



## Methods paper

# Assessing the thermal dissipation sap flux density method for monitoring cold season water transport in seasonally snow-covered forests

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Productivity of conifers in seasonally snow-covered forests is high before and during snowmelt when environmental conditions are optimal for photosynthesis. Climate change is altering the timing of spring in many locations, and changes in the date of transition from winter dormancy can have large impacts on annual productivity. Sap flow methods provide a promising approach to monitor tree activity during the cold season and the winter–spring and fall–winter transitions. Although sap flow techniques have been widely used, cold season results are generally not reported. Here we examine the feasibility of using the Granier thermal dissipation (TD) sap flux density method to monitor transpiration and dormancy of evergreen conifers during the cold season. We conducted a laboratory experiment which demonstrated that the TD method reliably detects xylem water transport (when it occurs) both at near freezing temperature and at low flow rate, and that the sensors can withstand repeated freeze–thaw events. However, the dependence between sensor output and water transport rate in these experiments differed from the established TD relation. In field experiments, sensors installed in two *Abies* forests lasted through two winters and a summer with low failure. The baseline (no-flow) sensor output varied considerably with temperature during the cold season, and a new baseline algorithm was developed to accommodate this variation. The *Abies* forests differed in elevation (2070 and 2620 m), and there was a clear difference in timing of initiation and cessation of transpiration between them. We conclude that the TD method can be reliably used to examine water transport during cold periods with associated low flow conditions.

**Keywords:** conifer, dormancy, freeze–thaw, sap flow, winter.

## Introduction

The timing of the shift from winter dormancy to activity of conifers in seasonally snow-covered forests can have a substantial impact on annual forest productivity and water balance. Understanding the environmental and physiological controls on trees during this transition period is important to predict how forests will respond to future change in climate. Many previous studies on seasonal patterns of tree activity have utilized eddy covariance to measure whole-forest gas exchange (Baldocchi et al. 2005, Monson et al. 2005, Kelly and Goulden 2016) or leaf-level methods to assess physiological changes (Ensminger

et al. 2004, Zarter et al. 2006a, 2006b, Koh et al. 2009). Observations at the whole-tree level have rarely been used to investigate transpiration during the cold season (late fall, winter and early spring) or seasonal transitions (e.g., Sevanto et al. 2006, Vanhatalo et al. 2015), but could provide valuable insight into the ecophysiology of overwintering evergreens.

The beginning of the active season is often a productive period for seasonally snow-covered forests because soil moisture is plentiful and air temperature is warm enough for photosynthesis to occur (Monson et al. 2005, Barr et al. 2009, Kelly and Goulden 2016, Winchell et al. 2016). For many forests,

variability in annual productivity is best explained by early season activity (Moore et al. 2008, Richardson et al. 2009, Clark et al. 2014). Flux tower studies have shown that the transition between dormant and active states for conifers is correlated with air temperature (Suni et al. 2003, Monson et al. 2005), but leaf-level studies show large variability in the rates of recovery of photosynthetic capacity in the spring for different tree species (Ensminger et al. 2004, Nippert et al. 2004, Verhoeven 2014). Furthermore, because tree activity in this period is related to both temperature and snowmelt, climate change effects on the length of the active season are likely to be driven by changes in spring events (Aerts et al. 2006).

In the western USA, there has been a general trend toward increasing winter and spring temperature (Barnett et al. 2005) that has been accompanied by decline in annual snowfall (Mote et al. 2005, Scalzitti et al. 2016). Variation in timing of snowmelt can influence the degree of summer soil moisture limitation for plants (Hu et al. 2010, Blankinship et al. 2014). Large-scale tree mortality events have become more frequent in response to drought and related insect infestation (van Mantgem et al. 2009, Adams et al. 2012, Anderegg et al. 2013), with important legacy effects for forest health (Anderegg et al. 2015). In light of these changes, there is a pressing need to understand the seasonal patterns of activity of different tree species to predict how forests will respond to future climatic variability and ecosystem disturbances.

Sublimation can be a dominant component of the total water vapor flux from snow-covered forests (Molotch et al. 2007, Broxton et al. 2015), but because eddy covariance cannot distinguish between transpiration and sublimation, the technique does not allow assessment of cold season transpiration. Sap flow methods are promising as an alternative, but few studies have employed them for periods greater than 1 year (Clausnitzer et al. 2011, Yoshifuji et al. 2011, Link et al. 2014), in part because the sensors are easily damaged. No long-term studies have occurred in seasonally snow-covered environments, and very few in the cold season (e.g., Vanhatalo et al. 2015, Liu et al. 2016). Year-round sap flow observations could provide extremely useful information on tree physiology during the transitions from fall activity to winter dormancy and return to activity in spring. Because transpiration is closely linked to photosynthesis through stomatal conductance, sap flow measurements can also be used to estimate canopy photosynthesis (Schäfer et al. 2003, Wang et al. 2014). Monitored over multiple years, such observations could increase our understanding of the controls on winter dormancy versus activity and how they vary inter-annually for different tree species.

Measurements of stem sap flux or sap flux density (SFD, sap flux per unit area) provide a direct means to monitor water use of individual trees. While there are currently many techniques available (Vandegehuchte and Steppe 2013), the thermal dissipation (TD) sap flux density method developed by Granier

(1985) has the advantage of low cost and relatively easy sensor assembly and field installation (Smith and Allen 1996, Lu et al. 2004). Using the TD method, transpiration rates are determined using a simple empirical relation based on the temperature difference between one continuously heated and one unheated probe installed in the hydro-active xylem of a tree.

