The ^{18}O content of leaf water and of CO_2 and water vapor exchanged by leaves

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for
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1. Introduction

1.1 General introduction of the research area.

Terrestrial vegetation plays an important role in the global climate system. This is primarily because it exchanges large quantities of gases including CO_2, H_2O and O_2 with the atmosphere: during photosynthesis, respiration and evapotranspiration. Moreover, it provides a sensitive record of climatic changes, in tree rings and plant remains (Epstein et al., 1977; DeNiro & Epstein, 1979).

The oxygen isotopic composition of these gases exchanged between terrestrial vegetation and the atmosphere and the organic matter deposited in plants, are all imprinted with the oxygen-18 (^{18}O, expressed as $\delta^{18}O$, where δ (‰) = $(R_{sample}/R_{standard} - 1)\cdot 10^3$, R is $^{18}O/^{16}O$, the standard is Vienna Standard Mean Ocean Water (VSMOW)) signature of leaf water (Dongmann et al., 1972; DeNiro & Epstein, 1979; Francey & Tans, 1987; Yakir, 1992). As a result, the $\delta^{18}O$ value of leaf water is a useful signal for studies related to global carbon cycle, hydrological cycle, oxygen cycle, and paleoclimate reconstruction.

1.1.1 The ^{18}O content of leaf water.

Leaf water becomes enriched in the heavy isotopes of deuterium and ^{18}O relative to plant stem (or soil) water during transpiration. Weshburn & Smith (1934) were the first to observe deuterium enrichment at the natural abundance levels in leaf water. Since then, with the development of the modern mass spectrometer, this phenomenon was firmly established for $\delta^{18}O$ (Gonfiatini et al, 1965; Dongmann et al., 1972, 1974; Förstel 1978; Farris & Strain, 1978; Zundel et al., 1978). The physical bases for the fractionation of these isotopes are two isotopic fractionation effects occurring as water evaporates from a surface (or transpires from a leaf): one is an "equilibrium" effect, resulting from the phase change from liquid to gaseous vapor, i.e., water containing the lighter isotopes have higher vapor pressures and thus preferentially escape during evaporation (Wahl and Urey, 1935); and the other is a "kinetic" effect, caused by the different diffusion rates of the light and heavy water vapor in air, i.e., water containing the heavier molecules moves at a slower rate. The equilibrium effect usually takes place at the interface between liquid and vapor, and has been shown to be inversely related to temperature at the evaporating site (Majoube, 1971). The kinetic effect usually takes place above the water surface and its magnitude is determined by the aerodynamic nature of the boundary layer (Dongmann et al., 1974).

Traditionally, leaf water has been treated as a thin, well mixed and isotopically uniform water pool. A model incorporating these isotope fractionation and exchange processes, originally developed by Craig & Gordon (1965) for studying evaporation from the ocean, has been applied

to leaf water under transpiration (Dongmann et al. 1974; Schiegel, 1974; Farris & Strain, 1978; Förstel, 1978; Zundel et al., 1978; Burk & Stuiver, 1981; Bariac et al., 1983; 1989; Allison et al., 1985; Leaney et al., 1985; Farquhar & Lloyd, 1993; Walker et al, 1989; White, 1989; Yakir et al, 1990; Flanagan et al. 1991; Farquhar et al., 1993). Assuming leaf water is under isotopic steady-state condition, the $\delta^{18}O$ value of the leaf water at the sites of evaporation (δ_{ss}) may be approximated as:

$$\delta_{ss} = \delta_s + \varepsilon_{eq} + \varepsilon_k + h \cdot (\delta_a - \varepsilon_k - \delta_s) \quad (1)$$

where, δ_s is the $\delta^{18}O$ value of the source water, δ_a is the $\delta^{18}O$ value of the ambient air moisture; ε_{eq} is derived from the equilibrium fractionation factor α_{eq} as follows:
$\varepsilon_{eq} = (1-\alpha_{eq})\cdot 10^3$, which is 9.38‰ at 25°C (Majoube, 1971); ε_k is derived from the kinetic fractionation factor α_k as follows: $\varepsilon_k = (1-\alpha_k)\cdot 10^3$, which is influenced by leaf stomatal conductance and air boundary layer conductance (Merlivat, 1978; Farquhar & Lloyd, 1993; Buhay et al., 1996); h is relative humidity of the ambient air at the leaf surface temperature, or equals to the ratio of partial vapor pressures in the ambient air (e_a) and inside leaf intercellular spaces (e_i) (Flanagan & Ehleringer, 1991). To predict the $\delta^{18}O$ value of the leaf water, the model requires inputs of the $\delta^{18}O$ values of the water that feed plants and atmospheric water vapor, together with other meteorological parameters, such as leaf surface temperature and relative humidity of the ambient air. Since the hydrological and meteorological systems greatly influence the regional scale temperature, relative humidity and $\delta^{18}O$ signatures of the source input water and air moisture (Dincer, 1968; Gat, 1981; IAEA, 1990), the $\delta^{18}O$ value of the leaf water should also be influenced by the regional environmental conditions.

Under controlled environment conditions, the observed isotopic composition of bulk leaf water is usually less enriched than that predicted by the Craig & Gordon model for the evaporative sites within leaves (Dongmann et al., 1974; Leaney et al., 1985; Bariac et al., 1989; Walker et al., 1989; Yakir et al, 1989; Flanagan & Ehleringer, 1991; Farquhar & Lloyd, 1993). Much of the difference between the modeled and observed leaf water isotopic compositions was first proposed to be incomplete mixing of two water pools within the leaf (Leaney et al., 1985; Bariac et al., 1989; Walker et al., 1989): one is the isotopic composition of an unfractioned source water (δ_s), and the other is the isotopic composition of the leaf water at isotopic steady state (δ_{ss}):

$$\delta_L = f \cdot \delta_{ss} + (1-f) \cdot \delta_s \quad (2)$$

where δ_L is the $\delta^{18}O$ value of the measured bulk leaf water, f is the fraction of the leaf water equilibrated with the atmospheric moisture. When the rate of transpiration is lower, there will be more diffusion of highly enriched water away from the evaporating site within the leaf, which will result in a higher f value. The factor f has been shown empirically to be reversibly correlated with the rate of transpiration (Leaney et al., 1985; Walker et al., 1989), but there is no mathematical formula to describe the relationship.

Later on, studies have shown that leaf water is not isotopically homogeneous (Yakir et al, 1989, 1994; Yakir, 1991; Walker and Lance, 1991; Luo & Sternberg, 1992). It has been suggested that there are two or three distinct water compartments within a leaf: one is the vein water which is not isotopically enriched; another is mesophyll water which undergoes evaporation and thus becomes isotopically enriched; the mesophyll water can be even further divided into apoplastic water which becomes isotopically enriched and symplastic water which lags in isotopic enrichment because of slow mixing with the apoplastic pool (Yakir et al, 1989). It has also been suggested that there are patches of leaf water that do not become enriched because of areas of stomatal closure with the subsequent cessation of transpiration (Flanagan et al, 1991). But few reports are available now on the mechanism of the isotopic heterogeneity in the leaf water, and what the relation is between the heterogeneous isotopic composition of the leaf water and that of the average bulk leaf water.

To improve the prediction of the $\delta^{18}O$ value of the leaf water, Farquhar & Lloyd (1993) developed a one-dimensional advection-diffusion model, based on a continuous gradient within the leaf caused by a shifting balance between the bulk flow of unfractioned water into the leaf (convection) and the back diffusion of heavy isotope molecules away from the enriched evaporating sites. This model incorporates the rate of transpiration (E) and an "effective" anatomical dimension (L) into the Craig & Gordon model:

$$\delta_L = \delta_s + (\delta_{ss} - \delta_s) \cdot \frac{1 - e^{-p}}{p} \qquad (3)$$

where $p = (E \cdot L)/(C \cdot D)$ stands for the peclet number, C for mole concentration of water (5.56×10^4 mol m^{-3}); D for diffusivity of $H_2{}^{18}O$ (2.66×10^{-9} m^2 s^{-1}) in water (Wang, 1954). By selecting a value for the peclet number, this model can be adjusted to fit the observed $\delta^{18}O$ value of the leaf water; this adjustment is empirical and depends on assigning a value for the anatomical dimension (L) which is not to be determined directly at present.

In using these modified models or any other addition to the Craig & Gordon model, one has to assume an upper limit to the $\delta^{18}O$ value of leaf water defined by Eq. 1 under isotopic steady state condition. In contrast to these assumptions, some plants may not reach the isotopic

steady state due to a slower response to the change in ambient conditions during the diurnal cycle (Förstel, 1978); and in several cases leaf water $\delta^{18}O$ values may be higher than predicted by Eq. 1 (Förstel, 1978; Walker & Lance 1991; Flanagan et al., 1993; Flanagan & Varney, 1995). Nevertheless, it is notable that the modified equations (Eq. 2 & 3) are transpiration dependent and the extent of the difference between modeled and observed leaf water isotopic compositions (δ_{ss}-δ_L) has been shown to be positively related to leaf transpiration rate (White 1989; Walker et al., 1989; Flanagan et al., 1991).

Since most of the models for predicting the $\delta^{18}O$ value of the leaf water depend on the assumption of isotopic steady state attained within the leaf water (Dongmann et al., 1974; Allison et al., 1985; Yakir et al., 1990; Farquhar et al., 1993), there seems to be a need to test the assumption of the attainment of isotopic steady state in the leaf water. In addition, there is a need to characterize the global scale variability in leaf water $\delta^{18}O$ values due to species diversity and due to plant characteristics which are not associated with climatic variations.

1.1.2 The ^{18}O content of atmospheric CO_2.

The $\delta^{18}O$ value of atmospheric CO_2 can provide useful information for studies of the global carbon cycle (Francey & Tans, 1987; Tans et al., 1990; Farquhar et al., 1993). This is because CO_2 exchange processes that occur between the ocean and the atmosphere are associated with very different isotope effects from those between terrestrial vegetation and the atmosphere. In CO_2 exchange between the ocean and the atmosphere, the $\delta^{18}O$ value of atmospheric CO_2 is influenced by the $\delta^{18}O$ value of the ocean water, which is relatively constant throughout the globe, with only small variations associated with ocean salinity (Craig & Gordon, 1965). In contrast, in CO_2 exchange between terrestrial vegetation and the atmosphere, the $\delta^{18}O$ value of atmospheric CO_2 is strongly influenced by plants leaf water (will be further addressed below), which have substantially large variations throughout the globe due to variations in the hydrological and meteorological systems across the globe (Craig, 1961; Dansgaard, 1964); superimposed on this climatic signal is the $\delta^{18}O$ enrichment in leaf water associated with evapotranspiration.

During terrestrial photosynthesis, air CO_2 entering the leaf is fixed into carbohydrates, while oxygen is produced and emitted, together with transpired water. The photosynthetic reaction can be schematically represented by:

$$nCO_2 + nH_2O \xrightarrow{\text{sunlight}} [CH_2O]_n + nO_2$$

where $[CH_2O]_n$ stands for carbohydrate.

All the CO_2 that diffuses into the leaf is likely to be hydrated with leaf water. However, only a fraction of CO_2 diffused from the atmosphere is fixed into carbohydrates, while the rest is dehydrated and diffuse back to the atmosphere carrying the $\delta^{18}O$ signature of the leaf water. The quantity of CO_2 that diffuses out of the leaf depends on the concentration of CO_2 in the chloroplast and conductances to diffusion within and from the leaf (Farquhar et al., 1993). This $\delta^{18}O$ value of CO_2 is different from that of the ambient atmospheric CO_2 since rapid exchange of oxygen between CO_2 and leaf water is facilitated by the high activity of the enzyme carbonic anhydrase which is predominantly localized in chloroplasts of the leaf cell (Jacobson et al., 1975).

Since the $\delta^{18}O$ value of leaf water has a strong influence on the isotopic signature of atmospheric CO_2, two laboratory studies (Farquhar *et al*, 1993; Yakir *et al*, 1994) have considered the source $\delta^{18}O$ signature of leaf water responsible for ^{18}O exchange with atmospheric CO_2. One concludes that this $\delta^{18}O$ signature can simply be estimated with the conventional evaporation model (that applies to the evaporating surfaces); the other concludes that the $\delta^{18}O$ value of the water fraction relevant for CO_2 exchange is more depleted than that predicted by the original evaporation model.

In a study of the latitudinal $\delta^{18}O$ variations in atmospheric CO_2, Francey & Tans (1987) observed large latitudinal ^{18}O variations of atmospheric CO_2, and tried to predict such variations. The predicted latitudinal $\delta^{18}O$ gradient is about +1 to +2‰ higher than the observed, when assuming, among other things, full ^{18}O equilibrium between CO_2 and leaf water predicted by the Craig & Gordon model. The discrepancy between the observed and predicted latitudinal $\delta^{18}O$ gradient would diminish if it was assumed that $C^{18}O^{16}O$ equilibrium occurred with more depleted water, or there was not full equilibrium between $C^{18}O^{16}O$ and leaf water predicted at the evaporating sites.

The $\delta^{18}O$ value of atmospheric CO_2 may potentially provide useful information on partition the net CO_2 exchange flux between terrestrial vegetation and the atmosphere into gross primary productivity (GPP) and soil and stem respiration (R) (Yakir, 1992; Flanagan & Varney, 1995). An advantage to use the $\delta^{18}O$ signature of atmospheric CO_2 is that there exists large $\delta^{18}O$ difference between CO_2 related to GPP and CO_2 related to R, because of the large $\delta^{18}O$ enrichment in leaf water relative to soil and water. Long term monitoring of the changes in the $\delta^{18}O$ value of atmospheric CO_2 may in turn provide information on long term changes in GPP and R, which may subsequently provide insights relevant to the CO_2 "missing sink", an imbalance between sources and sinks of CO_2 in the global carbon budget (Sundquist, 1993).

The quantitative use of the $\delta^{18}O$ signal in atmospheric CO_2 for assessing terrestrial vegetation—atmosphere interactions depends on the identification of the source isotopic signals in both leaf water and soil water, both quantities are not well characterized at present.

1.1.3 The ^{18}O content of atmospheric O_2

The $\delta^{18}O$ value of atmospheric O_2 is also a useful tracer in studies of global response of the terrestrial and marine biospheres to climate change (Bender et al., 1994). Similar to $\delta^{18}O$ variations in atmospheric CO_2 during exchange with the terrestrial ecosystem and the ocean, atmospheric O_2 has also large $\delta^{18}O$ variations due to $\delta^{18}O$ differences in source substrate for O_2 production. Since there is no oxygen isotope fractionation during O_2 production (Guy et al., 1993), the $\delta^{18}O$ value of the O_2 produced from marine photosynthesis should have an identical $\delta^{18}O$ value as the standard mean ocean water (SMOW), which by definition equals to 0‰. While in the global scale, about 54 percent of the annual O_2 flux into the atmosphere comes from terrestrial plants photosynthesis (Keeling & Shertz, 1992; Farquhar et al, 1993). Water in leaf chloroplasts is the substrate for the water splitting reaction and controls the $\delta^{18}O$ signature of the O_2 produced. As stated earlier, leaf water becomes isotopically enriched during transpiration. Assuming the $\delta^{18}O$ value of the chloroplast water is similar to that predicted by the conventional evaporative enrichment model, Farquhar et al (1993) predicted a global average of +4.4‰ for the $\delta^{18}O$ value of plants leaf water, which should apply to terrestrial photosynthetic O_2 since this is the same source without isotopic fractionation (Guy et al, 1993). On the global scale, this signal represents the terrestrial contribution to the so-called Dole effect (the observed difference of +23.5‰ between the $\delta^{18}O$ value of atmospheric O_2 and that of seawater; Dole et al, 1954), which reflects a balance of the oxygen isotope signature of water during photosynthesis, the isotope fractionation in respiration (Guy et al., 1993) and hydrologic processes. In general, increases in the ratio of terrestrial to marine production will increase the $\delta^{18}O$ value of atmospheric O_2; in contrast, decreases in the ratio of terrestrial to marine production will decrease the $\delta^{18}O$ value of atmospheric O_2.

Accurate determination of the global average $\delta^{18}O$ enrichment in chloroplast water of the terrestrial vegetation may aid in studies of the Dole effect. However, similar to atmospheric CO_2 studies, estimating the $\delta^{18}O$ signal of terrestrial photosynthetic O_2 is still hampered by uncertainties in estimating the source $\delta^{18}O$ signal within leaves. Thus there is a need to identify the source $\delta^{18}O$ value of the leaf water for O_2 production.

1.1.4 The ^{18}O content of atmospheric H_2O.

The $\delta^{18}O$ value of atmospheric moisture within the canopy boundary layer can be used to estimate the rate of evapotranspiration (ET) and provide information on water exchange between terrestrial vegetation and the atmosphere (Brunel et al, 1992).

Terrestrial vegetation exchanges large quantities of heat and water with the atmosphere. Quantitative estimation of the ET flux on a regional scale is important as to provide insights into

studies, among other things, of global warming and the global hydrological cycle (Loaiciga et al., 1996). Conventionally, there are two major approaches to estimate ET (Jarvis & McNaughton, 1986). One is a physiological method that pays more attention to the stomata's regulation of transpiration process (Burrows & Milthorpe, 1976); and the other is a meteorological method that emphasizes the energy needed for evaporating large quantities of water (Brutsaert, 1982). Normally, the rate of transpiration resulting from a single leaf or plant estimated by the physiological method, under controlled environmental conditions or in the field, is quite different from the ET estimate obtained from a large field of the same plant by meteorological methods, since the former point measurement is not representative of its large natural surroundings. As a result, the meteorological method has an advantage over the physiological method and has been widely used to estimate ET in large scale studies (Penman, 1948; Thom, 1975; Brutsaert, 1982; Massman, 1992; Wofsy et al., 1993; Morton, 1994; Monteith, 1995; Grace et al., 1995; Goulden et al., 1996).

There are basically three techniques to estimate the ET flux by the meteorological method, namely, aerodynamic technique, energy balance and Bowen ratio (EBBR) technique, and eddy correlation (EC) technique. Based on the assumption that momentum, heat and mass are transported by eddies of turbulent air which makes the coefficient much larger than for molecular diffusion, the aerodynamic technique requires high precision measurements of specific humidity within the CBL. The Bowen ratio is defined as the ratio of the sensible heat flux over the latent heat flux, and the EBBR technique is based on total energy balance and the assumption that the eddy diffusivities for sensible heat and water vapor are equal (Brutsaert, 1982). The EC technique, which integrates the products of vertical velocity and absolute humidity in the over passing air, is based on the assumption that the anemometers, thermometers, and hygrometers can respond to up- and down-ward vertical velocity over the entire range of eddy magnitudes and frequencies (Brutsaert, 1982). Though providing reliable estimates of the ET flux from land vegetation, the EBBR and EC methods require sophisticated instruments for precise measurements of the temperature, specific humidity, or vertical wind velocity within the CBL. Moreover, difference in ET estimates exist among different techniques (Morton, 1994). As a result, there is clearly a need to develop a complementary method that would simplify field instrumentation and also help constrain the ET estimates.

While accurate estimation of the ET flux from land vegetation is difficult, it was recently suggested that it could be improved with the application of $\delta^{18}O$ signature of water by focusing on the isotopic interaction of different water vapor sources in the soil-plant-atmosphere continuum (Bariac et al., 1983, 1989; Walker and Brunel, 1990; Brunel et al., 1992). As air moves above the surfaces of transpiring leaves, water vapor, as well as other masses and energy, would be added to the flow by eddies of the turbulent air. Consequently, the influence of the

$\delta^{18}O$ value of plant transpiration water would be superimposed on the $\delta^{18}O$ signature of the ambient atmospheric moisture within the CBL. Therefore, the $\delta^{18}O$ value of the atmospheric moisture above the vegetation must contain information on the size of the ET flux. Such isotope approach has some advantages by avoiding sophisticated field instrumentations and allowing increased spatial sampling resolution (does not need special towers, etc.). Moreover, the isotope approach would be useful to constrain other meteorological methods and provide additional information and insights, notably, in partitioning evaporation (E) and transpiration (T), and in estimating water use efficiency (WUE).

Basically, there are three major factors which control the distribution of the ^{18}O isotope within the CBL: one is the fetch length of an interested plot, another is the $\delta^{18}O$ difference between the input water vapor and the background air moisture, and the third is the aerodynamic natures of the air flow. The spatial scale of the fetch length determines the height of the CBL (Thom, 1975). If the fetch length of the plot is small, such as 100 m, the height of the CBL will be about 1 m, which will consequently reduce the potential for effective measurement. As heterogeneity in input water and species diversity increase with the enlargement of the fetch length, such as in a regional scale, the isotopic signals contributed by each water source have to be characterized before applying the isotope approach.

The $\delta^{18}O$ values of the input water vapor and the background air moisture is usually controlled by the geographical location and the associated climatic conditions of the studied area (for a recent review, see Gat, 1996). The $\delta^{18}O$ difference between the input water vapor and the background air moisture will be a major factor influencing the isotope mixing gradient.

The aerodynamic natures of the air flow, which can be static, laminar, or turbulent, is another important factor which controls the stable isotopes distribution within the CBL. If the air flow is static or laminar, the movement of the stable isotopes would be slow within the CBL, since the mass transport would be more dominated by molecular diffusion (Merlivat, 1978). If the air flow is turbulent, large eddies of the turbulent air would carry the stable isotopes, together with momentum, heat and other mass, much quicker from one air layer to another above the land vegetation (Thom, 1975). Wind speed, as a measure of the aerodynamic natures of the air flow, has been found to increase logarithmically with height above the land or canopy surface (Brutsaert, 1982). As affected directly by the wind speed, the distribution of the stable isotopes of water vapor, with the lower boundary value controlled by the input water and the upper boundary value controlled by background air moisture, should also have a logarithmic relationship with height within the CBL. Such a logarithmic relationship holds true also for temperature, specific humidity, and other masses, such as CO_2, under neutral conditions (see also Brutsaert, 1982; Brunel et al., 1992).

