

## CHAPTER 2

### ESTABLISHING A GRASSLAND SIGNATURE IN VEINS: $^{18}\text{O}$ IN THE LEAF WATER OF $\text{C}_3$ AND $\text{C}_4$ GRASSES

#### **Abstract**

It is shown that  $^{18}\text{O}$  evaporative enrichment of bulk leaf water in grass species can be significantly more enriched than predicted by the Craig-Gordon model, with  $\text{C}_4$  grasses considerably more enriched than  $\text{C}_3$  grasses. The results suggest that the unanticipated  $^{18}\text{O}$  leaf water enrichment of grasses is attributable to the progressive evaporative enrichment along parallel veins (a function of both leaf length and interveinal distance), a pattern which does not occur in Dicotyledonous species. It is proposed that the differential  $^{18}\text{O}$  enrichment of grasses will result in distinct  $\text{C}^{18}\text{O}^{16}\text{O}$  biospheric signals from grassland and forest ecosystems, allowing for further partitioning of terrestrial carbon fluxes.

## Introduction

The evaporative isotopic enrichment of  $^{18}\text{O}$  in surface waters (such as lakes) was described by Craig and Gordon (1965) more than three decades ago and later applied to leaves, which also undergo an isotopic enrichment during transpiration (Dongmann et al. 1974, Farris and Strain 1978, Flanagan et al. 1991a, Yakir et al. 1990). Application of the Craig-Gordon model to leaves from trees and herbs has been shown experimentally to be robust over a wide range of environmental conditions (Flanagan et al. 1991a, Flanagan et al. 1991b, Roden and Ehleringer, 1999). Capitalizing on this established relationship, Farquhar and colleagues (1993) showed that the equilibration of  $^{18}\text{O}$  between  $\text{CO}_2$  and leaf water could be used to partition the gross fluxes of  $\text{CO}_2$  from vegetation on a global basis.

Previous studies showed that there were no differences in bulk leaf water  $^{18}\text{O}$  enrichment of Dicotyledonous plants possessing either the  $\text{C}_3$  or  $\text{C}_4$  photosynthetic pathways and that leaf water behaved similar to predictions of the Craig-Gordon model (Flanagan et al. 1991a,b). Based on these consistent results, global modeling efforts have used the Craig-Gordon model for estimates of leaf water  $\delta^{18}\text{O}$  ( $\delta^{18}\text{O}_{\text{wl}}$ , SMOW scale throughout) values that are needed to calculate the  $\text{C}^{18}\text{O}^{16}\text{O}$  retrodiffusing from terrestrial ecosystems to the atmosphere (Farquhar et al. 1993, Ciais et al. 1997, Ciais et al. 1998, Flanagan et al. 1994). However, the Craig-Gordon model of evaporative leaf-water enrichment has remained untested on grasses and other Monocotyledonous species. A fundamental difference between monocots and dicots which hinted that

$\delta^{18}\text{O}_{\text{wl}}$  might be different is the veinal structure of leaves, with monocot leaves having parallel veins while dicot leaves primarily have a reticulate veinal structure. It was hypothesized that grass leaves will exhibit a leaf-water  $^{18}\text{O}$  evaporative enrichment pattern that is not consistent with the commonly used single-water-source version of the Craig-Gordon model, because the nature of water movement along parallel veins in a grass leaf is more analogous to movement of water through a sequence of pools in series.

I investigated possible  $^{18}\text{O}$  differences in leaf water enrichment with a variety of  $\text{C}_3$  and  $\text{C}_4$  grasses, because grasses with different photosynthetic pathway are known to have different interveinal differences (Kawamitsu et al. 1985, Dengler et al. 1994). The hypotheses were (a) leaf water  $^{18}\text{O}$  enrichment of grasses should be greater than observed in dicots and (b) leaf water  $^{18}\text{O}$  enrichment of  $\text{C}_4$  grasses (narrower interveinal distances) should be greater than leaf water  $^{18}\text{O}$  enrichment of  $\text{C}_3$  grasses (wider interveinal distances).

## Methods

### *Plant material and growth conditions.*

In the pilot experiment, we grew *Lolium multiflorum* ( $\text{C}_3$  grass), *Muhlenbergia wrightii* ( $\text{C}_4$  grass), and *Helianthus annuus* ( $\text{C}_3$  dicot) ( $n = 6$  for each species) from seed under identical environmental conditions with an average midday relative humidity of 40

%). In the survey experiment, five C<sub>3</sub> grasses and five C<sub>4</sub> grasses were used (species listed in Table 1). For the survey experiment n=3 for each species and average midday relative humidity was 35 %. The growth conditions and experimental protocol were identical for both experiments except where specified below.

