Diurnal variations of needle water isotopic ratios in two pine species

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Received: 13 November 2009 / Revised: 5 February 2010 / Accepted: 24 February 2010 / Published online: 14 March 2010
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Abstract Diurnal fluctuations of leaf water isotope ratios ($\delta^{18}$O and $\delta$D) were measured for Jeffrey (Pinus jeffreyi Balf.) and lodgepole (Pinus contorta Douglas ex Loudon) pine. Two trees per species were sampled every few hours on 15–16 October 2005 and 19–20 June 2006. Diurnal gas exchange was measured during the summer sampling. In fall 2005, leaf water $\delta^{18}$O ranged from 0.7 to 9.0‰, and leaf water $\delta$D ranged from $-70$ to $-50$‰. In summer 2006, leaf water $\delta^{18}$O ranged from 7.7 to 20.7‰, and leaf water $\delta$D ranged from $-61$ to $-24$‰. Diurnal variation of leaf water isotope values typically reached a maximum in early afternoon, began decreasing around midnight, and reached a minimum in mid-morning. Both periods showed a high degree of enrichment relative to source water, with leaf water–source water enrichments ranging up to 37.8‰ for $\delta^{18}$O, and up to 95‰ for $\delta$D. Leaf water enrichment varied by season with summer enrichment being greater than fall enrichment. A steady-state model (i.e., modified Craig–Gordon modeling) for leaf water isotope compositions did not provide a good fit to measured values of leaf water. In summer, a non-steady state model provided a better fit to the measured data than the steady-state model. Our findings demonstrate substantial leaf water enrichment above source water and diurnal variations in the isotopic composition of leaf water, which has application to understanding short-term variability of atmospheric gases (water vapor, CO$_2$, O$_2$), climate studies based on the isotopic composition of tree rings, and ecosystem water fluxes.

Keywords Hydrogen and oxygen isotopes · Leaf gas exchange · Pinus jeffreyi · Pinus contorta

Introduction

Controls on the isotopic composition of leaf water have received increasing attention because of their importance for evaluating fluxes from different ecosystem pools of water (Wang and Yakir 2000), affecting the stable isotopic ratios of O$_2$ produced during photosynthesis (Hoffmann et al. 2004), determining climatic change from plant tissue (Gessler et al. 2007), and for understanding variations in $\delta^{18}$O of atmospheric CO$_2$ that are driven by vegetation (Barbour et al. 2007). During plant water uptake, there is generally no isotopic fractionation as water moves from the soil to the roots and through the xylem (Flanagan and Ehleringer 1991). However, evapotranspiration causes residual leaf water to become isotopically enriched because the lighter isotopes (containing $^{16}$O or $^1$H) are preferentially evaporated from the leaf surface to the atmosphere (Flanagan and Ehleringer 1991). This difference in isotopic composition of leaf water versus source water is important if leaf water is used for understanding water fluxes (Wang and Yakir 2000). Additionally, CO$_2$ diffusing in and out of...
the leaf equilibrates with leaf water thus influencing the oxygen isotopic ratios of CO₂ in the atmosphere and is useful for understanding global carbon cycles. Controls on the stable isotopic composition of leaf water are complex, and influenced by temperature, humidity, isotopic variability in soil moisture, differences in water uptake between C3, C4, and CAM plants, leaf boundary layers (Cooper and DeNiro 1989), the degree of stomatal control, and heterogeneity within a single leaf (Farquhar and Gan 2003; Luo and Sternberg 1992). Heterogeneity in leaf water evaporative enrichment can occur within a single leaf because the back diffusion of enriched leaf water away from the site of evaporation co-occurs with the convection of un-enriched xylem water towards the site of evaporation (Farquhar and Gan 2003), known as the Péclet effect. Furthermore, bulk leaf water may differ from vapor in the stomatal cavity because of the effects of fractionation associated with the phase transition from liquid water to vapor, and the diffusion of vapor through stomatal and boundary layers (Farquhar and Lloyd 1993; Farquhar and Cernusak 2005). The isotopic composition of water at the sites of evaporation within the leaf stomatal cavity, rather than bulk leaf water, impacts control upon the isotopic composition of gases (e.g., water vapor, CO₂, O₂) that exit through the stomata because this is where CO₂ equilibrates with leaf water (Farquhar and Cernusak 2005). Bulk leaf water isotope compositions are potentially highly variable (Cernusak et al. 2002, 2004; Flanagan and Ehleringer 1991), and because it is difficult to directly measure vapor isotope compositions in the stomatal cavity and liquid water only at the sites of evaporation, empirical studies of in situ bulk leaf water enrichments are needed to determine which models of evaporative enrichment are applicable.

