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Spatial and temporal patterns of xylem sap pH derived from stems and twigs of *Populus deltoides* L.

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ABSTRACT

Xylem sap pH (pH_X) is critical in determining the quantity of inorganic carbon dissolved in xylem solution from gaseous [CO_2] measurements. Studies of internal carbon transport have generally assumed that pH_X derived from stems and twigs is similar and that pH_X remains constant through time; however, no empirical studies have investigated these assumptions. If any of these assumptions are violated, potentially large errors can be introduced into calculations of dissolved CO_2 in xylem and resulting estimates of internal carbon transport. We tested the validity of assumptions related to pH_X in *Populus deltoides* L. with a series of non-manipulative experiments. The pH_X derived from stems and twigs was generally similar and remained relatively constant through a diel period. The only exception was that pH_X derived from lower stem sections at night was higher than that derived from twigs. The pH_X derived from stems was similar on clear days when solar radiation and vapor pressure deficit (VPD) were similar, but higher on an overcast day when solar radiation and VPD were lower. Similarly, cloudy conditions immediately before an afternoon thunderstorm increased pH_X derived from twigs. The pH_X derived from twigs remained similar when measured on sunny afternoons between July and October. Our results suggest that common assumptions of pH_X used in studies of internal carbon transport appear valid for *P. deltoides* and further suggest pH_X is influenced by environmental factors, such as solar radiation and VPD that affect transpiration rates.

1. Introduction

The concentration of hydrogen ions ([H^+]), commonly referred to as pH, is fundamental to numerous plant physiological processes. Within plant cells and organelles, pH gradients drive critical processes such as ATP synthesis (Mitchell, 1966), energy dissipation (Demmig-Adams and Adams, 2006), and photosynthetic electron transport (Schonknecht et al., 1995). Enzyme activity is also largely dependent on pH with different enzymes exhibiting different optimal pH values. The pH within specific organelles varies among species and organs, but the pH of cytosol appears to be homeostatically maintained around 7.2–7.5 under non-stressful conditions (Felle, 2001), whereas the pH in vacuoles is more acidic (Kurkdjian and Guern, 1989; Smith and Raven, 1979). The apoplastic pH of roots has been shown to range from 4.8 to 5.9, whereas the apoplastic pH of leaves has been shown to range from 4.6 to 6.7 (Felle, 2001). The pH of xylem sap (pH_X), which has been reported to range from 4.5 to 7.4 (Teskey et al., 2008), can influence leaf elongation (Bacon et al., 1998), xylem hydraulics (Gascó et al., 2008), and changes in pH_X have been associated with root to shoot stress signaling (Wilkinson, 1999). The pH_X also affects the solubility of ions and molecules. For example, pH_X is important in determining the quantity of dissolved CO_2 that can be transported internally through the transpiration stream of trees—a pathway for CO_2 movement that is gaining appreciation as an integral component of forest carbon dynamics (Friend, 2010; Hanson and Gunderson, 2009; Holtta and Kolari, 2009).

Although direct measurement of dissolved CO_2 in the xylem sap of woody tissue cannot currently be made *in situ*, dissolved CO_2 concentration ([CO_2]) can be calculated from gaseous [CO_2], temperature, and pH_X. According to Henry's gas law, equilibrium exists between the [CO_2] of the gaseous and liquid phases when the phases are in contact, as is the case in xylem (Hari et al., 1991; Levy et al., 1999). Solubility of CO_2 in solution decreases with increasing temperature and increases with increasing pH (see Teskey et al., 2008). For example, at a pH of 6.5 and gaseous [CO_2] of 10%, a 20 °C increase in temperature from 15 °C to 35 °C decreases dissolved [CO_2] approximately 35% from 10.19 to 6.56 mmol CO_2 L^{-1}. At a temperature of 25 °C and a gaseous [CO_2] of 10%, a change in pH across the range of values previously reported for tree

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xylem sap (4.5–7.4; Teskey et al., 2008) increases dissolved [CO_2] nearly twelve-fold (from 3.43 to 40.95 mmol CO_2 L^{-1}). Therefore, in addition to measuring gaseous [CO_2], knowledge of xylem sap temperature and pH_X are critical to determining the quantity of dissolved CO_2 in the xylem.