Plants for all experiments were grown in the greenhouse in a manner that kept the root systems completely submerged in an isotopically constant water source, while the aerial portions of the plants experienced greenhouse conditions. Plants were grown from germination in 1 L pots in a sterilized soil/perlite/vermiculite (1:1:1) mixture and the pots were placed in 190 L open top plastic chambers (stock tanks, Rubbermaid Inc., Wooster, OH). To avoid evaporative enrichment and to keep source water constant, a flow-through system was developed in which the chambers were fitted with water entry and exit ports. Tap water was gravity fed to the chambers via a system of hoses from a 658 L buffer tank. Closed cell foam was placed at water level to further assure that no evaporative enrichment of source water occurred. The water level within the chambers was kept constant and at a level which submerged all but the top 1 cm of soil in each pot. Source water was oxygenated by two aquarium pumps, with the air flow separated to ten diffusers distributed evenly throughout the bottom of the chambers. A nutrient solution was supplied to the plants once a week by addition to the large buffer tank. Oscillating fans were placed next to the chambers to mitigate the propagation of leaf boundary layer differences between species. Wet bulb/dry bulb thermocouples were situated at plant height to measure air temperature and relative humidity throughout the experiments. A light sensor (Licor, Lincoln, NE, USA ) was placed at plant height to

measure irradiance which averaged approximately  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the pilot experiment and  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the survey experiment, both for a sixteen hour photoperiod. Thermocouples and light sensor readings were recorded at fifteen minute intervals with a datalogger (Campbell Scientific, model 21X, Logan, UT, USA).

### *Sample collection.*

Immediately prior to sampling leaf material, water samples of inlet and outlet water were taken to ensure no evaporative enrichment was occurring within the chambers. During this time water vapor in air was collected by drawing air through an ethanol dry ice trap (see Chapter 6). Leaf temperature was measured prior to sampling leaf material. In the pilot experiment, samples were taken for leaf water  $\delta^{18}\text{O}$  once per week for a three-week period. In the survey experiment leaf water was sampled at one time period. With the exception of Figure 2.2, all leaf water  $\delta^{18}\text{O}$  values represent the entire leaf blade from ligule to tip.

### *Anatomical measurements*

Immediately prior to sampling for leaf water in the survey experiments, leaf blade length was measured. Using a second set of leaves, measurements of interveinal distance were made by excising the whole, fully expanded leaves of three plants per species and removing the middle, widest portion of the leaf. The middle portions were free-hand sectioned into 1-mm sections and placed in petry dishes filled with a  $\text{H}_2\text{O}/\text{Clorox}$  solution (80:20) for approximately one hour until fully cleared. Sections

were mounted in glycerol and interveinal distance measured with a light microscope coupled with a drawing tube and bit pad (Donsanto Microplan II, Natuck, MA).

### *Isotope analysis*

Leaf water was extracted by cryogenic distillation as detailed in Ehleringer and Osmond (1989). For  $\delta^{18}\text{O}$  analysis of all water samples the equilibration technique of Socki et al. (1992) was used with the following modifications: 20-500  $\mu\text{l}$  of water was introduced by syringe into a 6-ml headspace vial, sealed with a 20-mm Hycor Septum (Alltech, Deerfield, IL). Prior to injection of water, the headspace vial was evacuated and backfilled to atmospheric pressure (measured at the time of vial preparation) with air of 9:1,  $\text{N}_2:\text{CO}_2$ . The  $\text{CO}_2$  and water samples were allowed to equilibrate for 48-72 hours within the headspace vials. A 250- $\mu\text{l}$  gas tight lockable syringe (VICI Precision Sampling, Baton Rouge, LA) was used to inject 50-150  $\mu\text{l}$  of air from the headspace vial into the helium carrier stream of a Varian GC (Model 3300, Walnut Creek, CA) via a split/splitless on-column injector (approximately a 1:10 split). The sample was carried through a 50-m plot column (Porapak-Q) and on through a 1:2 fixed post column split interface to the MS (Model 252, Finnigan MAT) in continuous flow mode. Prior to injection of the sample into the column, the syringe was filled and evacuated with a 'pre-sample' to purge the syringe. After purging the syringe with the pre-sample, the syringe was filled with a sample and over-pressurized to ensure that the needle dead space volume was cleansed before injection into the PreCon. Following every ten samples analyzed, one each of two laboratory standards (BSLC, -15.5 ‰ and SHOW, -3.2 ‰,

VSMOW scale). The two laboratory water standards were analyzed in volumes from 10-500  $\mu\text{l}$  with this modified  $\text{H}_2\text{O}:\text{CO}_2$  equilibration headspace vial technique and showed no significant variation related to water volume. Precision for all isotope analysis was 0.2 ‰.

## Results and Discussion

In applying the Craig-Gordon model to leaves, two primary assumptions are made: (a) the leaf represents a single well-mixed pool into which water enters from the petiole and from which water exits solely by evapotranspiration; (b) the leaf is at isotopic steady-state, where the isotopic composition of the transpiration stream is identical to the water entering the leaf through the petiole. The isotopic composition of water in the leaf is then a function of known fractionation factors which are controlled by leaf temperature, the evaporative gradient, the leaf boundary layer and the isotopic composition of atmospheric water vapor. All previous studies using the Craig-Gordon model to predict leaf water  $\delta^{18}\text{O}$  have shown the model to work well, or in some cases the predicted  $\delta^{18}\text{O}_{\text{wl}}$  was more enriched than what was actually observed (Flanagan et al. 1991a,b, Yakir et al. 1990, Yakir et al. 1994, Wang and Yakir 1995, Roden and Ehleringer 1999). The explanation for the latter observation is that the leaf does not represent a perfectly-mixed pool and that some contribution of unenriched vein water in bulk leaf water tends decrease the observed  $^{18}\text{O}$  enrichment. If the primary assumptions

are met and accurate measures of all parameters necessary to calculate predictions are made, the  $\delta^{18}\text{O}$  of leaf water cannot exceed predictions of the Craig-Gordon model.