One question is whether to apply steady-state or non-steady state assumptions for modeling leaf water isotopic composition through time. The steady-state model (i.e., modified Craig–Gordon model) modified the equations for evaporation from open bodies of water to fit evaporation from leaves and takes into account the above-mentioned diffusion of vapor through the stomatal and boundary layers (Craig and Gordon 1965; Dongmann et al. 1974; Farquhar et al. 1989; Flanagan and Ehleringer 1991). Steady-state modeling requires that the isotopic composition of transpired water be equal to that of the source water (Flanagan and Ehleringer 1991; Yakir 1992), and implies that plants instantly adjust to changes in environment. Increased transpiration resulting from decreased relative humidity leads to increased leaf water enrichment. However, if increased transpiration is the result of increased stomatal conductance, this would reduce enrichment because kinetic fractionation effects and leaf temperature effects predominate (see review by Farquhar et al. 2007). Therefore, the expected relationship is that decreased stomatal conductance will lead to increased enrichment.

The non-steady state model does not require transpired water and source water isotopic values to be equal at a given air humidity (Cernusak et al. 2002; Farquhar and Cernusak 2005; Flanagan and Ehleringer 1991; Lai et al. 2006; Yakir and Sternberg 2000). Assumptions of steady state under field conditions may not be valid because of rapid changes in humidity, leaf energy balance, and water availability that affect plant stomatal conductance and transpiration rates, leaf water volumes and leaf water residences times (Cernusak et al. 2002; Farquhar and Cernusak 2005; Lai et al. 2006). In this way, changes in stomatal conductance, transpiration and leaf water content are propagated into changes in leaf water enrichment.

Diurnal processes can significantly change the isotopic composition of leaf water, and hence the isotopic composition of transpired water vapor, CO₂, and organic compounds (Cernusak et al. 2005, 2002; Gessler et al. 2007; Lai et al. 2006; Seibt et al. 2006, 2007). During photosynthesis, enriched leaf water is used to synthesize carbohydrates, which are then exported and used to build stem tissues. It is primarily daytime leaf water enrichment that is recorded, but post-photosynthetic exchange can also influence the isotopic composition recorded by plant organic material (Shu et al. 2005). The expectation is that for plants growing in the same environment, enrichment will decrease as transpiration increases (Farquhar et al. 2007). However, this expectation is based on steady-state assumptions and may not hold when there are large departures from steady state.

Studies on plant species other than Pinus performed in controlled environments found that diurnal fluctuations in leaf water isotopic values were better predicted under non-steady state assumptions than steady-state assumptions (Cernusak et al. 2002; Farquhar and Cernusak 2005; Farquhar and Gan 2003; Flanagan and Ehleringer 1991; Wang and Yakir 1995). In situ field studies are uncommon for other species, but recent field studies of conifer species found that non-steady state conditions more robustly predicted leaf water enrichment values in needles than steady-state conditions (Pendall et al. 2005; Lai et al. 2006; Seibt et al. 2006, 2007; Barnard et al. 2007). Of the two field studies performed on the genus Pinus, one considered a full 24 diurnal cycle (Barnard et al. 2007), while the other did not (Pendall et al. 2005).

We investigated the diurnal variation of bulk leaf water stable isotopes (δ¹⁸O and δD), in two Pinus species that have not been previously measured, Jeffrey pine (Pinus jeffreyi Balf.) and lodgepole pine (Pinus contorta Douglas ex Louden). The emphasis was on natural patterns of diurnal variations and how these patterns were described by both a steady-state and non-steady state model.
Sampling was conducted during summer and fall. Gas exchange parameters were measured during the summer. Steady-state results were compared against measured values for both seasons, and with non-steady state results during the summer. Because the steady-state model is easier to estimate than the non-steady state model, it is instructive to know when the steady-state model is a reasonable approximation of leaf water enrichment.