The stable isotope interactions between vegetation and the atmosphere can be better understood when applying a mass balance equation to an air sample above the vegetation surface. Considering an air moisture sample at a height z within the CBL, we have:

$$q_z = q_a + q_b \tag{4}$$

where q is specific humidity (mmol H_2O mol^{-1} air), subscript a stands for ambient atmosphere, b for biology. Since q_a is relatively constant, gradients in q_z (Δq_z) should be similar to gradients in q_b (Δq_b).

When considering the mass balance for ^{18}O isotope in water vapor above the canopy, we obtain:

$$q_z \cdot \delta_z = q_a \cdot \delta_a + q_b \cdot \delta_b \tag{5}$$

where δ_b is the isotopic signature of the water vapor contributed from both the plants and the soils. Note that there will be a small error in the order of 0.01‰ when using the "δ" notation instead of real ^{18}O concentration of water vapor (Hayes, 1982).

Substituting $q_b = q_z - q_a$ from Eq. 4 into Eq. 5 and rearranging the equation, we can express the specific humidity at level z_1 in terms of the isotopic compositions of water from different sources:

$$q_{z1} = \frac{\delta_b - \delta_a}{\delta_b - \delta_{z1}} \cdot q_a \tag{6}$$

Similarly, we can obtain q_{z2} at another level z_2 within the CBL. From Eq. 6, it is clear that we can obtain specific humidities of the air at any two levels above the canopy, based on a single measurement of the specific humidity of the ambient air (q_a) outside the CBL together with several isotope measurements of the water samples. The specific humidity of the ambient air outside the CBL and the $\delta^{18}O$ values of air moisture at two different levels (δ_{z1}, δ_{z2}) could be directly measured, but the isotopic signature of water vapor contributed from both soils and plants, δ_b, is difficult to measure in the field, since it is mixed with the ambient atmosphere soon after the water is evapotranspired. The best way to obtain the δ_b value is to use a mixing analysis of Eqs. 4 & 5, and rearranging the equation in the way developed by Keeling (1961):

$$\delta_z = \delta_b + M / q_z \tag{7}$$

where $M = (\delta_a - \delta_b) \cdot q_a$.

This intercept approach requires measurements of q_z and δ_z at several heights z_i (i=1, 2, 3, ..., n) within the CBL. When δ_z is plotted against $1/q_z$ over profiles of both isotopes and specific humidity above the canopy, the resulting intercept of the mixing line should be the δ_b value. To get a reliable intercept, this practice requires high precision measurements of the isotopic composition of the water vapor, together with high precision measurements of the specific humidity within the CBL.

After the specific humidity gradient Δq_z (or Δq_b) is reconstructed based on the isotope measurements within the CBL, the ET flux (E_b) can be calculated according to:

$$E_b = -\overline{K} \cdot \frac{\Delta q_z}{\Delta z} = -\overline{K} \cdot \frac{\Delta q_b}{\Delta z} = -(\overline{K} \cdot \frac{q_a}{\Delta z}) \cdot f \qquad (8)$$

where $\overline{K}$ is the eddy diffusivity of the air in layer Δz, which is calculated from wind speed profiles and surface characteristics by meteorological methods (Thom, 1975); and factor $f = (\delta_b - \delta_a)[1/(\delta_b - \delta_{z2}) - 1/(\delta_b - \delta_{z1})]$.

To test the isotope approach, there is a need to conduct ET measurements together with other meteorological techniques in the field; in addition, there is a need to partition total ET estimate into soil evaporation (E) and plant transpiration (T).

1.2 Scope of the present research work

Quantitative analysis of the $\delta^{18}O$ value of leaf water is essential for the utilization of the stable oxygen isotope in environmental sciences. We made the following studies in understanding the $\delta^{18}O$ value of leaf water and applied the isotope technique to study CO_2 and water exchanged between terrestrial vegetation and the atmosphere.

In chapter 3.1, we monitored changes in the $\delta^{18}O$ values of transpiration water over time in a variety of plant species in a non-destructive way, in order to test the assumption of the attainment of the isotopic steady state attained in leaf water. We also studied in detail environmental influences on diurnal changes in the $\delta^{18}O$ value of leaf water in different plant species.

In chapter 3.2, in order to acquire a global perspective on climate-independent variability in leaf water $\delta^{18}O$ values among various plant species, we made an isotopic survey in 90 different plant species collected from all the continents and grown within a restricted area in the Jerusalem Botanical Garden, under similar environmental conditions. To further address the uncertainty of leaf water $\delta^{18}O$ signature responsible for ^{18}O exchange with that in atmospheric CO_2, we conducted on-line measurements of ^{18}O discrimination against $C^{18}O^{16}O$ under both low-

light regime in the laboratory and natural light irradiance in the botanical garden. We discussed several possibilities for adjusting the Craig & Gordon model to predict $\delta^{18}O$ values of chloroplast water. To characterize the displacement of the $\delta^{18}O$ value of leaf water (or chloroplast water) from the meteorologically predicted $\delta^{18}O$ value associated with plant anatomy, all the 89 C_3 plants were divided into several sub-groups according to their ecological or morphological types or to their ratios of water use efficiency (A/E), and possible relationships among various parameters were analyzed.

In chapter 3.3, I developed a two-dimensional model for predicting the $\delta^{18}O$ value of total leaf water. The model is transpiration dependent and considers a leaf as a series of evaporating pools with the ^{18}O content continuously enriched from the beginning pool. The model describes well the temporal and spatial $\delta^{18}O$ variations of leaf water under natural conditions.

In chapter 3.4, we applied the isotope method and measured fluxes of CO_2 and H_2O exchanged between terrestrial vegetation and the atmosphere. We demonstrated that the net CO_2 flux could be partitioned into gross primary productivity (GPP, downward) and respiration (R, upward), based on small vertical gradients in $\delta^{13}C$ and $\delta^{18}O$ values in atmospheric CO_2 above vegetation at the local scale. The results provide a basis for evaluating plant response to environmental change at the field scale, and have the potential to be scaled up to regional ecosystem and ultimately to global scales.

In chapter 3.5, we evaluated the isotopic interaction between terrestrial vegetation and the atmosphere associated with evapotranspiration. We have examined the water exchange process at different time scales, e.g., mid-day, diurnal and seasonal variations; and compared the isotope method with the conventional meteorological techniques, including energy balance and Bowen ratio method, and eddy correlation method; and we carried out the comparison of all the techniques above completely different substrates, including crops fields and a planted forest in an extremely arid region.

In appendix A1.1, I describe a method developed for analyzing the $\delta^{18}O$ value of a micro size water sample with an external precision of $\pm 0.1‰$, which made it possible for short term, high temporal and spatial resolution measurements of leaf and atmospheric water samples (chapters 3.1 and 3.4).

2. Objectives

The main goal of the present research was to provide a better understanding of environmental influence on the $\delta^{18}O$ value of leaf water, and to apply leaf water $\delta^{18}O$ signal in studies of CO_2 and H_2O exchanges between terrestrial vegetation and the atmosphere. This involved several specific objectives: (1) To test the assumption that leaf water during the day is at, or close to, isotopic steady-state under natural conditions; (2) To acquire a global perspective on non-climatic variability in leaf water $\delta^{18}O$ values among plant species, and obtain a $\delta^{18}O$ relationship between leaf water and leaf cellulose; (3) To study the ^{18}O exchange mechanism between atmospheric CO_2 and leaf water under laboratory and natural conditions; (4) To improve model predictions of the isotopic composition of leaf water by incorporating the effects of isotopic gradients in leaf water; (5) To test the potential of using ^{18}O to partition CO_2 flux into photosynthetic and respiratory fluxes, and H_2O flux into soil evaporation and plant transpiration fluxes.

3. Results

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3.1

Temporal and spatial variations in the oxygen-18 content of leaf water in different plant species

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ABSTRACT

Temporal variations in the δ^{18} oxygen ($\delta^{18}O$) content of water transpired by leaves during a simulated diurnal cycle fluctuated around the $\delta^{18}O$ content of the source water. Reconstructed variations in the $\delta^{18}O$ values of leaf water differed markedly from those predicted by conventional models. Even when transpiring leaves were maintained under constant conditions for at least 3 h, strict isotopic steady-state conditions of leaf water (equality of the $^{18}O/^{16}O$ ratios in the input and transpired water) were rarely attained in a variety of plant species (*Citrus reticulata*, *Citrus paradisi*, *Gossypium hirsutum*, *Helianthus annuus*, *Musa musaceae* and *Nicotinia tabacum*). Isotopic analysis of water transpired by leaves indicated that leaves approach the isotopic steady state in two stages. The first stage takes 10 to 35 min (with a rate of change of about 3·3‰ h^{-1}), while in the second stage further approach to the isotopic steady state is asymptotic (with a rate of change of about 0·4‰ h^{-1}), and under conditions of low transpiration leaves can last for many hours. Substantial spatial isotopic heterogeneity was maintained even when leaves were at or near isotopic steady state. An underlying pattern in this isotopic heterogeneity is often discerned with increasing $^{18}O/^{16}O$ ratios from base to tip, and from the centre to the edges of the leaves. It is also shown that tissue water along these spatial isotopic gradients, as well as the average leaf water, can have $^{18}O/^{16}O$ ratios both lower and higher than those predicted by the conventional Craig and Gordon model. We concluded, first, that at any given time during the diurnal cycle of relative humidity the attainment of an isotopic steady state in leaf water cannot be assumed *a priori* and, secondly, that the isotopic enrichment pattern of leaf water reflects gradual enrichment along the water-flow pathway (e.g. as in a string of pools), rather than a single-step enrichment from source water, as is normally assumed.

Key-words: isotopic enrichment; isotopic heterogeneity; leaf water; oxygen-18; stable isotopes; transpiration.

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INTRODUCTION

Quantitative treatment of the isotopic composition of leaf water is essential for the utilization of stable isotopes of oxygen and hydrogen in environmental sciences. Notably, on a landscape scale the $^{18}O/^{16}O$ ratio of leaf water, or of a certain part of it, influences the $^{18}O/^{16}O$ ratios of atmospheric CO_2 and O_2 (Bender *et al.* 1985; Francey & Tans 1987; Farquhar *et al.* 1993; Yakir *et al.* 1994a). The isotopic composition of leaf water associated with photosynthesis also influences that of plant organic matter (Epstein, Thompson & Yap 1977; Sternberg, DeNiro & Savidge 1986). It is increasingly being suggested that plant organic matter, in turn, represents a permanent, time-integrated, isotopic record of climatic conditions (e.g. Epstein *et al.* 1977; Edwards & Fritz 1986), and of plant–atmosphere interactions (e.g. Yakir 1992; Farquhar *et al.* 1993). It has also been shown that the isotopic composition of the water transpired by leaves can be used for estimating evapotranspiration of vegetation on a landscape scale (Bariac *et al.* 1989, 1991; Brunel *et al.* 1992).

Evapotranspiration from leaves is associated with enrichment of heavy isotopes (^{18}O and ^{2}H) in leaf water due to the preferential loss of water molecules containing the lighter isotopes (^{16}O and ^{1}H) (Washburn & Smith 1934; Dongmann *et al.* 1974). Under constant conditions, isotopic enrichment of a continuously fed water surface, such as occurs in a leaf, is assumed to reach steady-state conditions defined by the conventional Craig and Gordon (1965) model and approximated by

$$\delta_L = \delta_{in} + \varepsilon_{eq} + \varepsilon_k + h\,(\delta_a - \varepsilon_k - \delta_{in}), \qquad (1)$$

where δ (‰) $= [(R_{sample}/R_{standard}) - 1] \times 10^3$, R is $^{18}O/^{16}O$ and the standard is Standard Mean Ocean Water (SMOW). Subscripts 'L', 'in' and 'a' represent leaf water, input water and air humidity, respectively, ε_{eq} is the equilibrium fractionation factor (9·4‰ at 25 °C; Majoube 1971), ε_k is the kinetic fractionation factor, which is influenced by the aerodynamic nature of the boundary condition (Merlivat 1978), and h is the relative humidity of the ambient air at the surface temperature, a term that is used throughout the present paper for historical reasons. This model is attractive as a means of predicting the isotopic composition of leaf water because it uses only easily measurable environmental parameters and a few well-defined constants. If it is

to be applied to leaves, however, one must first assume that the leaf is in the isotopic steady state. The main objective of the present study was to test this assumption.

The Craig and Gordon (1965) model relates to a well-mixed, isotopically homogenous evaporating water surface. It is now generally recognized that leaf water does not represent such a water body, and in several studies substantial isotopic heterogeneity of leaf water has been observed (Yakir, DeNiro & Rundel 1989; Luo & Sternberg 1992; Yakir *et al.* 1994a) or inferred (Farris & Strain 1978; Leaney *et al.* 1985; Walker *et al.* 1989; Yakir, DeNiro & Gat 1990). Although various additions to the Craig and Gordon (1965) model, mainly considering the relationships between the evaporating surface and bulk leaf water, have been suggested, a faithful model that predicts the isotopic composition of leaf water has yet to be developed (cf. Flanagan & Ehleringer 1991). A recent modification of the mixed, one-pool model has been advanced by Farquhar & Lloyd (1993), who suggest that isotopic gradients are formed by the opposing convective and diffusive fluxes of depleted source water to and enriched water away from the evaporating surfaces, respectively. The average $\delta^{18}O$ value of bulk leaf water therefore represents an integration of that gradient:

$$\delta_L = \delta_{in} + (\delta_{ss} - \delta_{in})\frac{1 - e^{-p}}{p}, \quad (2)$$

where subscript 'ss' denotes the steady state (Eqn 1) and $p = (EL)/(CD)$, where C represents the mole concentration of water ($5{\cdot}56 \times 10^4$ mol m^{-3}), D is the diffusivity of $H_2{}^{18}O$ ($2{\cdot}66 \times 10^{-9}$ m^{2}s^{-1}) in water (Wang 1954), E is the transpiration rate (mol m^{-2}s^{-1}) and L is the 'effective' mixing length (m).

The Farquhar and Lloyd model accounts for the observations that the $\delta^{18}O$ value of leaf water is often lower than that predicted by Eqn 1 (e.g. Farris & Strain 1978; Walker *et al.* 1989; Yakir *et al.* 1990; Flanagan & Ehleringer 1991), and there is an inverse relationship between the extent of enrichment and the rate of transpiration (Walker *et al.* 1989; Flanagan, Comstock & Ehleringer 1991). In using this or any other addition to the Craig and Gordon (1965) model, one also assumes isotopic steady state and an upper limit to $\delta^{18}O$ values of leaf water defined by Eqn 1. In contrast to these assumptions, some plants may have turnover rates of leaf water (the ratio of leaf water volume to the rate of transpiration; V/E) that are much slower than the rate of change of ambient conditions during the diurnal cycle, and in several cases leaf water $\delta^{18}O$ values higher than those predicted by Eqn 1 have been reported (e.g. Walker & Lance 1991; Flanagan, Marshall & Ehleringer 1993). The effect of V/E on the time course of the leaf water $\delta^{18}O$ value while approaching the steady state has been described by Farris & Strain (1978; see also White 1989):

$$\delta_L = \delta_{ss} - (\delta_{ss} - \delta_{1})\, e^{-bt}, \quad (3)$$

where δ_1 is the initial isotopic value of leaf water at time $t = 0$; $b = \alpha_{eq}\alpha_k^q E/[V(1-h)]$, $\alpha_{eq} = (1 - \varepsilon_{eq}/1000)$ and $\alpha_k^q = (1 - \varepsilon_k/1000)$. It is clear from Eqn 3 that, under the same conditions, the discrepancy between δ_{ss} and δ_L would increase with the leaf V/E ratio.

MATERIALS AND METHODS

Plant material

Experiments were carried out on leaves of banana (*Musa* sp. *Musacea*), mandarin (*Citrus reticulata* cv. clemantine), cotton (*Gossypium hirsutum* cv. vered), pomelo (*Citrus paradisi*), sunflower (*Helianthus annuus* cv. DY3) and tobacco (*Nicotiana tabacum*). Plants were grown for 1 month in a glasshouse at the Weizmann Institute in 5 dm^3 pots containing a soil mixture of 50% volcanic dust, 30% vermiculite and 20% turf (Sphlagnum-Turf, Klasmann-Deilmann, Germany), and using standard horticultural practices. Three-month-old citrus plants and 1-month-old banana plants were purchased at local nurseries, and all other plants were grown from seed. Irrigation was with tap water which had a mean $\delta^{18}O$ value of $-4{\cdot}5$‰.

Gas-exchange measurements

Experiments were performed in a closed gas-exchange system similar to that described by Yakir *et al.* (1994a). An intact leaf was sealed in the leaf chamber and the relative humidity was controlled by continuously adjusting the flow rate (500–1500 cm^3 min^{-1}) of a dry air mixture, and by monitoring with an in-line CO_2/H_2O analyser (LiCor-6262, Lincoln, NE, USA). The CO_2 concentration in the input air was adjusted to 450–500 μmol mol^{-1} with a mixture of CO_2-free air and 1% CO_2, monitored by the in-line CO_2/H_2O analyser. Light was provided by a 250 W halogen lamp (Thorn Lighting Ltd, UK) placed above a 10-mm-thick distilled water container to remove infrared light, and a 50 μm sieve (CellMicronSieves, Carmel, NY, USA) to enhance uniform light distribution. The irradiance flux at the leaf level was 800–1000 μmol m^{-2}s^{-1}. Air and leaf temperatures were monitored by two T-type thermocouples connected to a calibrated readout device (OMEGA Digital Thermometer, HH82, Stamford, CT, USA). The leaf temperature was regulated by water circulating through a cooling bath, and was maintained at 24–25 °C. Air moisture leaving the leaf chamber was collected by passing the air through a dry ice-acetone trap (16 cm in height and 3·5 cm in diameter, filled with 5 mm glass beads).

There were two experimental protocols. In the first, 3–4 samples of transpired water were collected during a 3 h time period. In each case a sample was cryogenically trapped for 15 min. At this stage, the air humidity in the leaf chamber was either increased or decreased, and 4–6 samples were collected over the next hour. In this case, trapping was for 3–5 min per sample (40–60 mm^3 H_2O, depending on the transpiration rate). An additional 4–6 samples were collected over the next 2 h, but with a trapping time of 10–15 min per sample. In some cases, an

additional final sample of transpiration water was collected 10–60 min after the originally planned time course.

In the second protocol, a diurnal cycle of relative humidity was simulated inside the leaf chamber. Relative humidity was reduced from 88% to 68% in four steps of 1 h each, and was then increased to 95% in a similar way. At each humidity step, two samples of transpiration water were collected for 20 min each, with 10 min intervals between samples. A total of 12 samples were collected over a period of about 6 h.

Extraction of water samples

Leaf water volume was determined by sampling 10 leaf discs taken from along the leaf. The fresh weights of the leaf discs were measured immediately, and their dry weights were measured after drying at about 90 °C for 12 h. The precision of the weighing was within ±0·001 mg. At the end of each experiment, the leaves were immediately covered with saran film (Saran wrap, DowBrands Inc., IN, USA) on both sides before leaf discs (1 cm in diameter) or pieces (of area 0·9–16·9 cm^2) were cut out, avoiding the veins, and sealed in Vacutaineres (Becton Dickinson, NJ, USA). Samples of petioles and stems were also sealed in Vacutaineres and stored together with all leaf tissue samples at –20 °C until further analysis. The duration of the sampling process for each plant was about 2 min.

Water from all tissue samples and water traps was extracted quantitatively by vacuum distillation at 60 °C into 100 mm×6 mm (outside diameter) tubes. About 20–30 mm^3 of the distilled water samples were placed at the centre of the 50 mm^3 pyrex capillaries (Monoject Scientific, St Louis, MO, USA), which were then sealed at both ends with a micro-torch flame (Blazer, Piezo micro torch, Japan).

Isotopic analysis

Oxygen isotopic analysis was essentially as described by Santrock & Hayes (1987). Water samples of 2 mm^3 each were withdrawn by gas-tight syringe from the storage capillaries and injected into the helium flow of a Carlo-Erba elemental analyser (EA1108, Carlo-Erba Instruments, Fison Inc., Milan, Italy). After pyrolysis in the analyser, the CO produced was converted to CO_2 by passing it over I_2O_5 at 120 °C. The CO_2 was then collected cryogenically and analysed in a Finnigan MAT-250 mass spectrometer for the determination of $\delta^{18}O$ values (relative to SMOW). The external precision of the $\delta^{18}O$ measurement for laboratory standards was within ±0·3‰.

