thinned them to a single seedling once one appeared healthy enough to survive. During the first week after planting, the pots were misted to keep the top of the soil moist. After that, the seedlings were bottom watered. To control for soil moisture, we monitored soil weight daily and watered when the soil weight was greater than 2 grams below the calculated saturated soil weight taking into account an estimate of plant weight based on the previous harvest.

**Light treatments**

To manipulate stem length and diameter, source leaf activity, and sink root activity, three light treatments
(hereafter referred to as high, medium, and low) were created by positioning shelves in the growth chamber at different distances from the lights. Every week before harvesting the seedlings, the photosynthetic photon flux density (PPFD) was measured over each seedling using a PAR meter (LI-250A, LiCor, Nebraska, USA) to see how the light environment changed as the seedlings grew. The high light treatment’s PPFD was 1999 ± 1296 (SD) μmol m⁻² s⁻¹. The medium PPFD was 593 ± 99 μmol m⁻² s⁻¹. The low light treatment’s PPFD was 52 ± 33 μmol m⁻² s⁻¹. As the plants grew, we adjusted the shelves to maintain each treatment within the expected light intensity range.

Measuring plant size and growth

Ten seedlings were randomly selected from each light treatment (n = 30) and harvested during weeks two, four, six, seven, and eight. All except for four seedlings died in the low light treatment by week eight. After each harvest, stem length, stem diameter at the base, and root length were measured to the nearest 0.1 millimeter after the roots were rinsed with water and patted dry. Leaves were scanned (Epson Perfection V39, Tokyo, Japan) and leaf area was calculated using ImageJ in both cohorts (Rasband, 1997). After scanning, the leaves were dried at 71°C for at least seven days and then weighed. For the first cohort, the stems were stored in 70% ethanol to preserve for subsequent vascular anatomy measurements.

Quantifying source activity

To examine the relationship between leaf activity and vascular anatomy, we estimated net daily carbon assimilation of seedlings in cohort 1. After the leaves had fully expanded (week four for the high and medium seedlings and week six for the low light seedlings), dark respiration and photosynthesis were measured the day before the seedlings were harvested using a portable infrared gas exchange machine (LI-6800, LiCor, Nebraska, USA) with a multiphase flash fluorometer head (6800-01A). To measure dark respiration, the seedlings were removed from the growth chamber in the morning before the lights turned on. A leaf was measured under dark conditions and the cuvette conditions were configured to match the environmental conditions of the growth chamber with the lights off (400 ppm CO₂, 21.5°C, 49-50%, flow 500 μmol s⁻¹). We then moved the seedlings underneath a grow light and placed them in a bucket of water to prevent them from drying out. Photosynthesis was measured between 11:00 and 17:00 under the same environmental conditions as the morning with the addition of light set to match the light level in the growth chamber of the individual seedling being measured. By week six, some of the high light seedlings were experiencing light levels well over 3000 μmol m⁻² s⁻¹, the maximum light level that can be achieved in the LiCOR chamber. For these plants, we kept the chamber at 3000 μmol m⁻² s⁻¹.

Net daily carbon assimilation (mmol C per day) was calculated by adding daily net photosynthesis (μmol m⁻²) and daily dark respiration (μmol m⁻²) assuming 15 hours of light, and then multiplying this by leaf area (m²). We also created light response curves in weeks seven and eight to determine the maximum photosynthetic rate and saturating light level for each light treatment (Fig. S1).

Measuring vascular anatomy

To identify sieve elements, we immunolabeled sieve element cell walls with the LM26 monoclonal antibody, which binds to a β-1,6-galactosyl substitution of β-1,4-galactan pectin, in actively conducting sieve element walls. We visualized the LM26 (Kerafast, Boston, MA) with Alexa Fluor 555 goat anti-rat secondary antibody (ThermoFisher Scientific, Waltham, MA) (Ray & Savage, 2020; Torode et al., 2018). Transverse sections were taken by hand from the base of each ethanol-preserved seedling stem at least one millimeter aboveground from the roots to avoid sectioning the transition zone from root to stem. Because samples were collected at the stem base, stem length is equivalent to its distance from the tip of the plant. After staining, the sections were imaged on an epifluorescence microscope (Nikon, ECLIPSE Ts2, New York, USA) and a charge-coupled device (CCD) camera (Teledyne Photometrics, CoolSNAP Dyno, Arizona, USA) using the TRITC/Cy3 filter set (560 nm) to image the sieve elements and the DAPI filter (385 nm) to image vessel elements (autofluorescence). Negative controls (no primary antibody) exhibited little or no sieve element fluorescence in the red range from nonspecific binding of the secondary antibody control, while positive controls showed fluorescing sieve elements (Fig. 1). We only examined the vascular anatomy in weeks four.
through eight due to the small size of the week two seedlings.

