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Influence of nitrogen fertilization on xylem traits and aquaporin expression in stems of hybrid poplar

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Summary We studied the influence of nitrogen (N) on hydraulic traits and aquaporin (*AQP*) expression in the stem xylem of hybrid poplar saplings (*Populus trichocarpa* (Torr. & Gray) × *deltoides* Bartr. ex Marsh clone H11-11). Plants were grown in a controlled environment and were kept well watered throughout the experiments. Hydraulic measurements were done on basal and distal stem segments of plants receiving high N fertilization (high N plants) versus plants receiving only adequate N fertilization (adequate N plants). High N plants grew faster and exhibited more leaf area than adequate N controls. These morphological differences were paralleled by wider vessels and higher specific conductivities (K_S) in high N plants. However, stems of high N plants were more vulnerable to xylem cavitation, at least in one of two experiments, and showed lower wood densities than stems of adequate N plants. Leaf area was strongly correlated with cross-sectional xylem area in both plant groups. Since higher K_S in high N plants was accompanied by concomitant increases in leaf area, leaf-specific conductivities were similar in both plant groups. Influences of N on hydraulic traits were paralleled by changes in *AQP* expression. Seven *AQP*s were upregulated in the stem xylem of high N plants, five of which have been identified recently as water transporters. The enhanced growth of secondary xylem of high N plants has been shown to result from both increased cambial activity as well as increased cell size. We suggest that some of these water-transporting AQPs could play a role in xylogenesis, facilitating the influx of water into the zone of differentiating and maturing cells in secondary xylem, including expanding vessels.

Keywords: hydraulic conductivity, vessel diameter, water transport, wood density, xylem cavitation, xylogenesis.

Introduction

Populus species and their hybrids are increasingly cultivated to meet the demand for wood products while reducing the exploitation pressure on native forests (Harvey and van den Driessche 1997, DesRochers et al. 2006). High rates of productivity in poplar require an abundant and continuous supply of moisture as well as high nutrient uptake. Nitrogen (N) availability is often a limiting factor to growth both in natural forests and in sites favoured for cultivation of poplar (Heilman et al. 1996). *Populus trichocarpa* (Torr. & Gray) × *deltoides* Bartr. ex Marsh clone H11-11 hybrids showed considerable morphological and physiological plasticity in response to N availability and rapidly incorporated N from the soil (Cooke et al. 2005). Among the responses to an increased N supply were increased photosynthesis and leaf area on both per-leaf and whole-plant bases. This was accompanied by a shift in biomass allocation away from root and stem tissue towards leaves, which was in large part driven by N-induced sylleptic branching (Cooke et al. 2005).

N-induced changes to wood fibre properties have been examined in detail (e.g., Pitre et al. 2007*a*, 2007*b*, 2010), but the effects of N fertilization on xylem structure and hydraulic traits are less well understood. While Harvey and van den Driessche (1999) observed larger vessel diameters of high N fertilized hybrid poplars than in low N trees, Luo et al. (2005) found no consistent differences in vessel diameters of three poplar species and hybrids in response to N fertilization. Nitrogen additions resulted in increased specific conductivity in savannah trees (Bucci et al. 2006), while roots of loblolly pine showed lower specific conductivity (Ewers et al. 2000). In *Eucalyptus grandis*, high N did not affect shoot hydraulic properties (Clearwater and Meinzer 2001). The preceding examples highlight that there is not a single, predictable response of hydraulic traits to N fertilization. One reasonable explanation for these variable results is that different plant groups (angiosperms versus conifers) and different plant organs (stems versus roots) were examined in these studies. Differences in plant growth conditions—e.g., natural stands, plantations or greenhouses—likely also contribute to the different findings, as do water availability and duration of N fertilization prior to measurements.

Recently, microarrays were used to profile N-mediated gene expression changes in secondary xylem of poplar (Pitre et al. 2010). An aquaporin (*AQP*) was among the genes found to be upregulated by high N. AQPs are membrane-spanning proteins involved in the transport of water, small neutral solutes such as N compounds or gases across plasma and intracellular membranes (Maurel et al. 2008). As such, *AQP*s are interesting candidates to investigate for their role in N-induced changes in secondary xylem. Several *AQP*s are expressed in the secondary xylem of poplar stems (A. Almeida-Rodriguez et al., unpublished, Gupta and Sankararamakrishnan 2009), with some of these expressed in the cambial zone and differentiating xylem cells (Schrader et al. 2004). In the xylem of walnut trees, *AQP*s were found in paratracheal parenchyma and ray cells (Sakr et al. 2003). However, little is known about their function and their cellular and subcellular localization in this tissue, since the majority of work on plant *AQP*s has focused on roots and leaves. High expression levels of particular *AQP*s have been associated with high cell expansion rates and cell differentiation (Tyerman et al. 2002 and literature cited therein). However, it is not clear yet if *AQP*s play a role in xylogenesis. Even less is known about the role of *AQP*s in the transport of N compounds in secondary xylem of woody plants.