RESULTS

Simulated diurnal cycle

An attached sunflower leaf was maintained in the leaf chamber while the relative humidity of the air was decreased from 88 to 68% in four steps of 1 h each, and then increased back to 95% in a similar manner (Fig. 1a). The rate of transpiration was monitored continuously and was found to vary significantly during the experiment, although there was no consistent correlation with relative humidity (Fig. 1a). Based on the conditions in the leaf chamber, the steady-state $\delta^{18}O$ values were calculated according to Eqns 1, 2 and 3 (Fig. 1b), showing the expected diurnal trend. In a more direct approach, the variations in $\delta^{18}O$ of the leaf water were reconstructed by mass-balance calculation based on the $\delta^{18}O$ values of the water lost during transpiration (averaged over 1 h), $\delta^{18}O$ values of the input water, and the leaf water volume (Fig. 1b, and see also 'Discussion' section). At each humidity step, two samples of transpired water were collected (Fig. 1c), and in all cases the second sample had a $\delta^{18}O$ value nearer the $\delta^{18}O$ value of petiole

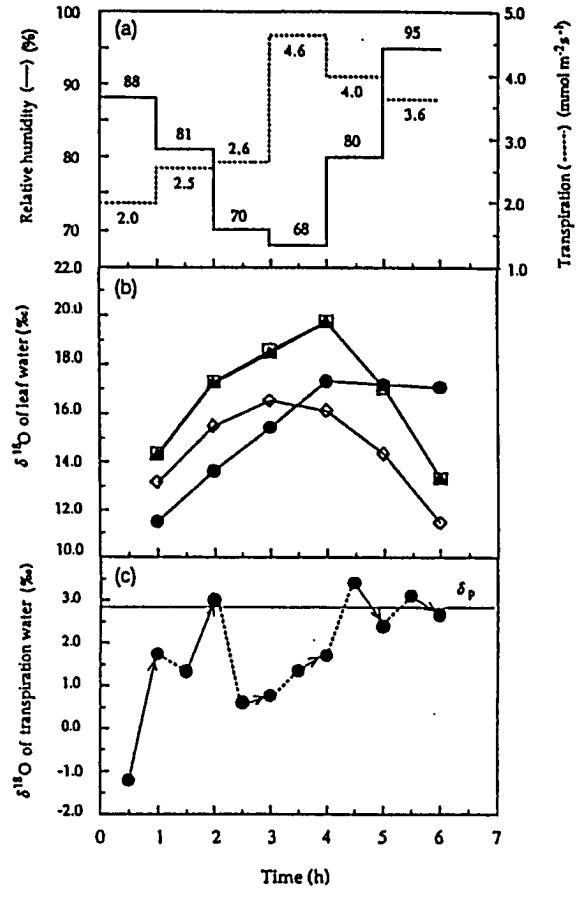

Figure 1. Changes in $\delta^{18}O$ values of transpiration water and of the expected $\delta^{18}O$ values of total leaf water of a sunflower leaf in response to a simulated diurnal cycle in relative humidity. (a) Transpiration rates (broken lines) and relative humidity (solid lines) at each time step. (b) Predicted $\delta^{18}O$ values of the leaf water according to Eqn 1 (□), Eqn 2 (◇) and Eqn 3 (▲), and based on isotopic mass-balance calculation (Eqn 4) (●). (c) The $\delta^{18}O$ values of transpiration water. Solid lines represent changes in $\delta^{18}O$ values within the same time step when conditions were kept constant. The $\delta^{18}O$ value of the petiole water (δ_p) is indicated.

water (δ_p). However, in many cases the $\delta^{18}O$ value of the transpiration water was markedly different from δ_p. These differences were most apparent at mid-cycle under the lowest humidity conditions (Fig. 1c). Under these conditions, the rate of change of the $\delta^{18}O$ value during each humidity step (i.e. the slope of the line connecting two points at the same humidity) was the lowest.

Approach to steady state

The approach of leaf water to isotopic steady state was monitored by comparing the $\delta^{18}O$ values of transpiration water (δ_T) to δ_p over time. A general pattern was apparent, as is shown schematically in Fig. 2; the specific results for a number of plant species are shown in Table 1. For example, in tobacco I there was a relatively rapid 'response stage' in which the $\delta^{18}O$ value of transpiration water decreased significantly from an initial $\delta^{18}O$ value (δ_1=−3·4‰) to an 'extreme point' (δ_{ex}=−6·3‰) within 10 to 35 min (with high transpiration plants such as tobacco, sunflower and cotton responding more rapidly than low transpiration plants such as banana and citrus plants). After δ_{ex} was reached, the trend in $\delta^{18}O$ values was reversed and a series of samples showed a gradual increase (from −6·3 to −4·6‰ in tobacco I) over the next 2 h, approaching the $\delta^{18}O$ value of the source water (δ_p=−3·6‰ in tobacco I). As expected, when the humidity was increased rather than decreased as in the above example, the opposite pattern was observed, with the $\delta^{18}O$ value first increasing (e.g. from +0·8 to +3·1‰ in banana I) and then decreasing, approaching or going beyond δ_p (e.g. from +3·1 to −3·4‰ in banana I). Only in two out of 11 cases did the $\delta^{18}O$ value of transpiration water differ by less than 1‰ from δ_p after 3 h under constant conditions. In almost all the other cases

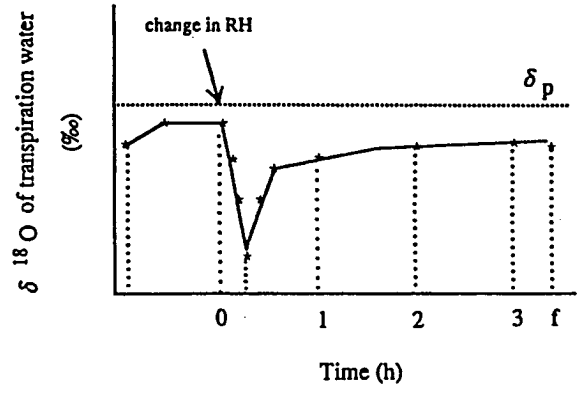

Figure 2. Schematic diagram of the protocol for sampling of transpiration water for the experiment shown in Table 1, indicating the typical shape of the isotopic response curve after a change in relative humidity (RH) at t=0. The sampling time (*) indicates the mid-point during the cryogenic trapping of transpired water. The sampling time between t=0 and t=1 was 3–5 min, and in all other cases it was 15–20 min (time=f indicates a final sampling time ranging from 10 to 60 min beyond the originally planned 3 h sampling period; the shape of the response curve when relative humidity was increased was the inverse of that shown).

the final $\delta^{18}O$ value (δ_f) was lower than that for petiole water by 1·5 to 3‰ (Table 1). It was also observed that in most cases the rate of change of the $\delta^{18}O$ of transpiration water was biphasic. During the first hour this rate was on average 3·3‰ h^{-1}, while after that time the rate of approach to isotopic steady state was almost an order of magnitude lower, becoming asymptotic at about 0·4‰ h^{-1}. The leaf water turnover rate (V/E) for each of the plants used in this study was also determined (Table 1). All plants had thin

Table 1. Variations in $\delta^{18}O$ values of transpiration water at time t (t=0–f; see Fig. 2), after a change in relative humidity (RH). An attached leaf was enclosed in the leaf chamber under constant initial RH for at least 3 h, after which time conditions were changed to the final RH. The time at which the extreme $\delta^{18}O$ value (δ_{ex}; see Fig. 2) was obtained is indicated, together with the difference between the $\delta^{18}O$ values of the petiole and final transpiration water samples ($\delta_p-\delta_f$), leaf water turnover rate (V/E) and the predicted $\delta^{18}O$ values based on Eqn 1 (δL^1) or Eqn 3 (δL^2). When no transpiration sampling was carried out after 3 h, δ_3 was considered to be equal to δ_f

Plant species	RH (%) (initial/final)	t_{ex} (min)	δ_1 (‰)	δ_{ex} (‰)	δ_1 (‰)	δ_2 (‰)	δ_3 (‰)	δ_f (‰)	$\delta_p-\delta_f$ (‰)	V/E (h)	δL^1 (‰)	δL^2 (‰)
Decreasing RH												
Cotton	85/63	15	+2·6	−0·1	+0·8	+1·5	+1·5	+1·6	+1·5	0·7	+20·9	+20·9
Sunflower I	85/80	20	−2·3	−3·9	−3·8	−3·7	−3·6	—	+1·8	1·2	+11·3	+11·3
Sunflower II	99/84	10	+1·5	+0·3	+2·5	+3·1	+3·5	—	−1·6	0·5	+16·8	+16·8
Tobacco I	65/49	30	−3·4	−6·3	−6·0	−4·8	−4·7	−4·6	+1·0	2·1	+18·5	+17·6
Tobacco II	80/35	15	−2·4	−8·1	−6·5	—	—	—	+3·0	2·4	+21·7	+17·7
Increasing RH												
Banana I	59/75	33	+0·8	+3·1	+1·7	−0·7	−2·7	−3·4	+0·6	25·6	+13·1	+2·7
Mandarin	48/70	35	−4·6	+0·7	+0·2	−2·3	−4·0	—	+2·5	8·0	+13·9	+9·3
Pomelo	43/69	35	−3·9	+3·1	+2·5	+0·2	−0·6	−1·7	0	8·7	+15·5	+9·6
Sunflower III	65/78	17	−5·5	−2·8	−4·8	−5·3	−5·9	—	+1·5	2·4	+9·8	+9·7
Sunflower IV	81/84	10	−0·3	+1·0	−1·9	−5·4	−5·6	−5·7	+2·8	1·6	+9·0	+9·0
Tobacco III	57/68	15	−4·3	−1·3	−2·4	−3·5	−3·7	−3·8	−0·1	2·4	+13·6	+13·2

Table 2. Variation in $\delta^{18}O$ values within leaves. The $\delta^{18}O$ values of water extracted from the petiole (δ_P), along the vein (δ_V) and from leaf discs (δ_d; see Fig. 3) are shown. The average $\delta^{18}O$ value of the leaf discs is compared with the predicted $\delta^{18}O$ value of total leaf water shown in Table 1. All samples were collected after the leaf had been maintained in the leaf chamber for at least 3 h under constant conditions. Tobacco IIIa and IIIb represent two halves of the same leaf

			δ_V		δ_d					
Plants		δ_P	I	II	1	2	3	4	5	Mean $\delta_d \pm$SD
Cotton	A	+3·1			+22·6	+22·3	+20·7	+20·6		+21·5±1·5
	B				+18·9	+21·2	+24·3	+22·0	+20·9	
Sunflower II	A	+1·9			+15·2	+15·7	+16·3			+15·0±1·0
	B				+13·9	+14·0	+14·6			
Tobacco I	A	−3·6			+18·6	+19·8	+13·9			+16·4±2·6
	B				+13·4	+15·5	+17·0			
Banana I	A	−2·8			+10·1	+17·7	+19·9			+13·7±5·6
	B				+14·6	+4·3	+15·3			
Banana II	A	−3·9	−2·0		+12·8	+3·9	+17·1			+11·2±6·1
	B				+3·7	+12·2	+17·2			
Mandarin	A	−1·5	−1·6		+13·7	+16·3	+20·6	+18·2		+17·2±2·9
	B									
Pomelo	A	−1·7	−2·7		+14·6	+18·1				+15·6±4·5
	B				+8·4	+13·5	+17·3	+21·5		
Sunflower III	A	−4·4	−2·1	−1·8	+5·9	+7·4	+7·4	+8·2		+6·5±1·3
	B				+4·4	+5·1	+5·7	+6·3	+7·7	
Sunflower IV	A	−2·9			+5·4	+6·5	+5·1	+5·3		+5·6±0·9
	B				+3·8	+5·9	+5·3	+5·8	+6·9	
Tobacco IIIa	A	−3·9	−3·6	−2·4	+12·8	+16·4	+18·2	+18·1		+16·4±1·9
	B				+14·1	+17·4	+16·4	+18·3	+15·9	
Tobacco IIIb	A	−3·9			+8·1	+13·7	+15·9	+16·0	+16·0	+15·3±3·0
	B				+16·6	+18·5	+15·0	+17·5		

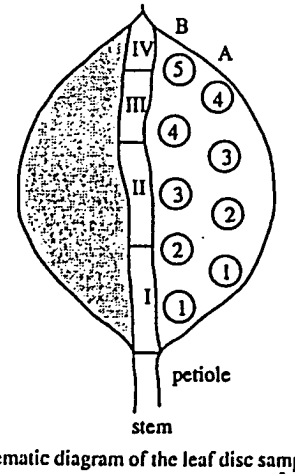

Figure 3. Schematic diagram of the leaf disc sampling for the experiment shown in Table 2. Leaf discs (0·9 cm^2) or slices (16·9 cm^2 in the case of banana leaves only) and vein sections were excised while the leaf was covered on both sides with Saran membrane. Sampling was carried out in a random order bearing no relation to the numbers in the diagram, and after the leaf had been maintained for at least 3 h under constant conditions.

(non-succulent) wide leaves. Variations in leaf water volume between species were relatively small (mean $V = 12{\cdot}3 \pm 2{\cdot}8$ mol m^{-2}), but turnover rates ranged from 0·49 h to 25·6 h as E ranged from 6·34 mmol $m^{-2} s^{-1}$ in sunflower to 0·16 mmol $m^{-2} s^{-1}$ in banana. The use of these turnover rates together with Eqn 3 (δL^2 in Table 1) showed that, in low transpiration leaves such as those of the citrus and banana plants, the $\delta^{18}O$ values of total leaf water should have deviated significantly from the steady-state value predicted by Eqn 1 (δL^1 in Table 1).

Spatial heterogeneity

Spatial isotopic heterogeneity was evaluated by analysing water samples from leaf discs or slices taken along and across the leaf as described schematically in Fig. 3 and reported in detail in Table 2. In one case, a more detailed mapping was carried out on the two halves of a tobacco leaf, with 18 leaf disc samples and four mid-vein samples (tobacco IIIa,b in Table 2). In this case, the $\delta^{18}O$ value of the transpiration water (−3·8‰) was determined before sampling and was found to be similar to that of the petiole

water (–3·9‰). In all cases, samples were taken only after the leaf had been maintained in the leaf chamber for at least 3 h under constant conditions. Monitoring of the precise conditions in the leaf chamber allowed estimation of the steady-state $\delta^{18}O$ value according to Eqn 1 (shown in Table 1), and its comparison with mean leaf water (shown in Table 2). As might be expected, the complex water pathway in leaves such as those used here resulted in widely differing patterns of variation in $\delta^{18}O$ values across leaves. However, in many cases a general trend could be clearly discerned with increasing $\delta^{18}O$ values from leaf base to tip and from leaf centre to leaf edges (Table 2). For example, the $\delta^{18}O$ values of disc water increased from 12·8 to 18·1‰ along the edges (row A) of the leaf in tobacco IIIa, and from 14·1 to 18·3‰ along the centre (row B) of the same leaf (Table 2). In sunflower III the $\delta^{18}O$ values increased from 5·9 to 8·2‰ along the edge (row A), and from 4·4 to 6·3‰ along the centre (row B) of the same leaf. Similarly, in several cases there was a clear trend of increasing $\delta^{18}O$ values from the centre to the edges of the leaves. For example, in sunflower III the $\delta^{18}O$ values increased from 4·4 to 5·9‰, from 5·1 to 7·4‰, from 5·7 to 7·4‰ and from 6·3 to 8·2‰ in a systematic progression from row B to row A (Table 2). It is important to emphasize that leaf disc sampling did not follow the veins or any known water-flow pathway in the leaves. It is not surprising, therefore, that a consistent enrichment pattern along the leaf could not always be observed. More significantly, it is important to note that in most cases at least some of the leaf water samples had a $\delta^{18}O$ value greater than that predicted by Eqn 1 (as reported in Table 1). In contrast to the general trends reported above, we also noted in some cases, especially in the banana, extreme patchiness that did not fit any pattern. In these cases variation of up to about 15·6‰ in the $\delta^{18}O$ value of adjacent leaf tissues could be observed (Table 2).

Large gradients in $\delta^{18}O$ values were also observed along the main vein of tobacco and sunflower leaves (Table 2). Two samples of tobacco vein water (for tobacco III) that are not reported in Table 2 had $\delta^{18}O$ values of –0·6‰ for the third and +7·5‰ for the fourth main vein sections (cf. Fig. 3). Here again the complex water pathway in the leaves was indicated. While a marked increase in the $\delta^{18}O$ value of water in the central vein was observed, tissue water adjacent to it, clearly not fed by this water, could be significantly more depleted. Relatively large variations in the $\delta^{18}O$ value of petiole water were also observed (Table 2), even though all plants were irrigated with the same water ($\delta^{18}O = -4·5‰$). The extreme (positive) δ_p values of the cotton and sunflower plants are representative of plants kept outside the greenhouse. It was also observed in many cases that the $\delta^{18}O$ value of stem water was slightly but consistently higher than that of the petiole or the base of the central vein.

DISCUSSION

Prediction of the $\delta^{18}O$ value of leaf water using any model based on the steady-state assumption of Eqn 1 is attractive because of its simplicity. The steady-state assumption is based on two major arguments. First, it is generally expected that leaf water volume (V) is small relative to the transpiration flux (E) passing through it, and changes in $\delta^{18}O$ values of leaf water can therefore be expected to take place rapidly. This expectation is in contrast to the observed turnover rates, ranging from 0·5 h to 25·6 h, for the plants used here. Secondly, on a large scale there is a requirement for long-term isotopic mass balance in the vegetation transpiration flux, $F_{in}\delta_p = E\delta_T$, where F_{in} and E are the input and output water fluxes through the leaf and δ_p and δ_T are the $\delta^{18}O$ values of these fluxes, respectively (the effect of leaf metabolism is assumed to be negligible; Yakir 1992). Clearly, on a long-term basis E must be matched by F_{in} and, consequently, δ_p must be equal to δ_T, consistent with the expected isotopic steady state. This long-term equality, however, can be misleading because it must be based on the complete diel cycle. For example, during the day, or parts of it, E can be larger than F_{in}, and δ_T can be smaller than δ_p, a situation that can be reversed during other parts of the day, or during the night (Wang & Yakir, unpublished results). In the present context, we are concerned only with the isotopic enrichment during the day, and there is therefore no requirement *a priori* for the steady-state assumption.

To test for the attainment of the isotopic steady state in leaf water in a variety of plant species in a non-destructive way, we measured the $\delta^{18}O$ values of transpiration water (Flanagan *et al.* 1991; Yakir 1991; Yakir *et al.* 1994a). As mentioned above, isotopic steady-state conditions can be determined when the $\delta^{18}O$ values of the output water (δ_T) and the input water (δ_p) are equal. In fact, assuming that at least one leaf water 'turnover' is necessary to approach a new isotopic steady-state condition, it was expected, based on the above-mentioned V/E rates, that at least in the slow transpiration plants the $\delta^{18}O$ value of leaf water would normally lag, to a certain extent, behind the expected steady-state value. The results presented in Fig. 1b for the simulated diurnal cycle showed that such a trend can be observed even in high transpiration plants.

Simulated diurnal cycle

As expected, δ_T fluctuated around the predicted steady-state value (δ_p) (Fig. 1c). Moreover, it was apparent that, even within each time step, the $\delta^{18}O$ value was approaching the expected steady-state value. However, δ_T differed markedly from δ_p over extended parts of the cycle, most notably at the lowest humidities at midday, when the slope of the line connecting two points within a time step (the 'rate of response') was also the lowest. The significance of the variations in δ_T can be evaluated by reconstructing the corresponding changes in the $\delta^{18}O$ value of leaf water from simple mass-balance calculations averaged over 1 h time steps and assuming constant leaf water volume (V):

$$\delta_{L,2} = \delta_{L,1} + (\delta_p - \delta_T)(E/V), \quad (4)$$

where $\delta_{L,1}$ is the $\delta^{18}O$ value of leaf water at time t_1, $\delta_{L,2}$ is the $\delta^{18}O$ value of leaf water at time t_2, and $t_2 - t_1$ is 1 h. The

results shown in Fig. 1b indicate that, although the sunflower leaf had a typical enrichment during the early part of the cycle, the overall pattern and extent of enrichment were markedly different from any of those predicted (Fig. 1b). Notably, in contrast to expectations there was a significant lag in enrichment during the first half of the cycle and only a slight decrease in $\delta^{18}O$ in the latter part. It is also important to note that, according to Eqn 3, the sunflower leaf had a sufficiently high *E/V* to maintain a steady-state $\delta^{18}O$ value of total leaf water throughout the simulated cycle (Fig. 1b). The results indicate, therefore, that caution should be exercised before assumptions regarding the isotopic steady state of leaf water are generally applied. It is important to emphasize, however, that under field conditions changes in humidity are gradual and the $\delta^{18}O$ values of leaf and transpiration water may fluctuate closer to the expected values than under laboratory conditions. Similarly, the potential effects of patchy stomatal closure due to humidity changes may be less under field conditions.

Approach to steady state

To characterize in more detail the kinetics of the approach to isotopic steady state, we monitored the change in δ_L over longer periods of time after an incremental change in relative humidity (Fig. 2 & Table 1). Here again, δ_L showed the expected approach to a steady-state value. A true steady-state value ($\delta_L = \delta_p$) was, however, rarely attained. The response of δ_L to a change in air humidity followed a typical pattern (Fig. 1) with a rather slow, asymptotic approach to a final steady-state value. The $\delta^{18}O$ value during the approach to the isotopic steady state can also be predicted by Eqn 3. This calculation shows that the various plant species used here can be clearly divided into high transpiration and low transpiration plants. The first group appears to have average *V/E* ratios sufficient to reach an isotopic steady state within the time frame of the experiment ($\delta L^1 \approx \delta L^2$ in Table 1). In contrast, the second group should, according to Eqn 3, be significantly removed from the isotopic steady state even after 3 h. Interestingly, however, the results for δ_L do not show this clear-cut distinction between plants. Even the high transpiration species did not, in many cases, give the expected equality of $\delta_L = \delta_p$. This observation is consistent with the results shown in Fig. 1, and cannot readily be explained by the current models of water distribution and fluxes within leaves. The most extreme example of the unpredictable nature of $\delta^{18}O$ in leaf water was observed in the banana (Table 2, and see below). In this case, patches of extremely low $\delta^{18}O$ values of leaf water were observed. If these observations reflect very low rates of transpiration in parts of the leaf (possibly associated with patchy stomatal closure), the approach to isotopic steady state of these parts, and consequently of the leaf as a whole, could be slow, as was reflected by δ_L. Similarly, it was postulated previously (e.g. White 1989; Yakir *et al.* 1990) that any internal compartmentalization of leaf water can result in a slow exchange between compartments and a slowing down of the response of the whole leaf to environmental changes.