Whereas both gaseous [CO_2] and xylem sap temperature can be monitored automatically and at high frequencies, pH_X cannot. Techniques for determining pH_X require extraction of xylem sap from tissue for analysis. Xylem sap can be extracted from tree stems, branches, or twigs, and extraction from each tissue type has advantages and disadvantages. An advantage of obtaining xylem sap from stems is that the sap is derived directly from the tissue where gaseous CO_2 measurements occur and dissolved [CO_2] is to be calculated. However, extraction of xylem sap from stems requires an increment core of xylem tissue be removed. Unfortunately, repeated increment coring of a stem could directly affect the underlying investigation of internal carbon transport. For example, repeated coring could reduce sap flux and wounding could increase the amount of CO_2 efflux from the stem surface to the atmosphere. After the core is removed from the stem, it is compressed in a vice to expel the sap which can then be collected and analyzed. The use of a vice could contaminate xylem sap with solution derived from ruptured cells which may have a very different pH than that of the xylem sap.

To avoid potential problems associated with collecting stem tissue, sap derived from twigs has been used as a surrogate for stem pH_X in some studies (McGuire and Teskey, 2002, 2004; Teskey and McGuire, 2007). Twig samples can be collected from tree canopies and placed into a pressure chamber to extract xylem sap. Compared to stem samples, twig samples are much easier to collect, xylem sap is much easier to extract, and the procedure allows repeated sampling of the same tree over time without concerns of influencing sap flux or CO_2 efflux. However, the literature is relatively devoid of information about pH_X derived from different tissues or spatial locations of the same tissue. Saveyn et al. (2008) reported that pH_X derived from stems and twigs of *Populus deltoides* L. was similar, but the evidence was based on sample means and did not include statistical hypothesis testing. Although restricted to stem tissue, Schill et al. (1996) found that pH_X decreased with stem height. To our knowledge, no empirical investigations have examined relationships in pH_X derived from different tissues and different spatial locations.

Studies investigating internal carbon transport generally occur over relatively short time periods. Although seasonal patterns of pH_X have been well described in the literature (e.g., Fromard et al., 1995; Glavac et al., 1990), pH_X dynamics at finer temporal scales have not been well studied. Internal carbon transport studies have generally assumed that pH_X remains constant throughout a diel period (Aubrey and Teskey, 2009; McGuire and Teskey, 2004; Saveyn et al., 2008; Teskey and McGuire, 2007). We are aware of only a few reports of diel pH_X patterns and the results are inconsistent. For example, Teskey and McGuire (2007) found that pH_X of *Platanus occidentalis* L. varied by <0.15 units when measured at 4 h intervals between 08:00 and 20:00 h, whereas Schurr and Schulze (1995) found an increase of 0.6 pH units from the beginning to the end of the light period in castor bean plants (*Ricinus communis* L.). Beis et al. (2009) found that pH_X derived from leaves increased throughout the morning compared to predawn measurements. Likewise, we are not aware of any reports that investigate pH_X across multiple days or in relation to environmental factors other than drought.

The purpose of this study was to test the validity of assumptions commonly made regarding tissue similarity and diel constancy of pH_X. If any of these assumptions are violated, potentially large errors are introduced into calculations of dissolved CO_2 in xylem and resulting estimates of internal carbon transport. Our specific objectives were to determine the relationship between pH_X derived from *P. deltoides* stems and twigs, and to investigate pH_X dynamics at a variety of temporal scales ranging from diel to growing season patterns. To this end, we tested the hypotheses that: (1) pH_X derived from stems and twigs are similar; (2) pH_X remains relatively constant over a diel period; and (3) pH_X remains relatively constant during the growing season.

2. Materials and methods

2.1. Study site, plant material, and microclimatic monitoring

We conducted experiments at the U.S. Department of Energy Savannah River Site, a National Environmental Research Park located in the Carolina Sand Hill physiographic region (33°23′N, 81°40′E). We selected eastern cottonwood trees (*P. deltoides*) in their 9th growing season. Sample trees were originally part of a productivity experiment (Coyle and Coleman, 2005; Coyle et al., 2008) and received fertilization, irrigation, and complete competition control throughout stand history. Potential evapotranspiration was calculated on a daily basis from a weather station located at the experimental site and was used to determine irrigation requirements. Irrigation was supplied daily, if necessary, via drip tubes throughout our experiments based on calculated irrigation requirements designed to eliminate the evapotranspiration deficit and ensure favorable soil moisture. Therefore, pH_X dynamics observed in this study should not have been influenced by drought stress signaling. The weather station also provided hourly measurements of temperature, relative humidity, vapor pressure deficit, and global radiation. Global radiation (hereafter referred to as solar radiation) includes both direct and diffuse solar radiation and was measured with a stationary pyranometer (LI-2100; Li-Cor Biosciences, Lincoln, NE, USA).