Materials and methods

Study area

Samples were collected at Little Valley, Nevada, which lies on the east slope of the Sierra Nevada and is part of the Whittell Wildlife Area managed by the University of Nevada, Reno. Sampled trees were spread over an area approximately 400 m² in size, at an elevation of 1,990 m. The annual climatic regime is summer-dry winter-wet, with most precipitation falling in the form of snow. Based on a 30-year average, mean monthly maximum temperature reaches 26°C in July, and mean monthly minimum temperature is as low as −9°C in December (Vander Wall 2005). Forest cover is dominated by conifers, mostly Jeffrey pine (P. jeffreyi) and lodgepole pine (P. contorta), with some white fir (Abies concolor) and some groves of quaking aspen (Populus tremuloides).

Field methods

A large (>80 cm in diameter at 1–1.4 m above ground) and a small (<25 cm in diameter at 1–1.4 m above ground) Jeffrey pine and lodgepole pine (for a total of four trees) were carefully selected so that they were healthy and free of recent disturbance, within the 400-m² plot, all trees were on the same aspect, the small trees were located downhill, of the larger trees on a lesser slope. Needles were hand collected using pruning shears, placed into glass vials, and quickly sealed with parafilm. These samples were collected from the lower crown, mixing needles from different fascicles at the terminal end of the branches, thus representing a bulk sample of recent needles. Canopy relative humidity and air temperature were measured using a handheld sensor. Xylem samples were collected using a 12-mm, battery-powered, drilling bit from adjacent trees (these large cores would have otherwise heavily damaged the trees that were sampled for needles). Xylem water was sampled in adjacent trees of both species between one and three xylem samples were taken. All samples were then placed into a cooler for transport and long-term storage.

Needle samples were obtained on 15–16 October 2005, and 19–20 June 2006. Observations on solar radiation, dew point, air temperature, and precipitation were available from the Little Valley weather station, located less than 1 km from the sampled area. Those weather data were downloaded from the Western Regional Climate Center (http://www.wrcc.dri.edu), and described by Monnar (2007). Weather conditions varied during the fall diurnal cycle, with rain on 15th October from a fast moving storm. Collection was delayed until humidity was less than dew point. Average air temperature on 15th October was 5.4°C, and the total precipitation for the day was 4.8 mm. 16th October was a little cooler (average air temperature of 3.8°C), and without precipitation. Weather conditions were less variable in the summer, with no precipitation on both days. 19th June was sunny, with average air temperature of 15.6°C, and on the following day average air temperature was 14.9°C.

During the summer sampling event, measurements of CO₂ gas exchange (μmol CO₂ m⁻² s⁻¹), transpiration (mmol H₂O m⁻² s⁻¹), and stomatal conductance (mol H₂O m⁻² s⁻¹) were made using a LI-6400 portable infrared gas analyzer (Li-Cor, Lincoln, NE). The LI-6400 analyzer was used with a clear conifer chamber adapter to maximize total leaf area within the chamber, and to track ambient light conditions. The conifer chamber is made of lexan lined with Teflon and approximates normal photosynthetically active radiation within 10% of ambient light conditions. The formulas used to determine gas exchange followed von Caemmerer and Farquhar (1981). Leaf area was measured by digital image analysis (O’Neal et al. 2002), and used in conjunction with the gas exchange measurements to assess net photosynthesis, transpiration, and stomatal conductance.

Laboratory methods and data analysis

Water extraction was performed by azeotropic distillation using toluene, after the method of Revesz and Woods (1990). Leaf water concentration was calculated from the weight of the extracted waters divided by the weight of the wet samples. Leaf water residence times were calculated by dividing the weight of water per unit leaf area by the transpiration rate measured with the LI-6400. Water-δ¹⁸O analyses were performed using the CO₂ equilibration technique (Epstein and Mayeda 1953), and water-δD analyses were performed using an on-line chromium reduction method (Morrison et al. 2001). Isotope analyses are reported in the usual δ notation versus VSMOW, and have precision (one standard deviation) of ±0.1‰ for δ¹⁸O and ±1‰ for δD.