The effect of cold and freezing, coupled with low flow rates, on TD sensor performance has not yet been evaluated. Previous studies with the TD technique have identified situations in which the original empirical formulation does not accurately predict actual water transport rates (Clearwater et al. 1999, Lu et al. 2004, Tatarinov et al. 2005, Bush et al. 2010, Steppe et al. 2010, Wullschleger et al. 2011). Errors can arise from thermal gradients within the sapwood, radial variation in water transport rate, sensor orientation and wounding effects from sensor installation. Additionally, the sensors can be slow to respond to changes in sap flow under low flow conditions (Hölttä et al. 2015), which are common in the cold season. Low temperature can further complicate TD sensor measurements by altering the thermal conductivity of the wood. The TD measurement relies on the temperature difference between a heated and unheated sensor; in low flow or low temperature conditions, the heat is not transported as efficiently away from the heated sensor and could make flow difficult to detect (Wullschleger et al. 2011). In sub-freezing conditions, the sensors might also experience mechanical damage or physical displacement from freeze–thaw cycles.

Here we describe a two-component study designed to examine the utility of the TD method for monitoring water transport during the cold season. We first conducted a laboratory experiment in which we artificially manipulated the temperature of detached conifer stems at and near the freezing point, with simultaneous gravimetric and TD measurements of water transport rate. Second, to evaluate the cold season application of this technique under actual field conditions, we conducted sap flow and environmental observations over a full annual cycle in two *Abies* forests in the Rocky Mountains with a continental temperate climate.

## Materials and methods

### *Thermal dissipation sap flux density*

Sap flux density was measured using the TD method (Granier 1985, 1987) in the laboratory and in the field. The general application of the technique is discussed here, and the specifics for each experiment in the next sections. Pairs of sensors were assembled in the lab and consisted of a reference sensor and a heated sensor. Both sensors contained a 36-gauge copper-constantan thermocouple (TT-T-36-1000, Omega Eng., Stamford, CT, USA) inside a 19-gauge (2 mm diameter) hypodermic needle (305,187, PrecisionGlide, Becton, Dickinson and Co., Franklin Lakes, NJ, USA) that was cut to 21 mm in length. The thermocouple was secured with ethyl cyanoacrylate glue in the

needle 10 mm from the end. The heated sensor was wrapped in 40-gauge constantan heating wire (TFCC-005-500, Omega Eng.) and was delivered a constant power of 0.2 W via commercially available electronics ([www.simplecircuitboards.com](http://www.simplecircuitboards.com)).

The sensors were wired to measure the temperature-dependent differential voltage between them,  $\Delta V$ , which was used to calculate the SFD,  $J_s$  ( $\text{g m}^{-2} \text{s}^{-1}$ ), according to the standard Granier (1985) equation

$$J_s = 119 \times \left( \frac{\Delta V_{\max}}{\Delta V} - 1 \right)^{1.231} \quad (1)$$

The daily maximum voltage difference between sensors,  $\Delta V_{\max}$ , occurs under zero flow conditions (night, or with no vacuum in the laboratory). This serves as a baseline to which voltage differences during the day ( $\Delta V$ ) are referenced. The quantity  $K = (\Delta V_{\max}/\Delta V - 1)$  was used to compare lab results to the relation in Eq. (1). Shifts in the baseline occur over time and can be affected by changes in temperature (as we show later); therefore, a proper assessment of the baseline was required for accurate calculations of  $J_s$ . We applied a continuously moving baseline that was calculated by using a piecewise cubic Hermite spline to interpolate between the nighttime differential voltages (Matlab 7.12.0.635 R2011a, The MathWorks, Inc., Natick, MA, USA). This approach assumes that there is no nocturnal transpiration, which is almost certainly valid in the cold season. However, this has the important advantage of accounting for day-to-day and seasonal variation in baseline voltage associated with temperature.

### Sap flux density measurements: laboratory experiment

To test the ability of the TD sensors to quantify water transport in cold stems and to withstand subfreezing stem temperature, we designed a laboratory experiment based on the system described in Bush et al. (2010). This allowed us to gravitationally measure stem water transport for comparison with concurrent TD measurements (Figure 1). The TD sensors were installed radially in stem

segments of *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr. (white fir), with 10 cm spacing and the heated sensor at the downstream end of the water flow direction (top in Figure 1).

A separate thermocouple was installed ~20 cm away to measure stem temperature at the same radial depth as the TD sensors. Stem diameters ranged from 5.5 to 6.5 cm. Approximately 1 m axial-length bole segments were cut from young trees in the field, bagged in dark plastic and returned to the lab. Immediately before experiments began, both ends of the stem segment were recut underwater such that the remaining segment was ~50 cm in length. The stem segment was suspended with a ring stand in a beaker of 20 mM potassium chloride (KCl) solution on a balance (LP34000P, Sartorius, Goettingen, Germany) and sensors were connected to a CR1000 datalogger (Campbell Scientific, Inc., Logan, UT, USA). Measurements were logged every 10 s.

Stem temperature was modified by wrapping a 6 mm diameter coil of copper tubing around the stem (Figure 1). The coil was attached to a recirculating fluid bath (VWR Scientific, Model 1157, Denver, CO, USA). Silicone polymer heat transfer fluid (Syltherm XLT, Dow Chemical Company, Midland, MI, USA), which maintains a low viscosity at subfreezing temperature, was used in the fluid bath. The entire stem-plus-coil system was covered in radiatively-reflective thermal insulation, and kept in a cold room at 4 °C to minimize the temperature gradient between it and its surroundings. For each trial, the fluid bath was set at a constant fluid temperature between -20 and +20 °C and the stem was left for several hours with fluid circulating through the coil to allow temperature equilibration. During this time a zero flow baseline voltage was established. After equilibration, a constant vacuum of 17 kPa was applied to the top of the stem for a period of 5–6 h using a vacuum pump (G8GCX, GE Motors, Fort Wayne, IN, USA). The KCl solution, kept at 4 °C, was periodically refreshed in the supply beaker to maintain a roughly constant water potential gradient across the stem segment.