2.2. Spatial and diel patterns of pH_X with respect to tissue origin

We randomly selected seven trees within a 15–17 cm diameter at breast height range (dbh; diameter at 1.4 m height). We divided tree canopies into approximately even upper and lower sections (hereafter referred to as upper and lower canopy sections) and collected a single twig from each stratum at three 8 h intervals in a single 24 h period on July 12th 2008. Sampling occurred in the morning (07:00 h), afternoon (15:00 h), and night (23:00 h). Microclimatic data for the sampling periods can be found in Table 1. Sunrise on July 12th was 06:26 h and sunset was 20:39 h. Twig samples were approximately 5 mm in diameter and included foliage. We expressed approximately 0.2 mL of sap from twigs with a pressure chamber (PMS Instruments, Corvalis, OR, USA) and collected it with a Pasteur pipette. We concurrently collected stem cores (5 mm radial diameter) at 1.0 and 5.0 m above ground level (hereafter referred to as upper and lower stem sections) using a stem increment borer (Suunto, Vantaa, Finland). Stem cores were placed into a vice and compressed to express sap which was collected with a Pasteur pipette. The two different pH_X extraction techniques were necessary for the different tissues. For example, xylem sap could not be expressed from stem increment cores with a pressure chamber. Although a vice could be used to extract xylem sap from twigs, contamination of sap from non-xylem cells (e.g., phloem cells) could occur. We immediately transferred the expressed sap to a solid state pH microsensor connected to a pH meter (Red-Line Standard Sensor, Argus meter; Sentron Europe BV, Roden, The Netherlands). The pH microsensor requires only 20 μL liquid sample for analysis.

2.3. Daily patterns of pH_X

To examine daily patterns of pH_X, we randomly selected five additional trees (i.e., $n = 12$) in the same diameter range as in the

Table 1
Microclimatic data corresponding to the investigation of spatial and diel patterns of pH_X with respect to tissue origin (day 193) and the investigation of daily patterns of pH_X (day 193, 195, and 197).

Day	Time	Temperature (°C)	Relative humidity (%)	Vapor pressure deficit (kPa)	Solar radiation ($W\,m^{-2}$)
	07:00	20.0	99.7	0.01	10
193	15:00	32.1	43.2	2.71	723
	23:00	22.4	84.7	0.41	0
195	07:00	21.0	99.0	0.02	5
	15:00	23.0	96.0	0.11	97
197	07:00	18.2	99.7	0.01	10
	15:00	31.7	32.5	3.15	866

Table 2
Microclimatic data corresponding to the investigation of seasonal patterns of pH_X.

Day	Temperature (°C)	Relative humidity (%)	Vapor pressure deficit (kPa)	Solar radiation ($W\,m^{-2}$)
183	31.0	40.7	2.66	677
205	27.7	67.1	1.22	356
226	26.3	72.5	0.94	412
247	24.7	59.4	1.26	581
272	20.6	43.4	1.37	558
295	18.9	69.2	0.67	458

first experiment. We analyzed xylem sap derived only from lower stem sections (because of unavailability of the equipment to reach the canopy) in both the morning and afternoon of July 12th, 14th, and 16th. Microclimatic data for the sampling periods can be found in Table 1.

2.4. Influence of cloud on pH_X

An afternoon thunderstorm on July 11th 2008 allowed for an opportunistic comparison of twig pH_X under heavy clouds and clear skies. The thunderstorm occurred on the afternoon of what was previously a clear day and cloud cover lasted approximately 1 h. The sky quickly cleared once the thunderstorm passed. We collected pH_X of twigs from upper, middle, and lower canopy strata of three trees during the cloudy period immediately before and the clear period immediately after the thunderstorm.

