Letters

Among-species variation in sap pH affects the xylem CO_2 transport potential in trees

Introduction

To understand the responses of forest carbon (C) cycling to environmental change, it is important to attribute the observed C release to the correct respiratory sources. One area where considerable uncertainty still exists is the role of the xylem in transporting CO_2 from the roots and/or soil to be released from, or assimilated by, the stems or the foliage (e.g. Hölttä & Kolari, 2009; Bloemen *et al.*, 2013; Stutz *et al.*, 2017; Salomón *et al.*, 2021; Tarvainen *et al.*, 2021). While several studies have quantified the magnitude of CO_2 transport by the xylem sap of different tree species, they have yielded contradictory results regarding the importance of this process for tree-scale C cycling. It has been consistently reported that xylem CO_2 transport rates can be considerable in ring-porous and diffuse-porous species with high sap flow rates and small conducting areas (McGuire & Teskey, 2004; Teskey *et al.*, 2008; Bloemen *et al.*, 2013, 2014; Salomón *et al.*, 2019; Mincke *et al.*, 2020). However, xylem transport has been found to be of limited magnitude in large conifers with slow sap flow rates (e.g. Ford *et al.*, 2007; Ubierna *et al.*, 2009; Tarvainen *et al.*, 2021).

While differences relating to the ease of water movement through the xylem, such as wood anatomy and sap flow rate, undoubtedly explain some of the among-species variation in xylem CO_2 transport, the chemistry of the sap also matters. The CO_2 partial pressure (pCO_2) of the sap has been reported to vary over a very broad range from near zero up to 26.3% among species and studies (e.g. Teskey *et al.*, 2008; Tarvainen *et al.*, 2014; Salomón *et al.*, 2021), and directly affects the amount of CO_2 dissolved in the xylem sap ([CO_2*], McGuire & Teskey, 2002) that is available for transport as described by Henry's law (Eqn 1). However, previous observations of pCO_2 show that both high and low pCO_2 can be found in species with generally higher xylem CO_2 transport rates, such as diffuse- and ring-porous species, as well as in those with generally lower transport rates, such as conifers (Teskey *et al.*, 2008).

The pH of the xylem sap is another factor that can affect the potential for xylem CO_2 transport through its effects on the solubility of CO_2 (Eqn 1; Levy *et al.*, 1999; Erda *et al.*, 2014) and is also known to vary among species (e.g. Teskey *et al.*, 2008). Previous studies on trees have reported diel (e.g. Aubrey *et al.*, 2011; Erda *et al.*, 2014; Salomón *et al.*, 2016) and seasonal (e.g. Aubrey *et al.*, 2011; Salomón *et al.*, 2016; Losso *et al.*, 2018; Pramsohler

et al., 2022) variation in xylem pH. Such patterns may be linked to several interacting factors known to affect xylem pH including within-tree variation in sap chemistry (Schill *et al.*, 1996), phenological stage (Pramsohler *et al.*, 2022), water and nutrient availabilities (e.g. Gollan *et al.*, 1992; Jackson *et al.*, 2003; Sharp & Davies, 2009; Wang *et al.*, 2012), site elevation (Pramsohler *et al.*, 2022), solar radiation, vapor pressure deficit, and air and soil temperatures (Wilkinson & Davies, 2002, 2008; Wan *et al.*, 2004; Pramsohler *et al.*, 2022). Although the magnitude of, and causes for, within-species variation in xylem pH has received considerable attention, few studies to date have compared xylem pH in tree species from different functional types growing under similar climatic and soil conditions. Thomas & Eamus (2002) studied six Australian savanna species, including two deciduous species, two semi-deciduous species and two evergreens. They found a wet to dry season difference in xylem pH that correlated with changes in leaf water potential in the semi-deciduous and the evergreen species, but not in the deciduous species, suggesting that among-species differences in sap pH are seasonally variable. However, they did not specifically analyze the among-species variation in pH. For nonwoody species, Sharp & Davies (2009) measured the xylem pH of 22 perennial species growing under similar conditions in pots and found a pH range of more than three units. Thus, the previous observations suggest that xylem pH varies over a broad enough range to matter for the CO_2 transport rates but do not directly address the question of whether different tree species operate at different xylem pH when exposed to similar conditions.