Steady-state modeling of leaf water isotopic compositions was performed using a modified Craig–Gordon
equation (Cernusak et al. 2002; Craig and Gordon 1965; Farquhar and Cernusak 2005; Muttiah et al. 2005):
\[ \delta_L = \delta_S + \delta_k + \varepsilon_e + (\delta_V - \delta_S - \delta_k) h \]  
(1)
where \(\delta_S\) is the isotopic composition of the source water (assumed to be the same as xylem water); \(\delta_k\) is the kinetic fractionation factor due to diffusion through stomata and leaf boundary layer (Farquhar and Lloyd 1993); \(\varepsilon_e\) is the equilibrium liquid–vapor fractionation factor at leaf temperature (Majoube 1971). Leaf temperature was assumed to follow canopy air temperature. \(\delta_V\) is the isotopic composition of vapor in the atmosphere, assumed to be in equilibrium with source water (Muttiah et al. 2005); and \(h\) is the leaf boundary relative humidity, assumed to be in equilibrium with the measured canopy relative humidity (%). The kinetic fractionation factor for oxygen can be estimated as:
\[ \varepsilon_k = \frac{(32r_s + 21r_b)}{(r_s + r_b)} \]
where \(r_s\) and \(r_b\) are stomatal and boundary layer resistances, and 32 and 21%o are the respective associated oxygen isotope fractionation factors (Cappa et al. 2003).
However, since there was no gas exchange data for the fall sampling, we used an approximation for both seasons. Since conifer needles have low stomatal conductance and leaf area is small, thereby increasing boundary layer conductance, the stomatal term will dominate and the equation will approach 32%, which we used as reasonable approximation. For hydrogen, the same equation was used with respective fractionation coefficients of 16 and 11%, and a reasonable approximation of 16% was used.

The gas exchange measurements performed in summer 2006 allowed for non-steady state modeling to be performed (Cernusak et al. 2002; Farquhar and Cernusak 2005; Farquhar et al. 2007; Pendall et al. 2005):
\[ \Delta_{en} = \Delta_{es} - [(d(W_{en})/dr)/(gw_i)] \]  
(2)
where \(\Delta_{en}\) is the calculated non-steady state value of isotopic composition at the site of evaporation; \(\Delta_{es}\) is the calculated steady-state value (from Eq. 1) of isotopic composition at the site of evaporation; \(W\) (mol m\(^{-2}\)) is the leaf water concentration; \(g\) (mol m\(^{-2}\) s\(^{-1}\)) is the total leaf conductance to water vapor; and \(w_i\) is the mole fraction of vapor in the intercellular spaces. The model was initialized with the measured leaf water enrichment values for hydrogen and oxygen at 1800 h on 19th June. This was used as the start time because it was when the first gas exchange measurements were made concurrent with more frequent measurements of leaf water isotopic composition (Cernusak et al. 2002; Pendall et al. 2005). The model was solved iteratively using the Microsoft Excel solver function (Cernusak et al. 2002; Pendall et al. 2005). The \(g\) parameter was determined from the gas exchange measurements, and \(w_i\) was calculated from leaf temperature at full saturation (see Pearcy et al. 1990).

A two-way ANOVA was used to determine the effect of species and tree size (and their interaction) on observed leaf water enrichment values (\(\delta D\) and \(\delta^{18}O\)). Data were inspected to meet the assumptions of ANOVA. Significance differences were reported (\(\alpha < 0.05\)). Due to small sample size (only four trees were sampled) these data are only indications of trends and are interpreted with caution. Linear correlation was used to determine if there was a relationship between leaf water enrichment and measured variables (relative humidity, photosynthetic rate, stomatal conductance, transpiration and canopy temperature).