Figure 1. Negative (A) and positive (B) controls of the cross-section of *Populus tremuloides* stems using the LM26 immunolabeling technique showed that minimal fluorescence of sieve elements occurred without the primary antibody using the TRITC/Cy3 filter set (560 nm). The arrows are pointing to just a few of the visible sieve elements in the positive control (B).

We analyzed micrographs of vascular traits with ImageJ (Rasband, 1997 Version 1.53a). We measured the entire phloem and xylem cross-sectional area in each stem section. To estimate the number of conduits (sieve and vessel elements) in each cross-section, we counted the conduits in a quarter of the section and multiplied it by four. In turn, we used our conduit estimate to calculate the density of sieve and vessel elements (n) by dividing each by the appropriate tissue cross-sectional area. We calculated the number of sieve and vessel elements needed to find the mean lumen area, which was 30 conduits for each (see Fig. S2 and S3 for sampling curves). For the vessel elements, all conduits in between two rays were measured, which often led to more than 30 vessels being measured. We derived the average sieve and vessel element diameters (D) using conduit area measurements and assuming a perfect circle. The conduit count and average conduit diameter were used to estimate the theoretical hydraulic conductivity (Kp and Kx), which is how easily sap flows through conduits in the phloem and xylem respectively, and percent conductive area in both types of vascular tissue.

\[
\text{Eq. 1} \quad K = \frac{\pi \rho \omega}{\eta} \times n \times D^4 \quad (\text{Poorter et al. 2010})
\]

\[
\text{Eq. 2} \quad \text{2% conductive area} = \left( \frac{\text{Conduit count} \times \text{Average Conduit Area}}{\text{Vascular Cross-sectional Area}} \right) \times 100
\]

**Measuring and modeling sink activity and organ biomass**

We modeled sink activity using plants from the second cohort. After the growth measurements (described above), the roots were scanned, oven dried at 71 for at least seven days and then weighed to assess biomass. During week seven, we measured root respiration for six plants from each light treatment (n = 18) using a portable gas exchange machine (LI-6400, LiCor, Nebraska, USA) and the clear-top conifer chamber (6400-05). We set conditions in the cuvette similar to those in the growth chamber (400 ppm CO₂, 21.5 °C, 60-80% RH, flow 500 μmol s⁻¹). To remove soil from the roots, they were washed, patted dry, and allowed to air dry for up to ten minutes. Then the entire root system was inserted into the conifer chamber and after respiration plateaued, measurements were taken every 30 seconds for three minutes according to Wiley, Hoch and Landhäusser (2017). Root biomass was used to estimate root respiration on a dry mass basis. Using the root scans, we estimated the root area using the ImageJ Root Image Analysis (RIA) package and protocol (Lobet et al., 2017).
Since we did not measure root respiration or root biomass on the plants in cohort 1, we modeled root biomass (g) and daily root respiration (nmol C g$^{-1}$ per day) for cohort 1 from the cohort 2 data. We completed a multiple linear regression using root, stem, and leaf traits as independent variables and root respiration and biomass as dependent variables. We included leaf, stem, and root traits because studies have found that these organs all interact and influence one another (Niklas, 2005; Cheng et al., 2014; Kramer-Walter et al., 2016; Bt and Kl, 2018). We used root length instead of root area in these models since we did not have root area measurements for the first cohort. Because light treatment was not significant in our analysis, we created a single model for all the light treatments. The best multiple linear regressions for root respiration and root biomass were chosen using the smallest Akaike information criterion (AIC; Table S1; Table S2). Then these equations were used to estimate root respiration and biomass for the cohort 1 plants.

**Piecewise SEM**

To test any relationships between sources, sinks, and stem length and diameter and phloem hydraulic conductivity and percent conductive area for the cohort one seedlings, we constructed a piecewise structural equation model (SEM) using the piecewiseSEM package (Lefcheck, 2016). Piecewise SEMs are probabilistic models that examine potential causal relationships between multiple predictor and response variables in one model by combining multiple linear regression equations (Lefcheck, 2016). The results of piecewise SEMs indicate whether there are significant relationships between the predictor and response variables and the strength of the relationship.