The main objective of this study was to evaluate the effect of N fertilization on xylem traits in poplar saplings. We hypothesized that plants subjected to high N availability (high N plants) will have wider vessels (Harvey and van den Driessche 1999, Watanabe et al. 2008) and more efficient xylem than plants given only adequate N (adequate N plants). A more conductive xylem would be better able to support the faster shoot growth and the greater leaf areas that are typically associated with N fertilization. Given that wider vessels and increased transport efficiency are often correlated with increased vulnerability to xylem cavitation (Hacke et al. 2006, Cai and Tyree 2010), we hypothesized that high N fertilization will also lead to greater xylem vulnerability. To test this hypothesis, *P. trichocarpa* × *deltoides* hybrid poplars were grown in a controlled environment under adequate N versus high N conditions and were well watered to reduce the influence of other factors besides N on hydraulic architecture. A long-term goal is to identify molecular mechanisms by which N alters the structure and water transport patterns of xylem. As a first step towards identifying roles for *AQP*s in the N response of secondary xylem, we examined the expression patterns of 11 *AQP*s in secondary xylem under adequate and high N availability, focusing on a subset of xylem-expressed *AQP*s recently shown to be water transporters (Secchi et al. 2009).

Materials and methods

Plant material

Rooted cuttings of *P. trichocarpa* × *P. deltoides* were produced and maintained in a controlled environment room with 16-h days/8-h nights, 25 °C constant temperature, ca. 70% relative humidity and ~325 μmol m^{-2} s^{-1} of photosynthetically active radiation. Prior to initiating each experiment, all plants received 0.5 g l^{-1} of N–P–K 20–20–20 fertilizer (Plant Products, Brampton, ON, Canada) once weekly, supplemented with calcium on a biweekly basis. Plants were at least 80 cm tall at the outset of the experiment, so that plants exhibited cessation of internode elongation and also showed substantial secondary growth for at least 60 cm of the lowermost portion of their height. A relatively uniform group of plants was selected, and plants from this group were randomly assigned to either the adequate N or high N treatment. The adequate N group received 0.5 mM NH_4NO_3 in 0.5× Hocking's complete nutrient solution (Hocking 1971), while the high N group received 10 mM NH_4NO_3 in 0.5× Hocking's solution. Plants were fertilized with either the adequate N or high N solution to field capacity three times weekly for 4–5 weeks and watered on each of the other days. Pots were placed on saucers to minimize the chance of the soil drying between water or nutrient solution application.

After 4–5 weeks of treatment, plants were harvested to generate materials for measurements. Two 15-cm segments of stem were harvested for measurement of hydraulic parameters: a 15-cm basal segment was harvested 15 cm above the soil height, and a 15-cm distal segment was harvested 45 cm above the soil height. Similar to the approach used by Pitre et al. (2007*a*, 2007*b*, 2010), the position of these stem segments was carefully chosen to avoid differences between the two treatments in the physiological age of their stems. The segments were sampled from the lower portion of the stem above the original rooted cutting but within the region that exhibited cessation of internode elongation and significant accumulation of secondary xylem prior to commencing treatment with differential N availability. As such, the transition from primary growth to secondary growth in these segments was completed prior to the onset of the experiment. In these stem segments, any effect of N treatment on xylem development would arise from the vascular cambium along the transverse axis rather than a delay in the transition to secondary growth along the longitudinal axis. A third segment of stem comprising three internodes was removed at 60 cm above the soil surface (i.e., just above the distal segment collected for measurement of hydraulic parameters). This section also represented wood that had ceased internode elongation and had developed significant amounts of secondary xylem prior to commencing the differential N treatments. The stem was debarked and the remaining secondary xylem rapidly frozen in liquid nitrogen for RNA analysis. Two independent experiments were conducted, referred to as set 1 and set 2.

Hydraulic conductivity

Stem segments were trimmed to a final length of 14.2 cm. The same segments were used for all hydraulic and anatomical measurements. For each set of plants, six segments were measured per treatment and position. Segments were flushed

with 20 mM KCl + 1 mM $CaCl_2$ solution at 50 kPa for 15 min to remove reversible embolism. The deionized water for this solution had been filtered through an E-Pure system (Barnstead, Dubuque, IA) and a capsule filter (0.2 µm). The segments were then fitted to a tubing apparatus (Sperry et al. 1988) where the initial hydraulic conductivity was measured, using the same solution that was used for flushing. Hydraulic conductivity was calculated as the quotient of flow rate through the segment and pressure gradient along the segment. Conductivity was based on the net flow rate through a segment. The net flow rate was determined by measuring the total flow under a pressure of 4–5 kPa and subtracting the 'background' flow, measured in the absence of a difference in hydraulic head across the segment. Background flow was measured before and after the pressurized flow, with the average used to calculate the net flow. Each flow measurement was the average of five successive 10-s measurements and was monitored gravimetrically using an electronic balance (CP225D, Sartorius, Göttingen, Germany) interfaced with a computer (using Collect 6 software, Labtronics, Guelph, Canada). Conductivity was corrected to 20 °C to standardize for temperature-dependent viscosity effects. The maximum hydraulic conductivity was used to determine the specific conductivity (K_S), which is the hydraulic conductivity per transverse sectional sapwood area (Tyree and Zimmermann 2002). Xylem area (excluding pith) was determined from a cross-section through the middle of a segment. The area was measured with a stereomicroscope (MS5, Leica, Wetzlar, Germany) equipped with a digital camera (Infinity 1, Lumenara, Ottawa, Canada) and interfaced with a computer for image analysis. Leaf-specific conductivity (K_L) was measured by dividing the maximum hydraulic conductivity by leaf area. Leaf areas were measured using a scanner (set 1) or a LI-3100 leaf area meter (LI-COR, Lincoln, NE; set 2). The leaf area of all the leaves distal to the segment was added to one-half the leaf area of those leaves attached to the segment (Woodrum et al. 2003).