2.5. Growing season patterns of pH_X

To examine growing season patterns of pH_X, we randomly selected 18 trees within a 16–22 cm dbh range. We analyzed pH_X derived from twigs collected from the lower portion of tree canopies between 10:00 and 12:00 h on clear days. Sampling began July 2nd 2009 (i.e., during the tenth growing season for this stand) and was repeated five times at approximately two or three week intervals until October 22nd. Different trees were sampled at each sampling interval. Microclimatic data for the sampling periods can be found in Table 2.

2.6. Statistical analysis

We used repeated measures ANOVA to examine diel patterns of pH_X derived from different tissues and spatial strata. There was no biologically meaningful way to include spatial strata as a factor in the ANOVA model as canopy sections and stem core heights were not biologically equivalent stratifications. Therefore, we entered each tissue-by-location combination (hereafter referred to as sample location; $n = 4$) into the model as our overall fixed treatment factor and included diel period ($n = 3$) as our repeated factor. We considered individual tree ($n = 7$) as a random subject factor in our model.

We used a similar repeated measures ANOVA to examine pH_X derived from lower stem sections ($n = 12$) in morning and afternoon ($n = 2$) across three days ($n = 3$). Repeated measures ANOVA was also used to examine pH_X derived from twigs at three different canopy strata on three trees ($n = 3$) during the cloudy period immediately before and the clear period immediately after a thunderstorm. One-way ANOVA was used to examine mid-day pH_X dynamics throughout the growing season ($n = 6$).

We performed all analyses using the mixed model procedure (PROC MIXED) of SAS (Version 9.1.3, SAS Inc., Cary, NC, USA) with a type-I error rate of 0.05. To model the correlation within experimental units over time, we analyzed each response using common covariance structures appropriate for data collected at equal temporal spacing within and among experimental units and used AIC_C (Burnham, 1998) to determine which structure best fit each model. Denominator degrees of freedom were estimated according to the Kenward–Roger method (Kenward and Roger, 1997). We investigated main effects using Fisher's Least Significant Difference Tests (LSD) with a type-I error rate of 0.05. When interactions occurred, we performed tests of simple main effects using the SLICE option in the LSMEANS statement (Littell et al., 2006; Schabenberger et al., 2000). Prior to ANOVA, we assessed normality of pH_X data using the Shapiro–Wilks goodness-of-fit-tests as well as normality and box plots in the univariate procedure of SAS (PROC UNIVARIATE). We examined potential relationships between pH_X and temperature using Pearson product moment correlation (PROC CORR).

3. Results

Our examination of spatial and diel patterns of pH_X with respect to tissue origin found few differences in pH_X derived from stems or twigs regardless of the strata from which they were collected and pH_X remained relatively constant over the diel period (Table 3). The only differences we observed in pH_X depended on tissue type, location, and diel period (i.e., sample location × diel period interaction; Table 3); specifically, differences resulted from pH_X dynamics in the lower stem. The pH_X derived from lower stem cores was

Table 3
ANOVA results for testing assumptions related to spatial and diel patterns of pH_X with respect to tissue origin.

Effect	F	P
Diel period	2.97	0.0575
Sample location	1.08	0.3619
Diel period × sample location	2.38	0.0371

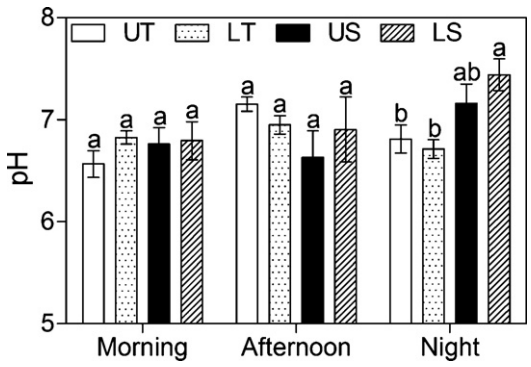

Fig. 1. Mean ± SE pH_X expressed from upper (UT) and lower canopy twigs (LB) and from upper (US) and lower stem cores (LS) obtained from seven randomly selected trees at 07:00 (morning), 15:00 (midday), and 23:00 h (night) on July 12th 2008. Means sharing a letter within a diel period are not significantly different (Fisher's LSD, $\alpha = 0.05$).