For our SEM, the chosen response variables were vascular hydraulic conductivity and percent vascular conductive area because these variables are more emergent traits related to function in the phloem and xylem. The predictor variables were net daily carbon assimilation, stem length, stem diameter, and daily root respiration (Fig. 2). For net daily carbon assimilation and daily root respiration, we created multiple linear regressions to determine what other plant traits might impact these variables (Table S3; S4, Fig. 2). We did not include root length as a direct predictor variable because root length was used to estimate root respiration in cohort one. Finally, we allowed the phloem and xylem traits to have correlated errors since the phloem and xylem may be impacted by the same underlying driver (Fig. 2). To ensure that we accounted for all possible associations, we ran tests of directional separation on the overall piecewise SEMs as well as each model. Models with the lowest AIC were chosen to incorporate into the piecewise SEM (Table S3; S4; S5; S6; S7; S8).

![Figure 2: Variables included in the original multiple linear regression models for the piecewise SEM for xylem hydraulic conductivity (kg μm MPa$^{-1}$ s$^{-1}$) or percent conductive area (mm$^2$) and phloem hydraulic conductivity (kg μm MPa$^{-1}$ s$^{-1}$) and percent conductive area (mm$^2$) with random effects of age and light level (μmol m$^{-2}$ s$^{-1}$). Arrows indicate a predicted directional positive relationship between the predictor and response variables.](image)

**Statistical analyses**

All statistical analyses were performed in R v. 4.02 (R Core Team, 2020) using an alpha of 0.01. To test if plant growth and vascular anatomy significantly differed between the light treatments, we used an ANCOVA, with light treatment as the independent variable and age as the covariate. Pairwise comparisons
were completed using Tukey’s honestly significant difference test. To study the allometric relationships between phloem and xylem hydraulic conductivity and percent conductive area, we used a reduced major axis regression (RMA) since we did not want to assume that one variable was dependent on the other (Smith, 2009). Since stem length is known to be the main factor affecting conduit diameter, we calculated the residuals of phloem and xylem hydraulic conductivity and percent conductive area and stem length to remove the effect of length using lmmod2 (Olson et al., 2014, 2020; Rosell, Olson and Anfodillo, 2017; Legendre, 2018).

To compare our conduit diameter scaling relationships with previous data on mature plants, we plotted conduit diameter versus stem diameter and length relationships on a log$_{10}$ graph to normalize and linearize the data using an ordinary least squares regression (OLS). We used the slope.test function in the smatr package to test if the slopes were different from those found in the literature (Warton et al., 2012). We tested for compliance of the assumptions of regressions and ANCOVAs using the R packages stats and multcomp (Hothorn, Bretz and Westfall, 2008; R Core Team, 2020).

RESULTS

Stem length and diameter, source size and activity, and sink size and activity under different light treatments

To understand how light level influences stem, source, and sink traits, we tested whether there were significant differences in growth between the light treatments during the last week of growth (week 7; Table 1) and throughout the entire experiment with an ANCOVA with age as a covariate (Table 2). Light altered all the plant growth traits we examined, particularly in the low light. By week seven, the low light seedlings had the shortest and narrowest stems, and they exhibited the smallest source size (leaf area) and activity (photosynthetic rate and net daily carbon assimilation). In terms of sinks, the low light seedlings were also the smallest with the shortest roots, the lowest root respiration rates, and the lowest estimated daily root respiration. However, the low light leaf respiration rates were only significantly higher than the medium light seedlings in week seven. By week seven, the only difference between the high and medium light treatments was that the high light seedlings had longer stems.

Table 1. Means and standard deviations of source, sink, and stem traits. Bolded letter superscripts indicate statistically significant differences (p < 0.01) from the other treatments using a Tukey HSD test with light treatment as an independent variable and age as a covariate.