After several weeks of growth in the pilot experiment, bulk leaf water (from ligule to leaf blade tip in the grasses)  $\delta^{18}\text{O}$  values were sampled as well as other source and atmospheric parameters necessary to evaluate the Craig-Gordon model (Roden and Ehleringer 1997) one day a week for three successive weeks. The  $\delta^{18}\text{O}_{\text{wl}}$  value for the  $\text{C}_4$  grass ( $14.7 \pm 3.0 \text{ ‰}$ ) was significantly and consistently more enriched than that of the  $\text{C}_3$  grass ( $7.2 \pm 2.0 \text{ ‰}$ ;  $p < 0.01$ ), which was significantly more enriched than the  $\text{C}_3$  dicot ( $3.9 \pm 1.0 \text{ ‰}$ ;  $p < 0.01$ ) under midday growth conditions. The observed  $\delta^{18}\text{O}_{\text{wl}}$  values for the dicot leaf were consistent with predictions of the Craig-Gordon model (4.1 ‰). However, the observed  $\delta^{18}\text{O}_{\text{wl}}$  values in both grasses were consistently more enriched than predicted by the Craig-Gordon model: 7.2 ‰ versus 4.7 ‰ for the  $\text{C}_3$  grass, and 14.7 ‰ versus 6.1 ‰ for the  $\text{C}_4$  grass. The differences in predicted values among plants reflected measured differences in leaf temperatures.

After six weeks growth in the survey experiment, bulk leaf water  $\delta^{18}\text{O}$  values and all other parameters as detailed above were sampled. The patterns observed were similar to those of the pilot experiment: whole leaf  $\delta^{18}\text{O}_{\text{wl}}$  values of all  $\text{C}_4$  grasses were > 4 ‰ more enriched than all  $\text{C}_3$  grasses, and the observed whole-leaf  $\delta^{18}\text{O}_{\text{wl}}$  of all but one grass species were more enriched than predicted Craig-Gordon values (Table 2.1). In analyzing the residuals (observed minus predicted values or deviations from a single-source-water version of the Craig-Gordon model), a significant positive correlation with leaf length and a significant negative correlation with interveinal distance was observed

**Table 2.1**Observed and predicted values of  $\delta^{18}\text{O}_{\text{wl}}$  for bulk leaf water

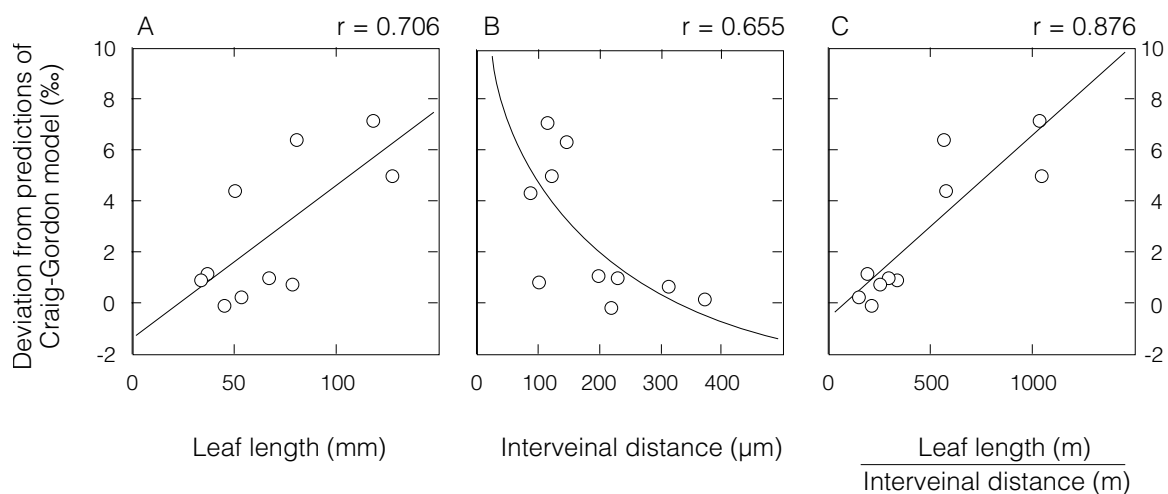
	Observed $\delta^{18}\text{O}_{\text{wl}}$ (‰)	Craig-Gordon Predicted $\delta^{18}\text{O}_{\text{wl}}$ (‰)
<i>Agropyron desertorum</i> (C <sub>3</sub> )	7.7 ± 1.6	7.8
<i>Agrostis stolonifera</i> (C <sub>3</sub> )	9.2 ± 2.8	8.1
<i>Alopecurus pratensis</i> (C <sub>3</sub> )	8.3 ± 3.6	7.6
<i>Bromus inermis</i> (C <sub>3</sub> )	8.2 ± 1.7	7.6
<i>Lolium multiflorum</i> (C <sub>3</sub> )	8.0 ± 1.2	7.8
<i>Andropogon gerardii</i> (C <sub>4</sub> )	15.1 ± 3.7	8.0
<i>Bouteloua curtipendula</i> (C <sub>4</sub> )	12.7 ± 0.3	7.6
<i>Eragrostis curvula</i> (C <sub>4</sub> )	12.4 ± 4.2	8.0
<i>Leptochloa dubia</i> (C <sub>4</sub> )	14.2 ± 2.8	7.8
<i>Muhlenbergia wrightii</i> (C <sub>4</sub> )	9.0 ± 2.6	8.1
C <sub>3</sub> mean	8.3 ± 0.5a	7.8
C <sub>4</sub> mean	12.6 ± 3.2b	7.8