Many tree species have been found to have xylem pH values that can be near the point where solubility of CO_2 increases strongly, pH *c.* 6–6.5 (e.g. Levy *et al.*, 1999; Erda *et al.*, 2014; Fig. 1). Thus, the accuracy of the pH estimates is of key importance for studies analyzing xylem CO_2 transport with the commonly-used mass balance approach (e.g. McGuire & Teskey, 2004; Bowman *et al.*, 2005; Salomón *et al.*, 2018; Tarvainen *et al.*, 2021), which relies on Henry's law to calculate [CO_2*].

A potential issue with the accuracy of the pH data is the method used for sap extraction. If the sap is forced out of the xylem sample in a way that damages the parenchyma cells, such as crushing the tissue using a vice, the sample may be contaminated by leakage from the damaged cells. If such contamination occurs it will cause an overestimation of sap pH, given that the cytoplasmic pH is in the 7.0–7.4 range (Madshus, 1988). As an alternative, it has been suggested that the displacement method (e.g. Bennett *et al.*, 1927; Glavac *et al.*, 1990), where a water column is used to create pressure to force out the sap from, for example, a cut branch, leads to minimal sample contamination due to the large sap volume collected (Dambrine *et al.*, 1995). However, this method also requires the cutting of cells and is likely to result in some contamination of the first sap collected. To our knowledge, it has not been previously determined whether, and by how much,
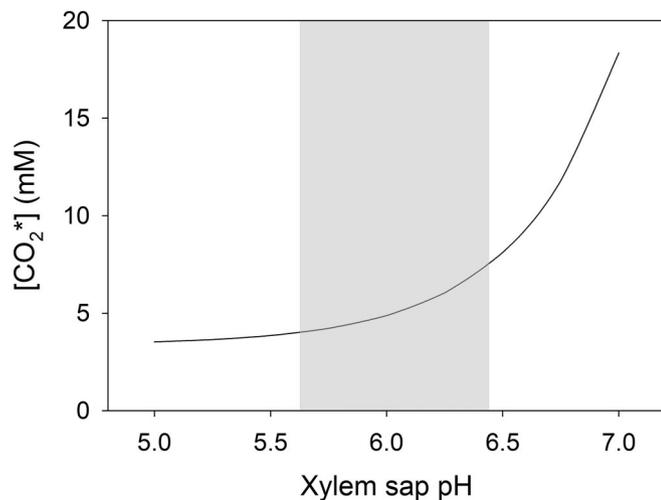
Fig. 1 Change in the amount of CO_2 dissolved in the xylem sap ($[CO_2*]$) with xylem sap pH at CO_2 partial pressure of 10% and stem temperature of 25°C calculated with Henry's law (Eqn 1). The shaded area indicates the range of pH values observed (at 23°C) in this study.

parenchyma damage during sampling may affect the pH estimates in different tree species, and how this in turn affects the estimates of $[CO_2*]$ and xylem CO_2 transport.

In this study, we hypothesized that the contradictory estimates regarding the magnitude of CO_2 transport in the xylem sap can, in part, be explained by among-species differences in sap pH. To assess this hypothesis, we measured pH from the xylem sap extracted using a displacement time series from four tree species, including *Pinus sylvestris* (needle-leaf evergreen), *Larix sukaczewii* (needle-leaf deciduous), *Sorbus aucuparia* (broadleaf deciduous), and *Alnus incana* (broadleaf deciduous, symbiotic with nitrogen fixing bacteria *Frankia alni*), growing in close proximity to each other under the same soil and climatic conditions. We then used the pH data and Henry's law to calculate the potential amount of dissolved CO_2 in the xylem sap of these trees for different combinations of xylem CO_2 concentrations and temperatures. The following research questions were investigated:
(**1**) How much does contamination from damaged parenchyma cells influence sap pH estimates?
(**2**) Does xylem sap pH vary sufficiently among species growing under similar conditions to explain the contradictory reports on CO_2 transport in the xylem?