<table>
<thead>
<tr>
<th>Trait</th>
<th>High</th>
<th>Medium</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm$^2$)</td>
<td>204.3 ± 56.0$^a$</td>
<td>192.8 ± 42.6$^a$</td>
<td>23.3 ± 14.9$^b$</td>
</tr>
<tr>
<td>Leaf photosynthesis (μmol m$^{-2}$ s$^{-1}$)</td>
<td>10.9 ± 3.9$^a$</td>
<td>8.97 ± 3.0$^a$</td>
<td>1.02 ± 1.4$^b$</td>
</tr>
<tr>
<td>Leaf respiration (μmol m$^{-2}$ s$^{-1}$)</td>
<td>-1.0 ± 0.39$^a$</td>
<td>-1.25 ± 0.37$^a$</td>
<td>-0.78 ± 0.28$^b$</td>
</tr>
<tr>
<td>Net daily carbon assimilation (mmol C)</td>
<td>7.9 ± 4.9$^a$</td>
<td>6.1 ± 3.5$^a$-0.07 ± 0.21$^b$</td>
<td></td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>25.0 ± 2.61$^a$</td>
<td>22.7 ± 1.86$^a$</td>
<td>10.7 ± 5.95$^b$</td>
</tr>
<tr>
<td>Root respiration (μmol m$^{-2}$ s$^{-1}$)</td>
<td>-0.006 ± 0.003$^a$</td>
<td>-0.006 ± 0.002$^a$</td>
<td>-0.0004 ± 0.0005$^b$</td>
</tr>
<tr>
<td>Estimated net daily root respiration (mmol C g$^{-1}$)</td>
<td>-0.008 ± 0.002$^a$</td>
<td>-0.006 ± 0.002 1.5 x 10$^{-6}$ ± 0.0006$^b$</td>
<td></td>
</tr>
<tr>
<td>Stem diameter (mm)</td>
<td>3.02 ± 0.62$^a$</td>
<td>2.68 ± 0.39$^a$</td>
<td>1.03 ± 0.22$^b$</td>
</tr>
<tr>
<td>Stem length (cm)</td>
<td>17.9 ± 3.5$^a$</td>
<td>12.4 ± 1.5$^b$</td>
<td>4.4 ± 1.6$^c$</td>
</tr>
</tbody>
</table>

Table 2. ANCOVA of plant growth traits with light treatment as the independent variable and age as a covariate (df = 2, 103).
Dependent variable F-value p-value

Leaf area (cm²) 78.61 < 0.0001
Leaf photosynthesis (μmol m⁻² s⁻¹) 37.16 < 0.0001
Leaf respiration (μmol m⁻² s⁻¹) 9.81 < 0.0002
Net daily carbon assimilation (mmol C) 25.39 < 0.0001
Root length (cm) 192.0 < 0.0001
Root respiration (μmol m⁻² s⁻¹) 16.47 < 0.0002
Estimated net daily root respiration 122.1 < 0.0001 (mmol C g⁻¹)
Stem diameter (mm) 163.6 < 0.0001
Stem length (cm) 100.2 < 0.0001
Age as a covariate was significant (p < 0.01)

Vascular anatomy under different light treatments

We found that all measured traits at the base of the stem, apart from sieve element density and percent xylem conductive area, were significantly different between the light treatments when age was a covariate (Table 3; ANCOVA). When breaking down data by light treatments and age, we found that the low light seedlings had the narrowest conduit diameters, the highest density of conduits, and the smallest vascular cross-sectional areas across all the weeks (Fig. 3). Though the low light seedlings did have a significantly smaller percent phloem conductive area, there was no significant difference between the percent xylem conductive area in any of the light treatments (Fig. 3). In both vascular tissues, the high light seedlings had wider conduits, higher conductive areas, and larger cross-sectional areas than the medium light seedlings at week four, but by the end of data collection there were no significant differences in any vascular anatomical traits between these treatments (Fig. 3).

The low light seedlings had significantly lower phloem and xylem hydraulic conductivity (Kₓ and Kₓ) than the other two treatments (Table 3). Though there were no significant differences in phloem hydraulic conductivity between the high and medium light seedlings, the high light seedlings did have significantly higher xylem hydraulic conductivity than the medium light seedlings (Table 3).

Table 3. ANCOVA of phloem sieve element (SE) and xylem vessel element (VE) traits with light treatment as the independent variable and seedling age as a covariate (df = 2, 103).