Each value represents a mean of three plants and the associated standard deviation. C<sub>3</sub> and C<sub>4</sub> means are significantly different at  $p < 0.01$ . Predicted  $\delta^{18}\text{O}_{\text{wl}}$  values (SMOW scale) are based on a single-water-source Craig-Gordon model. Sample conditions were:  $\delta^{18}\text{O}_{\text{atm w vapor}} = -27$  ‰; RH = 35%; air temp = 25 °C; leaf temperatures varied but were not significantly different from the mean of 27 °C. Sample conditions for pilot experiment (data in text):  $\delta^{18}\text{O}_{\text{atm w vapor}} = -24$  ‰;  $\delta^{18}\text{O}_{\text{source}} = -15.6$  ‰; RH = 40%; air temp = 35 °C; leaf temperatures were C<sub>3</sub> dicot = 34 °C, C<sub>3</sub> grass = 35 °C and C<sub>4</sub> grass 37 °C.

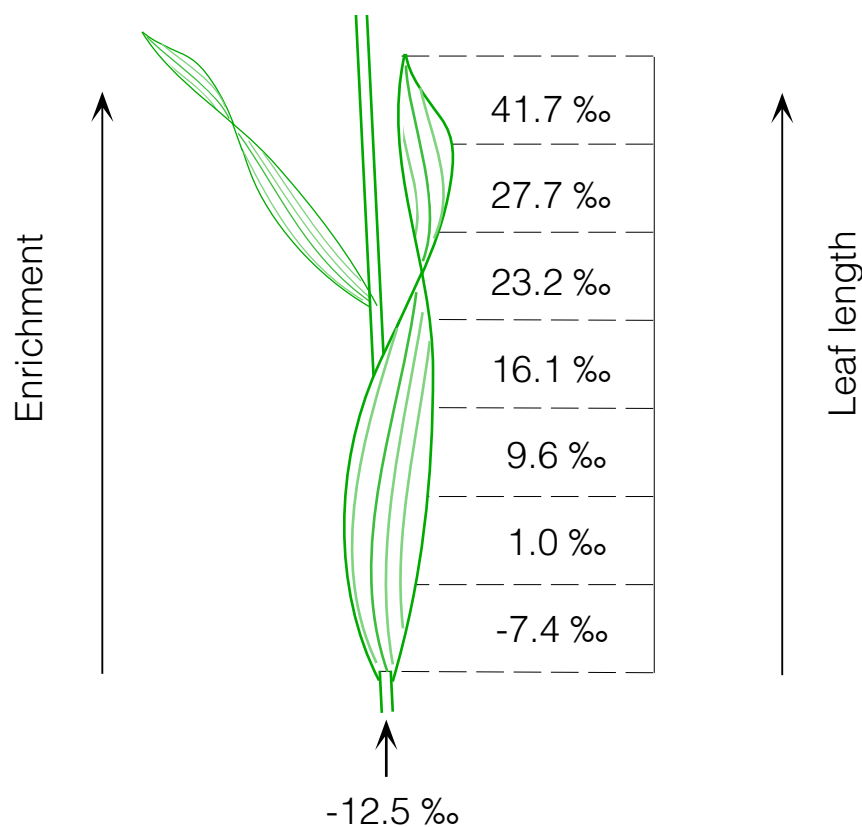
(Figure 2.1A, B;  $p < 0.01$ ). When the residual was plotted versus leaf length divided by interveinal distance (Figure 2.1C), there was a highly significant correlation that explained most of the deviation of the bulk grass blade  $\delta^{18}\text{O}_{\text{wl}}$  from the Craig-Gordon model ( $r = 0.876$ ,  $p < 0.001$ ).

To assess the pattern of  $^{18}\text{O}$  enrichment in grasses, whole grass blades were collected, sectioned from base to tip and the  $\delta^{18}\text{O}_{\text{wl}}$  value was measured in each section. A large and distinct progressive  $\delta^{18}\text{O}_{\text{wl}}$  enrichment was observed along the length of the grass leaf (Figure 2.2). The base-to-tip  $\delta^{18}\text{O}_{\text{wl}}$  difference for this grass blade was 49 ‰. All grass species analyzed exhibited a similar pattern of progressive enrichment, with some base-to-tip differences exceeding 50 ‰. Such large and distinct  $^{18}\text{O}$  enrichment patterns did not occur in dicot leaves (Wang and Yakir 1995).