Stem water transport rate was independently calculated from the change in liquid mass on the balance. After TD measurements

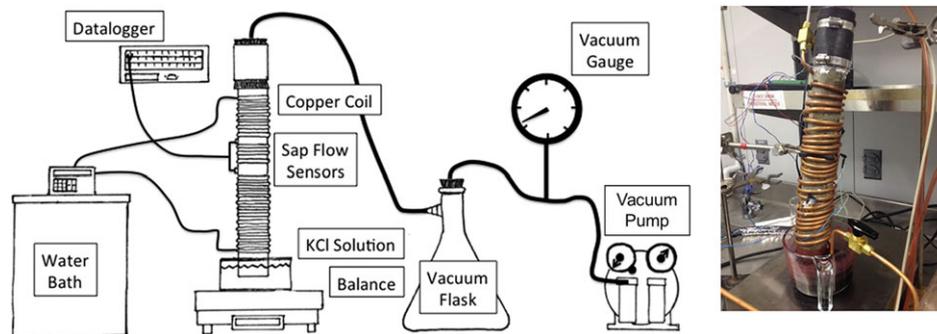


Figure 1. Laboratory system used to test the function of TD sap flux density sensors at cold and subzero stem temperature. A stem segment of *A. concolor* was cut and suspended in a beaker of KCl solution on a balance. A rubber gasket attached to the top of the stem was connected to a vacuum pump via an Erlenmeyer flask. A copper coil around the stem connected to a recirculating fluid bath allowed modification of stem temperature. Thermocouples and TD sensors were installed in the stem segment and SFD measurements were compared with water transport rate based on the loss of mass from the balance as KCl solution was pulled through the stem.

were complete for a given stem, the vacuum was used to pull 0.05% safranin dye through the stem to facilitate measurement of hydro-active xylem area and sapwood depth. These were used to convert the gravimetric measurements to SFD following Clearwater et al. (1999).

### Field study locations

For the field component of this study, sap flow measurements were made at two forests at different elevations in the Wasatch Mountains of northern Utah, USA. These sites are part of the iUTAH EPSCoR GAMUT Network (<http://iutahepscor.org>). The lower elevation site was located on a southeast-facing slope in the Knowlton Fork drainage of Red Butte Canyon, ~8 km northeast of Salt Lake City, Utah, USA (2070 m elevation, 40.81°N, -111.77°W). The dominant tree species in this mixed stand were *A. concolor* and *Populus tremuloides* Michx. (aspen), and *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir) was also present. Twelve *A. concolor* located in a 60 m<sup>2</sup> area were instrumented for TD measurements, with diameter at breast height (DBH) of study trees 29.5 ± 7.4 cm (mean ± standard deviation).

The higher elevation site was located at the T.W. Daniel Experimental Forest maintained by Utah State University (2620 m, 41.87°N, -111.51°W), ~30 km northeast of Logan, Utah, USA. The site is underlain by karst bedrock and contains a mixed conifer forest dominated by *Abies lasiocarpa* (Hook.) Nutt. (subalpine fir) and *Picea engelmannii* Parry ex Engelm. (Engelmann spruce). Twelve *A. lasiocarpa* in a 25 m<sup>2</sup> plot were instrumented for TD measurements, with DBH 24.6 ± 8.5 cm. Both sites were equipped with weather stations to monitor air temperature, snow depth and other environmental parameters. Snow water equivalence data were obtained from the USDA Natural Resources Conservation Service Snowpack Telemetry sites Louis Meadow (low elevation site, 2.5 km distant) and Doc Daniels (high, 0.1 km distant) that are located at nearly identical elevations to the field sites.

### Sap flux density measurements: field experiment

In the field, a small area of bark/cambium (~3 cm diameter) was removed for each sensor and pairs of TD sensors were installed 10–15 cm apart, with the heated sensor directly above the reference sensor, in the outermost sapwood layer. Sensor pairs were installed 1.3 m above the ground, in random radial orientation. One pair of sensors was installed in each tree. Sensors were covered in radiatively reflective thermal insulation to minimize energy balance influences on measurements. All instrumented trees were greater than 15 cm DBH, and visually selected to capture the range of stem diameters present at each site.

Measurements at the low elevation site began on 20 February 2014, at the high elevation site on 24 March 2014, and continued over a full annual cycle through the end of the following winter. Sap flux density measurements were taken every 10 s and 15 min averages were logged on a CR1000 datalogger (Campbell Scientific, Inc.).

### Sap flux density data filtering for field experiment

Baseline voltages for each tree were determined as described above. Because unusual diel patterns occasionally occurred in the cold season during periods of abrupt ambient temperature change, raw field data were then processed with three filtering steps. First, the data for each species were separated into four periods (winter, fall, spring and summer), defined based on timing and magnitude of transpiration for each species. Second, a typical diel pattern for each tree for each period was established, and the distributions of the data were used to statistically remove outliers. For each of the four seasonal periods, all data from each tree were compiled for every 15 min period, such that all diel cycles within the period were combined and compared for every tree individually. Points outside the 98th and 2nd percentiles of the combined data for each 15-min period were removed for each tree. Third, days with less than 95% of 15-min periods remaining were then omitted entirely. The SFD was calculated on a daily basis using the filtered data and Eq. (1). Sapwood area and depth were not measured in the field trees. Two *A. lasiocarpa* at the high elevation site exhibited inconsistent daily patterns that did not match the other trees, and data from these were not used.

## Results

### Laboratory experiment

We found a strong linear relation ( $R^2 = 0.86$ ) between the raw TD sensor differential voltages (referenced to zero flow baseline voltage) and independent gravitationally based flow rates across many stems under steady-state laboratory conditions (Figure 2). This held true for stems with measured temperature as low as 0.5 °C. Variation in stem temperature did not influence the relation (Figure 2A), but there was variability between stems. Some of the lowest flow rates observed occurred at the lowest temperature (Figure 2A, -1 °C trial). Difference in flow rates across trials was due both to variation between stem segments and possibly to embolism or occlusion of tracheids that occurred over the course of the trials.

When stem temperature dropped below freezing, water was not transported through the stem via the relatively small vacuum applied. Lack of water movement was evident as constant liquid mass on the balance and as constant voltage output of the TD sensors. Multiple freeze–thaw events did not cause water transport to stop, unless the stem was frozen, nor did they lead to failure of the sensors. Rates of water transport were sometimes lower after a freezing event, but were still detectable following freeze–thaw cycles.