Materials and Methods

Study site

The study was carried out at the Svartberget research station located in Vindeln, northern Sweden (64°42′N, 19°77′E, 260 m asl). The 30-yr mean annual temperature and precipitation in at the site are 1.8°C and 614 mm, respectively (Laudon *et al.*, 2013).

The sampling was conducted at an experimental site set up as a blocked design (three blocks) with several tree species. Each sub-block was *c.* 0.12 ha in size and included 289 trees. Six trees from four species growing at the site were included in this study:

Pinus sylvestris (Scots pine), *Larix sukaczewii* (Siberian larch), *Sorbus aucuparia* (rowan), and *Alnus incana* (grey alder). The trees chosen for this study were also used as a part of a nitrogen fertilization experiment where KNO_3 fertilizer (corresponding to a single dose of 70 kg N ha^{-1}) was applied around three individuals of each of the four studied species. Thus, the overall design consisted of a total of six individuals of each species, divided into three control and three fertilized trees. The experiment was initiated on 26 August 2015 when the fertilizer was applied. The sampling was carried out 6 d later in the morning of 1 September 2015. We note that nitrogen (especially nitrate) content has been shown to correlate with sap pH in several plant species (e.g. Gollan *et al.*, 1992; Wang *et al.*, 2012). Because of this, the data are presented here separately for the control and fertilized trees. During the sampling, the Scots pine and larch branches were collected from mature trees growing at an experimental site. Since alder and rowan were not included in the original blocked design, the alder branches were collected from mature trees growing adjacent to the blocked experiment and the rowan samples consisted of main stems of younger trees interspersed within the blocked experiment. The selected branches were cut near the trunk and had similar diameters for all species (*c.* 25 mm at the proximal end). The site is located on a gentle westward facing slope with all the sampled trees thus exposed to similar soil and climatic conditions. After collection, the samples were placed in large opaque plastic bags and transported by car, for *c.* 1 h, before being stored in cold room (8°C) in darkness until sap extraction.

Sampling and data analysis

The sap was extracted by the displacement method (e.g. Glavac *et al.*, 1990) as described by Tarvainen *et al.* (2021). Briefly, the sample branches were recut under water at both ends and the bark removed with a knife before the xylem sap extraction. The length of the branch sections used for the analysis varied among the samples but was generally > 30 cm to allow for sufficient amount of sap to be collected. The branch was fastened to one end of a 25-mm-diameter PVC tube that was mounted vertically above the branch and *c.* 600 ml deionized water including food colouring (Dr Oetker Sverige AB, Gothenburg, Sweden; blue, pH 3.37) was poured into the tube. The xylem sap was pushed out of the branch by the resulting pressure and collected in a series of successive 1.5-ml Eppendorf vials until colored water arrived. Despite the use of similar external pressures, the sap extraction rates and the total amount of sap extracted per branch were variable, likely in response to the among-species differences in wood anatomy. The pH of each sample was measured immediately following sap extraction with a PHM202 pH meter (Radiometer, Copenhagen, Denmark). The room temperature during the sap extraction and pH measurement was *c.* 23°C. Analysis of the pH in each vial allowed us to determine the potential biasing effect of the initial parenchyma cell damage during sap extraction on the pH estimates. When analyzing the change in pH with sample number, each pH observation was normalized relative to the average pH of the last five samples collected before dye breakthrough, representing asymptotic steady-state pH. For some branches (one rowan and one larch branch), the dye broke through so early that such averaging was not possible. For

three pine branches, some pH observations were likely affected by pitch (which was visible at the cut surface and appeared to cause the pH to increase) and were removed from further analyses. In these cases, we used the pH observed in the single last sample before dye breakthrough or appearance of pitch for all analyses. The steady-state data, showing no evidence of dye breakthrough, were used for the among-species comparison of xylem pH.