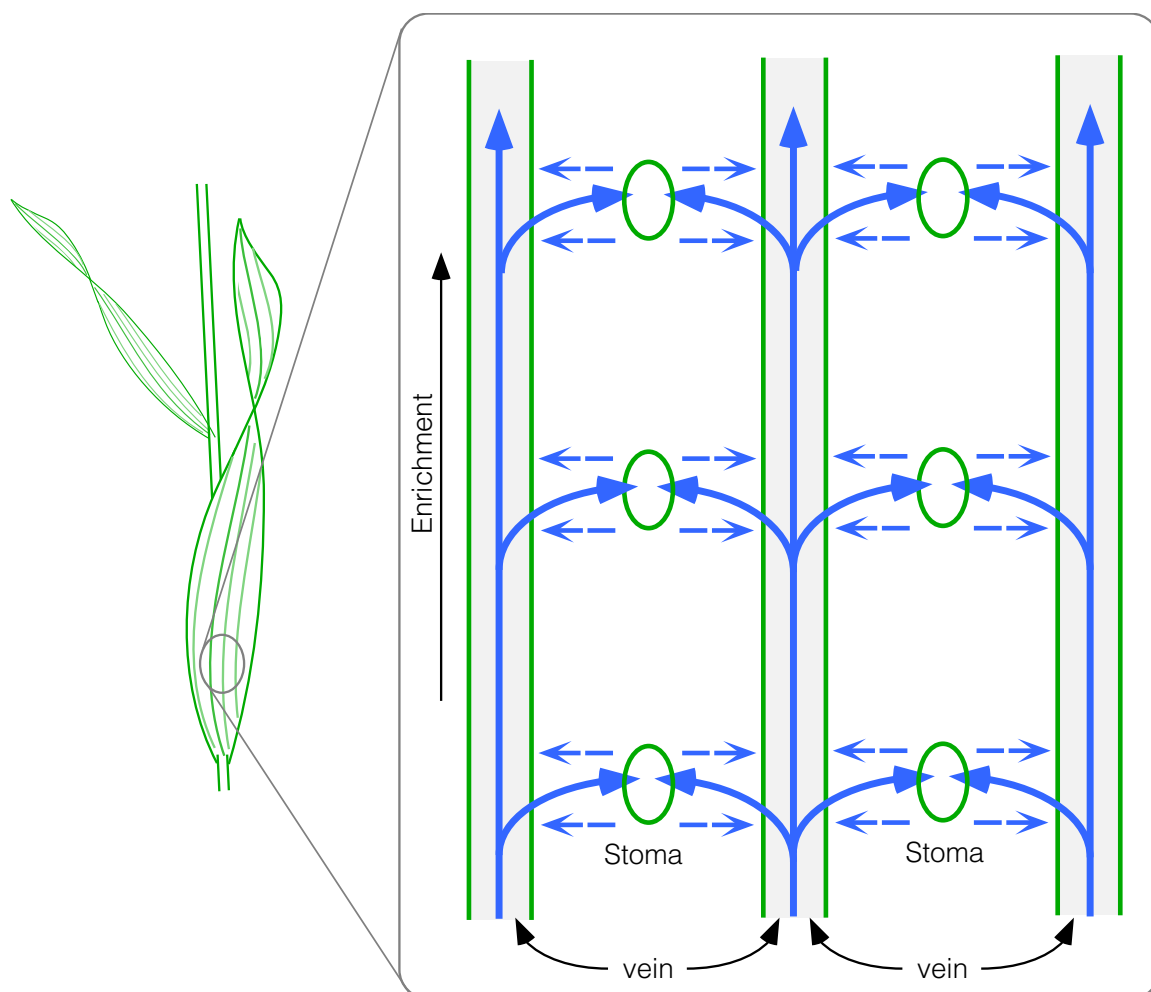
I propose a simple explanation that accounts for both the progressive enrichment and the effect of interveinal distance on grass  $\delta^{18}\text{O}_{\text{wl}}$  (Figure 2.3). As water moves out of leaf veins and towards the stomatal apertures, leaf water becomes evaporatively  $^{18}\text{O}$ -enriched. As a result, an  $^{18}\text{O}$  gradient develops between the stomatal complex (region of evaporatively enriched water) and vein (water source) as expected by the Craig-Gordon model. Despite the large advective transpirational flux towards the stoma, back diffusion of  $^{18}\text{O}$ -enriched leaf water towards the vein should occur (Peclet effect; Farquhar and Lloyd 1993) and any back diffusion ultimately enriches vein water. This enriched vein water travels up the leaf providing source water to the next stomatal complex and



**Figure 2.1** The relationships between  $\delta^{18}\text{O}_{\text{wl}}$  residuals (difference between the observed and predicted  $\delta^{18}\text{O}_{\text{wl}}$  values) versus grass leaf morphological characteristics. (A) residual versus leaf length ( $p < 0.01$ ), (B) residual versus interveinal distance ( $p < 0.01$ ), (C) residual versus leaf length divided by interveinal distance ( $p < 0.001$ ). Each point represents a different grass species and is the mean of three plants.