Cavitation resistance

Vulnerability curves were generated to evaluate the xylem's resistance to embolism. The centrifuge method was used (Alder et al. 1997). Segments were spun in a custom-built rotor to increasingly negative xylem pressures. Segments were held at each target pressure for 10 min to ensure complete embolism before being taken out of the rotor and before hydraulic conductivity was re-measured. The percentage loss in conductivity from the original value was plotted versus the negative pressure, and curves were fitted using a Weibull function. The xylem pressure corresponding to 50% loss of conductivity (P50) was calculated for each segment.

Vessel diameter measurements

Transverse sections of five to six stem segments per treatment (adequate N versus high N fertilized) and position (basal versus distal) were prepared with razor blades or with a sliding microtome. Sections were stained with toluidine blue for 3 min, rinsed in water and mounted on glass slides with glycerin. Photographs of xylem sectors spanning from pith to cambium were taken with a Leica DFC420 C digital camera mounted on a DM3000 microscope (Leica Microsystems, Wetzlar, Germany). Magnification was ×200. The diameter of each vessel lumen in a radial sector of functional xylem was measured with image analysis software (ImagePro Plus version 6.1, Media Cybernetics, Silver Spring, MD). A minimum of two radial sectors per stem was measured to obtain an average based on a minimum of 100 vessel diameters per stem. Based on the Hagen–Poiseuille equation, the diameter of the average vessel of a stem segment was calculated as $D = [(\Sigma d^4)/n]^{1/4}$, where n is the number of vessels measured, and d is the individual vessel lumen diameter. Hence, D represents the diameter of a vessel of average lumen conductivity (Tyree and Zimmermann 2002). The mean for each treatment and position was the mean of five to six segments.

Wood density

To study whether hydraulic parameters were correlated with wood density, the dry weight per fresh volume of wood samples was measured. One ~2-cm-long wood sample was prepared per segment, and was cleaned of any pith and bark material. The sample was submersed in water to measure its volume displacement. The displacement weight was converted to sample volume. Samples were subsequently dried at 70 °C for 3 days, and their dry weight was measured.

Quantitative reverse transcription–polymerase chain reaction of AQPs

Total RNA was extracted using the hexadecyltrimethylammonium bromide (CTAB) extraction protocol of Chang et al. (1993). Two micrograms of total RNA were treated with DNase I (New England Biolabs, Ipswich, MA) and used as template for first-strand cDNA synthesis using an anchored oligo$(dT)_{23}$ primer (Superscript® III Reverse transcriptase, Invitrogen, Carlsbad, CA).

Eleven *AQP*s representing different *AQP* subfamilies were selected for quantitative reverse transcription–polymerase chain reaction (qRT–PCR) analysis in secondary xylem of trees subjected to adequate N or high N treatments (Table S1 available as Supplementary Data at *Tree Physiology* Online). Since an expression analysis of all 56 *AQP* genes identified in v2.0 of the *P. trichocarpa* genome (http://www.phytozome.net/poplar) was beyond the scope of this work, we chose representative *PIP1*, *PIP2*, *TIP* and *SIP* genes that were known to be expressed in N-treated secondary xylem of *P. trichocarpa* × *deltoides* based on expressed sequence tag (EST) evidence (Pitre et al. 2010), with a focus on five of the *PIP2* genes that were recently identified as water transporters (Secchi et al. 2009). The names used to denote these *AQP*s in this study are consistent with those used by Gupta and Sankararamakrishnan (2009). qRT–PCR was performed essentially as described in Voicu et al. (2009). *EF1-α* (GenBank acces-

sion no. GQ253565) and *TIF5A* (GenBank accession no. GU452541) were chosen as reference genes, since transcript abundance of the arithmetic mean corresponding to *EF1-α* and *TIF5A* did not show significant differences between adequate N and high N treatments for each independent experiment (set) when expressed as $2^{-\Delta Ct}$ ($P = 0.064$ for set 1 and $P = 0.438$ for set 2). Near full-length cDNAs for these reference genes were cloned by RT–PCR from *P. trichocarpa* × *deltoides* tissues using standard methods (Sambrook and Russell 2001). Primers used to clone *PtdEF1-α* were sense: 5′-ACGGTTACGCTCCAGTCCTTG-3′; antisense: 5′-CAAGACCGAAACCACGCCAC –3′; and *PtdTIF5A* were sense: 5′-CACGTCACCCGTACTGACTACCAGCTG-3′; antisense: 5′-GATGATGAGGAAAGGAACTGGAAC-3′.

Transcript abundance was quantified using standard curves for both target and reference genes according to Voicu et al. (2009), with slight modifications. Templates for the standard curves were generated by serial dilution of PCR amplicons obtained from plasmids corresponding to each target or reference gene. A pooled serial dilution series was made that included all target and reference genes, ranging from 4×10^7 to 4×10^1 copies for each gene. Gene-specific qRT–PCR primers for the target and reference genes were designed in Primer Express v3 (Applied Biosystems, Foster City, CA) and are listed in Table S1. Real-time PCR was performed on a 7900 HT Fast Real-Time PCR system (Applied Biosystems). Assays were carried out in 384-well plates. At least four biological replicates, each with three technical replicates, were assayed for each nitrogen treatment in the two experiments. The two reference genes and pooled standard curves were included on every plate. PCR was carried out in a volume of 10 µl including a final concentration of 50 ng of cDNA, 1×