We used Henry's law (Eqn 1; McGuire & Teskey, 2002) to calculate the amount of CO_2 dissolved in the xylem sap ($[CO_2^*]$) at the observed pH and CO_2 partial pressures (pCO_2) of 1% and 10% and stem temperatures (T_s) of 10°C and 25°C:

$$[CO_2^*] = \left(1 + \frac{K_1}{10^{-pH}} + \frac{K_1 K_2}{(10^{-pH})^2}\right) K_H pCO_2, \quad \text{Eqn 1}$$

where the T_s dependent solubility constants; K_1, K_2 and K_H were calculated according to McGuire & Teskey (2002):

$$K_1 = \left(2.5764 \times 10^{-7}\right) + \left(3.3742 \times 10^{-7}\right)\left(1 - e^{-0.0318 T_s}\right), \quad \text{Eqn 2}$$

$$K_2 = \left(2.3777 \times 10^{-11}\right) + \left(9.0041 \times 10^{-13}\right) T_s, \quad \text{Eqn 3}$$

$$K_H = 0.0114 + 0.0661 e^{-0.0433 T_s}. \quad \text{Eqn 4}$$

The effect of species and soil nitrogen availability on sap pH was analysed by two-way independent ANOVA with species and treatment (i.e. control or fertilizer addition) as fixed factors. The data were checked for normality and homogeneity of variance, and a Bonferroni correction was used in the post hoc analyses. All statistical analyses were performed with IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA).

Results and Discussion

Destructive sampling of xylem sap can cause cell damage, leading to cytoplasmic contamination of the sample, potentially biasing the pH measurement (e.g. Glavac *et al.*, 1990). Accordingly, in the current study, the pH estimates based on the first extractions from the larches and pines were up to 8% higher than for latter samples (Fig. 2), corresponding to *c.* 0.45 pH units.

Such an error would have had two major consequences for the continued data analysis and the interpretation of the results had we not continued sampling until a pH asymptote was reached. First, the error is large enough that it would have led us to incorrectly conclude that the xylem pH did not vary significantly among the four studied species. Second, because the studied trees all varied around pH = 6, where bicarbonate solubility begins to increase exponentially (Fig. 1), even small increases in pH could have considerable effects on the estimated amount of dissolved CO_2 present in the sap (Table 1; Levy *et al.*, 1999; Erda *et al.*, 2014).

For example, the observed +0.45 pH-unit error would have led us to overestimate the $[CO_2^*]$ of the two conifer species by up to 50% under the T_s and pCO_2 (which is linearly related to $[CO_2^*]$ under constant T_s according to Eqn 1) used in our calculations. This would,
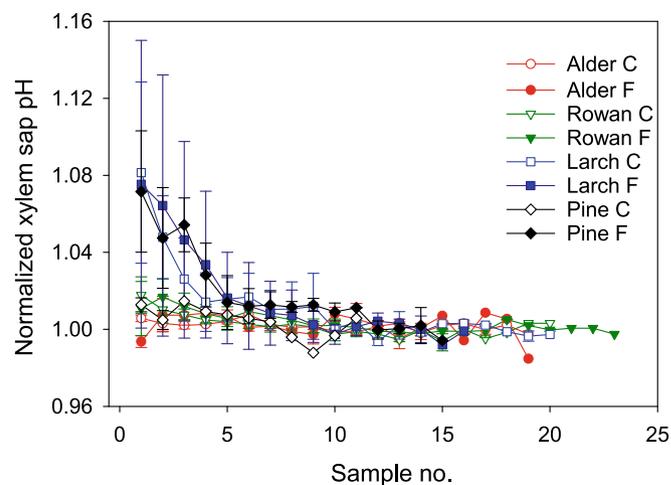

Fig. 2 Change in observed pH (normalized) with sample number in the four studied species (means ± SE, $n = 1$–3 per sample number). The room temperature during the sample extraction was 23°C. C, control trees; F, fertilized trees.