In some of the slow transpiration leaves, a δ_L value essentially identical to δ_p was observed (apparently indicating steady-state conditions). Application of Eqn 3, however, strongly indicated that these leaves should have been markedly removed from the isotopic steady state. It is possible that, in these slowly responding cases, such an agreement between predicted and measured $\delta^{18}O$ values is due to 'memory' effects associated with the conditions and $\delta^{18}O$ values that prevailed before the beginning of the experiment.

Spatial heterogeneity

Spatial isotopic heterogeneity in leaf water was reported previously (e.g. Yakir 1991; Luo & Sternberg 1992). In the present study we have shown that spatial heterogeneity is maintained even after at least 3 h under constant conditions in a well-stirred leaf chamber, and in high transpiration crop plants (Table 2). Furthermore, we have shown that in many cases this spatial heterogeneity has a clear underlying pattern. In these cases, after a large incremental increase in $\delta^{18}O$ values from stem to leaf blade, the $\delta^{18}O$ value of tissue water continued to increase from the leaf base toward the tip. These trends, consistent with previous observations made along a corn leaf (Yakir 1991), were maintained over extended time periods under constant conditions, and could not be explained by gradients in external conditions in the well-stirred leaf chamber. We concluded that the leaves behaved essentially as a string of pools in which each element was fed by water already enriched by evaporation in the preceding element (see Diagram 3C in Yakir 1991). Furthermore, we assumed that the trend is not always clear because the elements in such a string are not discrete and its track along a typical C_3 leaf is not obvious. The 'string' hypothesis is strongly supported by the observation that $\delta^{18}O$ values of specific leaf sections were below and above the values predicted by Eqn 1 (Table 2). This is exactly what would be expected in a string of interconnected evaporating lakes (Gat & Bowser 1991):

$$\delta_n = \delta_{n-1} + \frac{(\delta_a + \frac{\varepsilon}{h}) - \delta_{n-1}}{1 + \frac{F_+(1-h)}{Eh}}, \qquad (5)$$

where subscript n represents the nth element in the series, $\varepsilon = \varepsilon_{eq} + (1-h)\,\varepsilon_k$, and F_+ is the flux into and E is the evaporation from the element.

According to Eqn 5, water fractions become increasingly enriched as they are fed by the preceding already enriched elements, up to a limit defined by the ambient conditions, and which can be much higher than that predicted by Eqn 1 (Gat & Bowser 1991). Although at steady state the average $\delta^{18}O$ value of such a 'string' would be expected to approach the value predicted by Eqn 1, it is important to note that in plants (particularly with low tran-

spiration flux) the first element in the 'string' may in fact be located in the petiole or stem itself (see e.g. Dawson & Ehleringer 1993). In this case, the water in the leaf blade alone (which is normally measured) could have an average $\delta^{18}O$ value higher than that predicted by Eqn 1. Indeed, such high $\delta^{18}O$ values have been reported previously (Walker & Lance 1991; Flanagan *et al.* 1993; cf. Cotton or Mandarin in Table 2). The application of Eqn 5, or its integrated form, to leaves is not simple and will be dealt with elsewhere. This is because of the non-discrete nature of the string elements in a leaf, and because the effects of back diffusion between the string elements, which are irrelevant in a lake system (Gat & Bowser 1991), must be included when applying Eqn 5 to leaves.

Although an underlying pattern consistent with the 'string' approach is apparent, we note that it can be obscured by complex water pathways, and by additional patchiness such as that observed in banana leaves. In this extreme case, differences in $\delta^{18}O$ values of leaf water as large as 15·6‰ were observed between adjacent leaf sections (Table 2; and consistent with other preliminary observations in this species). Such effects may be associated with patchy stomatal closure, but they also indicate that there was little water communication between adjacent parts of the same leaf, a situation similar to findings in some succulent leaves (Tissue, Yakir & Nobel 1991; Yakir *et al.* 1994b).

The results of these experiments are consistent with the conclusions drawn above with regard to the attainment of the isotopic steady state, but they also show that an underlying pattern in the distribution of $\delta^{18}O$ values of leaf water may be discerned. Thus, isotopic enrichment in leaves of a variety of plant species appears to occur along a 'string' of water elements, all of which are exposed to evaporative enrichment, in contrast to the one-step enrichment which is usually assumed. Whether this pattern reflects symplastic water movement or simply isotopic enrichment of vein water has yet to be determined.

In conclusion, we note that the results of the present study, if substantiated, indicate that it may be necessary to include more species-specific physiological parameters in future models of leaf water $\delta^{18}O$. Alternatively, the $\delta^{18}O$ value of a constituent of leaf organic matter (starch, cellulose, etc.) may prove useful in providing a time-averaged record of the $\delta^{18}O$ value of leaf water.

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3.2 Non-climatic variations in the oxygen isotopic compositions of plants

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Abstract

The ^{18}O enrichment of leaf water, relative to local meteoric water, is known to reflect environmental conditions and to uniquely label atmospheric CO_2 and O_2 exchanged with leaves. Less is known, however, to what extent variations in the ^{18}O content of leaf water are influenced by plant species-specific characteristics. Here we evaluate the non-climatic variations in the isotopic composition of plant water and cellulose, and other related parameters, in a collection of 90 plant species from all continents grown under the same climatic conditions in the Jerusalem Botanical Garden. Variations of about 9‰ were observed in the $\delta^{18}O$ values of stem water, δ_S, and of about 14‰ in the mid-day $\delta^{18}O$ enrichment of bulk leaf water, $\delta_{LW} - \delta_S$. Differences between $\delta^{18}O$ values predicted by a conventional evaporation model, δ_M, and δ_{LW} ranged between -3.3‰ and +11.8‰. The $\delta^{18}O$ values of water in the chloroplasts (δ_{ch}) in leaves of 10 selected plants were estimated from on-line CO_2 discrimination measurements. Although much uncertainty is still involved in these estimates, the results indicated that δ_{ch} can significantly deviate from δ_M in species with high leaf peclet number. The $\delta^{18}O$ values of bulk leaf water significantly correlated with $\delta^{18}O$ values of leaf cellulose (directly) and with instantaneous water use efficiency (A/E, inversely). Differences in isotopic characteristics among conventionally defined vegetation types were not significant, except for conifers that significantly differed from shrubs in $\delta^{18}O$ and $\delta^{13}C$ values of cellulose and in their peclet numbers, and from deciduous woodland species in their $\delta^{18}O$ and $\delta^{13}C$ values of cellulose. The results indicate that predictions of the $\delta^{18}O$ values of leaf water (δ_{LW}, δ_M and δ_{ch}) could be improved by considering plant species-specific characteristics.

Introduction

The ^{18}O signature of leaf water of land plants is increasingly implemented in various biogeochemical models that investigate the exchange of CO_2 and O_2 between the land surface and the atmosphere (e.g. Francey & Tans, 1987; Berry, 1992; Ciais et al., 1997; Bender et al., 1994; Farquhar et al., 1993). The incorporation of ^{18}O is expected to be useful in addressing questions associated with the partitioning of net fluxes of these gases between photosynthetic uptake and respiratory release (for CO_2, e.g. Ciais et al., 1997; Yakir & Wang, 1996), or between land and ocean productivities (for O_2, e.g. Bender et al., 1994). The oxygen isotopic composition of leaf water is, however, not well characterized and is difficult to accurately predict. It is known to represent an isotopically heterogeneous system (Yakir et al 1989; Walker & Lance, 1991; Luo & Sternberg, 1992; Bariac et al., 1994; Wang & Yakir, 1995), only part of which participate in labeling atmospheric CO_2 and O_2, i.e. the leaf water fraction contained within the chloroplasts. The leaf chloroplasts is where oxygen is evolved from water during photosynthesis, and where, predominantly, the enzyme carbonic anhydrase (CA) facilitates the isotopic exchange of oxygen between water and CO_2 (cf. Yakir et al., 1994).

The Craig and Gordon (1965) model that describes the isotopic composition of water bodies undergoing evaporation has been widely applied (Gonfiantini et al, 1965; Dongmann et al., 1974; Förstel, 1978; Farris & Strain, 1978; Flanagan & Ehleringer, 1991) to predict the ^{18}O enrichment in leaf water under steady state conditions according to:

$$\delta_M = \delta_s + \varepsilon_{eq} + \varepsilon_k + h^* \cdot (\delta_a - \varepsilon_k - \delta_s) \qquad (1)$$

where, δ (‰) = $(R_{sample}/R_{standard} - 1)\cdot 10^3$; R is $^{18}O/^{16}O$ ratio, the standard is Vienna Standard Mean Ocean Water (VSMOW). Subscripts M, s and a stand for modeled leaf water, source water and air humidity, respectively; ε_{eq} is the equilibrium fractionation factor, which at 25°C is 9.38‰; ε_k is the kinetic fractionation factor, which is influenced by the leaf stomatal and boundary conductances (Farquhar & Lloyd, 1993; Buhay et al., 1996); h* was traditionally used for air relative humidity at the leaf surface temperature, and can be represented also by the ratio of the partial vapor pressures in the ambient air and inside the leaf (e_a/e_i ; Flanagan & Ehleringer, 1991).

This model, based primarily on meteorological data such as temperature, relative humidity and $\delta^{18}O$ values of source water and atmospheric moisture, generally overestimates to various extents the $\delta^{18}O$ value of bulk leaf water (Dongmann et al., 1974; Farris & Strain, 1978; Walker et al., 1989; Flanagan & Ehleringer, 1991; Yakir et al., 1994) and does not account for observed differences among plant species growing under the same environmental conditions (e.g. Förstel, 1978).

Several ways to improve predictions of the $\delta^{18}O$ values of the bulk leaf water (δ_{LW}), have been proposed (Walker et al, 1989; White, 1989; Buhay et al, 1996; Farquhar & Lloyd, 1993). The modification of Farquhar & Lloyd (1993) assumes that Eq. 1 predicts the $\delta^{18}O$ value of water at the evaporating surfaces, but the ^{18}O enriched water diffuse back into the leaf tissue against the advective evaporative flux and δ_{LW} represents the integration of the isotopic gradient that is formed over a mixing pathlength, L:

$$\delta_{LW} = \delta_s + (\delta_M - \delta_s) \cdot \frac{1 - e^{-p}}{p} \qquad (2)$$

where $p=(E \cdot L)/(C \cdot D)$ is the so-called peclet number, E stands for the rate of evapotranspiration, C for the mole concentration of water, and D for the diffusivity of $H_2{}^{18}O$ in water. This modification links the isotopic composition of leaf water to plant-dependent parameters such as E and L, and consequently p, and predicts that bulk leaf water, δ_{LW}, will be less enriched than the evaporating surfaces, δ_M. Variations in plants p or L, are, however, not well characterized at present.

Uncertainties still exist also as to the specific $\delta^{18}O$ signature of chloroplast water (δ_{ch}) which can be the same as that of the evaporating surfaces (Eq. 1; Farquhar et al., 1993), or closer to that of bulk leaf water (Yakir et al., 1994). This is important since, as indicated above, the δ_{ch} is the specific quantity that influences the $\delta^{18}O$ of CO_2 and O_2 exchanges by leaves, and its distinction from δ_M or δ_{LW} may well be species specific.

The objective of this study was to assess the non-climatic variations in δ_{LW} and related parameters using a global scale collection of plants grown in close proximity in the field under similar environmental and isotopic conditions.

Materials and Methods

Plant material

Plants used in this study are grown in the Jerusalem and University Botanical Garden in Jerusalem which holds an outdoor collection of major plant species representative of all continents. Gas exchange measurements and collection of leaf samples from 90 different plant species, from all continents, were carried out during the same week in July 1995 between 10:20 and 13:30 under very stable climatic conditions. Only young, fully expanded and sunlit leaves were used. A list of the plant species used in this study is given in the Appendix. Three five months old potted plants purchased from a nearby nursery were also used for lab measurements of on-line discrimination. In these cases, plants were adapted to lab environments for one week before measurements.

Gas-exchange measurements and plant sampling

Rates of photosynthesis and transpiration, stomatal conductance, as well as micro-meteorological conditions (relative humidity, photon flux density and air and leaf temperatures), were measured by means of a portable leaf gas exchange system (ADC-3 with PLC-3 leaf cuvette, ADC, Hoddesdon, Herts, England) and calculated according to von Caemmerer and Farquhar (1981). Boundary layer conductance in the leaf cuvette was estimated to be about 2 mol $m^{-2}s^{-1}$ (Parkinson, 1985). Leaf area was determined from traces made for each leaf in the field. Subsequent to each gas exchange measurement, a similar, nearby leaf and a woody (non-green) stem sample were sealed, separately, in test tubes (Vacutainers, Rutherford, NJ, USA). Atmospheric moisture samples were simultaneously collected by flowing air through a cryogenic trap.

The above mentioned leaf cuvette was also used for measurements of leaf discrimination against $^{13}CO_2$ and $C^{18}O^{16}O$ by attaching it to a 1/4" stainless steel vacuum line (better than $1.0x10^{-3}$ torr) used for cryogenic drying (-80°C) and trapping of CO_2 at liquid nitrogen temperature (Fig. 1). CO_2 and water vapor in the air flow (ca. 250 mL min^{-1}) entering and exiting the cuvette was measured with an infra-red gas analyzer (LiCor-6262, Lincoln, NE, USA). CO_2 samples were trapped for 2 min, after which the vacuum line was evacuated and the CO_2 transferred and sealed in a glass ampoule. After each measurement, the leaf was cut and sealed in a test tube. Ambient environmental conditions at the time of the measurements were also recorded to allow estimation of leaf water $\delta^{18}O$ values (Eq. 1).

Isotopic analysis

Leaf and stem water were extracted by vacuum distillation at 60°C in the lab. $\delta^{18}O$ values of the water samples were determined by equilibration with CO_2 at 25°C over night followed by cryogenic separation of CO_2 for the mass spectrometric analysis (Finnigan MAT-250). Precision for the water analysis was ±0.1‰. Samples of CO_2 from the on-line discrimination measurements were directly analyzed on the mass spectrometer with an external precision of ±0.05‰ for $\delta^{13}C$ and ±0.1‰ for $\delta^{18}O$.

Leaf cellulose was purified according to Epstein et al. (1977), freeze dried and ground to fine powder with a pestle. $\delta^{13}C$ values of the cellulose were determine by combusting about 0.5 mg samples in an elemental analyzer (EA1108, Carlo-Erba Instruments, Fison Inc., Milan, Italy), and passing the CO_2 generated, on-line, to an isotope ratio mass spectrometer (OPTIMA, Micromass, Manchester, England). $\delta^{18}O$ values of cellulose were determined by pyrolyzing about 0.5 mg samples in the same elemental analyzer, converting the CO produced to CO_2 (over I_2O_5)

which was cryogenically trapped and used for mass spectrometric analysis as above (Santrock & Hayes, 1987). Precision of leaf cellulose analyses were ±0.06‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{18}O$.

Estimating $\delta^{18}O$ values of chloroplast water

Discrimination against $C^{18}O^{16}O$ ($^{18}\Delta$) was calculated, similarly to that for $^{13}CO_2$, according to (Farquhar & Lloyd, 1993):

$$^{18}\Delta = \bar{a}_l + \varepsilon \cdot (\delta^c_{ch} - \delta_e) \quad (3)$$

where $\bar{a}_l$ is the weighted total discrimination against $C^{18}O^{16}O$ due to diffusion at various steps from the atmosphere to the chloroplast (usually 7-8.8‰; cf. Farquhar and Lloyd, 1993); $\varepsilon = c_{cs}/(c_a - c_{cs})$, where c_{cs} is the CO_2 concentration at the chloroplast surface (see also Discussion); δ_e the $\delta^{18}O$ value of CO_2 entering the leaf chamber; $\delta^c_{ch} = \delta_{ch} + (\alpha - 1) \cdot 1000$ is the $\delta^{18}O$ value of CO_2 after full isotopic equilibrium with chloroplast water (δ_{ch}), α is the temperature corrected equilibrium fractionation for ^{18}O exchange between CO_2 and water (Brenninkmeijer et al., 1983). Estimates of δ_{ch} values were obtained using Eq. 3 by directly measuring $^{18}\Delta$ during leaf gas exchange according to Evans et al. (1986):

$$\Delta = \frac{\xi * (\delta_o - \delta_e)}{1000 + \delta_o - \xi * (\delta_o - \delta_e)} * 1000 \quad (4)$$

where δ_o and δ_e are the $\delta^{13}C$ or $\delta^{18}O$ values of the CO_2 leaving or entering the leaf chamber, respectively; $\xi = c_e/(c_e - c_o)$; c_e and c_o are CO_2 concentrations of the air entering and leaving the leaf chamber, respectively. Estimates of c_{cs} were based on an intermediate value between c_i and c_c, obtained from concurrent measurements of gas exchange parameters and of ^{13}C discrimination according to (Evans et al., 1986; von Caemmerer & Evans, 1991):

$$\frac{c_c}{c_a} = \frac{c_i}{c_a} - \frac{\Delta_i - \Delta_{obs} - (eR_d / k + f\Gamma_*)/c_a}{b - a_w} \quad (5)$$

$$\Delta_i = a_b \frac{c_a - c_s}{c_a} + a \frac{c_s - c_i}{c_a} + b \frac{c_i}{c_a} \quad (6)$$

where Δ_{obs} refers to ^{13}C discrimination determined as in Eq. 4, c is CO_2 concentration and subscripts a, s, i, and c stand for atmosphere, leaf surface, intercellular spaces, and chloroplast, respectively; a_b is the ^{13}C fractionation occurring during diffusion in the boundary layer (2.9‰); a

is the isotopic fractionation during diffusion through stomata (4.4‰); a_w is the combined ^{13}C fractionation due to dissolution and diffusion of CO_2 in water (1.8‰); b is the net ^{13}C fractionation of Rubisco which can vary in the presence of other carboxylases (27‰ to 29‰, Farquhar & Lloyd, 1993; Guy et al., 1993); R_d is the rate of dark respiration and e is the fractionation associated with it (assumed to be negligible); f is the fractionation associated with photorespiration (that may vary between 7‰ [Rooney, 1988] and almost zero [Gillon & Griffiths, personal communication]); k is the carboxylation efficiency of Rubisco and Γ_* is the CO_2 compensation point in the absence of dark respiration during the day (Brooks & Farquhar, 1985).

In Eq. 3, full isotopic equilibrium between CO_2 and water in the chloroplast is assumed. Considering incomplete ^{18}O equilibrium between CO_2 and chloroplast water due to low activity of the enzyme carbonic anhydrase, Eq. 3 can be modified as:

$$\Delta^{18}O = \frac{\bar{a}_1(1+3\rho)+\varepsilon\cdot[(\delta^c_{ch}-\delta_e)+3\rho\cdot b_1]}{1-\varepsilon\cdot(\delta^c_{ch}-\delta_e)+3\rho\cdot\varepsilon} \qquad (7)$$

where ρ is the ratio of the rate of carboxylation by Rubisco to rate of hydration of CO_2 by carbonic anhydrase; b_1 is the discrimination by Rubisco against $C^{18}O^{16}O$, which is presumably negligible (Farquhar & Lloyd, 1993).

Statistical analysis

Statistical analyses were performed using StatView 512+ (Abacus Concepts, Inc.). All curves were fitted using least squares regression. When a correlation between any two parameters was significant (*P < 0.05, **P < 0.01, ***P < 0.001), a linear regression equation was given; otherwise, only regression coefficient and probability were given. Unpaired t-test with a 2-tail probability or one factor Anova were also carried out for comparative purposes.

Results and Discussion

Bulk leaf water

The $\delta^{18}O$ value of leaf water is influenced by environmental conditions and the $\delta^{18}O$ values of input water and atmospheric moisture (Eq. 1). Sampling of the 90 plant species surveyed in this study were conducted under similar environmental conditions. Photon flux density was on average (± s.d.) 1652±80 μmol m^{-2} s^{-1}, leaf surface temperature was 36.2±1.8°C, relative humidity of the ambient air was 37.6±4.4% (Fig. 2). During the sampling period from 10:20am to 13:30pm, air temperature increased and relative humidity decreased to some extent with contrasting effects expected on δ_{LW}. Such effects were accounted for in calculations using Eq. 1. The $\delta^{18}O$ value of

air moisture during the sampling period (measured once during sampling) was typical for the region (-9.7‰).

Relatively large variations (ca. 9‰) were observed in the $\delta^{18}O$ value of stem water (Fig. 3a), although all plants are grown in a restricted area with no known source of water that may have $\delta^{18}O$ different than that of local precipitation.

The long term mean $\delta^{18}O$ value of local precipitation is -4.5‰ and the mean $\delta^{18}O$ value of precipitation during the proceeding winter was -4.9±0.8‰ (I. Carmi, personal communication). Only about half of the plants sampled (43 species) had δ_s consistent with these values, i.e. between -5.7 and -4.1‰. Most of the other species had higher δ_s values. This most likely reflected evaporative enrichment of soil water and variations in the depths at which water is taken up by different plant species (Dawson, 1993). In one extreme example, a plant (*Scyrpus*) growing near a small evaporating pond had a δ_s value of +3.3‰. Interestingly, a few plants showed δ_s values lower than mean precipitation. This is likely due to temporal variations in δ(precipitation) combined with incomplete mixing of water in the soil (c.f. Yakir & Yechieli, 1995).

While effects of evaporation from the soil were likely to be greater here than in a forest (as plants grow in small groups), perennial plants and trees are also usually expected to draw water at depths which are less sensitive to soil evaporation. The results indicate therefore that improving knowledge of the deviations of δ_S from mean δ(precipitation) are likely to improve predictions of δ_{LW}.