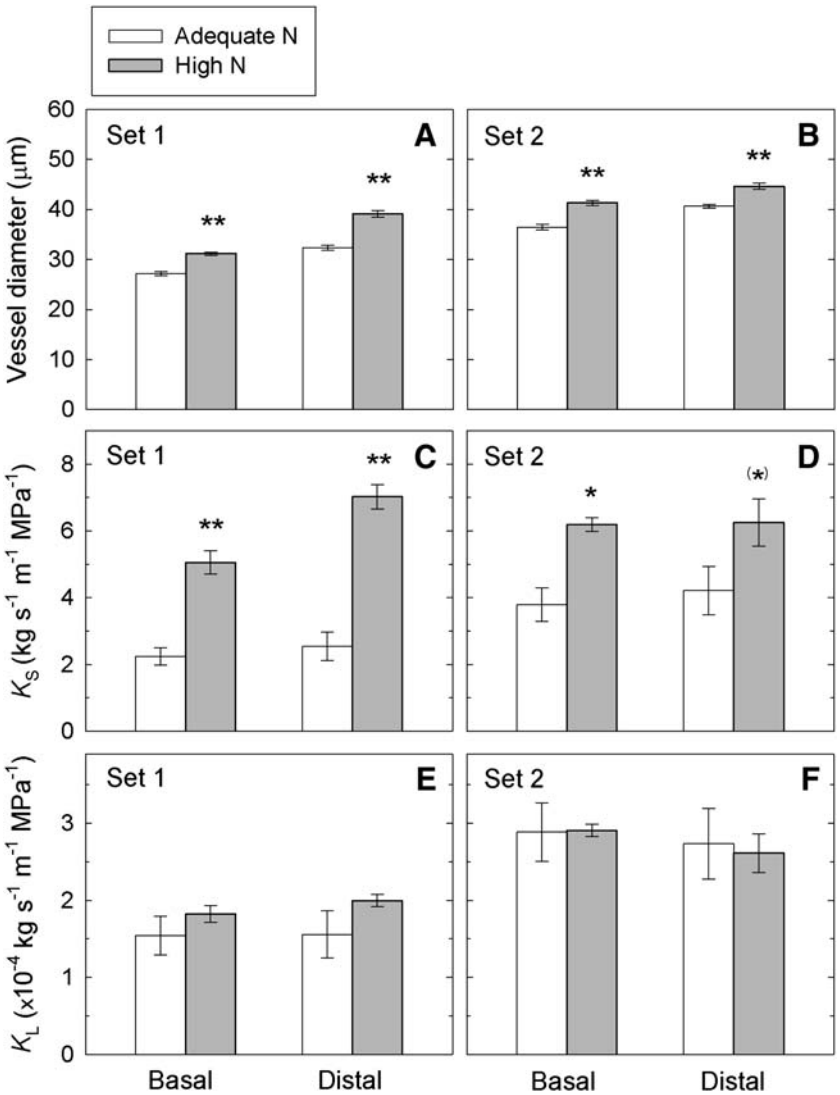

Figure 1. Average vessel diameter (**A**, **B**), xylem area-specific hydraulic conductivity (**C**, **D**) and leaf-specific conductivity (**E**, **F**) of basal and distal stem segments of adequate N (white bars) and high N (grey bars) plants. The experiment was repeated once; hence, there are two sets of plants, labelled set 1 (**A**, **C**, **E**) and set 2 (**B**, **D**, **F**). Means and SE, $n = 6$. $^{**}P \leq 0.01$ indicates significant differences between adequate and high N plants; $^{*}P \leq 0.05$ indicates significant differences; and $^{(*)}P = 0.07$ marks a marginally significant difference.

master mix containing 0.2 mM dNTPs, 0.3 U Platinum *Taq* polymerase (Invitrogen), 0.25× SYBR Green and 0.1× ROX. PCR conditions were as follows: one cycle at 95 °C for 2 min, 40 cycles at 95 °C for 15 s, 60 °C for 1 min and a dissociation stage including two cycles of 95 °C for 15 s, 60 °C for 1 min. Samples were subjected to auto Ct (cycle threshold) for analysis, and dissociation curves were verified for each of the genes. Transcript abundance for each target gene was normalized against the arithmetic mean of transcript abundance corresponding to *EF1-α* and *TIF5A*, and data were expressed as number of molecules.

Statistics

To separate means of hydraulic parameters within basal and distal stem segments of adequate and high N plants, *t*-tests were performed. The effects of nitrogen availability on the gene expression of each selected *AQP* were independently evaluated for the two sets of experiments by a *t*-test using SigmaStat version 3.5 (Point Richmond, CA).

Results

Xylem structure and hydraulic conductivity

Two independent experiments were conducted in which the effects of 0.5 mM NH_4NO_3 versus 10 mM NH_4NO_3 on xylem anatomy and water transport properties were examined. These two independent experiments are referred to as sets. The data for these two sets are presented separately to illustrate that although the results for the two experiments are very similar, subtle differences exist between the experiments that point to the dynamic and pliable nature of the hydraulic adjustment response. Statistical analyses were conducted on each set as well as on the combined dataset from the two independent experiments.