Dependent variable F-value p-value

SE lumen area (mm²) 45.57 < 0.0001
SE density (conduit mm⁻²) 2.96 N.S.
Phloem conductive area (mm²) 45.57 < 0.0001
Percent phloem conductive area (%) 10.94 < 0.0001
Kₓ (kg μm MPa⁻¹ s⁻¹) 19.23 < 0.0001
Phloem cross-sectional area (mm²) 47.79 < 0.0001
VE lumen area (mm²) 39.10 < 0.0001
VE density (conduit mm⁻²) 15.17 < 0.0001
Xylem conductive area (mm²) 56.78 < 0.0001
Percent xylem conductive area (%) 0.25 N.S.

$K_x$ (kg μm MPa$^{-1}$ s$^{-1}$) 40.06 < 0.0001

Xylem cross-sectional area (mm$^2$) 47.24 < 0.0001

Age as a covariate was significant ($p < 0.01$).

N.S. Not significant when $p > 0.01$

Figure 3. Comparisons of (A) average vessel element (VE) diameter, (B) average sieve element (SE) diameter, (C) vessel element density, (D) sieve element density, (E) xylem cross-sectional area, and (F) phloem cross-sectional area in each light treatment at different ages for the first cohort of seedlings ($n = 140$). Light levels are noted with different colors. Significant difference between the light treatments at each specific age is depicted with asterisks and was found using a Tukey HSD test. Each point represents a different plant.
Figure 4. Boxplots of (A) the percent xylem conductive area, and (B) the percent phloem conductive area (n = 110). Light levels are noted with different colors. Letters indicate differences between the light treatments based on a Tukey HSD test. Each point represents a different plant.

Impact of the carbon fixation, root respiration and stem traits on phloem hydraulic conductivity and percent conductive area

Our piecewise SEM allowed us to answer the question of how source activity (net daily carbon assimilation), sink activity (daily root respiration), and stem size (length and diameter) influenced vascular hydraulic conductivity and percent vascular conductive area in the phloem and xylem (Fig. 1). The piecewise SEM models were valid for phloem hydraulic conductivity (Fisher’s C = 14.99, df = 18, p = 0.66) and percent phloem conductive area (Fisher’s C = 8.83, df = 18, p = 0.96). For the phloem hydraulic conductivity component, stem widening led to an increase in phloem hydraulic conductivity (Fig. 5A). However, stem diameter did not have a significant impact on percent phloem conductive area (Fig. 5B). Net daily carbon assimilation positively impacted both phloem hydraulic conductivity and percent phloem conductive area.
and, in each case, explained around 20% of the variance in these traits (Fig. 5). There was no evidence that sink activity impacted phloem anatomy.

The piecewise SEM model was also valid for xylem hydraulic conductivity (Fisher’s C = 14.99, df = 18, p = 0.66) and percent xylem conductive area (Fisher’s C = 8.83, df = 18, p = 0.96). More factors impacted xylem hydraulic conductivity and percent conductive area than phloem hydraulic conductivity and percent conductive area (Fig. 5). Like phloem hydraulic conductivity, stem diameter and net daily carbon assimilation positively impacted xylem hydraulic conductivity. However, stem diameter negatively affected percent xylem conductive area whereas stem length positively affected both xylem hydraulic conductivity and percent xylem conductive area.

Figure 5. Piecewise SEM used to determine what traits influence (A) phloem and xylem hydraulic conductivity and (B) percent phloem and xylem conductive area. Black arrows indicate significant positive relationships, dashed red arrows indicate significant negative relationships, and gray arrows indicate non-significant relationships (? = 0.01). Arrow width signifies the magnitude of the path strength (i.e., standardized regression coefficient), which are the numbers on the arrows. The gray shaded boxes indicate traits that significantly influenced the phloem hydraulic conductivity or percent phloem conductive area. The R² indicates the goodness of fit of the associated multiple linear regression model.
Conduit scaling relationships with plant size and vascular allometric relationships

Conduit diameter scaled with stem diameter and length though the intercepts depended on the treatment (Fig. 6). The low light seedlings had conduit diameter and stem diameter and length relationships (for stem diameter: b = 0.33 – 0.36, for stem length: b > 0.2 – 0.33) similar to those found in previous studies on mature plants for sieve elements (Fig. 6C) and vessel elements (Fig. 6A and B) (Olson and Rosell, 2013; Olson et al., 2014; Jyske and Hölttä, 2015; Fajardo et al., 2020).