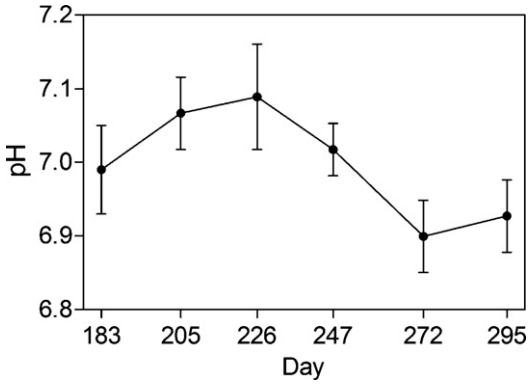

Fig. 3. Mean ± SE pH_X expressed from canopy twigs obtained from 18 randomly selected trees beginning on July 2nd 2009 (i.e., day 183) and continuing until October 22nd (i.e., day 295).

higher than that derived from either twig location, but this difference only occurred at night (Fig. 1) with pH_X of tissue derived from the lower stem being higher at night than in the morning and afternoon. With the exception of samples derived from the lower stem, pH_X remained similar throughout the diel period.

Our experiment examining daily patterns of pH_X derived from lower stems found that pH_X was not constant across days (Fig. 2; $P = 0.0003$), but remained similar between morning and midday sampling intervals ($P = 0.2729$). Our examination of the influence of clouds on pH_X derived from twigs found pH_X was lower when measured under clear skies immediately after a thunderstorm (mean 6.93 ± 0.05 SE; $P < 0.0001$) than under cloudy skies immediately before (7.44 ± 0.08) a thunderstorm, and the pattern was consistent in upper, middle, and lower canopy strata ($P = 0.0844$). Our investigation of growing season patterns of pH_X derived from twigs found pH_X remained statistically similar when sampled on relatively clear days throughout the 2009 growing season (Fig. 3; $P = 0.0911$). Although temperature varied from 18.2 to 32.1 °C over the course of our experiments (see Tables 1 and 2), we found no evidence of a correlation between pH_X and temperature ($P = 0.5853$; Pearson coefficient = −0.12308).

4. Discussion

The range of mean pH_X measured in our experiments (6.94–7.18) was within the range of previously reported pH_X for trees (4.5–7.4; Teskey et al., 2008). The only other reports of pH_X measured in *P. deltoides* were 6.3 (Stringer and Kimmerer, 1993), 6.8 (Saveyn et al., 2008), and 7.2 (Aubrey and Teskey, 2009). The highest individual pH_X observed in our study (8.45) surpassed the maximum of previously published reports (7.4; Schill et al., 1996). Exceedingly high pH_X values were measured in the lower stem at night, but not any other time during the diel period, or in the upper stem or twigs.

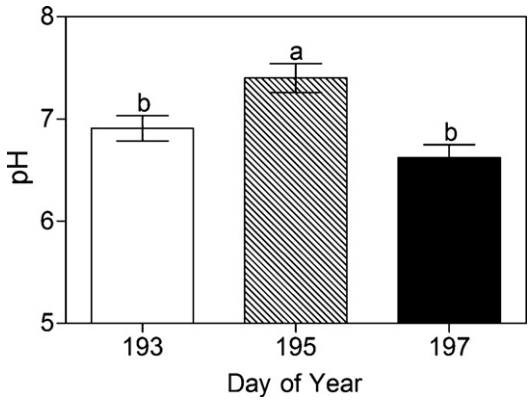

Fig. 2. Mean ± SE pH_X expressed from stem sections obtained from 12 randomly selected trees (averaged across morning and midday measurements) beginning on July 12th 2008 (i.e., day 193) and continuing over two additional non-consecutive days. Means sharing a letter are not significantly different (Fisher's LSD, $\alpha = 0.05$).