Vessel diameters varied from 27.16 ± 0.41 µm (mean ± SE) in basal segments of adequate N plants from set 1 to 44.62 ± 0.63 µm in distal segments of high N plants from set 2 (Figure 1A and B). High N plants consistently exhibited wider vessels than their adequate N counterparts, a trend that was highly significant in both basal and distal segments. Differences in vessel diameter between the two treatments were also statistically significant when the two datasets were combined for analysis ($P < 0.05$). Differences in D corresponded to trends in K_S (Figure 1C and D). In set 1, segments of adequate N plants had specific conductivities that were less than half those of high N plants. In set 2, the K_S of adequate N plants was about one-third lower than in high N segments. This trend was significant in basal segments and marginally significant ($P = 0.07$) in distal segments (Figure 1D). Analysis of the combined dataset also yielded significant differences in K_S between treatments ($P < 0.001$). While the high N treatment resulted in higher transport efficiencies on a xylem area basis, there were no differences in leaf-specific conductivity between adequate N and high N plants (Figure 1E and F). This was due to higher leaf areas in high N plants. Similarly, there were no significant differences in leaf-specific conductivity between N treatments for the combined dataset. In both adequate and high N plants, leaf areas were closely correlated with increasing sapwood area (Figure 2). However, stem segments of high N plants supported much more leaf area per sapwood area than segments of adequate N plants (Figure 2, compare black and open symbols).

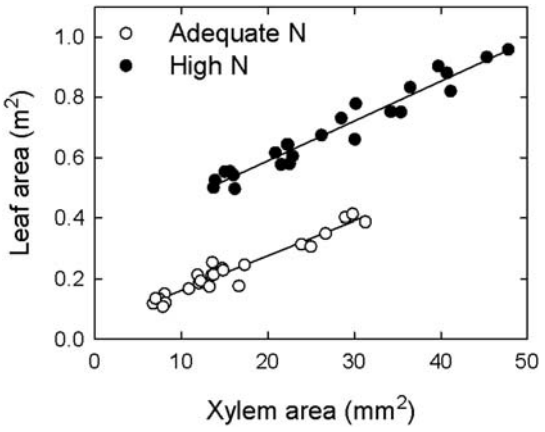

Figure 2. Leaf area supported by stem segments as a function of cross-sectional xylem area of the same segments. Closed circles represent high N plants ($r^2 = 0.95$; $P < 0.001$), open circles show adequate N plants ($r^2 = 0.94$; $P < 0.001$). The data from two experiments were combined.

In set 1, the P50 varied from −1.88 ± 0.05 MPa in basal segments of adequate N plants to −1.43 ± 0.05 MPa in distal segments of high N plants (Figure 3A). Adequate N segments were more resistant to cavitation than their high N counterparts. In set 2, basal segments of adequate N plants also tended to be more resistant to cavitation than basal segments of high N plants. This trend was marginally significant (Figure 3B, $P = 0.06$). Distal segments of plants from set 2 exhibited no difference in P50. However, the P50 values for the combined dataset showed significant differences ($P < 0.05$) between adequate N- and high N-treated plants. Adequate N segments consistently showed higher wood densities than their high N counterparts (Figure 3C and D). Analysis of the combined dataset also demonstrated significant differences in wood density between adequate N- and high N-treated stems ($P < 0.05$).

The P50 was correlated with D (Figure 4). Xylem with wider vessels was more vulnerable to cavitation than xylem with narrower vessels. This trend existed across all treatments and positions within a plant. Specific conductivity showed a similar trend with P50 across all segments ($r^2 = 0.51$, $P < 0.001$, data not shown). Higher conductivities were associated with greater vulnerability.

AQP transcript abundance

The effect of N availability on *AQP* transcript abundance is shown in Figure 5. All *AQP*s exhibited consistent expression patterns in sets 1 and 2, and except for *PtdPIP2;3*, *AQP* expres-