**Results**

**Diurnal variations, fall 2005**

Leaf water \(\delta^{18}O\) ranged from 0.7 to 9.0%, and leaf water \(\delta D\) ranged from \(-70\) to \(-50\%). Xylem water \(\delta^{18}O\) and \(\delta D\) was characterized at \(-13.6\) and \(-101\%), respectively, for Jeffrey pine and \(-13.9\) and \(-104\%) for lodgepole pine. In general, measured leaf water \(\delta^{18}O\) decreased in the morning between 7 and 9 am, reached a minimum between 9 and 11 am, and then increased to a maximum in the afternoon around 2 pm. Measured leaf water \(\delta D\) showed very similar patterns, decreasing in the morning between 7 and 9 am, reaching a minimum between 9 and 11 am, and then increasing to a maximum around 2 pm (Fig. 1). Overall for all four trees, there was no significant correlation between needle water isotopic ratios (\(\delta^{18}O\) and \(\delta D\)) and canopy temperature or relative humidity. This lack of correlation held true when correlations were run for subsets of the data by species and size. In addition to showing pronounced diurnal variation, \(\delta^{18}O\) and \(\delta D\) were substantially enriched above source water (Fig. 1) ranging from 14.3 to 22.9%o for \(\delta^{18}O\) and 31–53%o for \(\delta D\). There was no main effect of species or tree size on leaf water isotope values (\(p > 0.14\)), but there was a significant interaction effect of size and species (\(p < 0.003\)), which is likely due to the small sample size.

**Diurnal variations, summer 2006**

Leaf water \(\delta^{18}O\) ranged from 7.7 to 20.7%, and leaf water \(\delta D\) ranged from \(-61\) to \(-24\%). Xylem water \(\delta^{18}O\) and \(\delta D\) was characterized at \(-13.3 \pm 0.1\%) (1 SE) and \(-100 \pm 1.0\%), respectively, for Jeffrey pine and \(-17.1 \pm 0.6\) and \(-119 \pm 7.0\%) for lodgepole pine. Measured leaf water \(\delta^{18}O\) decreased in the morning, reaching a minimum between 8 and 10 am, then increased...
in the in mid-morning to early afternoon, reaching a maximum by 2–3 pm. Measured leaf water δD followed similar patterns, decreasing in the morning from 8 to 9 am, reaching a maximum between 12 and 1 pm, and then decreasing (Fig. 2). There was also another pronounced minimum on 19th June in the afternoon between 5 and 6 pm (Fig. 2). Similar to the fall sampling, leaf water was substantially enriched above source water (Fig. 2) ranging from 21.0 to 37.8‰ for δ18O and 39 to 95‰ for δD. A significant difference (p value < 0.0002) was detected between species, with lodgepole pine being more enriched than Jeffrey pine in both δD and δ18O.

Summer gas exchange data showed photosynthetic activity during daytime, with a maximum of 6 μmol CO2 m⁻² s⁻¹ at 1 pm (Fig. 3). One data point erroneously indicated nighttime photosynthesis and was likely due to a transient leak in the chamber for that particular measurement point. Transpiration increased from near zero in two of the trees at 7 am and was above zero in all trees by 10 am. Transpiration continued to increase in the afternoon in all but one tree. Stomatal conductance peaked at 7 am in two of the trees at 75 mmol H₂O m⁻² s⁻¹, was above zero in all trees by the 9 am sampling period, and remained above zero in all but one of the trees until the last measurement period at 3 pm. In general, stomatal conductance was near zero during dark hours, although two trees showed evidence of nighttime stomatal conductance.

The δ18O of leaf water for all trees sampled had a significant correlation with relative humidity (p = 0.004). Significant correlations (p < 0.05) of δ18O and relative humidity held for most groupings (small trees, each species), but not for large trees. However, δ18O of leaf water was not correlated with photosynthetic rate, stomatal conductance, transpiration or canopy temperature for all the data or any subset of the data (by size or by species). The δD of leaf water was not significantly correlated with any variable for any grouping. Relative humidity, leaf water concentration and calculated leaf water residences times are plotted for the summer diurnal event (Fig. 4). Relative humidity increased at night and sharply declined throughout the day reaching values of approximately 10%. There was no consistent pattern in leaf water concentration. Leaf water residences times (calculated as the concentration of leaf water per unit area divided by the transpiration rate) were generally between 500 and 100 min, except under low-light or dark conditions when transpiration rates were very slow or zero, then residence times increased and approached infinity as transpiration rate approached zero. In order to conveniently plot all residence time calculations in a single figure, the very long residence times calculated when transpiration rates were very slow or zero, have therefore been assigned and plotted with a residence time of greater the 5,000 h. (see gray box in Fig. 4.)