**Figure 2.2** Representative distribution of bulk leaf water  $\delta^{18}\text{O}_{\text{wl}}$  values of grass leaf sections for greenhouse grown plants. For this specific grass blade (*Miscanthus sinensis*), the stem source water  $\delta^{18}\text{O}$  value was -12.5 ‰, all other sampling parameters were the same as in Table 2.1. All grass species mentioned in this study were sampled in the same manner and showed similar results.



**Figure 2.3** Schematic of progressive  $^{18}\text{O}$  enrichment of leaf water as expected in grass leaves. As source water (solid arrowed lines) exits a vein and moves towards a stoma, it becomes evaporatively enriched in  $^{18}\text{O}$ . This creates a concentration gradient of enriched water at the stoma relative to water in the vein, which results in a back diffusion of enriched water (dashed arrowed lines). The mixing of the isotopically distinct water pools results in a slight enrichment of vein water, which then influences  $^{18}\text{O}$  enrichment of leaf water in the next leaf segment, where the process occurs again further enriching vein water.

the vein water is further enriched (explaining observation in Figure 2.2). We also expect that at any particular point along a grass blade, the extent to which vein water mixes with water from evaporative sites should be a function of the distance from the vein to the evaporative site (back diffusion path length). If interveinal distances are short, there is more mixing and the vein water enrichment should be greater for every leaf segment. Interveinal distances are known to be significantly shorter in  $C_4$  grasses than  $C_3$  grasses (Kawamitsu et al. 1985, Dengler et al. 1994). Consistent with this pattern, we observed that  $\delta^{18}O_{wl}$  values were more enriched in  $C_4$  grasses.

The patterns of leaf water enrichment we describe here require successively enriched pools in contrast to the Craig-Gordon model, which considers only one pool. Our hypothesis for grass blade enrichment is analogous to the string-of-lakes model developed by Gat and Bowser (1991):

$$\delta_n = \delta_{n-1} + \frac{\left( \delta_a + \frac{\varepsilon}{h} \right) - \delta_{n-1}}{1 + \frac{F_+(1-h)}{Eh}} \quad (2.1)$$

where  $\delta_a$  and  $\delta_n$  refer to the  $\delta^{18}O$  of atmospheric water vapor and water entering an evaporating surface (i.e., stoma), respectively.  $F_+$  represents the flux into a segment and  $E$  represents the evaporative flux out of a segment. Humidity is represented by  $h$ , and  $\varepsilon = \varepsilon_{eq} + (1-h) \varepsilon_k$ , where  $\varepsilon_{eq}$  and  $\varepsilon_k$  represent the equilibrium and kinetic fractionation factors.

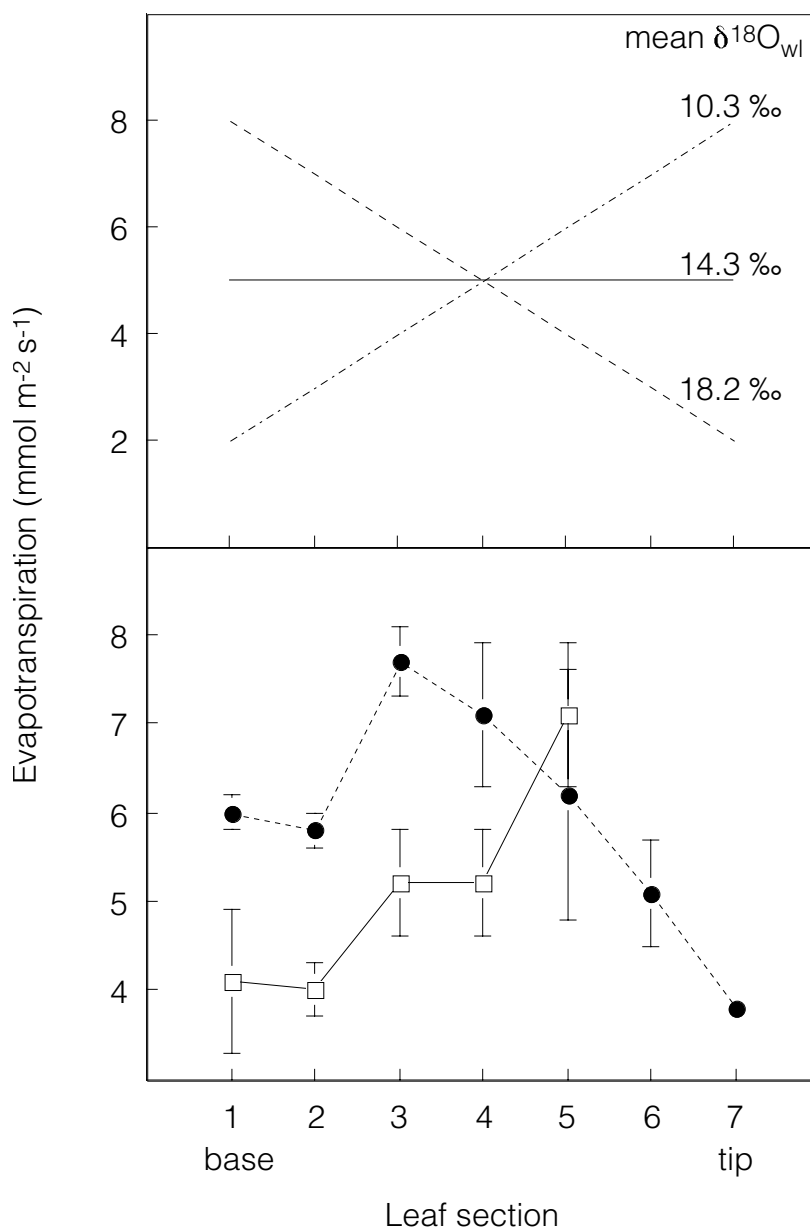
If one assumes that  $E$  is the same for all leaf segments, then the *mean*  $\delta^{18}\text{O}$  value of water from all segments has the same value as predicted by the single-water-source Craig-Gordon model. Under this assumption, we do predict progressive  $^{18}\text{O}$  enrichment along the leaf for successive leaf segments, but the predicted  $\delta^{18}\text{O}$  values of each segment do not match observed values (Table 2.2). Actual  $E$  along a grass blade is known to vary (Meinzer and Saliendra 1997). When values  $E$  for leaf segments were fitted to match the  $\delta^{18}\text{O}_{\text{wl}}$  data, close agreement was obtained (Table 2.2). There was not a fitted value of  $E$  that accurately predicted  $\delta^{18}\text{O}$  in the terminal segment. It is believed this is because the water volume in a leaf decreases as the leaf tapers toward the tip, a phenomenon not incorporated into the model. Note that by varying  $E$  between segments, the predicted whole leaf water  $\delta^{18}\text{O}_{\text{wl}}$  value is considerably more enriched than predicted by the single-water-source Craig-Gordon model, without violating steady-state assumptions.