The similarity of pH_X derived from the upper stem and twigs suggests pH_X derived from twigs is a suitable proxy for stem pH_X and that different pH_X extraction techniques used for stems (i.e., vice) and twigs (i.e., pressure chamber) do not produce different results. The only exception we observed was that pH_X derived from the lower stem at night was higher than that of twigs. Comparatively high pH_X values were not observed in the lower stem at other times during the diel period or in upper stem samples at night, suggesting that there is a diel pattern of pH_X in the lower stem that does not seem to occur in the upper stem or in twigs and is not a result of the extraction technique. Beis et al. (2009) found that the level of pressure applied with the pressure chamber could influence the origin of pH_X derived from leaves (i.e., petiole, midrib, major or minor vein). In our study, the level of applied pressure should not have influenced the origin of xylem sap extracted from twigs because such a small amount of sap (*ca.* 0.2 mL) was extracted and we applied only as much pressure as required to overcome the tension in the xylem. We were therefore confident that sap originated in twigs and not leaves. Saveyn et al. (2008) found that mean pH_X derived from *P. deltoides* stems and twigs, using a vice and pressure chamber, respectively, differed by only 0.03 pH units. Interestingly, standard errors were generally larger for pH_X derived from stems than twigs in our study (Fig. 1) and in the study by Saveyn et al. (2008). Larger standard errors could result from higher variation of pH_X in stems compared to twigs or from contamination of pH_X by cellular solution as cells may rupture when tissue is compressed in the vice. Our results suggest that even if cellular solution contaminates the xylem sap sample when stem tissue is compressed in a vice, the influence is minor and does not substantially affect pH_X measurements.

Growing season pH_X remained relatively stable between July and October with the range of mean pH_X spanning less than 0.2 pH units. Fromard et al. (1995) found a similar range (i.e., ≤0.2 pH units) of growing season pH_X in *Robinia pseudoacacia* L. between July and October. However, Glavac et al. (1990) found that pH_X of *Fagus sylvatica* L. increased *ca.* 0.8 pH units between July and October. Augé et al. (2000) investigated pH_X dynamics of 11 different deciduous tree species from May through September and found that pH_X of some species varied slightly across the growing season, but others varied up to 1.0 pH unit. Alves et al. (2004) concluded that seasonal dynamics of *Juglans regia* L. were not influenced by temperature.

Similarly, Correia et al. (1999) found that pH_X of the herbaceous plant *Lupinus albus* L. was not influenced by temperature. The temperature range did not vary greatly during our experiments and we did not find a relationship between temperature and pH_X.

The diel pattern observed in pH_X derived from the lower stem indicates xylem sap extracted during the day from higher in the stem or from twigs may not adequately represent xylem sap lower in the stem at night. Specifically, higher pH_X in the lower stem at night suggests that dissolved $[CO_2]$ would be underestimated when pH_X is derived from tissue or spatial locations other than the point of gaseous $[CO_2]$ measurement. Thus, previous studies of internal carbon transport that relied on pH_X obtained from daytime measurements likely underestimated night time dissolved $[CO_2]$ of xylem sap in the lower stem. However, night time measurements of dissolved $[CO_2]$, and thus pH_X, are not critical in studies investigating internal carbon transport as these studies focus on the movement of CO_2 with transpirational water demand (Aubrey and Teskey, 2009; Teskey and McGuire, 2007). Nonetheless, future investigations should aim to understand relationships between dissolved CO_2 and efflux both with and without transpirational water movement. Such studies will require quantification of dissolved CO_2 in the xylem at night and should therefore consider collecting pH_X directly from gaseous $[CO_2]$ measurement points both at night and during the day.

The long-term temporal similarity in pH_X observed among clear sunny days between July and October and the short-term differences observed over days and before and after a thunderstorm suggest pH_X can be influenced by environmental factors that dramatically affect transpiration rates, such a solar radiation or vapor pressure deficit (VPD). Large increases in pH_X (up to 2.5 units) have been associated with low transpiration rates in *Vitis vinifera* L. (Campbell and Strother, 1996). Beis et al. (2009) suggested that increases in pH_X between predawn and midmorning observations in their study and between 08:00 and 20:00 h in the study by Stoll et al. (2000) were related to the microclimate becoming more stressful. For example, high solar radiation, VPD, and temperature may increase pH_X (Wilkinson and Davies, 2002; Wilkinson, 2004). In our study across three non-consecutive days, total daily solar radiation was similar the first and third sampling day (i.e., sunny days) when pH_X derived from stems was similar (mean 24.7 ± 0.3 MJ m^{-2}), but more than 50% lower (10.82 MJ m^{-2}) on the second sampling day (i.e., cloudy day) when pH_X was higher (Fig. 2). The VPD at the time of pH_X measurement was also similar on sunny days (mean 2.93 ± 0.22 kPa), but VPD on the cloudy day was less than 4% of the mean VPD of the sunny days (Table 1). Similarly, solar radiation on the cloudy day was less than 13% of the mean solar radiation of the sunny days (794.5 ± 71.5 W m^{-2}). Since transpiration is positively correlated with microclimatic factors such as solar radiation and VPD when water stress is not occurring, transpiration rates would have been higher the first and third sampling day when pH_X was lower. Similarly, the cloudy conditions immediately before the thunderstorm would have resulted in dramatically reduced transpiration rates. Thus, it appears that any environmental factor capable of dramatically influencing transpiration, such as solar radiation, VPD, or temperature, may also dramatically affect pH_X.