Fig. 1 Diurnal variations of leaf water δ18O and δD, and leaf water–source water enrichments for Pinus contorta and Pinus jeffreyi on 15–16 October 2005. Gray shading indicates dark hours.
A comparison of measured versus steady-state model isotopic compositions for the fall 2005 sampling event is presented in Fig. 5. Comparisons of measured versus steady-state and non-steady state model isotopic compositions for the summer 2006 sampling event are presented in Figs. 6 and 7 for $\delta^{18}O$ and $\delta D$, respectively. Steady-state modeled isotopic compositions generally show poor agreement with measured leaf water values (Figs. 5, 6, 7). Typically, the steady-state values overestimate the amount of isotopic enrichment during the daylight hours, and underestimate the amount of isotopic enrichment during the nighttime hours. In contrast, the non-steady state model values generally show a much better correlation with the measured leaf water values (Figs. 6, 7), although this comparison could only be performed for the summer 2006 sampling.

Discussion

A striking result was the strong diurnal variation in leaf water isotopic values and the high levels of enrichment above source water. The magnitudes of these diurnal fluctuations were 4.8–6.8‰ for $\delta^{18}O$, and 7–18‰ for $\delta D$ in fall 2005; 6.5–11.5‰ for $\delta^{18}O$, and 12–34‰ for $\delta D$ in summer 2006 (Figs. 1, 2). The general pattern of mid-afternoon maxima, due to progressive enrichment of residual leaf water from continued loss of isotopically light evaporated water, followed by early morning minima, which was likely due to the recharge of isotopically light source water to the leaf, agrees well with the general pattern found in other studies (such as Cernusak et al. 2002). Some previous studies (such as Cernusak et al. 2002) showed smooth diurnal variation of isotopic compositions, whereas diurnal isotopic changes in this field study indicate a greater amount of non-systematic variation on short time scales.

The high degree of enrichment above source water (up to 37.8‰ for $\delta^{18}O$ and 95‰ for $\delta D$) is indicative of low stomatal conductance which produces progressive enrichment above source water. This is supported by the summer gas exchange data (Fig. 3). Published gas exchange rates for these species are uncommon, but a study performed in Montana and Idaho found that mean maximum photosynthesis rates of lodgepole pine averaged

1 The absence of LI-COR gas exchange measurements precluded calculation of the non-steady state model.
around 10 \( \text{mol m}^{-2} \text{s}^{-1} \) (Korol 2001) and conductance was 370 \( \text{mmol m}^{-2} \text{s}^{-1} \), which are greater than rates found in the current study (Fig. 3).

Leaf water residence times were generally between 1.6 and 8.4 h (Fig. 4), which compares to residence times of 2–82 h for pinyon pine (Pendall et al. 2005), and a residence time of 11 h for Douglas fir (Lai et al. 2006). The extreme variation in pinyon pine hampers comparison with the current study; however, it seems reasonable that these residence times are long enough so that trees are not operating under steady-state conditions. Some of the observed non-systematic variation in leaf water enrichment...
observed through time may be due to sampling on different pine needles where differences in the light environment and other abiotic factors lead to accumulated differences in enrichment within a single tree. However, even with this potential limitation, there was a consistent diurnal pattern across all sampled trees.

A high degree of isotopic enrichment has been documented in another pine species (*Pinus hartwegii*) where leaf water versus source water enrichment reached up to 40.6% for $\delta^{18}O$, and up to 104% for $\delta^D$ (Hartsough et al. 2008). Other studies on conifers have reported leaf water versus source water enrichments of $\delta^{18}O$ between 12 and 35% (Pendall et al. 2005; Lai et al. 2006; Seibt et al. 2006; Barnard et al. 2007). Isotopic enrichments of leaf water versus source water in the current study are high (especially during the summer), but fall within the range of other published studies.

Overall, comparison to other field studies on conifers showed that the diurnal isotopic variation measured in this study was much higher than reported by Pendall et al. (2005) for pinyon pine (*Pinus edulis* and *P. monophylla*) in the Great Basin and southwest US, and slightly higher than reported for Sitka spruce (*Picea sitchensis*) in Scotland (Seibt et al. 2007). Values in the current study were similar to that of Douglas fir (*Pseudotsuga menziesii*) in the Pacific Northwest (Lai et al. 2006) and Scots pine (*Pinus sylvestris*) in Germany (Barnard et al. 2007). We uncovered seasonal differences in diurnal cycles, with fall $\delta^{18}O$ and $\delta^D$ values being less enriched than summer values (Figs. 1, 2), which suggests that evaporative demand was greater in summer 2006 than fall 2005. Summer data showed that lodgepole pine was more enriched than Jeffrey pine. This suggests that stomatal conductance was lower in lodgepole pine, which is consistent with the gas exchange data (Fig. 3).