In the lab there was variability in the differential voltage baseline  $\Delta V_{\max}$  with temperature. Raw TD sensor output during baseline (zero flow) conditions was nearly constant until the temperature of the cooling fluid around the stem was reduced below -10 °C (Figure 3). Excursions in the raw TD sensor

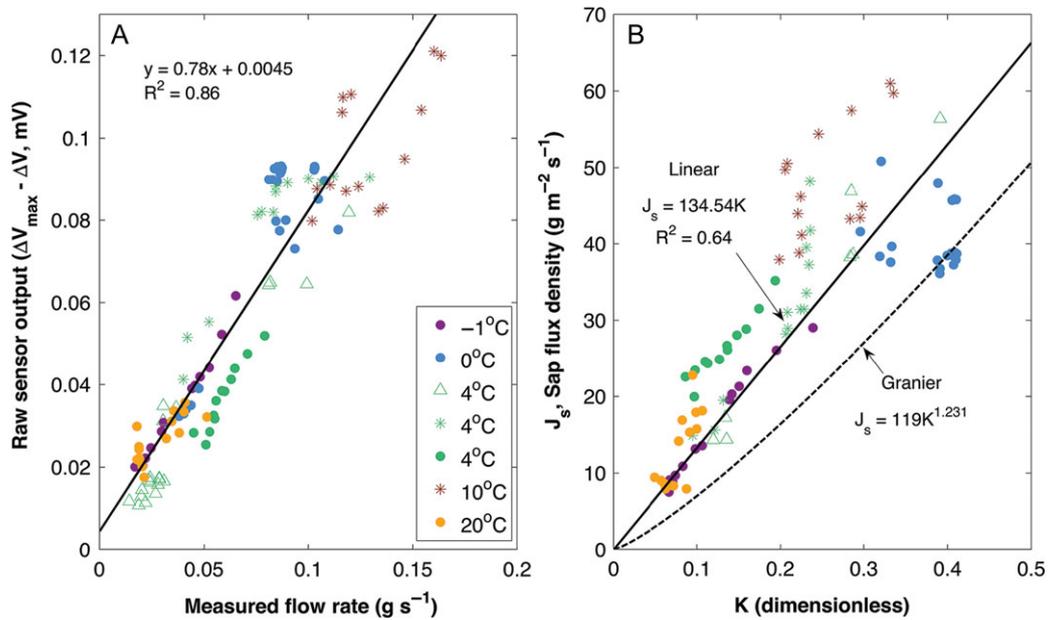


Figure 2. Laboratory test results under varying stem temperature. Each color represents a different fluid bath temperature, and symbols represent different stems. Stem temperature was above fluid temperature for the  $-1$  to  $4$  °C tests; the lowest stem temperature recorded for the data shown was  $\sim 0.5$  °C. (A) TD sensor output, shown as the difference from a baseline zero flow condition ( $\Delta V_{\max} - \Delta V$ ) versus water transport rate measured by loss of mass on balance (linear regression line shown). (B) Water transport rates measured from the balance were converted to SFD ( $\text{g m}^{-2} \text{s}^{-1}$ ) using the sapwood depth and area for each sample stem segment and are shown relative to  $K$  calculated from TD sensors. The original Granier TD calibration curve (Eq. (1)) and a linear fit forced through the origin are shown for comparison.

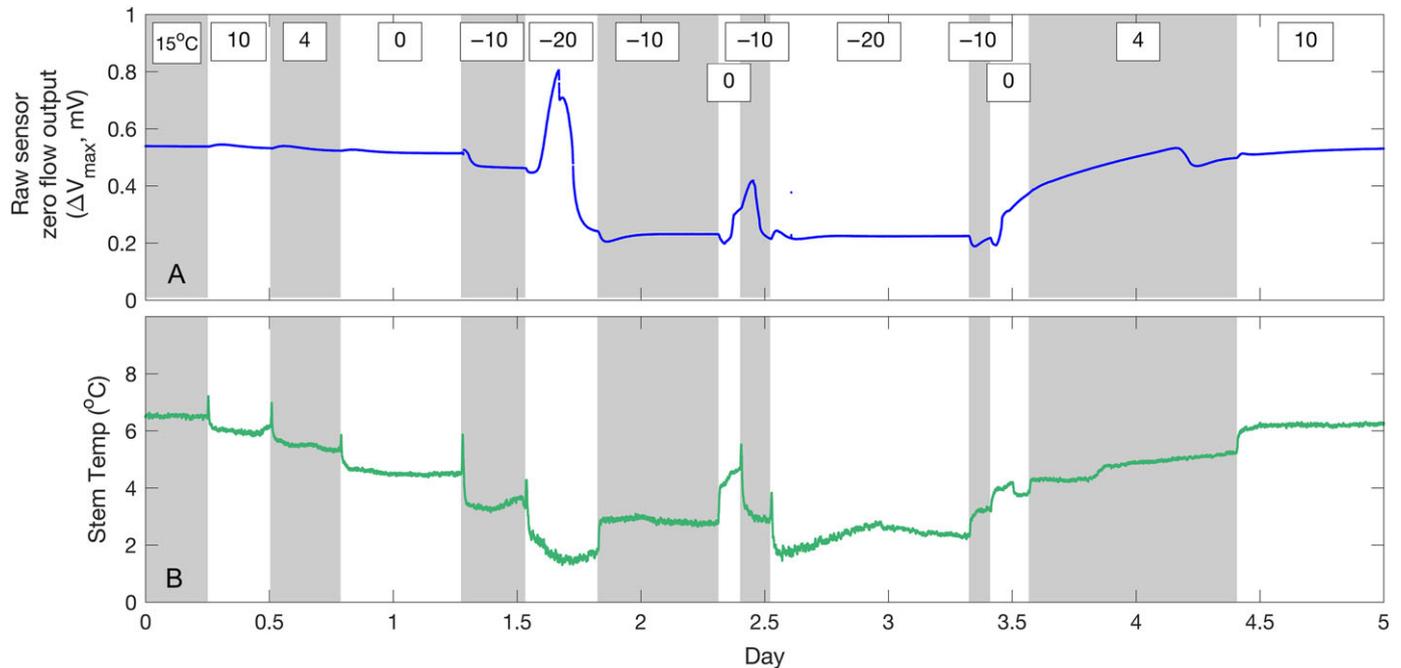


Figure 3. (A) Raw TD sensor output ( $\Delta V_{\max}$ ) during zero flow (baseline) conditions as temperature was varied in the laboratory. The temperature of the recirculating fluid surrounding the stem segment as it was varied from  $+15$  to  $-20$  and back to  $+10$  °C is indicated by boxes, and alternate shading patterns indicate time periods with constant fluid temperature setting. (B) Stem temperature measured at 1 cm depth during the same test.