However, some of our scaling exponents differed from what was expected, especially in the xylem. In the high light treatment, the scaling exponent for vessel diameter to stem diameter (OLS, b = 0.026, R = -0.58; Fig. 6A) and stem length (b = 0.049, R = -0.55; Fig. 6B) was lower than the expected exponents from the literature because it was not significantly different from a slope of 0. Though stem diameter was consistently an influential factor on percent phloem conductive area, the scaling exponent for sieve element diameter to stem diameter for the medium light plants (b = 0.54, R = 0.56, p < 0.001; Fig. 6C) differed from the expected value of 0.33 (Olson and Rosell, 2013; Jyske and Hölttä, 2015).

Between the light treatments, some of the intercepts were significantly different from each other for the observed scaling relationships (Fig. 6). For sieve element diameter versus stem diameter, there were no significant differences between the light treatments for the intercepts (ANCOVA, F = 1.1, p > 0.01) though stem diameter did have a significant effect on sieve element diameter (ANCOVA, F-value = 317.3, p < 0.0001). For sieve element diameter versus stem length, the medium light treatment had a significantly different intercept than both the high and low light treatments (ANCOVA, F-value = 11.33 p < 0.0001). For vessel element diameter, the low light seedlings had a significantly lower intercept than the high light treatment for vessel element diameter versus stem length (ANCOVA, F-value = 10.5, p-value < 0.0001). It is worth noting that even though the phloem and xylem are hydraulically linked, there were no significant relationships between percent phloem and xylem conductive area or theoretical hydraulic conductivity across any of the light treatments (Fig. 7).
Figure 6. Scaling relationships of (A) vessel element (VE) diameter to stem diameter, (B) vessel element diameter to stem length, (C) sieve element (SE) diameter to stem diameter, and (D) sieve element diameter to stem length for the week two to seven seedlings. Gray bars are 95% confidence intervals. Lines represent significant linear regressions for individual light treatments ($\alpha = 0.01$). Light levels are marked with different colors and symbols. Each point represents a different plant.
DISCUSSION

Though carbon transport is a vital part of plant physiology, we have a limited understanding of how environmental factors, like light, may directly and indirectly impact phloem anatomy. In our three light treatments there were clear differences in phloem cross-sectional area, conduit diameter, conduit density, and the percent phloem conductive area (Fig. 3 and 4). When we accounted for differences in light, stem diameter became an important explanatory variable for phloem hydraulic conductivity according to our pairwise SEM models (Fig. 5). However, the low light seedlings had larger sieve element diameters for a given stem diameter than the other two light treatments (Fig. 6), which indicates that size alone cannot explain variation in sieve element diameter. Net daily carbon assimilation appeared to explain the remaining variation in phloem hydraulic conductivity, and it was the only significant factor for percent phloem conductive area. Though light has direct effects on plant traits such as stem length and diameter, net daily carbon assimilation may have an additional impact on phloem anatomical traits that are important for carbon transport.

Differences in vascular anatomy, plant size, and source and sink activity

Our findings agree with other studies that found light intensity (PPFD) leads to changes in stem length and diameter, photosynthetic and respiration rates, and leaf area (Kappel and Flore, 1983; Armitage, 1991; Gloser, Schuurwater and Lambers, 1996; Lichtenthaler et al., 2007; Huang, Zhang and Hu, 2014; Zivcak et al., 2014). Similarly, in our experiment, light impacted vascular traits (Fig. 3), which is similar to the findings of other studies (Cochard, Lemoine and Dreyer, 1999; Lemoine, Cochard and Granier, 2002; Wang...
and Huang, 2003; Plavcová, Hacke and Sperry, 2011; Noyer et al., 2017). The narrower conduits at the stem base in turn led to a lower phloem and xylem hydraulic conductivity in the low light treatment (Eq. 2, Table 3). The narrower conduits also account for the smaller percent phloem conductive area in the low light seedlings because there was not a significant difference in sieve element density between the light treatments (Fig. 3). Our results illustrate that plants in a low light environment may have a lower transport capacity due to both lower conductivity and percent conductive area compared to individuals of the same species grown in a higher light environment.