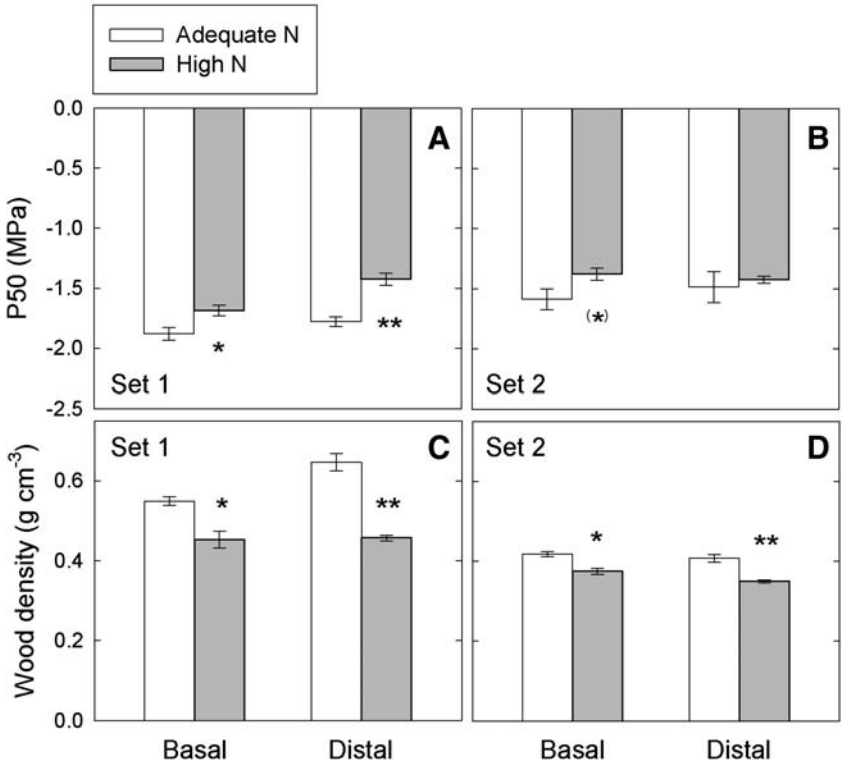

Figure 3. Cavitation resistance (**A**, **B**) and wood density (**C**, **D**) of basal and distal stem segments of adequate N (white bars) and high N (grey bars) plants. Cavitation resistance is expressed as the xylem pressure associated with 50% loss of hydraulic conductivity (P50), with more negative values representing more resistant xylem. Means and SE, $n = 6$. $^{**}P \leq 0.01$ indicates significant differences between adequate and high N plants; $^{*}P \leq 0.05$ indicates significant differences; and $^{(*)}P = 0.06$ marks a marginally significant difference.

sion patterns from sets 1 and 2 were both supported either with statistical significance or lack of significance. *PtdPIP2;5* was expressed at higher levels relative to the other *AQP*s that were assayed (Figure 5D, note the different *y* axis scale). Three different expression patterns were identified according to their response to N availability. First, seven *AQP*s—*PtdPIP2;2*, *PtdPIP2;3*, *PtdPIP2;4*, *PtdPIP2;5*, *PtdPIP2;7*, *PtdTIP1;6* and *PtdTIP2;1*—showed significantly greater transcript abundance under high N compared with adequate N conditions at least in one of the two independent experiments (Figure 5A–G). Second, *PtdPIP1;2* and *PtdSIP1;2* showed significantly lower expression levels in high N plants compared with adequate N plants (Figure 5H and I, respectively). Third, *PtdPIP1;3* and *PtdPIP2;7* did not show significant differences in transcript abundance across treatments in either experiment (data not shown).

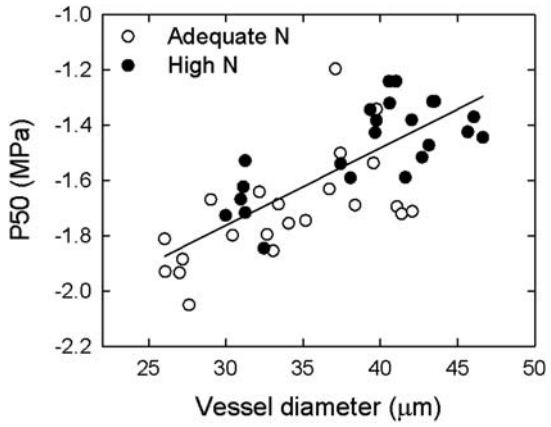

Figure 4. P50 as a function of average vessel diameter ($r^2 = 0.49$; $P < 0.001$). Symbols as in Figure 2. The data from two experiments were combined.

Discussion

Vessel diameters, hydraulic conductivity and leaf area

Although the same trends were exhibited in both independent experiments that were conducted (Figures 1 and 3), there was some variation in hydraulic parameters between the two sets of plants, demonstrating that, while the results are robust and replicable, the response to N fertilization is dynamic and thus subject to variation even under controlled conditions. Adequate and high N plants showed consistent differences in their xylem structure and function and shoot morphology. High nitrogen fertilization led to higher whole-plant leaf area, larger leaves and enhanced height growth as described previously (Cooke et al. 2005). In agreement with our first hypoth-