output occurred with temperature shifts below or above the freezing point (fluid temperature of  $-20$  and  $0$  °C, Figure 3) and likely represent phase changes occurring within the stem. Stem temperature was measured  $\sim 1$  cm into the xylem (Figure 3B),

and differed markedly from the circulating fluid temperature (Figure 3). The sudden apparent jumps in stem temperature immediately after changing water bath temperature were an electrical artifact due to the water bath temperature control

electronics adjusting to the new setting (Figure 3B). After jumps in the baseline following temperature excursions below the freezing point, the baseline stabilized, although the differential voltage was lower than when the stem temperature was above freezing. When the stem temperature returned above 0 °C, the baseline returned to a voltage similar to those prior to freezing (Figure 3A).

Despite the strong relation between measured flow rate and TD sensor output across the stems measured, our results deviated from the well-established Granier calibration, with almost all points well away from the Granier line (Eq. (1), Figure 2A and B, respectively). A simple linear regression provided a reasonable fit to the data.

### Field experiment

As temperature varied in the field, TD sensors responded with changing baseline as in the laboratory. Baseline patterns for different trees were consistent in diel pattern during warm periods when regular daily transpiration was occurring (Figure 4A). Shifts in the baseline occurred during cold periods and were qualitatively similar in different trees (Figure 4A). The baseline stabilized when sap transport resumed and daytime air temperature was consistently above freezing.

Environmental conditions varied between the two forests consistent with their difference in elevation (data mostly not shown,

except for air temperature and snow depth in Figure 5). Total precipitation at the low elevation site in the January–June period was 490 mm in 2014, of which 65.3% was snow and the rest rain. Peak snow water equivalent (SWE) was 401 mm and the site became snow-free on 7 May. Mean air temperature in January through March was –1.3 °C and in April through June was 9.5 °C. Total precipitation at the high elevation site in the January–June period was 815 mm, 95.3% snow. Peak SWE was 960 mm and the site became snow-free on 6 June. Mean air temperature in January through March was –5.3 °C and in April through June was 4.8 °C.

Across an annual period, we examined differences in seasonal patterns between low elevation *A. concolor* and high elevation *A. lasiocarpa*. Power limitation at the low elevation site prevented measurements before 20 February 2014. However, based on consistently subzero temperature prior to February, we are confident that we did not miss the start of the active season. From the beginning of SFD measurements, we first saw signs of sap transport on 25 February for *A. concolor* (Figure 5A). The magnitude of SFD (and presumably magnitude of transpiration) was sensitive to air temperature during this period (Figure 5A and B).

In contrast to the low elevation *A. concolor*, high elevation *A. lasiocarpa* did not transpire during the winter period (Figure 5C); no significant sap transport was observed at the high elevation