The steady-state model values showed a poor fit with measured values in both the fall and summer sampling events. Suggesting that the evaporative flux term ($g_w$), found in the non-steady state equation, was not relatively high in either sampling period (Cernusak et al. 2002), because if evaporative flux is high the non-steady state model approaches the steady-state value (see Eq. 2). Steady-state modeling overestimated enrichment during the day and underestimated enrichment at night similar to reports on other conifer species (Lai et al. 2006; Barnard et al. 2007). Lai et al. (2006) estimated the Péclet effect to

![Figure 5: Comparison of steady-state (modified Craig–Gordon) model values with measured values for leaf water $\delta^{18}O$ and $\delta^D$ for *Pinus contorta* and *Pinus jeffreyi* on 15–16 October 2005. Gray shading indicates dark hours.](Image)
see if it improved the steady-state model, but there was no marked improvement. Our findings indicate that interpretation of the $\delta^{18}O$ and $\delta D$ in organic material, especially in tree rings, may not follow steady-state predictions (Farquhar et al. 2007), as we found poor agreement with the steady-state model.

In contrast, non-steady state modeling estimations matched measured isotopic compositions much more closely than the steady-state model (Figs. 6, 7). The non-steady state model followed measured enrichment and depletion patterns relatively well, especially during the night. Better performance of non-steady state modeling over the steady-state model has also been observed in a number of previous studies (Cernusak et al. 2002; Farquhar and Cernusak 2005; Farquhar and Gan 2003; Flanagan and Ehleringer 1991; Lai et al. 2006; Pendall et al. 2005; Seibt et al. 2007; Wang and Yakir 1995).

Conclusions

This study revealed distinct diurnal variation in $\delta D$ and $\delta^{18}O$ of leaf water, and a high degree of isotopic enrichment of leaf water above source water for Jeffrey and lodgepole pines in the Sierra Nevada. Diurnal changes in isotopic composition were largest during the summer, but were also substantial in the fall. Relative humidity, canopy temperature and leaf gas exchange parameter were not well correlated with leaf water isotope values. A non-steady state model was a much better estimator of leaf water isotopic ratios than a steady-state (modified Craig–Gordon) model, in agreement with previous studies.

Our results illustrate the importance of considering the diurnal variation of the isotopic composition of leaf water, with consequent implications for studies of the isotopic composition of various atmospheric gases (water vapor, CO$_2$, O$_2$), climate studies based on the isotopic composition of plant tissues such as tree rings, and investigations of ecosystem water fluxes. Particularly for the genus Pinus, reconstruction of climatic records based on the $\delta^{18}O$ in tree rings may need to make adjustment for non-steady state conditions. Further consideration of the diurnal variation of the isotopic composition of leaf water (especially if these can be performed with increased spatial resolution), in conjunction with the rapid and accurate isotope analyses which can be performed at high temporal and spatial

Fig. 6 Comparison of steady-state (modified Craig–Gordon) and non-steady state model values with measured values for leaf water $\delta^{18}O$ for Pinus contorta and Pinus jeffreyi on 19–20 June 2006. Gray shading indicates dark hours
resolution using the newly developed technique of tunable diode laser absorption spectrometry, promises to yield improved insight into the complex behavior of water and other gases in natural environments.

Acknowledgments This work originated from the second author’s Master’s thesis at the University of Nevada, Reno, and was supported, in part, by the Whittell Forest Graduate Fellowship to R. Monnar and by National Science Foundation grant ATM-CAREER-0132631. We are grateful to Dr. Stephen B. Vander Wall for logistical support at the Whittell Forest (Little Valley). This manuscript benefited greatly from the comments of three anonymous reviewers.

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Fig. 7 Comparison of steady-state (modified Craig–Gordon) and non-steady state model values with measured values for leaf water δD for Pinus contorta and Pinus jeffreyi on 19–20 June 2006. Gray shading indicates dark hours.


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