Figure 2.4A shows three patterns of leaf water flux along a leaf and the associated mean  $\delta^{18}\text{O}_{\text{wl}}$  predicted by the Gat-Bowser. As mentioned previously, if  $E$  is constant along the leaf then predicted  $\delta^{18}\text{O}_{\text{wl}}$  from the Gat-Bowser model does not differ from Craig-Gordon predictions. If  $E$  is proportionally greater at the tip of a grass blade than the base, then  $\delta^{18}\text{O}_{\text{wl}}$  would be less than predicted by the Craig-Gordon model. Alternatively, if  $E$  is proportionally greater at the base than the tip, then  $\delta^{18}\text{O}_{\text{wl}}$  should be greater than that predicted by the Craig-Gordon model. Figure 2.4B and Table 2.3 show field data that generally support this

**Table 2.2**Observed and predicted leaf water  $\delta^{18}\text{O}$  values of leaf segments

Leaf Segment	Observed $\delta^{18}\text{O}$ (‰)	Craig-Gordon Model Predicted $\delta^{18}\text{O}$ (‰)	Gat-Bowser model			
			Nonvariable $E$		Fitted $E$	
			$E$ (mmol m <sup>-2</sup> s <sup>-1</sup> )	Predicted $\delta^{18}\text{O}$ (‰)	$E$ (mmol m <sup>-2</sup> s <sup>-1</sup> )	Predicted $\delta^{18}\text{O}$ (‰)
Base 1	-7.4	11.0	7.14	-7.5	7.40	-7.4
2	1.0	11.0	7.14	-2.4	12.25	1.0
3	9.6	11.0	7.14	3.2	10.60	9.6
4	16.1	11.0	7.14	9.4	6.05	16.1
5	23.2	11.0	7.14	16.4	5.70	23.2
6	27.7	11.0	7.14	24.6	2.40	27.7
Tip 7	41.7	11.0	7.14	35.3	5.60	37.3
mean	16.0	11.0	7.14	11.3	7.14	15.4

$\delta^{18}\text{O}_{\text{source}}$  was -12.5 ‰, all other environmental parameters as mentioned in Table 2.1. In the Gat-Bowser model, the mean value of  $E$  for the entire leaf remains constant. However, the proportion of  $E$  within a segment was either held constant (Nonvariable  $E$ ) or was varied (Fitted  $E$ ). The calculated variations in Fitted  $E$  across the length of the leaf are less than that of sugarcane (Meinzer and Saliendra 1997).



**Figure 2.4** (A) Three potential patterns of evapotranspiration along the leaf and the resultant bulk leaf water  $\delta^{18}\text{O}$  from Gat-Bowser model predictions. (B) Observed patterns of evapotranspiration along leaves of the  $C_4$  grass *Panicum virgatum* at a tall-grass prairie site in Oklahoma. Well-watered and drought conditions are represented by June (●) and July (●), respectively. The bulk leaf water  $\delta^{18}\text{O}$  values for these leaves are presented in Table 2.3. Model predictions for (A) were based on July environmental parameters: relative humidity, 45 %; atmospheric water vapor  $\delta^{18}\text{O}$ ,  $-11.6$  ‰; source water  $\delta^{18}\text{O}$ ,  $-4.1$ ; leaf temperatures,  $34$  °C.