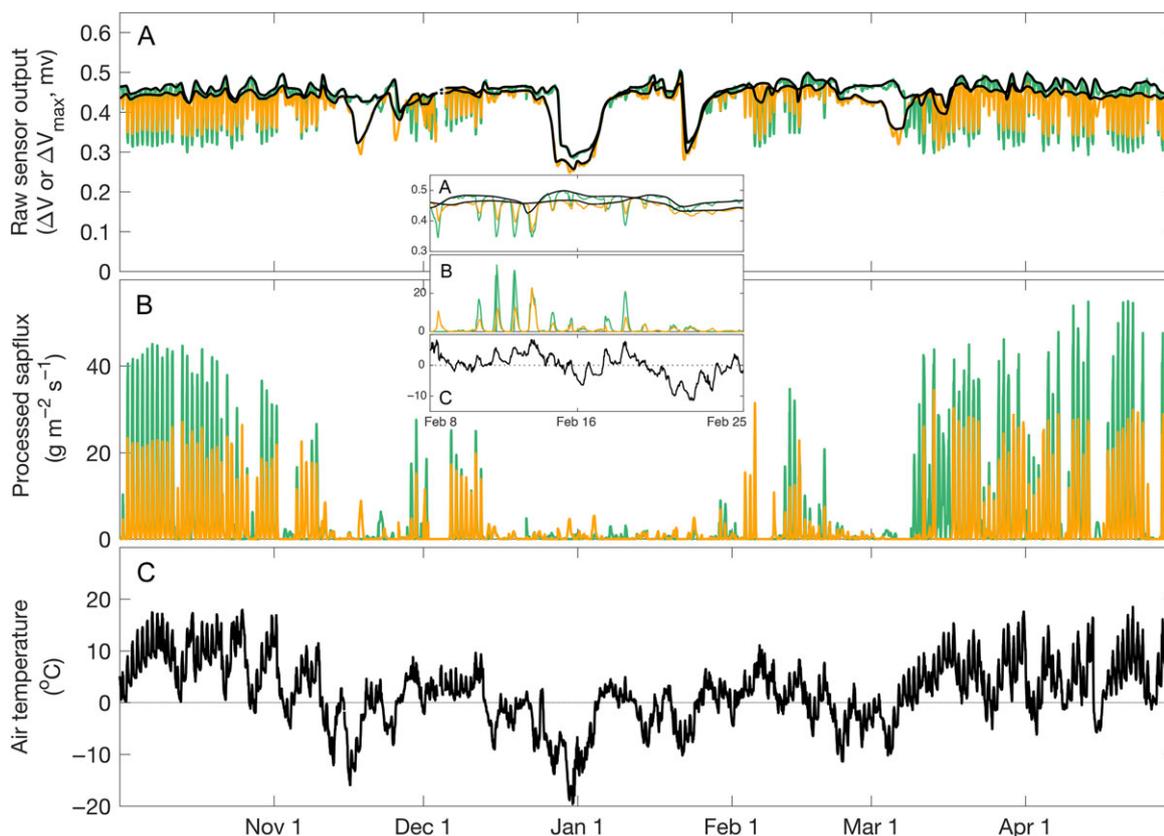


Figure 4. (A) Seasonal variation of raw TD sensor output ( $\Delta V$ ) for two *A. concolor* trees (colored lines) in the field, including the interpolated baseline ( $\Delta V_{max}$ ) for each tree (black lines). (B) Sap flux density for the trees in (A) calculated using Eq. (1). (C) Air temperature (dashed line indicates 0 °C).

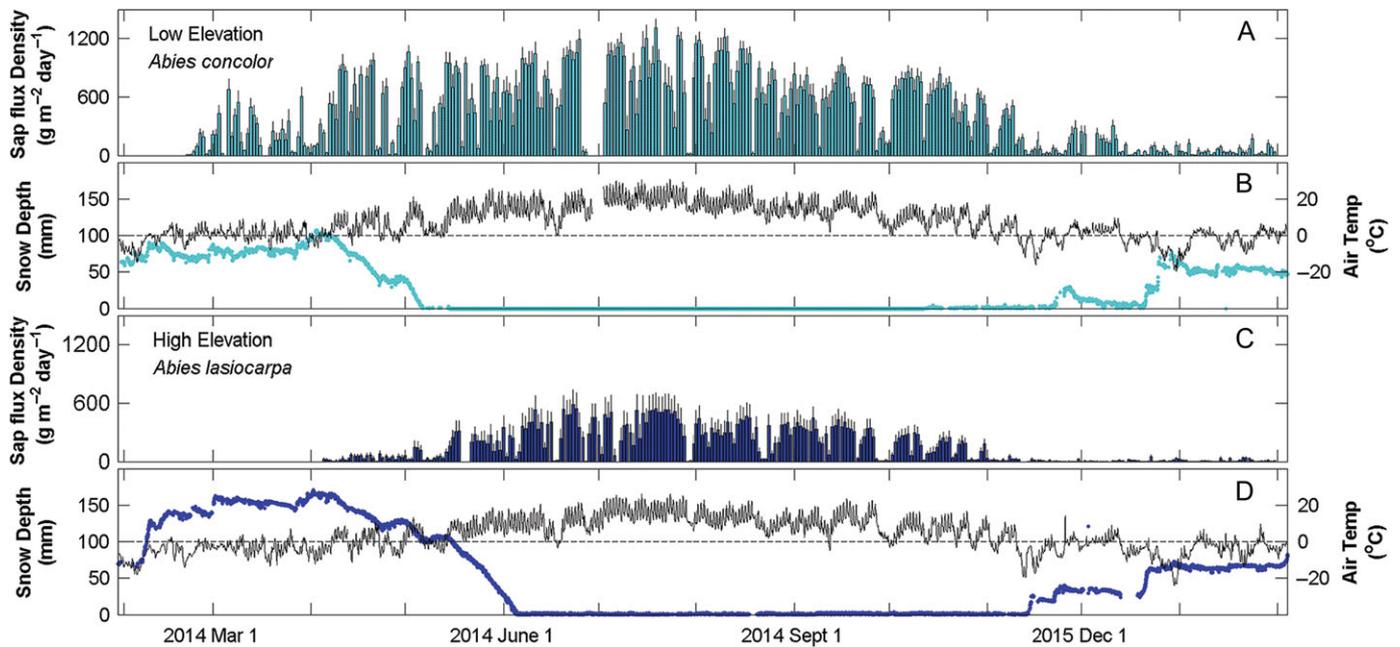


Figure 5. (A and C) Daily total SFD for low elevation *A. concolor* and high elevation *A. lasiocarpa* over an annual period. Error bars represent one standard error of the mean ( $n = 12$  trees for *A. concolor* and  $n = 10$  trees for *A. lasiocarpa*). (B and D) Concurrent air temperature (black line, right axis) and snow depth (cyan or blue line, left axis) for the low and high elevation sites, respectively (dashed lines indicate 0 °C).

site until 4 May. Average daily air temperature remained below zero until the beginning of May. Consistent daily sap transport was not observed until mid-May when average daily air temperature stayed above zero. As with the low-elevation site, sap transport began long before the snow fully melted (Figure 5C and D).

Sap flow rates measured in the lab were comparable to those measured in the field and ranged from  $\sim 10$  to  $60 \text{ g m}^{-2} \text{ s}^{-1}$  (Figures 2B and 4). In both settings, the sensors were able to detect flow as low as  $10 \text{ g m}^{-2} \text{ s}^{-1}$  when temperature was just above freezing. In the field there was a strong relationship between temperature and flow rate that remained relatively constant throughout the range of observed temperature (Figure 6), further corroborating our findings in the lab that temperature does not affect the ability of TD sensors to detect sap flow when it is occurring (Figure 2A).

## Discussion

The TD method appears to be a useful indicator of cold-season activity (assessed as SFD) and dormancy of montane evergreen trees in seasonally snow-covered forests. In both the laboratory and the field, we were able to reliably monitor water transport, including variation in magnitude and timing (when it did and did not occur), in boles with temperature ( $T$ ) ranging from typical summer conditions to below the freezing point and at low flow rate.