in turn, have considerably biased any further estimates of C cycling in the studied trees. Furthermore, given that the pH estimates for pine and larch did not stabilize until between 5 and 10 ml of sap were collected (Fig. 2), our results suggest that sample contamination issues likely will be exacerbated for small sample sizes, such as sap extracted from twigs using a Scholander type pressure bomb. Notably, the contamination effect was much smaller, < 2% deviation from the pH asymptote, in the two broadleaf species included in the study (Fig. 2). This raises the question of whether the pH effect from damage during sampling is especially large in species, such as Scots pine and larch, which may excrete large quantities of pitch in response to wounding. In addition, we note that the accuracy of the sap pH measurement is particularly important in studies where it is used for inferring xylem CO_2 transport rates, such as when using the mass balance approach (e.g. McGuire & Teskey, 2004; Bowman *et al.*, 2005; Salomón *et al.*, 2018; Tarvainen *et al.*, 2021). On the contrary, studies that utilize isotopic tracers (e.g. Ubierna *et al.*, 2009; Bloemen *et al.*, 2013; Salomón *et al.*, 2019; Tarvainen *et al.*, 2021) avoid these problems by directly tracking the CO_2 movement inside the stems.

There was no overall short-term xylem sap pH response to the fertilizer application detected on the samples collected once the pH had reached an asymptote, $F(1, 16) = 0.35$, $P = 0.56$ (Fig. 3). However, a significant main effect of species on the xylem sap pH, $F(3, 16) = 35.37$, $P < 0.001$, was found. The post hoc tests indicated that the xylem sap pH was similar for alder and rowan ($P = 1.00$), and for larch and pine ($P = 1.00$), but significantly higher for alder and rowan compared with larch and pine ($P < 0.001$; Fig. 3).

Furthermore, the calculations with Henry's law showed that, given the observed pH differences among the tree species, the amount of CO_2 dissolved in the xylem could differ by nearly 80% over a reasonable range of temperatures and CO_2 partial pressures (Table 1). A significant Fertilization × Species interaction was also detected ($F(3, 16) = 7.080$, $P = 0.003$), reflecting the lower pH in the fertilized larches compared to the controls. This may simply

Table 1 Effect of pH on CO_2 dissolved in xylem sap ($[CO_2*]$, mM) at different CO_2 partial pressures (pCO_2, %) and stem temperatures (T_s, °C).

| Species	Treatment	pH	pCO_2 (%)	T_s (°C)	$[CO_2*]$ (mM)	pCO_2 (%)	T_s (°C)	$[CO_2*]$ (mM)	pCO_2 (%)	T_s (°C)	$[CO_2*]$ (mM)
Larch	C	6.04	1	25	0.50	10	25	5.01	1	10	0.75
Larch	F	5.63	1	25	0.40	10	25	4.02	1	10	0.62
Pine	C	5.75	1	25	0.42	10	25	4.22	1	10	0.65
Pine	F	5.92	1	25	0.46	10	25	4.62	1	10	0.70
Rowan	C	6.30	1	25	0.63	10	25	6.35	1	10	0.92
Rowan	F	6.44	1	25	0.75	10	25	7.46	1	10	1.06
Alder	C	6.34	1	25	0.66	10	25	6.63	1	10	0.95
Alder	F	6.32	1	25	0.65	10	25	6.49	1	10	0.94

C, control trees; F, fertilized trees.
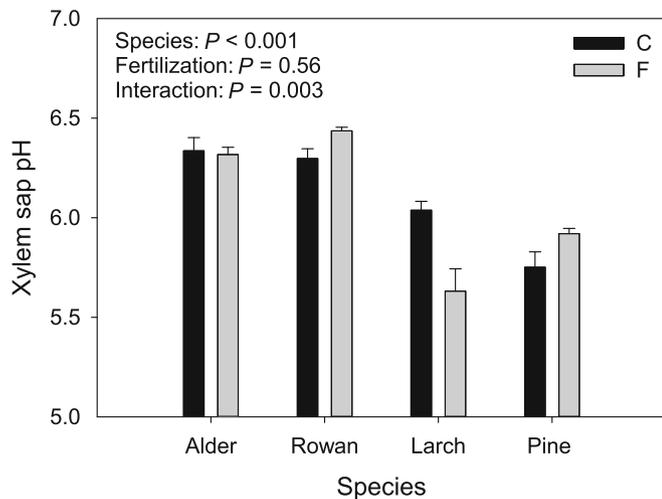
Fig. 3 Sap pH in trees of four species growing under the same climate and soil conditions with and without nitrogen fertilizer addition (means ± SE). C, control trees ($n = 3$); F, fertilized trees ($n = 3$). Reported results come from a two-way analysis of variance, Bonferroni correction was used in the post hoc analyses.