Although very large changes in environmental conditions that dramatically influence transpiration rates appeared to affect pH_X at short time scales, pH_X was relatively similar (deviating less than 0.2 pH units) among measurement days between July and October (Fig. 3). Although microclimatic conditions varied across these measurement dates (Table 2), all of the days were sunny and allowed for relatively high transpiration rates. For example, VPD on days when pH_X was measured between July and October was never less than 25% of the maximum value during that time period and solar radiation was never less than 52% of the maximum. Thus, even though microclimatic conditions varied across these measurement periods, the differences in microclimate were not nearly as dramatic among the sunny days between July and October as they were between sunny and cloudy days. It is also important to recognize that pH_X can be influenced by more than just microclimate conditions that influence transpiration rates. For example, pH_X dynamics have also been related to factors such as soil water content and nitrate concentration (Gollan et al., 1992). Intracellular pH dynamics can be influenced by nitrogen assimilation (Smith and Raven, 1979; Taulavuori et al., 1997) and pH_X may be influenced by nitrogen assimilation occurring in the shoot (Kirkby and Armstrong, 1980).

The pH_X depends on the difference between the sum of cations and anions, the concentration of ionizable groups, and the partial pressure of CO_2 (Gerendas and Schurr, 1999). McGuire and Teskey (2002) observed that a rain event rapidly increased the gaseous $[CO_2]$ in *Liriodendron tulipifera* L. stems and Saveyn et al. (2008) made a similar observation in *P. deltoides* stems. The gaseous $[CO_2]$ increase presumably occurs when transpiration slows or stops and CO_2 accumulates in the xylem sap. We would expect that accumulation of gaseous CO_2 in stems would cause a decrease in pH_X (Gerendas and Schurr, 1999). However, pH_X was higher on an overcast day compared to clear days and higher under cloud cover immediately before than immediately after a thunderstorm. Although we did not measure CO_2 in this study, Aubrey and Teskey (2009) showed that dissolved $[CO_2]$ at the base of *P. deltoides* stems increased at night. Their calculations were based on a constant diel pH_X, so night time increases in dissolved $[CO_2]$ resulted from increased gaseous $[CO_2]$. However, we observed higher pH_X in the lower stem at night. Thus, it appears that pH_X dynamics are not very sensitive to gaseous $[CO_2]$ and must therefore be more sensitive to cations, anions, and the concentration of ionizable groups in the xylem that are influenced by water transport.

5. Conclusion

Our results suggest pH_X derived from twigs was a suitable proxy for pH_X derived from stems and could therefore be used for calculating liquid phase $[CO_2]$ from gaseous phase $[CO_2]$ measurements in studies of internal carbon transport. We also found that pH_X remained relatively constant over the diel period, indicating high frequency pH_X measurements may not necessarily be critical for accurate measurement of liquid phase $[CO_2]$ over individual days. Thus, our data provide a good indication that common assumptions of pH_X used in studies of internal carbon transport are valid for *P. deltoides*. That pH_X derived from lower stems during the night was higher than pH_X derived elsewhere and showed a diel pattern should be recognized, but should not be a major impediment to internal carbon transport studies focused on dissolved CO_2 movement with the transpirational stream. We do, however, recommend that relationships between the tissue components, as well as diel patterns, be examined before making this assumption as other tree species may not exhibit similar relationships. Environmental conditions that dramatically influence transpiration affect pH_X—presumably by altering the ratio of cations and anions but not by influencing the gaseous $[CO_2]$. Studies designed to understand how transpiration, gaseous $[CO_2]$, and pH_X interact at short temporal scales will improve our understanding and further our ability to measure and predict internal carbon transport.

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