In lab experiments, water transport was detectable at near-freezing conditions using the TD method, and confirmed

independently with a gravimetric method. The strong linear relation between the raw sensor output voltage (referenced to zero flow baseline voltage) and water transport rate was consistent across all stems measured and across a wide range of applied fluid  $T$  and flow rate (Figure 2A). The measured stem  $T$  differed strongly from the circulating fluid  $T$  (Figure 3), reflecting the manner in which the fluid in the coil interacted with the stem. Since the fluid flow was unidirectional, the stem was coldest at the end where fluid first encountered the stem and the fluid-stem  $T$  difference was highest. There were likely axial and radial  $T$  gradients within the stems, differing from the way stem  $T$  would change (primarily radially) in an outdoor winter setting. Additionally, the KCl solution where it entered the stem segment was at 4 °C (the ambient  $T$  of the cold room), which further complicated our ability to precisely control stem  $T$ . Hence the measured stem  $T$  in the lab was a good relative, but not absolute, indicator of overall stem  $T$ .

The zero flow baseline voltage was generally stable under constant  $T$  conditions, except during phase change of xylem sap. The baseline voltage was highly variable when  $T$  was changed, especially as the fluid was cooled to  $-10$  and then  $-20$  °C, and again when it warmed above 0 °C (Figure 3). Measured stem  $T$  was coldest when the fluid was  $-20$  °C but remained above freezing. Regardless, the abrupt larger changes in baseline voltage (Figure 3) are most likely explained by freezing and then thawing of the xylem sap between or adjacent to the TD sensors (which were 20 cm away from the stem thermocouple location). Under the coldest conditions, no flow was detected using the TD

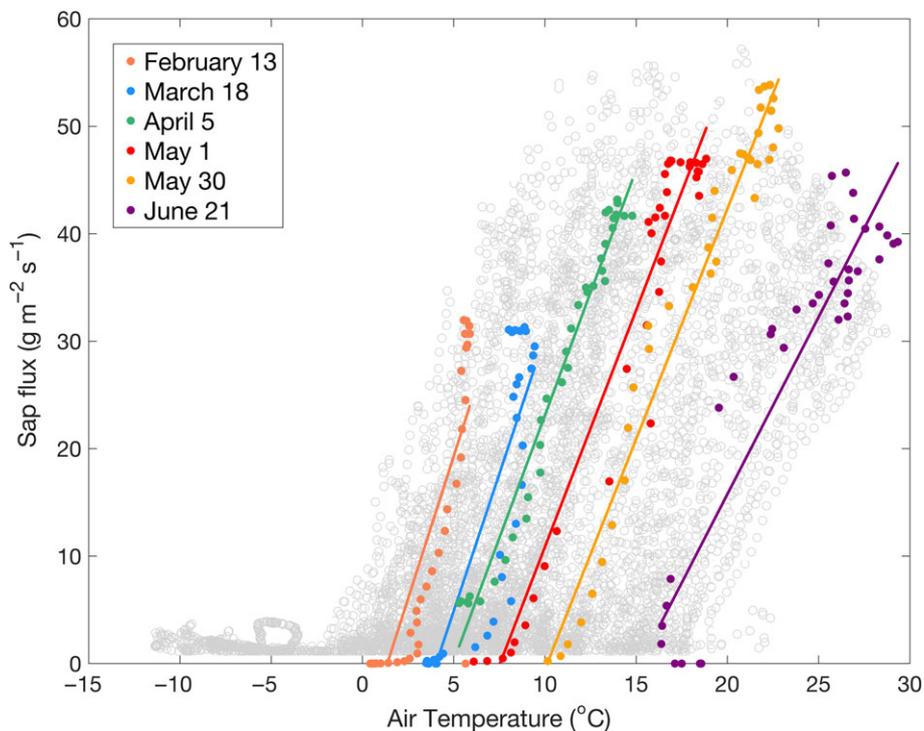


Figure 6. Sap flux density versus air temperature for one representative *A. concolor* tree during January to July 2015, shown in gray. Colors highlight daily patterns between 06:00 and 15:00 h on days with varying air temperature.

sensors (i.e., no change in voltage compared with the baseline value), and there was no change in mass on the balance, indicating blockage by ice in the stems. Note that this was true despite the constant energy input from the heated TD sensors.

The relation between TD measurements and actual water transport rate during the lab experiments was variable between stems, and within a stem over time (Figure 2A). When the gravitational measurements were converted to SFD using measured sapwood depth and area and compared with the  $K$ -value derived from the TD sensors, the results differed from the standard Granier formulation (Figure 2B). This is consistent with other studies conducted in warmer conditions that have shown the standard TD calibration equation is not applicable for all species and in many cases underestimates actual water transport rate (e.g., Bovard et al. 2005, Bush et al. 2010, Hultine et al. 2010, Steppe et al. 2010).

In field experiments, cessation of water transport in the fall, winter dormancy, and initiation of water transport in the spring were detected with the TD method. Clear diel patterns of sap flow were apparent in fall and in spring, but during most of the winter there was a lack of diel pattern (Figure 4). During cold periods in fall and spring, sap transport ceased, then returned when conditions were more favorable (e.g., February and March in Figure 4). Measuring sap flux during cold periods can be complicated since the energy balance of the upper canopy is highly variable (Ohta et al. 1999) and spatial patterns of water potential within a tree can be quite complex (Mayr and Charra-Vaskou

2007). At our temperate latitude, there may be substantial liquid water present in the main stem even when ice is present (Sparks et al. 2001). However, the diel patterns in early December and in February (Figure 4) highlight that despite these complexities, whole-tree water transport can be observed during cold periods with sensors located only in the main stem. The results of our lab experiment showing that TD sensors can reliably measure flow rates as low as  $10 \text{ g m}^{-2} \text{ s}^{-1}$  give us confidence in our early season field measurements displaying similar rates (Figure 4B).

The baseline (zero flow) differential voltages in the field were variable as temperature changed, with minor variation in fall and spring but much larger variation during the cold season (Figure 4). This was not a consistent relation with air temperature that could be used to empirically remove the variation (data not shown), and the variation was different for each tree (Figure 4). A standard approach for the TD method has been to estimate a baseline voltage every 7–10 days (Oishi et al. 2017, e.g., Lu et al. 2004, Clausnitzer et al. 2011), but we found this did not work well with the variable voltages, as it led to spikes and erroneous diel patterns of SFD.