Large variations in δ_{LW} (from +6.1 to +20.2‰) among plant species were observed (Fig. 3b; excluding the only C4 plant, *Scyrpus*, growing near evaporating pond, for which δ_{LW} was +28.0‰). These results, consistent with previous observations (Förstel, 1978), can not be explained by the variations in δ_s as similar variations, +11.1 to +24.7‰, were also observed in $[\delta_{LW}-\delta_S]$. Similarly, variations in δ_{LW} could not be explained by changes in environmental conditions during the measurements time (Fig. 2), as those would have an effect smaller than 5‰ and no correlation was observed between δ_{LW} and time of measurement (not shown). Mean bulk leaf water enrichment ($\delta_{LW}-\delta_S$) was about +16.9‰, with one extreme case of +24.7‰ (*Scyrpus*).

Assuming that leaf water was in all cases near isotopic steady state (Wang & Yakir, 1995), variations in δ_{LW} values could also be estimated by Eq. 1. Ideally, such estimates should be based on regional climatic and meteorological conditions yielding a single, climatically derived, value for our restricted sampling area. Predictions can be improved, however, by using actual leaf temperature, stomatal and boundary layer conductances and δ_s values. Using these measured values produced a range of predicted δ_M values with a mean value representative of the sampling site (+18.2±1.7‰; n=89; Fig. 3c). Notably, the diffusional isotopic fractionation (ε_k) used in the prediction of δ_M varied among plant species (Buhay et al, 1996) and significantly contributed to the

variations in δ_M. A rough estimate of the variations in ε_k, +21.1 to +27.7‰ (mean=+25.2±1.3‰, n=90; Fig. 3d) was obtained for leaves within the gas exchange system.

The differences between predicted and measured bulk leaf water values ranged between -3.3 to +11.8‰ (mean difference of +5.3±2.5‰; n=90; Fig. 3e). Notably, in four cases δ_{LW} values were higher than predicted δ_M. Such observations were made before (e.g. Walker & Lance, 1991; Flanagan et al., 1993) and can be due to enrichment of stem or petiole water or decreasing leaf water volume. In most cases δ_M was, as expected, higher than δ_{LW} (see Introduction). These differences can be used to characterize a plant-specific peclet number (Eq. 2; Fig. 3f). The range of peclet numbers, p, for the plant species sampled here was 0.1-1.6 (mean=0.64±0.28, n=86; Fig. 3f). This range is equivalent to 0.6-0.9 in the ratio of observed/predicted leaf water enrichment, $(\delta_{LW}-\delta_s)/(\delta_M-\delta_s)$. Note that as defined in Eq. 2, p is influenced primarily by two variables, the rate of the advective flux, E, and the effective mixing length, L (estimated to range here between 0.4-16.6 cm; mean= 4.3±2.9cm, n=86). Since E and L can vary independently, it is not surprising that in comparing different species, correlation between δ_{LW} and E was very poor (not shown) but with p was very high (see below).

Chloroplast water

As noted in the introduction, the specific $\delta^{18}O$ value of water in the chloroplast of transpiring leaves (δ_{ch}) is of particular interest. There is still uncertainty, however, about the relationships between δ_{ch} and δ_{LW} or δ_M. Here we estimated δ_{ch} values from measurements of discrimination against ^{18}O in CO_2 carried out on 10 selected plants. Three of the plant species were measured more extensively in the lab to test for effects of leaf enclosure in the leaf-cuvette on measured leaf discrimination. In these tests (data not shown), discrimination was measured on leaves that were maintained under constant conditions in the leaf cuvette for several hours and then again ~3 min following a change in conditions (e.g. increase in humidity, as is likely to occur during field measurements). Comparing the two measurements indicated that our rapid field measurements did not introduce a measurable change in discrimination.

Measured on-line leaf discriminations were then used to estimate δ_{ch} using Eq. 3 (Fig. 4). The range in δ_M values in Fig. 4 reflects the fact that environmental conditions were not identical for all measurements and co-variations in δ_M and δ_{LW} was generally observed. Notably, estimates of δ_{ch} are sensitive to some of the assumptions made in its calculation. First, estimating ^{18}O discrimination according to Eq. 3 requires knowledge of the CO_2 concentrations in the chloroplast, which is estimated from concurrent measurements of gas exchange and ^{13}C discrimination (Eqs. 4-6). These equations, in turn, are sensitive to the values assumed for b, the biochemical discrimination and f, the discrimination associated with photorespiration. The biochemical discrimination of Rubisco 29‰ (Guy et al., 1987; including in a green tissue), is sometime

assumed to effectively be lower (~27‰) due to activity of other, low discrimination, carboxylases in the same tissue (Farquhar & Lloyd, 1993). Little information is also available on the discrimination associated with photorespiration. A value of f=7 (Rooney, 1988) is often adopted, but it may vary with plant species and can, at least in some cases, be close to zero (Gillon & Griffiths, personal communication). As noted above, while ^{13}C discrimination provides an estimate of c_c, it may not be the appropriate parameter for ^{18}O measurements. This is because CA activity and the rapid oxygen exchange between CO_2 and water cancel out any ^{18}O gradients due to diffusion within the chloroplast (any CA activity outside the chloroplast membrane could do the same for cytoplasm). Since chloroplast resistance to diffusion can be a significant component of the liquid phase diffusion pathway (Evans & von Caemmerer, 1996; Nobel, 1991), some intermediate value, c_{cs}, between c_i and c_c may be more appropriate. Uncertainties associated with c_{cs} also contribute to variations in the kinetic discrimination, $\bar{a}_1$, which for ^{18}O in CO_2 has a maximum value of 8.8‰ (Hesterberg & Siegenthaler, 1991). The effective value, however, can be significantly lower (e.g. 7.4‰, Farquhar & Lloyd, 1993) mainly because of small discrimination (~0.8‰) in the liquid phase. $\bar{a}_1$ is therefore influenced by the extent of the liquid phase resistance that is considered. Correcting for c_{cs} in both $\bar{a}_1$ and ε in Eq. 3 will result in lower estimates of δ_{ch} (0.5-1‰). In contrast, Eq. 3 assumes c_{cs} to be at full equilibrium with chloroplasts water. It was previously noted, however, that such complete equilibrium may not be always attained (Farquhar et al. 1993; Flanagan et al, 1994; Williams et al., 1996), suggesting the use of Eq. 7 and a value of ρ=0.02. This would lead to lower estimates of $^{18}\Delta$ and higher δ_{ch} values.

Considering the possible uncertainties currently associated with estimating δ_{ch} is demonstrated in Fig. 4a. Maximal δ_{ch} values are obtained by using in Eq. 3,5,6,7 the parameters b=29, f=0, $\bar{a}_1$=7.4, ρ=0.02 and $c_{cs} = c_c$; while the minimum δ_{ch} values are obtained using b=27, f=7, $\bar{a}_1$=8.8, ρ=0 and $c_{cs} = (c_i + c_c)/2$. However, assuming that δ_M is generally the upper limit for leaf water enrichment our best estimate for δ_{ch} was obtained based on b=29; f=4; $\bar{a}_1$=7.7; ρ=0 and $c_{cs} = (c_i + c_c)/2$ (Fig. 4b).

The variations observed in δ_{ch} among plants were considerably larger than those in δ_M. A good agreement between δ_{ch} and δ_M was observed in some cases (e.g. conifers), but a significant discrepancy was observed in others (e.g. deciduous). Such discrepancies (as large as 11‰ which could not be reconciled with any combination of the parameters considered in Fig. 4a) tended to increase with increasing peclet number (Fig. 4b; cf. Eq. 2). Although calculated p can be influenced to some extent by changes in E with time of measurement, variations in p among plant species clearly reflected an intrinsic plant characteristics (Fig. 3f). The results indicated, therefore, that while a good prediction of δ_{ch} may be achieved by Eq. 1 for some species, considering specific species-specific characteristics are likely to improve such predictions.

Other leaf characteristics

Estimates of leaf water isotopic enrichment may be aided by additional plant-related information. For example, it is known that the $\delta^{18}O$ of leaf cellulose (δ^{18}_{cell}) is influenced by the $\delta^{18}O$ of leaf water (DeNiro & Epstein, 1979; Yakir, 1992), and therefore has the potential to be used as a record of it. $\delta^{13}C$ values of leaf cellulose (δ^{13}_{cell}) can be used to estimate leaf ^{13}C discrimination, substituting on-line measurements such as used here (Farquhar & Lloyd, 1993). Similarly, short term measurements of rates of photosynthesis, evapotranspiration and stomatal conductance, can help characterize different plant species. Here we measured such parameters, evaluating their variations and considered interactions among them first in the pooled group of plants, and then after dividing it to several subgroups.

Considering first variations in the pooled collection (Fig. 3), δ^{13}_{cell} values varied (excluding a single C_4 species) from -28.2‰ to -20.5‰ (mean=-24.1± 1.6‰, n=89) (Fig. 3g). The δ^{18}_{cell} values ranged from +23.2 to +41.3‰ (mean=+31.3±3.9‰, n=89; Fig. 3h). Differences between δ^{18}_{cell} and corresponding δ_{LW} (δ^{18}_{cell}-δ_{LW}) ranged from 9.2 to 27.9‰ (mean=+18.5±4.0‰, n=89; see Appendix). Leaf stomatal conductance in the 90 species ranged from 0.1 to 6.5 mol $m^{-2}s^{-1}$, (mean=1.1±0.9 mol $m^{-2}s^{-1}$, n=90), and the ratio of c_i/c_a had a mean value of 0.56±0.11, n=87. Variations in the kinetic fractionation factor (ε_k), adjusted for these variations in leaf stomatal conductance ranged from +21.1 to +27.7 (mean=25.2±1.3‰, n=90, Fig. 3d). This estimate of variation served only as an indicator for the extent of the possible variations as detailed measurements of leaf boundary layer conductances were not carried out (Buhay et al., 1996). Instantaneous, mid-day, water use efficiency (A/E, μmol CO_2 $mmol^{-1}$ H_2O) ranged from 0.1 to 6.9 (mean=2.4±1.1, n=89; see Appendix).

Regression analyses were carried out among different variables including instantaneous parameters such as E, A/E, δ_{LW}, p and long term, integrated parameters such as δ^{18}_{cell} and δ^{13}_{cell}. As expected, a significant linear correlation (p=0.002) was observed between δ_{LW} and δ^{18}_{cell} (Fig. 5a). The $\delta^{18}O$ values of cellulose were also correlated (p=0.04) with the $\delta^{13}C$ values of leaf cellulose (Fig. 5b). δ^{13}_{cell} was significantly correlated with E (p=0.01, Fig. 5c), but not with A/E for the pooled group (Fig. 5d). A highly significant inverse linear correlation was observed between A/E and δ_{LW} (P=0.0001, Fig. 5e). This was unexpected based on Eq. 2, as leaves with high E would tend to have lower δ_{LW}, and may indicate that in this study plants with high A/E were also generally more productive. There was also significant inverse linear correlation between E and δ^{18}_{cell} (P=0.11, Fig. 5f). As expected (Eq. 2), a highly significant inverse linear correlation was also observed between δ_{LW} and the peclet number (P=0.0001, Fig. 5g).

Sub grouping plant species

Any attempt to use conventional vegetation-type groupings (e.g. Farquhar et al., 1993; Gamon et al., 1995; Sellers et al., 1996) to identify unique isotopic characteristics, indicated that variations among plant species within each group is too large (data not shown). However, a more coarse sub-division to shrubs, deciduous woodland, evergreen woodland and conifer species did indicate some significant differences among vegetation types (Table 2). Based on this sub-grouping, conifers were found to be significantly different from deciduous woodland and from shrubs species in their δ^{18}_{cell} and δ^{13}_{cell}. Conifer species also differed significantly from shrubs in their computed peclet numbers (Table 2).

No clear interactions between $\delta^{18}O$ of leaf water (δ_{LW}, δ_M or the difference) and vegetation type were discerned. This reflected the relatively large variations in δ_{LW} values among plants in any vegetation-type group. However, the distinction among vegetation types in δ^{18}_{cell} and the significant linear correlation between δ^{18}_{cell} and δ_{LW} reported above should be noted in this context. This is because δ^{18}_{cell} may in fact provide a better record of the long-term mean δ_{LW} (δ_{LW} represented here only mid-day spot measurements).

Regression analyses similar to those reported in Fig. 5, were conducted in each of the four sub-groups and the results are reported in Table 3. As expected (Eq. 2), a clear correlation between δ_{LW} and p was observed in all groups and the correlation between A/E and δ_{LW} was significant in shrubs and deciduous woodland species. All other relationships were not statistically significant.

Using A/E as an independent, physiological, indicator for subgrouping plant species could improve correlation coefficients in some cases. For example, if plant species with extreme A/E values (higher than 3.33 or lower than 1.25) were separated out (Table 3), correlations within the remaining main group (66 species) were generally more significant. This included correlations between A/E and $\delta^{18}O_{LW}$ values (P=0.001), between δ^{18}_{cell} and δ^{13}_{cell} (P=0.007), between δ_{LW} values and δ^{18}_{cell} (P=0.0001), between A/E and δ^{13}_{cell} (P=0.08), or between E and δ^{13}_{cell} (P=0.01) (not shown) and between E and δ^{18}_{cell} (P=0.03) (not shown).

For the extreme cases of A/E <1.25, only correlation between A/E and δ_{LW} values was significant (P=0.07, Table 3). For plants having A/E >3.33, correlations were significant between E and δ_M-δ_{LW} (P=0.07) (not shown), and between δ_{LW} values and δ^{18}_{cell} (P=0.009). Varying the vegetation type grouping based on A/E did not provide new insights.

Conclusion

Large, non-climatic variations among different plant species were observed in the oxygen isotopic composition of plant water and organic matter. The results indicated that considering plant physiological and morphological characteristics is likely to improve predictions of the isotopic

composition of leaf water and related parameters. This will require better definition of the relevant plant traits, and their characterization. The large variations in the oxygen isotopic composition of leaf water and related parameters observed in this study did not allow the isotopic characterization of conventionally-defined vegetation types, but some more broad distinctions are possible, particularly using the isotopic composition of leaf cellulose. Limited data obtained on the $\delta^{18}O$ values of chloroplast water suggested that δ_{ch} could be well estimated by δ_M in low p leaves (e.g. conifers) but can be significantly overestimated by δ_M in high p leaves (e.g. deciduous). Using empirical plant characteristics, such as leaf peclet numbers, or proxy indicators, such as δ^{18}_{cell}, can help reconcile these differences and allow more effective use of the meteorologically based estimates of δ_M in regional scale estimates.

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Table 1. On-line gas exchange and isotopic measurements of arbitrarily selected plant species used in the botanical survey (measurements were conducted both in the lab and in the field as indicated). Leaf to atmosphere vapor pressure difference (VPD) and the ratio of intercellular to atmospheric CO2 concentrations (c_i/c_a) were estimated from conventional gas exchange measurements. Discriminations against $^{13}CO_2$ ($^{13}\Delta$) and $C^{18}O^{16}O$ ($^{18}\Delta$) during gas exchange were determined from isotopic analysis of input and output air from the leaf cuvette (Fig.1). Leaves were collected for isotopic analysis of bulk leaf water (δ_{LW}) at the end of each measurement. Modeled δ18O values of evaporating leaf water (δ_M) was estimated using Eq.1 and used together with δ_{LW} to estimate leaf peclet number (p). The δ18O value of chloroplast water (δ_{ch}) was estimated based on the above parameters using Eq.3.

Plant	VPD (kPa)	c_i/c_a	$^{13}\Delta$ (‰)	$^{18}\Delta$ (‰)	δ_{LW} (‰)	δ_M (‰)	δ_{ch} (‰)	P
Lab measurements (irradiance PAR=500 µmol photos $m^{-2}s^{-1}$)								
Osteospermum fruticosum	1.56	0.73	13.00	15.14	+14.8	+14.7	+6.2	--
Pittosporum tobira, var. *fol. variegata*	1.60	0.85	19.10	25.01	+13.9	+15.1	+6.1	0.13
Ficus benjamina	1.65	0.83	15.29	22.85	+16.6	+15.6	+8.3	--
Field measurements (irradiance PAR=1650 µmol photos $m^{-2}s^{-1}$)								
Santolina chanaecyprisus	1.68	0.78	19.89	19.39	+12.0	+14.3	+8.7	0.26
Myrtus communis	2.28	0.65	15.10	12.66	+15.0	+16.4	+10.1	0.13
Crataegus momogyna	2.59	0.85	15.83	22.79	+13.7	+17.5	+9.2	0.38
Calocedrus decurrens var 1	2.84	0.79	22.04	45.02	+16.6	+18.7	+15.1	0.19
Calocedrus decurrens var 2	2.92	0.79	23.42	60.18	+16.8	+19.3	+18.2	0.25
Styrax officinalis	3.00	0.90	23.07	49.58	+12.3	+19.9	+9.0	0.78
Pinus cabiniana	3.09	0.69	22.84	44.96	+18.6	+20.5	+20.6	0.15

Table 2: Differences in selected plant traits (leaf peclet number and the δ18O and δ13C values of leaf cellulose) among vegetation types measured in this study. Results of unpaired t-test are given using the index numbers for each vegetation group. N denotes number of plant species in each group, * and ** denote increasing significance levels of 0.05 and 0.01.

Group		Mean ± s.d.			N	Probability (2-tail)			
		Peclet number	$\delta^{18}O$ cell	$\delta^{13}C$ cell		Variables	1.	2.	3.
1.	Shrub	0.68 ±0.23	+30.6 ±3.6	-24.4 ±1.5	30		—		
2.	Deciduous woodland	0.59 ±0.20	+30.5 ±2.8	-23.9 ±1.8	27	Peclet $\delta^{18}O$ cell $\delta^{18}O$ cell	0.12 0.60 0.24	—	
3.	Evergreen woodland	0.51 ±0.13	+31.6 ±5.5	-24.1 ±1.7	7	Peclet $\delta^{18}O$ cell $\delta^{13}C$ cell	0.07 0.47 0.64	0.33 0.23 0.74	—
4.	Conifer	0.49 ±0.22	+33.7 ±3.2	-22.8 ±1.3	8	Peclet $\delta^{18}O$ cell $\delta^{13}C$ cell	0.04* 0.03* 0.005**	0.24 0.01** 0.01**	0.84 0.36 0.06

Table 3. Linear relationships among selected plant characteristics measured in the present study within vegetation groups. Vegetation grouping was based either on type (shrubs, deciduous woodland, evergreen woodland and conifers), or on individual plant water-use-efficiency (A/E; mmol CO_2/mmol H_2O), where plants with extreme values ($1.25<A/E<3.33$) were separated out. R denotes regression coefficients and p denotes probability levels. Other parameters as in Table 2.

Variables		R P	Shrub	Deciduous woodland	Evergreen woodland	Conifer	$\frac{A}{E} < 1.25$	$1.25 < \frac{A}{E} < 3.33$	$\frac{A}{E} > 3.33$
x	y		(N=30)	(N=27)	(N=7)	(N=8)	(N=10)	(N=66)	(N=13)
A/E	$\delta^{18}O_{LW}$		y=14.9-0.9x	y=15.9-1.4x		y=17.9-2.2x	y=15.3-1.7x	y=17.7-2.0x	
		R	0.51	0.60	0.38	0.64	0.60	0.39	0.31
		P	0.004 **	0.001 ***	0.41	0.08	0.07	0.001 ***	0.30
A/E	$\delta^{13}C$ cell.							y=-22.5-0.6x	
		R	0.23	0.13	0.08	0.12	0.18	0.21	0.29
		P	0.22	0.53	0.85	0.78	0.62	0.08	0.34
$\delta^{18}O$ cell.	$\delta^{13}C$ cell.							y=-28.1+0.1x	
		R	0.08	0.01	0.41	0.12	0.16	0.33	0.38
		P	0.69	0.95	0.35	0.78	0.66	0.007 **	0.20
Peclet number	$\delta^{18}O_{LW}$		y=18.5-8.7x	y=18.1-9.5x	y=17.5-9.3x	y=19.7-11.2x	y=16.0-3.1x	y=17.9-8.1x	y=15.4-6.4x
		R	0.81	0.87	0.89	0.93	0.74	0.87	0.88
		P	0.0001 ***	0.0001 ***	0.007 ***	0.0007 ***	0.01 *	0.0001 ***	0.0001 ***
$\delta^{18}O_{LW}$	$\delta^{18}O$ cell.					y=23.0+0.8x		y=22.6+0.7x	y=43.0-1.1x
		R	0.08	0.28	0.002	0.63	0.06	0.46	0.69
		P	0.67	0.16	0.99	0.09	0.87	0.0001 ***	0.009 ***

Figure legends:

Figure 1: A schematics of the field gas exchange system used for on-line isotopic discrimination measurements. A modified leaf cuvette (8, LCA3, ADC) was used to enclose a sunlit, attached leaf. Ambient air was sucked by a vacuum pump through a guard trap (1) at liquid nitrogen (LN2) temperature and through the gas exchange system at a flow controlled by needle valves (2) and monitored by a flow-meter (6). The CO_2 and moisture concentrations in the air were determined by an infrared gas analyzer (IRGA, LiCor 6262) on air exiting the cuvette with and without a leaf. Air moisture was then trapped (-80°C, H_2O trap) and the CO_2 was cryogenically separated from the air (CO_2 trap). After trapping (~2 min.), the system was evacuated to 10^{-3} torr (3) and the CO_2 was transferred to a glass ampoule (4) that was flame-sealed and transported to the lab for isotopic analysis. Various components could be isolated by four-way brass valves (5), with all tubings made of 1/4" stainless steel , except for the connecting tube (7; isolating the measured leaf from the system and operators, 2-3m), and the air intake tube (positioned with a pole above the canopy, upwind of the measurement system) which were plastic (Bev-a-Line IV). Electric power was supplied by a portable generator. The system was transported on a wheelbarrow in the field and used as a benchtop system in lab measurements.