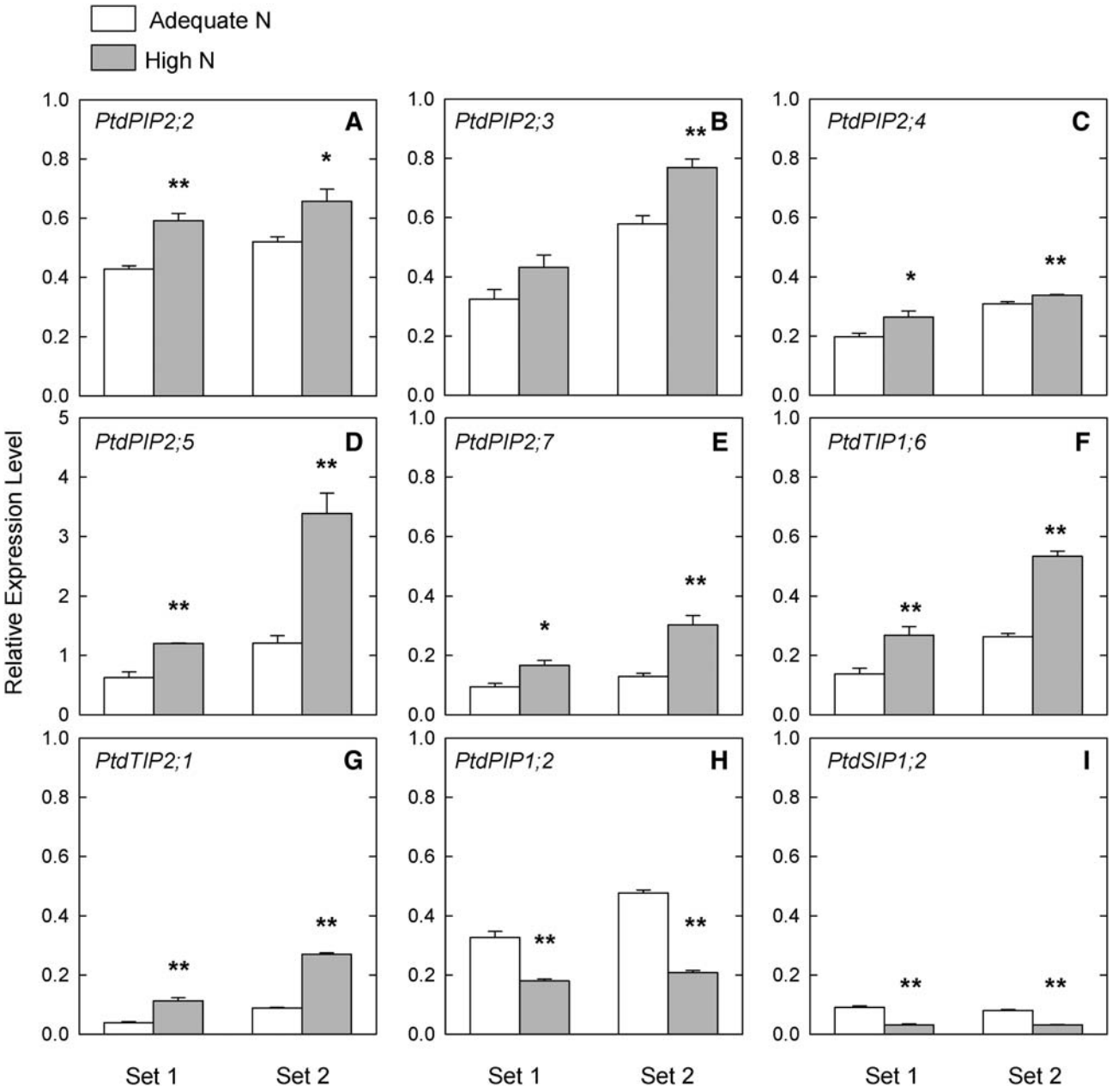

Figure 5. Transcript abundance corresponding to nine *AQP*s in secondary xylem of *P. trichocarpa* × *deltoides* grown under adequate N (white bars) and high N (grey bars) conditions. The experiment was repeated once; hence, there are two sets of plants labelled set 1 (left side of each plot) and set 2 (right side of each plot). Transcript abundance for each *AQP* was quantified by a standard curve and is expressed relative to the arithmetic mean of the transcript abundances corresponding to *TIF5A* and *EF1-α*, which were quantified simultaneously. Data are expressed as number of molecules. Means ± SE are shown. $n \geq 3$ biological replicates; each biological replicate was assayed in triplicate. $^{**}P \leq 0.01$ indicates significant differences between adequate and high N plants; $^{*}P \leq 0.05$ indicates significant differences.

esis, high N also resulted in higher D and K_S compared with adequate N fertilization (Figure 1). Wider vessels and higher xylem area-specific conductivities in high N-treated plants helped to support more leaf area. For a given xylem area, high N plants showed much higher leaf areas than adequate N plants (Figure 2). At a xylem area of 20 mm^2 for instance, stems of high N plants supported more than twice the leaf area than stems of adequate N plants. These high leaf-to-xylem area ratios reflect the fact that plants were kept well watered and that shoots were structurally supported by stakes. Without this added mechanical support, stems would have been prone to breaking or toppling. This is especially the case in high N trees, which produced most of their leaf area following the onset of the high N treatment, i.e., in the upper portion of the shoot (see figure 1 in Pitre et al. 2007*a*).

Despite large differences in leaf area and K_S between N treatments, leaf-specific conductivities were similar in adequate N and high N plants (Figure 1E and F). Such a

homeostasis in K_L will tend to maintain similar leaf water potentials in adequate N and high N plants, assuming that transpiration and embolism levels were similar in these plants. Native embolism was not measured, but since plants were kept well watered, it was probably low. Similar coordination of transport efficiency and leaf area was observed in a nutrient-limited mangrove species growing in natural settings (Lovelock et al. 2006). In contrast, fertilized loblolly pine trees showed a 50% reduction in whole-plant leaf-specific conductance compared with control trees, a trend that was driven by higher leaf area and a lower K_S of fertilized trees (Ewers et al. 2000). In savannah trees growing in natural conditions, K_L decreased by about 25% as a result of N fertilization (Bucci et al. 2006). This was caused by a large increase in total leaf area, which could not be fully compensated by a concurrent increase in K_S.

Cavitation resistance and wood density

As hypothesized, luxuriant levels of N tended to increase vulnerability to cavitation. A strong linear correlation between *D* and xylem vulnerability existed across all segments, regardless of experiment, treatment and position within the plant (Figure 4). Increased N fertilization of hybrid poplars moved xylem towards greater efficiency and reduced safety, a trade-off which has been previously observed across a large number of species (Hacke et al. 2006). In individual stems of aspen saplings, wider vessels were also found to be more vulnerable to cavitation than narrow vessels (Cai and Tyree 2010).

One of the most robust trends of this study was that high N plants consistently exhibited lower wood densities than adequate N plants (Figure 3C and D), which is likely due at least in part to the wider diameter fibres that are present in the secondary xylem of trees fertilized with luxuriant levels of N (Pitre et al. 2007*a*, 2010). This finding agrees with previous work showing that lower densities are often linked with fast growth (e.g., Telewski et al. 1996, Enquist et al. 1999, Wikberg and Ogren 2004, Curran et al. 2008) and greater vulnerability to cavitation (Hacke et al. 2001, Jacobsen et al. 2005). Hence, N fertilization did not alter what appears to be an inescapable trade-off between transport efficiency and safety. However, more work is necessary to evaluate to what extent common trade-offs between xylem traits will be reflected in intra-specific variation.