Instead we applied a Hermite spline fit to the nocturnal data, using the spline to predict a varying baseline voltage for each tree during the day. This worked well during spring, summer and fall when the raw voltages exhibited the typical diel pattern, and was also reasonably effective during cold periods (Figure 4), eliminating the erroneous spikes. However, some small peaks in SFD calculated with this approach remained, and they were

inconsistent between trees (e.g., near January 1 in Figure 4B). These tended to occur when the typical diel pattern of the raw voltages was absent (e.g., near January 1 in Figure 4A), and the calculated SFD displayed diel patterns that were not consistent with solar forcing. For these reasons, we developed additional filtering steps (described in Materials and methods) that were based on a seasonally relevant diel pattern in SFD, omitting data that did not converge to such a pattern. We used the filtered data to calculate daily sap flux totals for the field trees (Figure 5).

Baseline values stabilized again in spring, and measured SFD was similar in magnitude to values from the preceding fall (Figure 4). This indicates that in the field the sensors can withstand repeated freeze–thaw cycles without failure or serious physical displacement. We replaced sensors only when there was an obvious problem such as an electrical short or sensor failure. Over time, the contact area between the sensor and the wood may decline, affecting heat transport so new sensors should be periodically installed in new holes (Lu et al. 2004, Wullschleger et al. 2011). For example, Moore et al. (2009) observed a 45% decrease in SFD of Douglas fir in the second consecutive year of measurements with the same sensors, attributing it to growth affecting the sensor performance.

While the results from this exploratory study are encouraging, several limitations remain to be addressed. First, our laboratory experiments involved a single species, and the results were variable between stems and over time within a stem. Although we attempted to minimize embolism associated with cutting the stem by re-cutting underwater (Wheeler et al. 2013), it is likely that damage to xylem cells occurred at the cut surfaces which probably affected hydraulic conductivity differently in each stem. Freeze–thaw embolism may also have been induced by the experimental treatments, but the tension applied to the stem in the lab was minimal compared with actual cold-season water potential in the field (Sperry et al. 1994, Mayr and Charra-Vaskou 2007). Freeze–thaw embolism of course occurs naturally (Sperry and Sullivan 1992), but the lab experiments are a poor substitute for the way stems would freeze in nature.

Second, the accuracy of the TD method to reliably quantify the magnitude of transpiration in cold conditions remains to be established. The strong linear relation in Figure 2A is encouraging and suggests that the method is a reliable indicator of relative degree of activity, but the variability among the lab tests was high, and so the accuracy at this point should still be questioned. Other studies have highlighted that the original Granier equation is not universally applicable (see review by Vandegehuchte and Steppe 2013). This may be exacerbated under near freezing conditions since biophysical properties of viscosity, thermal diffusivity and solute concentration of xylem sap vary with  $T$  (Meinzer 2002, Hölttä et al. 2005, 2015, Wullschleger et al. 2011). Third, it would be very useful to examine alternative methods for measuring cold-season transpiration both in the lab and in the field, including variations on sap flux and SFD methods (Vandegehuchte and

Steppe 2013), and also stem diameter variation (Sevanto et al. 2006, De Swaef et al. 2015). Finally, the constant heating of the TD technique is an unavoidable disadvantage that may alter phase changes of sap within the stems in the field. However, this effect is probably highly localized near the heated sensor, and the overall whole-tree hydraulic limitation of ice in the stem in the cold season is not likely to be influenced. Alternative methods that use pulsed heating, such as the heat ratio or Sapflow+ methods (Burgess et al. 2001, Vandegehuchte and Steppe 2012), would be advantageous to minimize stem heating and also for reduced power consumption.

These results highlight the importance of biophysical controls on the cold season physiology of conifers. There was a clear distinction in the length of the growing season in the two *Abies* forests, with the lower elevation forest beginning sap transport more than a month before the high elevation forest, and continuing much later into the winter (Figure 5). Both forests began sap transport while the snowpack was at or near its maximum SWE (100–150 mm of SWE), fully 2 months before each snowpack was completely melted (Figure 5). The lower forest appeared to be more responsive to favorable warm periods during this time. These results may reflect species or elevational differences in biochemical downregulation of photosynthesis and associated non-photochemical energy dissipation (Nippert et al. 2004, Zarter et al. 2006a, Verhoeven 2014), hydraulic limitation via embolism of xylem (Sperry et al. 1994, Charra-Vaskou et al. 2011) or availability of liquid water for transpiration (Monson et al. 2005, Sevanto et al. 2006).

Climate directly influences vegetation phenology (Richardson et al. 2013), and as temperature rises, the magnitude and phase of precipitation, and the timing and dynamics of snowmelt are changing (Hamlet et al. 2005, Mote et al. 2005). These have influenced the seasonality of vegetation activity, with earlier spring greening (Barichivich et al. 2013). Changes in the seasonal cycle of CO<sub>2</sub> indicate that northern ecosystems are progressively taking up more carbon during this period (Barlow et al. 2015). The productivity of seasonally snow-covered evergreen forests in the temperate zone is sensitive to the environmental conditions during the winter–spring transition (Monson et al. 2005, Clark et al. 2014, Kelly and Goulden 2016, Winchell et al. 2016). Thus, predictions of biosphere response to climate change may improve by focusing more on the cold season and fall and spring transition seasons.

## Conclusions

We tested the utility of the TD sap flux density method to monitor water transport of conifers during winter and cold periods in fall and spring. In laboratory experiments the method successfully quantified water transport through detached conifer stems at and near freezing temperature, but the relation between water transport rate and SFD deviated from the traditional formulation.

Field observations highlighted temperature-induced variation in the no-flow baseline voltage, and we applied a simple algorithm to accommodate for this. The technique reliably identified periods of sap transport, and lack of sap transport, during the fall, winter and spring. Clear differences in the timing of activity in spring and cessation of it in fall were found in two *Abies* forests at different elevations. We conclude that the TD method can be reliably used to examine sap transport during cold periods.

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## Conflict of interest

None declared.

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