Figure 2. Variations in environmental conditions (photosynthetic photon flux density-PPFD, temperature, and relative humidity) during the sampling period in the Jerusalem Botanical Garden. Equations for the linear best fit lines and the correlation coefficients are indicated).

Figure 3. Frequency distribution (as percent of total number of plants) of selected plant parameters measured in this study. a. $\delta^{18}O$ values of stem water (δ_s); b. $\delta^{18}O$ values of bulk leaf water (δ_{LW}); c. predicted $\delta^{18}O$ values of evaporating leaf water at steady state (δ_M; using Eq. 1); d. kinetic fractionation factor due to diffusion of CO_2 based on leaf stomatal and cuvette's boundary layer resistances; e. differences between predicted (δ_M) and measured (δ_{LW}) $\delta^{18}O$ values of leaf water; f. peclet number derived from Eq. 3 based on measured δ_{LW} and estimated δ_M; g. $\delta^{13}C$ values of leaf cellulose, and h. $\delta^{18}O$ values of leaf cellulose.

Figure 4. Relationships between the $\delta^{18}O$ values of chloroplast water (δ_{ch}) and predicted $\delta^{18}O$ values of evaporating leaf water (δ_M) in ten selected plants used in the botanical survey. δ_{ch} was reconstructed based on on-line leaf ^{13}C and ^{18}O discrimination measurements and Eq. 3, a. taking into account known uncertainties associated with estimates of c_c and c_{cs} --CO_2 concentrations in the chloroplasts (cc) and at the chloroplast surface (ccs), and with estimate of the diffusional fractionation ($\bar{a}_1$) (see discussion for the range of values used); and b. Based on best estimates of

δ_{ch} with values of b=29, f=4 and $\bar{a}_1$=7.7. A general trend in leaf peclet number and the plant vegetation types are indicated.

Figure 5. Correlations, and their linear best fit regression lines, between selected plant variables measured in this study (equations of the lines are given for statistically significant correlations; P<0.05; R denotes correlation coefficient, NS denotes non-significant). Variables are as defined in Table 3.

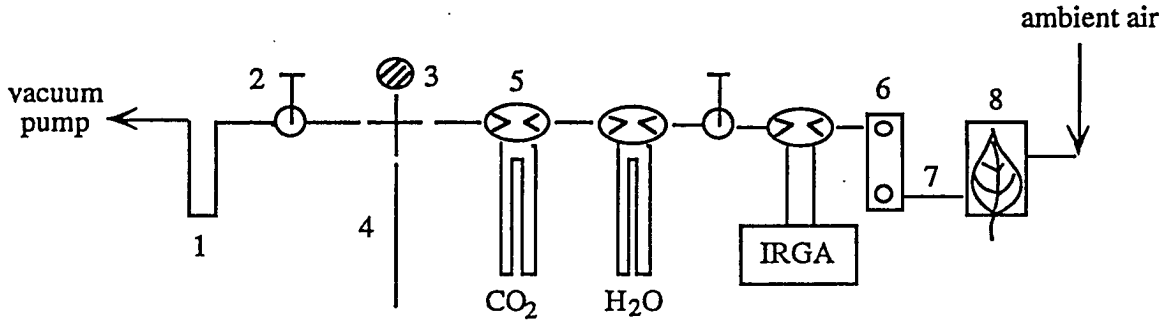


Fig.1

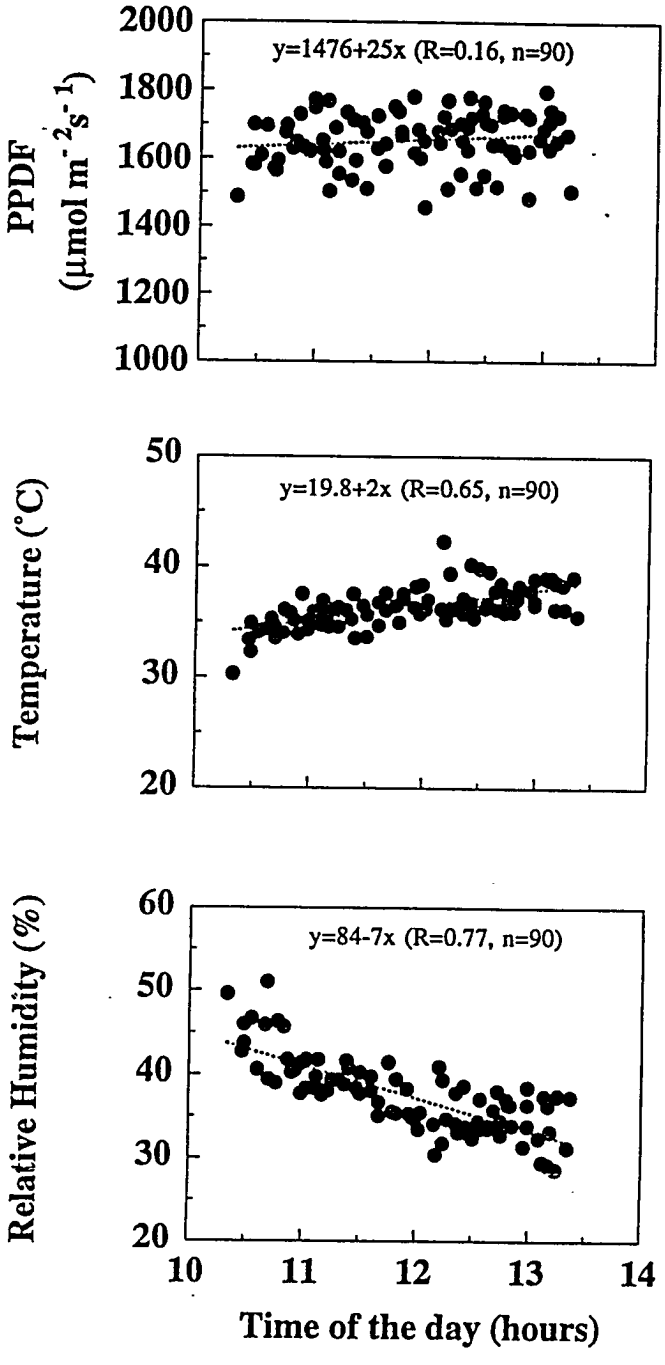

y=1476+25x (R=0.16, n=90)
PPDF (µmol m-2s-1)
2000
1800
1600
1400
1200
1000
y=19.8+2x (R=0.65, n=90)
Temperature (°C)
50
40
30
20
y=84-7x (R=0.77, n=90)
Relative Humidity (%)
60
50
40
30
20
10
11
12
13
14
Time of the day (hours)

Fig. 2

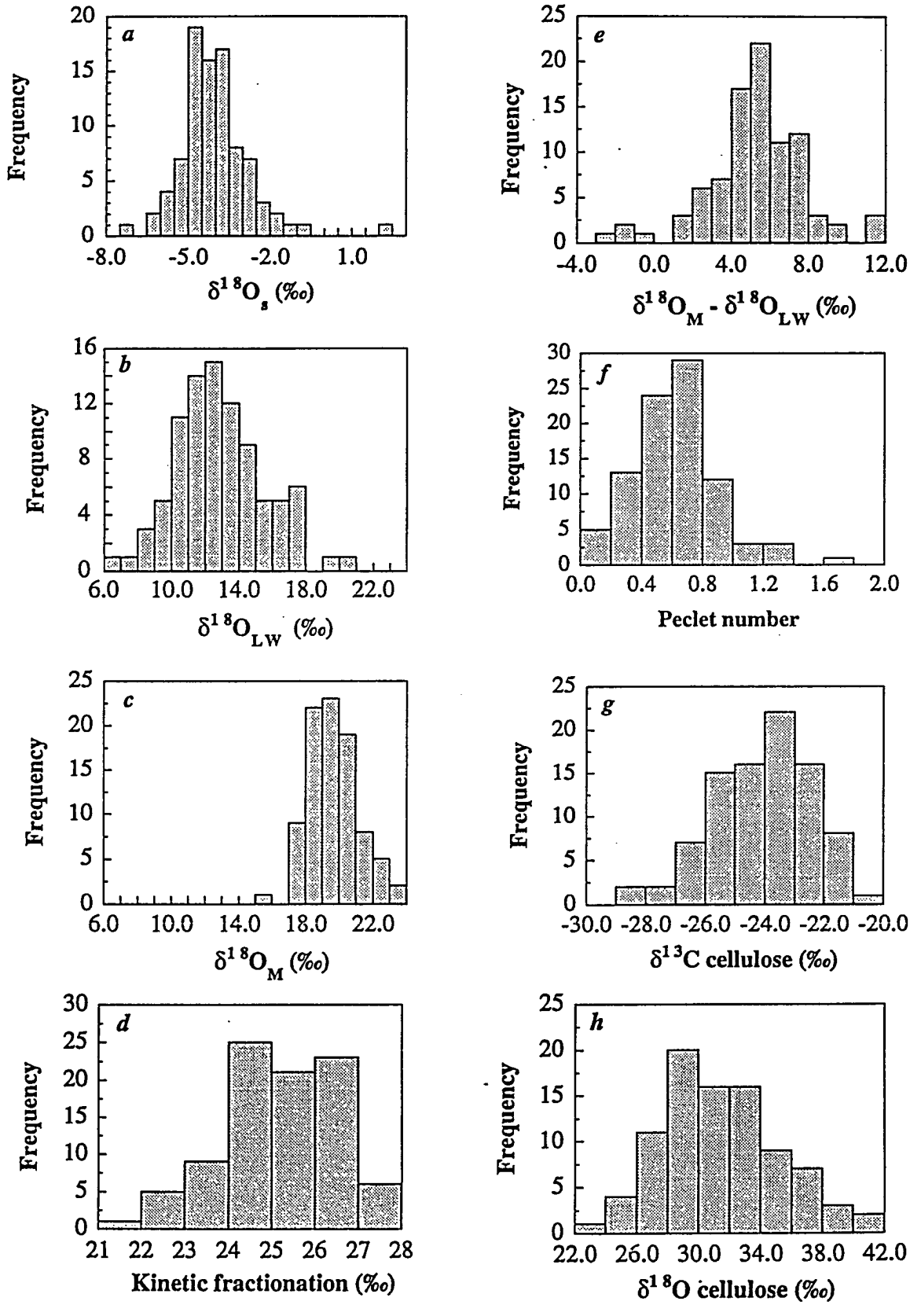

a
Frequency
$\delta^{18}O_s$ (‰)
b
Frequency
$\delta^{18}O_{LW}$ (‰)
c
Frequency
$\delta^{18}O_M$ (‰)
d
Frequency
Kinetic fractionation (‰)
e
Frequency
$\delta^{18}O_M - \delta^{18}O_{LW}$ (‰)
f
Frequency
Peclet number
g
Frequency
$\delta^{13}C$ cellulose (‰)
h
Frequency
$\delta^{18}O$ cellulose (‰)

Fig. 3

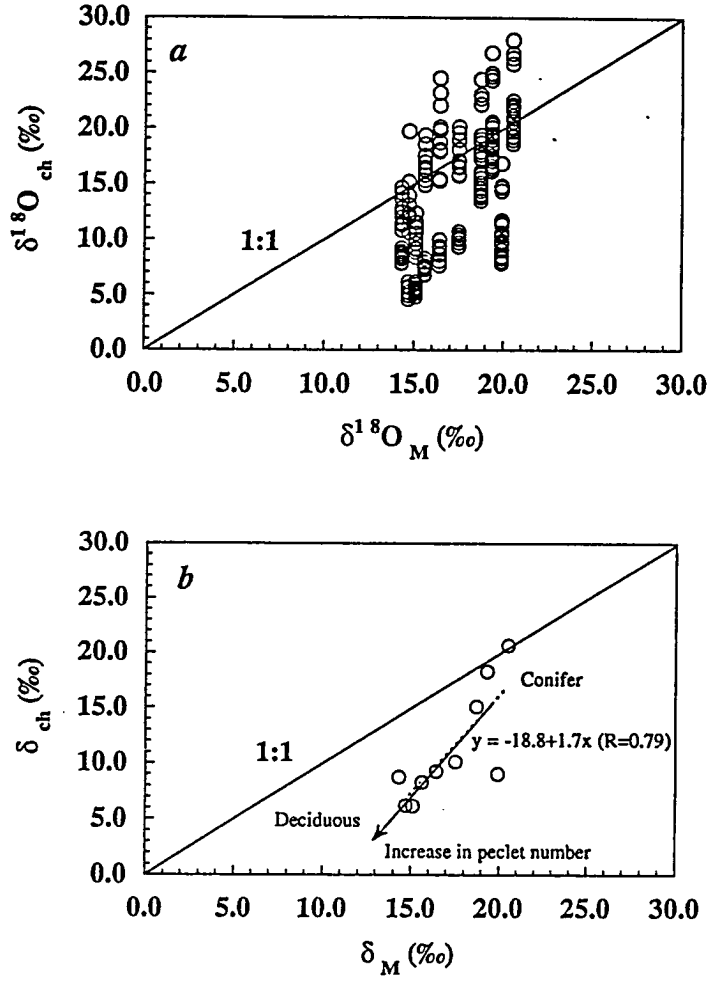
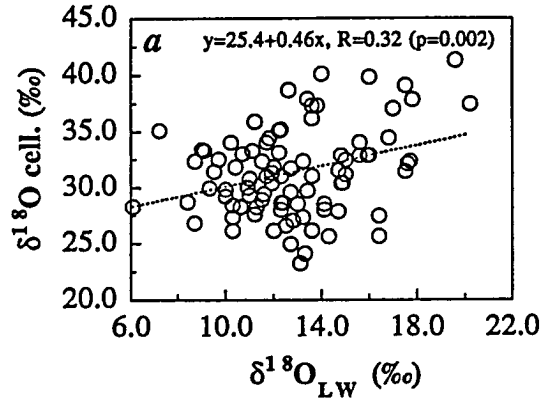
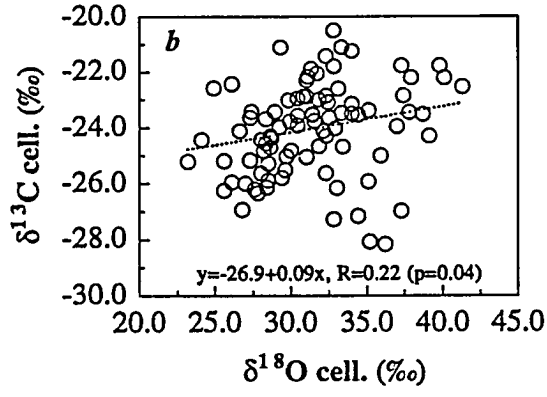
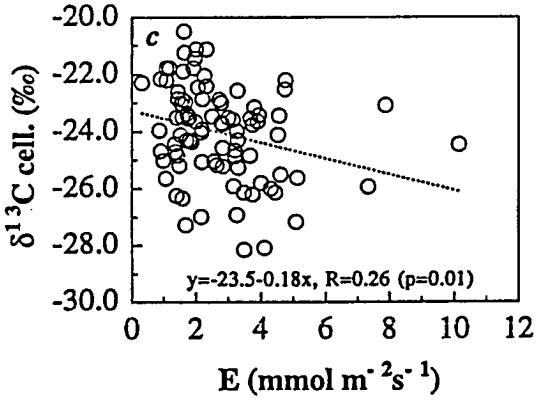
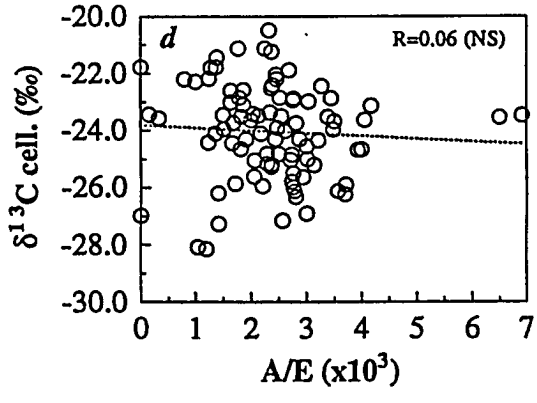

a
1:1
$\delta^{18}O_{ch}$ (‰)
$\delta^{18}O_M$ (‰)
0.0
5.0
10.0
15.0
20.0
25.0
30.0
b
1:1
Conifer
y = -18.8+1.7x (R=0.79)
Deciduous
Increase in peclet number
δ_{ch} (‰)
δ_M (‰)

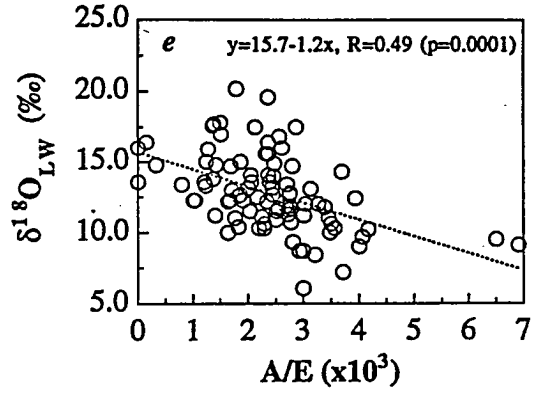
Fig. 4

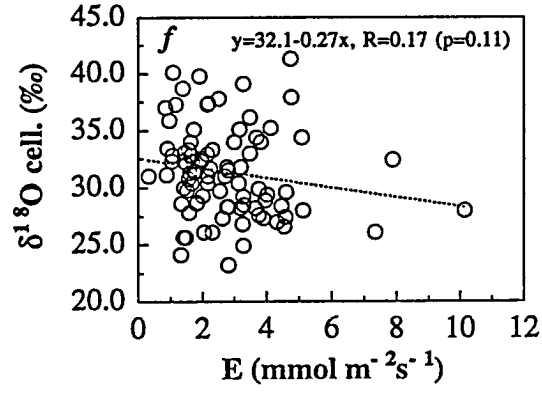

a
y=25.4+0.46x, R=0.32 (p=0.002)
δ18O cell. (‰)
δ18OLW (‰)


b
y=-26.9+0.09x, R=0.22 (p=0.04)
δ13C cell. (‰)
δ18O cell. (‰)


c
y=-23.5-0.18x, R=0.26 (p=0.01)
δ13C cell. (‰)
E (mmol m-2s-1)


d
R=0.06 (NS)
δ13C cell. (‰)
A/E (x10³)


e
y=15.7-1.2x, R=0.49 (p=0.0001)
δ18OLW (‰)
A/E (x10³)


f
y=32.1-0.27x, R=0.17 (p=0.11)
δ18O cell. (‰)
E (mmol m-2s-1)

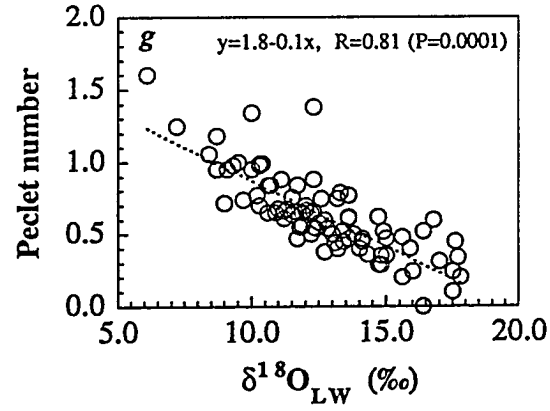

g
y=1.8-0.1x, R=0.81 (P=0.0001)
Peclet number
δ18OLW (‰)

Fig. 5

Appendix

Table A1. Plant species grown in the Jerusalem Botanical Garden used in this study, their geographical origin and vegetation type. Selected parameters measured on these plants during this study are reported [$\delta^{18}O$ values of leaf cellulose (Cell.) and water (LW), water use efficiency (A/E; µmol CO_2/ $mmol^{-1}$ H_2O) and computed leaf peclet numbers (p)].