present a statistical artifact given the small sample sizes and the short time between the fertilizer application and the sap collection in the current study. However, it is clear that sap chemical composition influences the xylem pH in general (e.g. Raven, 1985; Wilkinson & Davies, 2002) and previous studies have shown correlations between sap pH and nitrogen concentrations and forms in several plant species (e.g. Gollan *et al.*, 1992; Dodd *et al.*, 2003; Wang *et al.*, 2012). Based on these previous findings and the observation in this study of a significant among-species variation in sap pH in trees growing under the same conditions, it would seem useful to study further whether among-species differences in the forms in which nitrogen is present in the sap could explain the observed pH differences and the solubility of CO_2 in the xylem sap.

Given the limited scope of this study, two species per taxonomic group, it is important that the generality of the finding of among-species variation in xylem sap pH is further evaluated by other studies. The simplest way for accomplishing this would be to use a similar study design but increase the number of species and include greater within-species replication, for example, by utilizing arboretums or species trials established within forestry research. Such studies would greatly benefit from additional measurements to allow for full mass balance calculations over extended periods of time, including continuous observations of sap flow rates, sap CO_2 concentrations, and stem temperatures, as well as from repeated measurements of sap pH to account for its temporal variability and from continuous monitoring of environmental factors to link the changes in sap pH and CO_2 transport to their drivers. This would both allow for better evaluations of the among-species variation in xylem sap pH and provide information about the other potential physiological causes behind among-species differences in xylem CO_2 transport rates. The isotopic tracer approach (e.g. Ubierna *et al.*, 2009; Bloemen *et al.*, 2013; Salomón *et al.*, 2019; Tarvainen *et al.*, 2021) has great potential for studies of among-species variation in xylem CO_2 transport rate but needs to be supported by accurate measurements of other factors, including sap flow rate and sap pH, to provide improved mechanistic understanding. Furthermore, a meta-analysis approach would allow for utilizing large data sets to study the causes behind the observed among-species variation in the magnitude of xylem CO_2 transport. Notably, based on the findings presented here, such an analysis would need to be done with careful consideration of the methods used for sap pH determination in mass balance-based studies to account for the possible bias caused by cell damage during sampling.

Conclusion

The findings of this study suggest among-species variation in xylem pH to be large enough to significantly affect a species' capacity for stem CO_2 transport and hence be important for the tree-scale C cycling. However, we note that these results should be seen as preliminary given the limited number of species and trees investigated, and thus need to be corroborated by other studies. Detection of the among-species pH differences required careful elimination of cell contents from the analysed samples with continuous sap extraction. The two needle-leaf species, Scots pine and Siberian larch, had lower pH and, thus, weaker capacity for holding CO_2 dissolved in the xylem sap compared with the two broadleaves, rowan and alder, growing under similar soil and environmental conditions. Previous work has shown that gymnosperms exhibit lower sap flow rates than angiosperms (e.g. Flo *et al.*, 2021). Thus, the combination of low sap pH and low xylem transport rates may help to explain the limited magnitude of internal CO_2 transport observed for conifers.

Competing interests

None declared.

Author contributions

LT, NH, TN and JDM contributed to the design of the research; LT and NH performed the measurements; LT analysed the data and wrote the manuscript with input from the other authors.

ORCID

Nils Henriksson https://orcid.org/0000-0003-1088-9192
John D. Marshall https://orcid.org/0000-0002-3841-8942
Torgny Näsholm https://orcid.org/0000-0002-2275-2030
Lasse Tarvainen https://orcid.org/0000-0003-3032-9440

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Lasse Tarvainen[1,2]* , Nils Henriksson[2] ,
Torgny Näsholm[2] and John D. Marshall[2]**

[1]Department of Biological and Environmental Sciences, University of Gothenburg, PO Box 461, Gothenburg, SE-405 30, Sweden;
[2]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences (SLU), Skogmarksgränd, SE-901 83 Umeå, Sweden
(*Author for correspondence: email lasse.tarvainen@bioenv.gu.se)

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