AQP expression

Results from this study together with previous findings (Harvey and van den Driessche 1999, Luo et al. 2005, Pitre et al. 2007*a*) provide evidence that N availability can alter the xylem structure of poplar species. One of the manifestations of N fertilization on xylem structure is increased cell diameter of vessels, as demonstrated in this study. This would suggest that expansion of vessels occurs more rapidly and/or for a longer duration to achieve a greater final cell diameter in xylem of high N-treated plants. Cell expansion occurs mainly by water uptake, which in turn has been shown to be facilitated by *AQP*s (Maurel et al. 2008). Although not much is known about the function of *AQP*s in the secondary xylem of poplar, Pitre et al. (2010) recently showed that an *AQP* was upregulated in secondary xylem under conditions of high N in *P. trichocarpa* × *deltoides*. Many studies have demonstrated that regulation of *AQP*s can occur at least to some extent at the level of transcription (reviewed by Maurel et al. 2008). Accordingly, we reasoned that if *AQP*s play a role in mediating water transport to support xylogenesis, then *AQP*s in addition to the one identified by Pitre et al. (2010) might also show increased transcript abundance in the faster-growing high N plants than in the adequate N controls. We selected for transcript profiling a subset of *AQP*s that were known to be xylem expressed based on EST sequencing from the tissue of plants subjected to differential N availability (Pitre et al. 2010). Of the 11 genes that we examined, five *PIP2*s and two *TIP*s showed significantly higher transcript abundance under conditions of high N (Figure 5). Most PIPs and TIPs have been identified as functional water transporters in various plant species, conferring high water permeability to the membranes of the cell (Tyerman et al. 2002, Secchi et al. 2009). Lending support to our hypothesis, all five of these PtdPIP2s have recently been identified as water transporters by a swelling assay in *Xenopus laevis* oocytes (A. Almeida-Rodriguez et al., unpublished; Secchi et al. 2009). *PtdPIP2;5* exhibited the highest expression levels of the *AQP*s that were investigated (Figure 5D, note different scale on *y* axis), suggesting that *PtdPIP2;5* encodes a major water-transporting *AQP* in the vascular tissue of poplar.

Taken together, these data suggest that some members of the *PtdPIP2* subfamily may function as water transporters in secondary xylem. Possible functions of these *AQP*s might be to facilitate the radial transfer of water from functional vessels to the region of rapid cell expansion near the cambial zone as well as water influx into newly formed cells during their expansion and elongation phase. *AQP*s have been found in developing xylem vessels of tobacco (Otto and Kaldenhoff 2000) and generally appear to be enriched in areas of rapid cell expansion (Ludevid et al. 1992, Chaumont et al. 1998, Maurel et al. 2008, Okubo-Kurihara et al. 2009). Along similar lines, Schrader et al. (2004) found zone-specific expression patterns of *AQP*s in the cambial zone and in differentiating secondary xylem cells of poplar, suggesting that AQPs could be implicated in cell expansion within poplar xylem. Cellular-level localization of transcript and/or protein corresponding to these *AQP*s along with functional experimentation is required to validate this hypothesis.

Whereas PIPs are generally localized to the plasma membrane, TIPs are often localized to intracellular membranes such as vacuolar membranes. Although we do not have any functional information related to *PtdTIP1;6* and *PtdTIP2;1*, previous studies showed the association of *TIP1* transcript abundance with cell elongation and differentiation in the vascular tissue of *Arabidopsis thaliana* (Ludevid et al. 1992) and in the cambial zone and in differentiating xylem cells of *Populus tremula* (GenBank accession number

BI128934) (Schrader et al. 2004). Other studies have shown that TIPs are able to transport urea or ammonia in addition to water (Gerbeau et al. 1999, Liu et al. 2003, Jahn et al. 2004). Intracellular membrane-localized AQPs have also been shown to act as nitrate channels (Ikeda et al. 2002). Thus, possible roles for these two *PtdTIP*s include facilitation of cell expansion and/or transmembrane transport of N ions or small neutral molecules.

Both *PtdPIP1;2* and *PtdSIP1;2* showed reduced transcript abundance in secondary xylem under high N fertilization when compared with adequate N fertilization. We could expect differential regulation of the *AQP* isoforms in response to different N compounds available for the plant. It has been previously reported that some *AQP*s like *ZmPIP1;5b* are induced by nitrate (Gaspar et al. 2003), while others are induced under nitrogen starvation or ammonium supply, like *AtTIP2;1* (Loque et al. 2005).

In conclusion, this study has demonstrated that, although high N fertilization leads to higher K_S and higher leaf areas in *P. trichocarpa* × *deltoides* saplings, K_L remained relatively constant under controlled conditions. Importantly, treatment of these hybrid poplars with luxuriant levels of N tended to increase their vulnerability to cavitation. Increased K_S corresponded to wider vessels in high N plants, and we suggest that some of the water-transporting, N-responsive *AQP*s identified in this study may play a role in facilitating vessel expansion. Some of these *AQP*s may also be involved in transport of small N solutes. Future studies will focus on in-depth functional characterization of these *AQP*s to more precisely ascertain their role in xylogenesis and transport of small molecules in secondary xylem.

Supplementary data

Supplementary data mentioned in the text are available to subscribers at *Tree Physiology* online.

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