Species names	Sources	Ecological group	$\delta^{18}O$ (‰)			A/E	p
			Cell.	LW	Cell.-LW		
Banksia integrifolia	Australian vegetation	Evergreen	35.1	12.2	22.9	2.34	0.50
Eucalyptus woodwardii		Evergreen	28.7	8.4	20.3	3.21	1.06
Melaleuca nesophila		Shrub	33.1	12.2	20.9	1.64	0.65
Acacia turner		Evergreen	27.8	14.7	13.1	2.80	0.29
Callistemon phoeniceus		Shrub	31.4	9.5	21.9	6.50	1.00
Calocedrus decurrens	North America conifer & evergreen	Conifer	37.3	13.8	23.5	1.37	0.50
Pinus sabiniana		Conifer	31.3	11.9	19.4	2.68	0.65
Styrax officinalis		Deciduous	32.8	14.8	18.0	1.42	0.29
Cupressus arizonica		Conifer	30.0	10.9	19.1	2.50	0.65
Acacia farnesiana	Desert plants	Deciduous	31.5	14.7	16.8	1.69	0.62
Prosopis juliflora			37.9	13.4	24.5	0.80	0.45
Magnolia grandiflora		Evergreen	39.8	16.0	23.8	0.10	0.24
Acer negundo		Deciduous	37.3	13.6	23.7	2.61	0.47
Fraxinus pennsylvanica		Deciduous	34.0	11.7	22.3	2.55	0.47
Sequoia sempervirens	California woodland	Conifer	37.4	20.2	17.2	1.79	0.00
Ceanothus arboreus		Shrub	29.9	9.3	20.6	2.81	0.98
Pleigynium timoriense	Mediterran	Shrub	30.8	11.0	19.8	3.45	0.68
Caesalpinia palmeri	North Africa	Shrub	33.3	9.1	24.2	6.91	0.95
Amygdalvs communis		Deciduous	28.3	10.6	17.7	3.50	0.65
Cordiline australis		Grass	28.3	6.1	22.2	3.00	1.60
Cercis siliquastrum		Deciduous	32.5	9.7	22.8	4.06	0.74
Populus angulata	Deciduous forest	Deciduous	30.4	11.9	18.5	2.75	0.56
Platanus orientalis		Deciduous	32.8	15.6	17.2	2.32	0.20
Diospyros virginiana		Deciduous	31.1	15.0	16.1	1.24	0.35
Betula papyrifera var suberidata		Deciduous	28.9	11.5	17.4	2.04	0.76
Umbellularia californica	California woodland	Shrub	35.2	12.3	22.9	1.04	0.88
Salvia leucophylla		Shrub	26.1	10.3	15.8	2.20	0.99
Aesculus californica		Shrub	31.8	10.4	21.4	1.82	0.99
Fraxinus velutina		Deciduous	33.0	10.7	22.3	2.78	0.84
Olea africana	American montane forest	Evergreen	29.7	13.4	16.3	2.71	0.53
Podocarpus latifolius		Conifer	32.8	15.9	16.9	1.28	0.40
Dais cotinifolia	South African montane forest	Shrub	31.0	13.6	17.4	2.06	0.62
Podocarpus falcatus		Conifer	37.0	17.0	20.0	1.51	0.31
Rhus leptodictya		Shrub	28.5	13.0	15.5	1.71	0.50
Cussonia paniculata		Shrub	31.0	12.3	18.7	1.00	1.38
Celtis africana		Deciduous	32.3	17.7	14.6	1.38	0.34
Tamarix sp.	Australian tropical vegetation	Shrub	33.4	9.0	24.4	4.00	0.72
Acacia pendula		Evergreen	35.9	11.2	24.7	3.01	0.61
Paulownia tomentosa		Deciduous	32.3	11.5	20.8	2.52	0.64
Ailanthus glandulosa	Turanian section	Deciduous	26.8	8.7	18.1	3.00	0.95
Malus cerasifera		Deciduous	28.4	10.3	18.1	3.56	0.70
Ficus carica		Deciduous	29.3	11.0	18.3	1.77	0.68

Lycium barbarum	Turanian	Shrub	28.2	11.3	16.9	2.73	0.68
Rhamnus dolichophylla	desert	Shrub	28.2	10.6	17.6	2.29	0.84
Tamarix nilotica		Shrub	32.3	8.7	23.6	2.93	1.18
Malus orientalis		Deciduous	34.0	10.2	23.8	4.17	0.77
Pistacia vera	Central Asian	Deciduous	29.2	10.0	19.2	3.48	0.95
* unidentified	section		35.1	7.2	27.9	3.71	1.25
Crataegus songarica		Deciduous	27.3	10.3	17.0	2.29	0.98
Juniperus excelsa		Conifer	33.3	11.1	22.2	2.25	0.88
Halimodendron halodendron		Shrub	31.0	11.7	19.3	2.77	0.84
Anagyris foetida	Steppe forest	Shrub	28.0	14.1	13.9	2.05	0.47
Prunus divaricata		Deciduous	26.1	13.6	12.5	2.39	0.61
Quercus pedunculiflora	Mediteranean	Deciduous	28.5	14.1	14.4	2.36	0.45
Punica granatum	section	Shrub	30.4	14.9	15.5	2.47	0.52
Quercus boissierii		Deciduous	27.0	12.8	14.2	2.75	0.54
Celtis tournefortii		Deciduous	31.8	12.0	19.8	3.03	0.67
Lygos monosperma	Canary Island	Shrub	39.1	17.5	21.6	2.87	0.24
Bupleurum fruticosum	scrub	Shrub	32.4	15.0	17.4	1.86	0.47
Phoenix canariensis		Shrub	32.1	17.6	14.5	1.36	0.45
Typha angustata		Perennial herb	34.4	16.8	17.6	2.57	0.60
Scyrpus		Perennial herb	33.6	28.0	5.6	1.92	0.00
Jasminum odaratissimium	Mediteranean	Shrub	27.4	16.4	11.0	0.15	0.52
Laurus nobilis	section	Shrub	36.2	13.6	22.6	1.20	0.77
Pinus canariensis		Conifer	29.8	10.0	19.8	1.64	1.34
Myrsine africana		Shrub	24.1	13.3	10.8	1.23	0.79
Viburnum opulus	European	Shrub	25.6	14.3	11.3	3.69	0.36
Plantago lanceolata	section	Grass	25.6	16.4	9.2	2.37	0.00
Saponaria officinalis		Grass	29.6	12.7	16.9	2.75	0.38
Lonicera xylosteum		Shrub	28.6	12.4	16.2	3.93	0.54
Cynosorus cristatus			27.3	13.2	14.1	2.00	0.40
Ulmus glabra		Deciduous	26.1	12.0	14.1	3.27	0.70
Rhamnus cathartica		Shrub	34.0	15.6	18.4	2.37	0.48
Tilia platyphylla		Deciduous	31.7	12.7	19.0	2.45	0.60
Myosotis laxa		Grass	29.4	11.6	17.8	2.73	0.66
Cornus sanguinea		Shrub	34.4	11.8	22.6	3.39	0.55
Cedrus Libani		Conifer	30.4	14.8	15.6	0.33	0.35
Magnolia grandiflora		Evergreen	40.1	14.0	26.1	2.46	0.40
Ceratonia siliqua		Evergreen	27.6	11.2	16.4	1.41	0.66
Pistacia lentiscus	Mediteranean	Shrub	23.2	13.1	10.1	3.13	0.44
Olea europea var silvestris	section	Evergreen	24.9	12.7	12.2	1.86	0.60
Pistacia atlantica		Deciduous	28.0	12.3	15.7	1.67	0.55
Artemisia arborescens		Shrub	32.3	13.2	19.1	2.44	0.75
Spartium junceum		Shrub	41.3	19.6	21.7	2.37	0.00
Liquidambar syraciflua		Deciduous	32.9	16.0	16.9	2.61	0.20
Araucaria excelsa		Conifer	37.8	17.8	20.0	1.50	0.20
Metasequoia glyptostroboides		Deciduous	31.4	17.5	13.9	2.13	0.10
Paulownia tomentosa		Deciduous	28.6	12.3	16.3	1.91	0.66
Nerium var nanum		Shrub	26.6	12.5	14.1	2.18	0.58
Vitis vitifera			38.7	12.6	26.1	1.83	0.75

3.3 Temporal and spatial variations in the oxygen-18 content of leaf water in different plant species: Modeling and application

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Abstract

Quantitative analysis of the isotopic composition of leaf water is essential for the utilization of the stable oxygen isotope (^{18}O) in environmental sciences studies. In this study, we developed a 2D model for predicting the ^{18}O content of leaf water. The model is transpiration dependent and considers a leaf as a series of evaporating pools with the ^{18}O content progressively enriched along the series (Gat & Bowser, 1991). Taking into account the water exchange effects on both horizontal and vertical dimensions, the modeling showed that a plant with low transpiration rate might have greater isotopic enrichment than that predicted by a conventional Craig & Gordon model (1965) in parts of the leaf; in contrast, a plant with high transpiration rate might have lower isotopic composition in its leaf water compared to that predicted by the Craig & Gordon model, even under isotopic steady state conditions. A delay in the occurrence of the maximum leaf water ^{18}O enrichment is predicted relative to the occurrence of the minimum relative humidity of the ambient air. This 2D-model described therefore many of the temporal and spatial ^{18}O variations of leaf water under natural conditions.

Key words: isotopic enrichment, isotopic heterogeneity, leaf water, leaf modeling, oxygen-18, stable isotopes, transpiration.

Introduction

Quantitative analysis of the isotopic composition of leaf water is important for the utilization of stable isotopes of oxygen (^{18}O) and hydrogen (^{2}H) in environmental studies, since the $^{18}O/^{16}O$ ratio of leaf water, or of a certain part of it, influences the $^{18}O/^{16}O$ ratios of atmospheric CO_2 and O_2 (Bender et al., 1985; Francey & Tans, 1987; Farquhar et al., 1993; Yakir, 1992; Yakir et al., 1994; Yakir & Wang, 1996), as well as the $^{18}O/^{16}O$ ratio of plant organic (Epstein, Thompson & Yap, 1977; Sternberg, DeNiro & Savidge, 1986).

Leaf water becomes enriched in ^{18}O and ^{2}H during transpiration, because the lighter isotopes (^{16}O and ^{1}H) have higher vapor pressures and higher diffusion rates in air (Washburn & Smith, 1934). The leaf water has been treated before as a thin, well mixed and isotopically uniform water pool. A model, originally developed by Craig & Gordon (1965) for evaporation process, has been applied to predict isotopic enrichment of leaf water during transpiration (Dongmann et al. 1974; Schiegel, 1974; Farris & Strain, 1978; Förstel, 1978; Zundel et al., 1978; Bariac et al., 1989; Allison et al., 1985; Leaney et al., 1985; Farquhar & Lloyd, 1993; Walker et al, 1989; Yakir et al, 1990; Flanagan et al. 1991; Farquhar et al., 1993; Wang & Yakir, 1995). The steady state isotopic composition of bulk leaf water (δ_L) is approximated by:

$$\delta_L = \delta_{in} + \varepsilon_{eq} + \varepsilon_k + h \cdot (\delta_a - \varepsilon_k - \delta_{in}) \qquad (1)$$

where, δ (‰)=($R_{sample}/R_{standard}$ - 1)·10^3, R is the molar ratios of heavy to light isotope of oxygen, $^{18}O/^{16}O$, and the standard is Vienna-Standard Mean Ocean Water (V-SMOW). Subscripts L, in and a represent leaf water, input source water and air humidity, respectively. ε_{eq} is the equilibrium fractionation factor (9.38‰ at 25°C, Majoube, 1971), ε_k is the kinetic fractionation factor (Merlivat, 1978), h the relative humidity of the ambient air at the leaf surface temperature.

Recent studies have shown that leaf water is not isotopically homogeneous (Yakir et al, 1989; Walker and Lance, 1991; Luo & Sternberg, 1992; Yakir et al, 1994; Wang & Yakir, 1995). It is suggested that there are two or three distinct water compartments in leaf: one is the vein water which is not isotopically enriched; another is mesophyll water which undergoes evaporation and thus becomes isotopically enriched, or even further divided into apoplastic water which reaches isotopic steady state as described by Eq. 1, and symplastic water which lags in isotopic enrichment because of slow mixing with the apoplastic pool. It has also been suggested that there are patchiness of leaf water that do not become uniformly enriched because of areas of stomatal closure (Flanagan et al., 1991). Few reports are available now on the mechanism of the heterogeneity in the leaf water, and the relations between the heterogeneous isotopic composition of the leaf water and that of the average bulk leaf water.

At any given time during the day, the observed $\delta^{18}O$ value of bulk leaf water is usually less enriched than that predicted by the model (Eq. 1) (Farris & Strain, 1978; Walker et al., 1989; Flanagan & Ehleringer, 1991; Yakir et al, 1989, 1990). Much of the difference between the observed and modeled leaf water $\delta^{18}O$ value has been proposed to result from a shifting balance between the bulk flow of unfractioned source water into the leaf (convection) and the back diffusion of heavy isotopes away from the enriched evaporating sites (Farquhar & Lloyd, 1993). Based on Farquhar & Lloyd's (1993) interpretations, the $\delta^{18}O$ value predicted by the Craig & Gordon model is the upper limit in an isotopic enrichment gradient within the leaf water.

Farris and Strain (1978) reported however, measured $\delta^{18}O$ values higher than that calculated by Eq. 1 in water-stressed leaves of bush bean (*Phaseolus vulgaris* L.) (Fig. 12 in their paper). Water stressed leaves with lower transpiration rate also displayed greater $\delta^{18}O$ enrichment than did well-irrigated plants in the same environment. The observed nighttime leaf $\delta^{18}O$ levels were about 3‰ enriched above the predicted steady state value.

Such observations of the measured $\delta^{18}O$ values greater than predicted have also been reported in leaves of barley (Walker & Lance, 1991), juniper (Flanagan et al., 1993), and pine (Flanagan & Varney, 1995). Walker & Lance (1991) showed that the flag leaf had a much higher isotopic values than that calculated under field condition and they speculated the reason to be highly enriched water imported from the lower part to the flag leaf, instead of from the soil or

stem. Notably the observed $\delta^{18}O$ value of total leaf water, extracted from juniper leaves, was greater than that predicted for the evaporative sites throughout the experiment period (4:00~24:00) (Flanagan et al., 1993). Another study by Flanagan & Ehleringer (1991) also showed that measured $\delta^{18}O$ values of leaf water were higher than those predicted by the Craig & Gordon model both in the early morning (5:30~8:00) and late in the afternoon (17:30~22:00). The basis for such 'higher than predicted' $\delta^{18}O$ values of leaf water is not well understood at present.

According to the Craig & Gordon model, a similar ^{18}O enrichment can be expected in different leaves if meteorological conditions are identical. In contrast, significant variations were observed in the level of ^{18}O enrichment among leaves of birch (*Betula pubescens* L.), larch (*Larix decidua* Mill.) and spruce (*Picea abies* Karst.) at the same time and the same location under natural conditions, and with the same $\delta^{18}O$ value of the branch water (Förstel, 1978). Such climate-independent variation in the $\delta^{18}O$ values of leaf water in different plant species is not clear understood at present.

Concerning temporal variation, the Craig & Gordon model indicates that relative humidity is the most dominant factor which causes diurnal variations in $\delta^{18}O$ (or δD) of leaf water. Daily changes in $\delta^{18}O$ values of leaf water are well documented (Dongmann et al., 1974; Förstel, 1978; Zundel et al., 1978; Leaney et al., 1985; Walker et al., 1989; Yakir et al., 1990; Flanagan et al., 1993). But under natural conditions, however, these studies show that the diurnal $\delta^{18}O$ cycle of the leaf water become more complicated.

In Zundel et al. (1978) the position of the maximum $\delta^{18}O$ value coincided with the minimum relative humidity of the atmosphere (their Fig. 3a); while the position of the maximum $\delta^{18}O$ value was shifted by 2 hours towards the afternoon compared to the minimum relative humidity of the atmosphere (their Fig. 3b). The authors argue that the enrichment is lower or higher than predicted because of the slow response of the leaf water. Similar observations have also been reported in other studies where the maximum enrichment occurred either in advance or delay relative to the time of minimum relative humidity (Förstel, 1978; Leaney et al., 1985).

The objective of this study was to develop a 2D model that will improve the description of the temporal and spatial variations in leaf water $\delta^{18}O$ value, and provide a way to integrate plant specific characteristics.

Modeling Leaf Water Isotopic Composition

Farquhar & Lloyd (1993) developed a one-dimensional advection-diffusion model to improve the prediction of $\delta^{18}O$ value of leaf water. The bulk leaf water $\delta^{18}O$ value (δ_L) is then related to that predicted by Eq. 1 at the evaporation sites within leaves (δ_{ss}):

$$\delta_L - \delta_{in} = (\delta_{ss} - \delta_{in}) \cdot \frac{1 - e^p}{p} \qquad (2)$$

where, p is the ratio of convective to diffusive effects, or the peclet number, ($p = E \cdot L / (C \cdot D)$), E is the transpiration rate (mol m^{-2} s^{-1}); L the effective mixing length (m); C the molar concentration of water (5.56 x 10^4 mol m^{-3}); D the diffusivity of $H_2{}^{18}O$ (2.66 x 10^{-9} m^2 s^{-1}) in water (Wang, 1954).

Eq. 2 is similar to that developed by Zimmermann et al. (1967) and Barnes & Allison (1983), who used the equation to predict the distribution profile of the isotopic composition of soil water. Note, however that Eq. 2 predicts the interpreted, average isotopic composition over a distance, rather than at a single point along a gradient.

The practical range of the term $(1 - e^{-p}) / p$ in Eq. 2 is quite small (from 0.6 to 1.0) corresponding to a transpiration range at 0 to 15 mmol m^{-2} s^{-1} (e.g. Flanagan et al., 1993, 1994). This indicates that the isotopic composition of the averaged bulk leaf water (δ_L) is usually 0 to 40% lower than the enrichment at evaporating sites (δ_{ss}, or δ_L).

Discrete pools approach

Because of the observed spatial non-uniformity of the isotopic composition in leaf water (Luo & Sternberg, 1992; Wang & Yakir, 1995), we assume that bulk leaf water consists of a series of pools with different isotopic composition (i=1,2,3,···,N; N is the total number of pools) (Fig. 1a & b). Pools in the vicinity of each other within the leaf, allow for diffusion of isotopes. As a result, the $\delta^{18}O$ value of the bulk leaf water can be regarded as a mixture resulting from convection-diffusion fluxes of opposing directions within the leaf. In a two dimensional leaf, the convection flow is assumed to be from the xylem (bottom) and the evaporating surface (top) in the vertical direction; and to be from the stem toward the leaf tip in the horizontal direction.

In the vertical direction, the $\delta^{18}O$ gradient can be formed due to "back" diffusion of the heavy isotope from the evaporating surface against the convective flow. The average isotopic composition of the i-th pool (Fig. 1c), δ_i^z, can be formulated based on Farquhar & Lloyd (1993) model:

$$\delta_i^z = \delta_{i-1} + (\delta_{ss}^i - \delta_{i-1}) \cdot A \qquad (3)$$

where, superscript z stands for vertical direction; subscript (i-1) represents the previous pool relative to the i-th pool; δ_{ss}^i the isotopic enrichment at the evaporating sites of the i-th pool at steady state; $A = (1 - e^{-p_l}) / p_l$, $p_l = E \cdot L_l / C \cdot D$, L_l is the effective mixing length in vertical direction (m).

In the horizontal direction, a similar approach can be used to develop an equation for the isotopic enrichment caused by convection and diffusion fluxes. After resolving for the average isotopic composition of the i-th pool in the vertical direction (δ_i^z), the average isotopic composition of i-th pool over an effective distance of L_2 in the horizontal direction (δ_i) is obtained considering back diffusion effect from the next pool (δ_{i+1}) (Fig. 1d):

$$\delta_i = \delta_i^z + (\delta_{i+1} - \delta_i^z) \cdot B \tag{4}$$

where, $B = (1 - e^{-p_2}) / p_2$, $p_2 = F_{out} \cdot L_2 / C \cdot D$, F_{out} (mol m^{-2} s^{-1}) refers to the corrective outflow of the interested i-th pool.

Substituting δ_i^z from Eq. 3 into Eq. 4 and rearranging the equation, we obtain a general formula, which takes diffusion effects from both vertical and horizontal directions into account, for the prediction of the isotopic enrichment in the i-th pool of leaf water:

$$\delta_i = B \cdot \delta_{i+1} + A \cdot (1 - B) \cdot \delta_{ss}^i + (1 - A) \cdot (1 - B) \cdot \delta_{i-1} \tag{5}$$

Conceptually the isotopic composition of the i-th pool is influenced by 3 factors, one is the isotopic composition of the input water (δ_{i-1}); another is the "back" diffusion effect from the evaporating surface in vertical direction (δ_{ss}^i); and the last is the "back" diffusion effect from the forthcoming pool in horizontal direction (δ_{i+1}).

Starting from the first pool, the input is the source water and the $\delta^{18}O$ value at the evaporating sites can be calculated according to a model developed for the isotopic enrichment of water in coupled evaporative systems (Gat & Bowser, 1991). Based on the "String-of-lakes" model developed by Gat & Bowser (1991), the spatial $\delta^{18}O$ variation at the evaporating sites within a leaf (Fig. 2) can be expressed as:

$$\delta_{ss}^i = \frac{\frac{F_+}{E} \cdot \frac{1-h}{h} \cdot \delta_{ss}^{i-1} + (\delta_a + \frac{\varepsilon}{h})}{1 + \frac{F_+}{E} \cdot \frac{1-h}{h}} \tag{6}$$

where F_+ is the influx to the i-th pool, and $\varepsilon = \varepsilon_{eq} + (1-h) \cdot \varepsilon_k$.

The final average isotopic composition of the bulk leaf water (δ_L) is simply the average of the isotopic composition of all the individual pools:

$$\delta_L = \frac{\sum_{i=1}^{N} \delta_i}{N} \tag{7}$$

To solve all the individual δ_i (i=1,2,3,···,N) and then calculate the averaged isotopic composition of the total leaf water δ_L, one can first write all of the multi-variable linear equations and then use Gauss elimination method which includes two major steps, say, forward elimination and then back substitution, to reach the final solution.

Continuum approach

Considering convective and diffusive flow of heavy isotopes within the leaf is in 2-dimension, we can write a general convection-diffusion equation:

$$\frac{\partial \delta(x,y)}{\partial t} = -D_x \cdot \frac{\partial^2 \delta(x,y)}{\partial x^2} - D_y \cdot \frac{\partial^2 \delta(x,y)}{\partial y^2} + F_x \cdot \frac{\partial \delta(x,y)}{\partial x} + F_y \cdot \frac{\partial \delta(x,y)}{\partial y} \tag{8}$$

where, F_x, F_y are water fluxes in x and y directions, respectively.; D_x, D_y are diffusion coefficients of heavy isotopes in water in x and y directions, respectively.

Under isotopic steady state, the left hand term becomes zero. To solve Eq. 8 analytically, one needs to define the boundary conditions.