**Table 2.3**Observed and predicted bulk leaf water  $\delta^{18}\text{O}$  values

	June	July
Observed $\delta^{18}\text{O}_{\text{wl}}$	$8.1 \pm 1.0 \text{ ‰}$	$16.5 \pm 0.9 \text{ ‰}$
Craig-Gordon predicted $\delta^{18}\text{O}_{\text{wl}}$	8.9 ‰	14.3 ‰
Gat-Bowser predicted $\delta^{18}\text{O}_{\text{wl}}$	8.0 ‰	15.7 ‰

Observed values from a tall-grass prairie site in Oklahoma. Model parameters are listed in Figure 2.4.

idea. From June to July, the flux pattern along the leaf changed from being greater at the tip of the blade to greater at the base of the blade. Along with this change, observed  $\delta^{18}\text{O}_{\text{wl}}$  changed from being less enriched than Craig-Gordon predictions to more enriched than Craig-Gordon predictions (Table 2.3). Concomitant with this change in flux was the onset of a drought. It is possible that as the grasses became water-stressed, flux from the tip was reduced to either avoid xylem cavitation, or because of xylem cavitation.

The  $^{18}\text{O}$  differences in leaf water should affect  $\text{C}^{18}\text{O}^{16}\text{O}$  values of gross photosynthetic  $\text{CO}_2$  fluxes, because carbonic anhydrase assures rapid equilibration of  $^{18}\text{O}$  in leaf water and  $\text{CO}_2$  (Farquhar et al. 1993). Hence,  $^{18}\text{O}$  leaf-water differences should be reflected in the  $\delta^{18}\text{O}$  values of retrodiffused  $\text{CO}_2$  leaving the leaf and from the ecosystem. Farquhar *et al.* (1993) showed that most of the latitudinal variation in global  $\text{C}^{18}\text{O}^{16}\text{O}$  is a direct result of leaf water enrichment in terrestrial vegetation and implied that  $\text{C}^{18}\text{O}^{16}\text{O}$  values could be used to further distinguish terrestrial from oceanic  $\text{CO}_2$  fluxes. The observations presented here suggest that  $\text{C}_3$  dominated grasslands and  $\text{C}_3$  forests should be different in  $\delta^{18}\text{O}_{\text{wl}}$ . Consequently, the discrimination value of  $\text{C}^{18}\text{O}^{16}\text{O}$  fluxing from these ecosystems should differ according to the theory developed by Farquhar and colleagues (1993). Discrimination against  $\text{C}^{18}\text{O}^{16}\text{O}$  in  $\text{C}_4$  plants is distinguishable from  $\text{C}_3$  plants based on the inherent differences of  $[\text{CO}_2]$  in the air spaces of the leaf (Farquhar et al. 1993). However, based on the correlations shown here between  $\delta^{18}\text{O}_{\text{wl}}$  and leaf anatomy, the  $\text{C}^{18}\text{O}^{16}\text{O}$  discrimination values of major

grasslands, such as the short-grass and tall-grass  $C_4$  dominated prairies, may be distinct because of the large differences in leaf length and interveinal distance of the dominant species in these grasslands. Furthermore, the vast difference in  $C^{18}O^{16}O$  discrimination between  $C_3$  and  $C_4$  grasses, along with  $\delta^{13}C$  information from  $CO_2$ , may allow for separation of seasonal activity shifts in  $C_3/C_4$  dominance within these grasslands.

Grass-dominated ecosystems comprise nearly one fourth of total global vegetated land area (Sims 1988) and are among the most productive ecosystems. Yet there can be more than a fourfold difference in primary productivity on a year-to-year basis (Knapp et al. 1998). Given that the  $^{13}CO_2$  discrimination by  $C_4$  grasslands is nearly indistinguishable from oceanic discrimination (Ciais 1995), partitioning of the global carbon flux into grassland versus oceanic carbon sinks is difficult. The higher  $\delta^{18}O_{wl}$  and  $C^{18}O^{16}O$  discrimination values predicted for grassland ecosystems might be an approach for distinguishing grassland from oceanic productivity in the global  $^{13}C^{18}O^{16}O$  flask observations.

### **Acknowledgements**

I would like to thank J. Berry, G. D. Farquhar, J. Gillon, H. Griffiths, C. E. Martin, C. Still and D. Yakir for helpful comments on early drafts of this manuscript. I thank J. Roden for enlightening discussions during the early stages of this work.

J. Sperry and K. McCulloh were of great assistance with the anatomical measurements. C. Cook, M. Lott and Big Dog provided help for isotope analysis. This research was funded by the National Institute for Global Environmental Change through the U.S. Department of Energy (Cooperative Agreement No. DE-FC03-90ER61010). Additional funding was provided by a NASA Earth System Science Fellowship.

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