### Stem diameter impacts vascular hydraulic conductivity and percent xylem conductive area

Stem diameter was the most important factor in determining both phloem and xylem hydraulic conductivity according to our SEM model (Fig. 5A). This is not surprising considering that plants with wider stems generally have wider conduits (Niklas, 1993; Niklas and Spatz, 2004; Olson et al., 2013), and hydraulic conductivity is highly sensitive to small changes in conduit diameter. Though previous studies found a strong relationship between stem length and conduit diameter (Olson et al., 2013, 2014; Savage et al., 2017; Fajardo et al., 2020), we did not find evidence for this correlation in the phloem. However, stem length did have a significant effect on xylem hydraulic conductivity (Fig. 5), but there was only a small difference in the significance of stem diameter compared to stem length, which suggests that both stem length and diameter are important traits for vessel conduit width.

Despite our SEM models indicating that wider stems had a greater sieve element diameter regardless of light level, we found that for a given stem diameter low light seedlings had proportionally wider sieve elements compared to the medium and high light treatments (Fig. 6). This suggests that besides stem diameter, there is another factor that is impacting sieve element diameter, which may be particularly important or visible in our low light seedlings.

### Net daily carbon assimilation is tied to phloem hydraulic conductivity and percent conductive area

Net daily carbon assimilation was the other influential factor in explaining phloem hydraulic conductivity and the only explanatory factor for percent phloem conductive area in our SEM model (Fig. 5). Because our model considered the effect of light level measured above an individual seedling each week, it appears that any effects of net daily carbon assimilation on vascular anatomy are not driven by indirect effects of light. The impact of net daily carbon assimilation on vascular anatomy appears particularly obvious, and important, in the low light seedlings, which have large sieve element diameters for a given stem diameter (Fig. 6). This unexpected result indicates that seedlings with the lowest rate of net daily carbon assimilation have disproportionately wide sieve elements for their stem diameter.

We propose that these wider than expected sieve elements in low light plants could be a result of resource limitation in these stressed seedlings. By week six of the experiment, the majority of the low light seedlings had died or were dying, and only four low light seedlings survived to week eight. We are uncertain what led to seedling death, but with photosynthetic rates barely above their dark respiration rate (Table 1), it is possible that the low light seedlings died from carbon starvation. Carbon starvation is often discussed in terms of drought and how stomatal closure may lead to a depletion of stored carbon, which eventually kills the plant (McDowell et al., 2008; Sala, 2009; McDowell and Sevanto, 2010). However, it could happen in seedlings if their carbon stores are not enough to sustain them during times of low carbon fixation, as suggested in Savage, Zwieniecki and Holbrook (2013).

Though we did not study phloem pressure gradients in these plants, we hypothesize that the low light seedlings may have had a weak pressure gradient between the source and the sink because of their low net daily carbon assimilation. It is important to note that other compounds like potassium, might compensate and allow the seedlings to maintain a high pressure in the source tissue without much sugar loading as observed in mutant corn (Babst et al., 2022). However, if carbon was the main source of the pressure gradient between the source and the sink, then sap flow would decrease under these low pressure conditions and could even lead to phloem collapse (Sevanto et al., 2014), which could be fatal if the seedlings are not
able to transport enough carbon to maintain their sink costs. Because phloem sap flow rate is impacted by the pressure gradient and conductivity, larger sieve elements would allow low light seedlings to maintain a higher sap flow rate in the phloem to compensate for the lower pressure gradient.

The larger sieve element diameters in the low light could also be partially explained by the construction costs associated with building conduits. If for a given wall thickness, a wider lumen takes less carbon to construct, then for the carbon limited, low light seedlings, it would be beneficial to have wider lumens since they require less carbon. At least in the xylem, there is a relationship between vessel element lumen area fraction (like our percent vascular conductive area) and construction costs (Zanne et al., 2010). If vessel element lumen area fraction is fixed, then smaller vessel elements would have a greater total surface area compared to larger ones, which would require more wall material, but by altering the relationship between vessel element area and vessel wall thickness, these costs can be changed as well (Hacke and Sperry, 2001; Zanne et al., 2010). If sieve elements have similar relationships between conduit size and construction costs, then this could be another explanation why the low light seedlings had such wide sieve elements for their size.

The phloem is more responsive to light and changes in net daily carbon assimilation than the xylem

Although the phloem and xylem are hydraulically linked, we found several notable differences in how light, net daily carbon assimilation, root respiration, and plant size impacted the anatomy of the xylem differently than the phloem. Net daily carbon assimilation, which was the only significant explanatory factor for the percent phloem conductive area, had no impact on percent xylem conductive area (Fig. 2B). There also appeared to be a difference in how percent conductive area changed in response to light in the phloem and xylem. While percent phloem conductive area was smaller in the low light seedlings than the other two treatments, the percent xylem conductive area was consistent at around 15% in all light treatments (Fig. 4). These findings support the idea that percent xylem conductive area is often constant and controlled by a careful balance between structural and transport requirements of the tissue (Sperry, Meinzer and McCulloh, 2008; Savage et al., 2010). The phloem does not seem to have the same limitations and exhibits more flexibility in the percentage of conduits in the phloem compared to the xylem. Thus, future research on percent phloem and xylem conductive area should explore whether other environmental factors, like drought, may also lead to changes in phloem but not xylem anatomy.

Differences in phloem and xylem scaling and allometry

The light treatment impacted both scaling relationships between conduit diameter and stem length and diameter, but the effect was larger in the phloem (Fig. 6). In general, vessel elements in the high light seedlings did not get significantly wider as stem diameter or length increased while they did in the medium and low light seedlings (Fig. 6). The high light seedlings also had much lower scaling exponents than the hypothesized minimum (0.2 ≥ 0.33) found in the literature for stem diameter and length (Mencuccini et al., 2007; Savage et al., 2010; Olson and Rosell, 2013; Olson et al., 2014; Jyske and Hölttä, 2015). Since the high light plants were grown above their saturating light level, and thus likely not light limited (Fig. S1), selection may have favored increasing sieve element diameter to decrease carbon transport resistance. A decrease in resistance could potentially move more fixed carbon out of the leaves instead of down regulating photosynthesis, which may occur when excess sugars remain in the leaves (Nafziger and Koller, 1976; Azcón-Bieto, 1983). For example, conifers and species undergoing intense competition for water and nutrients have been found to have suboptimal xylem conduit widening, which means that the efficiency of water transport and in turn carbon gain may have been lowered (b < 0.33, Anfodillo et al., 2006; Petit, Anfodillo and Mencuccini, 2008; Jyske and Hölttä, 2015; Olson et al., 2021). Other studies have even found that vessel element diameter might be different than expected for a given stem length based on other variables such as temperature, wood density and porosity, and leaf phenology (Olson et al., 2021). These results show that even though the phloem is hydraulically linked to the xylem, phloem hydraulic conductivity is not necessarily limited by xylem hydraulic conductivity. Contrary to our expectations, we found no significant allometric relationships between the phloem and xylem in any of the light treatments (Fig. 7). Overall, our
model suggests that the phloem and xylem may respond differently to the factors of plant size and the light.

CONCLUSION

For the first time, we have measured phloem hydraulic conductivity and percent phloem conductive area of a large number of plants and were able to study how source leaves, sink roots, and stem length and diameter impact carbon transport. Net daily carbon assimilation impacted both phloem hydraulic conductivity and percent phloem conductive area in our seedlings. Though stem diameter was an explanatory factor for phloem hydraulic conductivity, when comparing between light treatments, seedlings with the same stem diameter had different sieve element diameters. This suggests that net daily carbon assimilation may be a more influential trait for sieve element diameter than previously thought and could be an important underlying relationship that has been overlooked. Phloem and xylem traits both changed under the different light treatments, yet xylem anatomy seemed more impacted by stem diameter and length than phloem anatomy, specifically when looking at the vascular hydraulic conductivity and percent vascular conductive area. Overall, it appears that while vessel diameter is indirectly impacted by the light environment due to changes in stem length and diameter, sieve element diameter is both directly impacted through carbon fixation and indirectly impacted by stem diameter. Our results suggest that phloem anatomy, and in turn carbon transport, may be more sensitive to environmental factors than previously supposed due to direct effects (i.e., carbon assimilation) and indirect effects (i.e., stem traits).

ACKNOWLEDGEMENTS

We appreciate the feedback we received on the manuscript from our reviewers. Additionally, we would like to thank Dr. Salli Dymond and Dr. Daniel Stanton for their input on the data analysis and writing. A special thanks to our lab assistants Britton Vandenheuvel and Lexi Wear for assisting with data collection. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. DGE-1840377, the National Science Foundation Grant No. 1942916, the Graduate Council Teaching Fellowship from the University of Minnesota Duluth, and the Bill Dahl Graduate Student Research Award from the Botanical Society of America.

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