Referring to Fig. 1a, the lower and upper boundary conditions are:

when x=0, or y=0, $\delta=\delta_{source}$=constant;
when $x=L_1$, $\delta=\delta_{max}$=constant;

The upper boundary condition at $y=L_2$, can be determined according to Gat & Bowser (1991). Considering the isotopic composition at the evaporating surface of the i-th pool water (at a position x) in a coupled evaporative system, Eq. 6 can be expressed as:

$$\delta(x) = [a \cdot \delta(x-1) + b] / (1+a) \tag{9}$$

where, $a = F_+ \cdot (1-h) / (E \cdot h)$; $b = \delta_a + \varepsilon / h$.

The overall enrichment of the heavy isotopes up to the (i-1) pool (at position (x-1)) along a series of lakes is (similar to Eq. 6 in Gat & Bowser, 1991):

$$\delta(x-1) = [1 + \sum_{1}^{n-2} c^x] \cdot c_1 + \delta_{in} \quad (10)$$

where, c=a/(1+a); c_1 is a constant ($=\Delta_{1,0}=(b-\delta_{in})/(1+a)$), δ_{in} is the isotopic enrichment of the source input water.

The sum of the geometric term in Eq. 10 is equal to $(c-c^x)/[c(1-c)]$. Substituting Eq. 10 into Eq. 9, we obtain:

$$\delta(x) = [c + \frac{c - c^x}{1-c}] \cdot c_1 + c \cdot \delta_{in} + \frac{b}{1+a} \quad (11)$$

Eq. 11 defines the upper boundary condition at $y=L_2$. Due to its complexity, it is difficult to solve the continuum equation (Eq. 8) analytically.

An example: a 3-pool-series

To test the nature of Eq. 5, we take a series with 3 pools (N=3) as a simplified example. Assuming leaf water is comprised of 3 pools in a series, and each pool has a distinct isotopic composition as δ_1, δ_2, and δ_3; and the corresponding isotopic compositions at the evaporating surface at δ_{ss}^1, δ_{ss}^2 and δ_{ss}^3. The isotopic composition of each pool can be expressed according to Eq. 5:

1st pool $$\delta_1 = B \cdot \delta_2 + (1-B) \cdot [A \cdot \delta_{ss}^1 + (1-A) \cdot \delta_{in}] \quad (12a)$$

2nd pool $$\delta_2 = B \cdot \delta_3 + (1-B) \cdot [A \cdot \delta_{ss}^2 + (1-A) \cdot \delta_1] \quad (12b)$$

3rd pool $$\delta_3 = [A \cdot \delta_{ss}^3 + (1-A) \cdot \delta_2] \quad (12c)$$

The δ_{ss}^1, δ_{ss}^2 and δ_{ss}^3 values can be calculated according to Eq. 6:

$$\delta_{ss}^1 - \delta_{in} = \frac{(\delta_a + \varepsilon / h) - \delta_{in}}{1 + 3 \cdot \frac{1-h}{h}} \quad (13a)$$

$$\delta_{ss}^2 - \delta_{ss}^1 = \frac{(\delta_a + \varepsilon / h) - \delta_{ss}^1}{1 + 2 \cdot \frac{1-h}{h}} \quad (13b)$$

$$\delta_{ss}^3 - \delta_{ss}^2 = \frac{(\delta_a + \varepsilon / h) - \delta_{ss}^2}{1 + 1 \cdot \frac{1-h}{h}} \tag{13c}$$

Unlike Gat & Bowser (1991) where F_+ / E is constant in all pools, here we allow E to vary along the series, say, $F_+ / E = 3$, 2 and 1 for the 1st, 2nd and 3rd pool, respectively, as would be expected in a leaf.

To predict the average isotopic composition at the evaporating surfaces of the leaf (δ_L^s), we summed up and averaged Eq. 13:

$$\delta_L^s = (\delta_{ss}^1 + \delta_{ss}^2 + \delta_{ss}^3) / 3 = (1 - h) \cdot \delta_{in} + \varepsilon + h \cdot \delta_a \tag{14}$$

As expected Eq. 14 has the same form as the Craig & Gordon model (Eq. 1) for predicting the isotopic composition at the evaporating sites in a single completely mixed leaf water pool.

Considering back diffusional effects on both the vertical and horizontal directions, the average isotopic composition of the leaf water is:

$$\delta_L = (\delta_1 + \delta_2 + \delta_3) / 3 \tag{15}$$

By forward elimination method, we get the isotopic composition for the 3rd pool:

$$x_1 \cdot \delta_3 = x_2 \cdot \delta_{ss}^3 + x_3 \cdot \delta_{ss}^2 + x_4 \cdot \delta_{ss}^1 + x_5 \cdot \delta_{in} \tag{16}$$

where, coefficients $x_1 = 1 - B \cdot (1 - A)(2 - B)$, $x_2 = A \cdot (1 - B \cdot (1 - A)(1 - B))$, $x_3 = A \cdot (1 - A)(1 - B)$, $x_4 = A \cdot (1 - A)^2 \cdot (1 - B)^2$, $x_5 = (1 - A)^3 \cdot (1 - B)^2$. Factors A and B were defined in Eqs. 3 & 4, respectively; and assumed constant for different pools within the leaf. After calculating δ_3, δ_1 and δ_2 can be solved in the same way by back substitution.

Under extremely low transpiration condition, factors A and B will approach unity. As a result, Eq. 12 can be simplified to:

$$\delta_1 = \delta_2 = \delta_3 = \delta_{ss}^3 \tag{17}$$

And Eq. 15 becomes:

$$\delta_L = (\delta_1 + \delta_2 + \delta_3)/3 = \delta_{ss}^3 \tag{18}$$

The isotopic enrichment at the evaporating surface of the last pool in a series (δ_{ss}^3) will approach in this case δ_{ss} of the bulk leaf water and any gradient will be minimized.

In contrast, under extremely high transpiration condition, factors A and B will approach zero. As a result, Eq. 12 will become:

$$\delta_1 = \delta_2 = \delta_3 = \delta_{in} \tag{19}$$

And Eq. 15 becomes:

$$\delta_L = (\delta_1 + \delta_2 + \delta_3)/3 = \delta_{in} \tag{20}$$

It is shown in Eq. 20 that the average isotopic composition of the bulk leaf water will approach that of the source input water under extremely high transpiration condition.

The conceptual extreme cases, as mentioned above in Eqs. 18 & 20, provided some insights as to why the average isotopic composition of the bulk leaf water is either smaller than or approaches that predicted by Craig & Gordon model under natural conditions. The model developed here is transpiration dependent and predicts the isotopic composition of the bulk leaf water at isotopic steady state. The transpiration rate affects the isotopic enrichment of the leaf water also through an exponential form in factors A (Eq. 3) and B (Eq. 4). We will find later that the Craig & Gordon model (Eq. 1) is suitable for predicting the isotopic composition of the leaf water at steady state only within a certain range of the transpiration rate. Out of this range, the heavy isotopes enrichment in the leaf water can be, even though at isotopic steady state, either higher or lower than that calculated by the Craig & Gordon model, as indeed was observed in previous works under natural conditions (e.g. Förstel, 1978; Zundel et al., 1978; Leaney et al., 1985; Flanagan et al., 1993).

If the leaf water is not at isotopic steady state, the approach of leaf water to steady state model (Farris & Strain, 1978) is:

$$\delta_L(t) = \delta_{ss} - (\delta_{ss} - \delta_0)\cdot e^{-b\cdot t} \tag{21}$$

where δ_{ss} refers to the isotopic steady-state value of leaf water given by Eq. 8; δ_o the initial isotopic composition of leaf water at time t=0; and factor b is given by:

$$b = \alpha_{eq} \cdot \alpha_k^q \cdot \left[\frac{F_{in}}{V} + \frac{E}{V} \cdot \frac{h}{(1-h)} \right] \qquad (22)$$

where, α_{eq} is equilibrium fractionation factor ($=1-\varepsilon_{eq}/10^3$); α_k^q is the kinetic fractionation factor ($=1-\varepsilon_k/10^3$); superscript q refers to the aerodynamic nature of the boundary layer (0.67 for laminar, and 1.0 for static conditions). In our experimental condition, the boundary layer resistance was estimated to be 25% of the total resistance (Parkinson, 1985). According to Farquhar and Lloyd (1993), the kinetic fractionation factor ε_k was calculated to be 25.75‰, then $\alpha_k^q = (1-\varepsilon_k/1000) = 0.97425$; F_{in} is the influx to the whole leaf water, which is equal to the transpiration rate (E); and V volume of the leaf water.

Diurnal variations of $\delta^{18}O$ value in leaf water can be examined by Eq. 21 (see below), which will help us understand better the daily behaviour of $\delta^{18}O$ in leaf water. However, Eq. 21 is not suitable to use during the night period, when the relative humidity approaches 100%.

Model Application

$\delta^{18}O$ enrichment in leaf water at isotopic steady state

The isotopic enrichment in a 3-pool-series model is similar to that for unspecified number of pools (Gat & Bowser, 1991), and we will apply the 3-pool-series model developed here as a simplification to predict $\delta^{18}O$ enrichment in the leaf water at steady state in response to diurnal variations in environmental parameters.

Diurnal variations in the environmental parameters were adapted from Yakir et al. (1990) (Fig. 3). The $\delta^{18}O$ value of the atmospheric vapor in Israel is taken as -11.0‰ (IAEA). The $\delta^{18}O$ value of the source input water (soil water) is -2.6‰ (Yakir et al., 1990). Assuming that plants began transpiration at about 7:00; and the transpiration rate increased linearly to the maximum value at 14:00, and then decreased linearly to zero at about 22:00 (Fig. 3).

With knowledge of the isotope data for the atmospheric vapor (δ_a) and the input water, together with daily variations in the temperature and relative humidity (Fig. 3), we can calculate the $\delta^{18}O$ values at evaporating surfaces in each pool based on Eq. 13, then calculate factors A and B (the effective mixing length is 23.6 mm for L_1 and 236 mm for L_2, based on Flanagan et al., 1994) and coefficients X_i (i=1,2,...,5). Finally, the averaged isotopic composition of the total leaf water (δ_L) is obtained, based on Eqs. 12, 13, and 15. Following changes in temperature, relative humidity and transpiration rate, the δ_L value changes accordingly. As a result, diurnal

changes in the $\delta^{18}O$ value of the bulk leaf water at isotopic steady state can be obtained (Fig. 4). The enrichment in $\delta^{18}O$ of leaf water in response to environmental changes, calculated independently by the Craig & Gordon model was also plotted in Fig. 4.

It is shown in Fig. 4 that the lower the transpiration rate of the plant, the higher the isotopic enrichment in leaf water. Below a certain value of the transpiration rate (e.g., less than 3.0 mmol $m^{-2}s^{-1}$ in Fig. 4, which depends on the effective mixing length taken), all of $\delta^{18}O$ values of the leaf water during the day will be higher than those predicted by the Craig & Gordon model, consistent with the previous observations (Farris & Strain, 1978; Flanagan et al., 1993). This is also the case for $\delta^{18}O$ values of the desert plants with low transpiration rate (Yakir et al., unpublished data).

Within a certain range of the transpiration rate, the diurnal variation curve of $\delta^{18}O$ value of the leaf water, calculated by the 3-pool-series model, will overlap with that predicted by the Craig & Gordon model. This is a somewhat fortuitous agreement and does not indicate any change in the system at these flux rates.

Above a certain range of the transpiration rate (e.g., greater than 3.0 mmol $m^{-2}s^{-1}$ in Fig. 4, which depends on the effective mixing length taken), the $\delta^{18}O$ values of the leaf water will be smaller than predicted by the Craig & Gordon model during the whole daily period. In the report by Förstel (1978, Fig. 4a), we postulate, the spruce tree probably had the lowest transpiration rate (highest $\delta^{18}O$ enrichment), while the birch tree had the highest transpiration rate (low $\delta^{18}O$ enrichment) (Fig. 4a in, 1978).

Diurnal variations of $\delta^{18}O$ values in leaf water under non-steady state conditions

The same data set (Fig. 3) was used for calculating diurnal $\delta^{18}O$ variations in leaf water under non-steady state conditions. It is hypothesized that for some low transpiring plants with low turnover rates, their leaf water does not reach isotopic steady state during the day (Wang & Yakir, 1995). It becomes necessary in such cases to apply Eq. 21 in order to examine the diurnal $\delta^{18}O$ changes in the leaf water under natural conditions. A daily cycling of the $\delta^{18}O$ value of the leaf water, in response to changes in the environmental parameters at different transpiration rates is shown in Fig. 5. The curves were obtained using a least square fit of the calculated data. It is shown in Fig. 5 that the maximum enrichment in the Craig curve, which is independent of the transpiration rate, occurred at 14:00 corresponding to the lowest relative humidity. For plants with low transpiring characteristics (E=0.3 or 1.0, Fig. 5), a delay was observed in the occurrence of the maximum $\delta^{18}O$ enrichment in leaf water relative to that of the minimum relative humidity, probably due to a slow mixing process between the highly enriched surface water and the total tissue water. Although the maximum enrichment in the case of E=0.3 during the day was the highest under steady state condition (Fig. 4), the maximum enrichment during the non steady-

state scenario was smaller than that in E=1.0 case. With increasing transpiration rates (e.g., to E=3), daily $\delta^{18}O$ variations in leaf water will approach those predicted by the Craig & Gordon model and within a certain range, these curves overlapped (Fig. 5). At higher rates of transpiration (e.g., E=6), the daily $\delta^{18}O$ variations in leaf water will be lower than those predicted by the Craig & Gordon model throughout the daily cycle (Fig. 5).

It is interesting to note that, between E=3 to E=6 in our specific case (which depends on the effective mixing length selected), the difference between leaf water $\delta^{18}O$ value predicted by the Craig & Gordon model and that by the approaching steady state model was linearly correlated to the transpiration rate (Fig. 5), this is in good agreement with previous observations (Leaney et al., 1985; Walker et al., 1989; Flanagan et al., 1991; Yakir et al., unpublished data).

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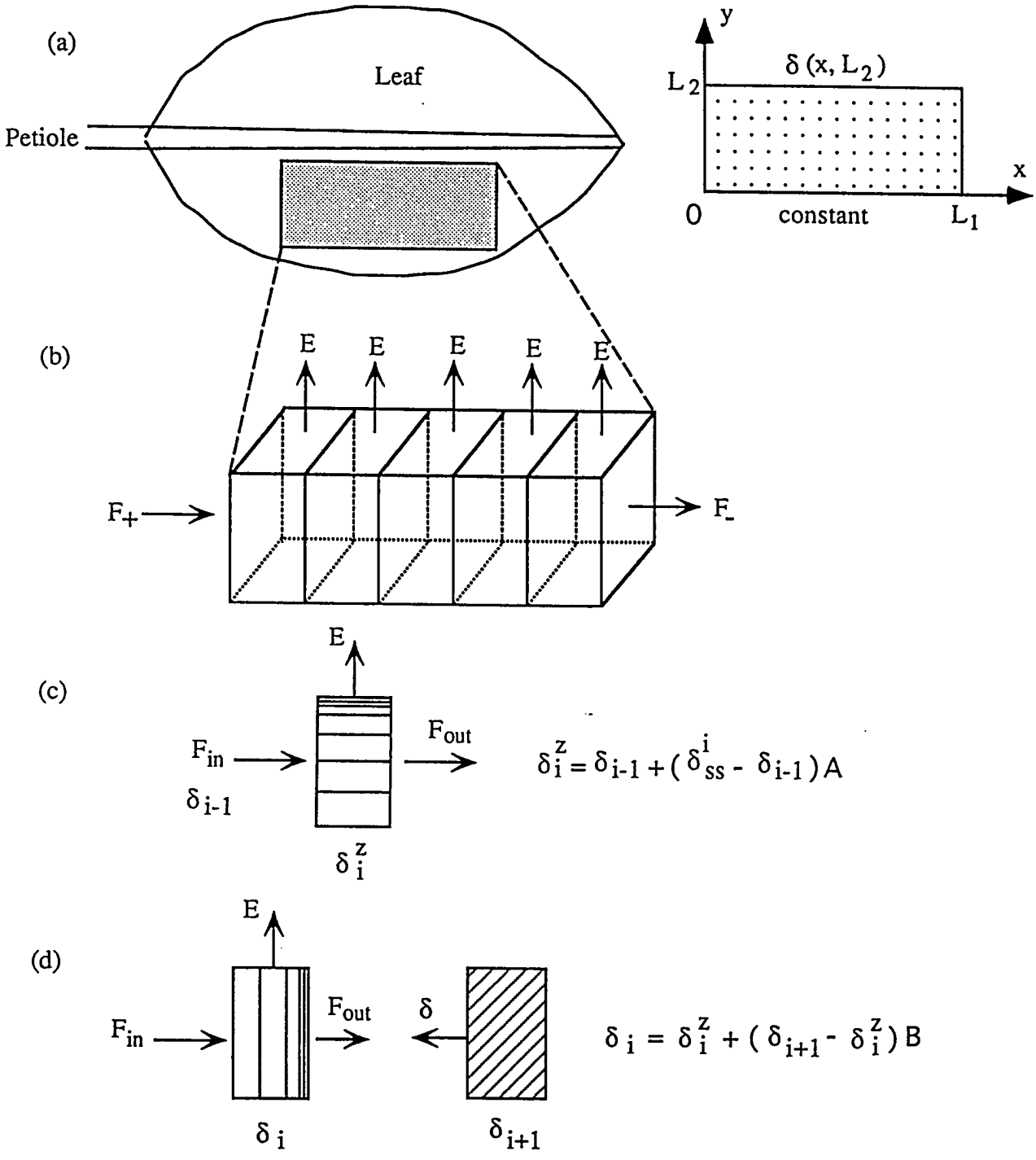

Fig.1 Schematic representation of water movement within a leaf. (a), (b) a series of pools in a leaf; (c) isotopic gradient in vertical direction in a single pool; and (d) isotopic gradient in horizontal direction in a single pool.

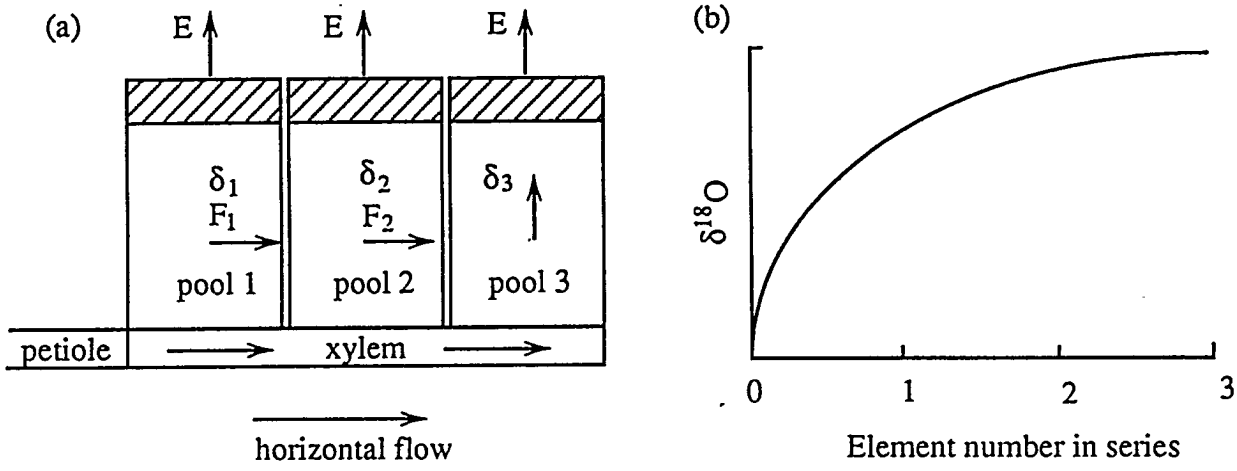

Fig.2 (a) Conceptual model showing different isotopic pools and flow direction in a leaf; (b)Schematic diagram of isotopic enrichment along a series of pools in a 3-pool model. The average isotopic composition of different pools is identical to that calculated by Craig & Gordon (1965) model, assuming the total leaf water is an uniform pool.

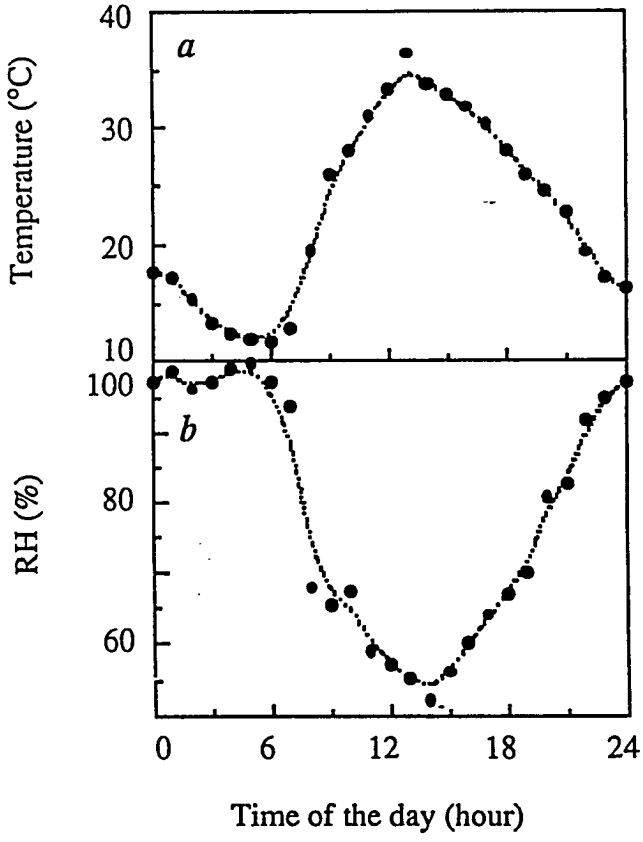

Fig. 3 Daily march of two environmental parameters (adapted from Yakir, et al, 1990): a. Temperature, b. Relative humidity.

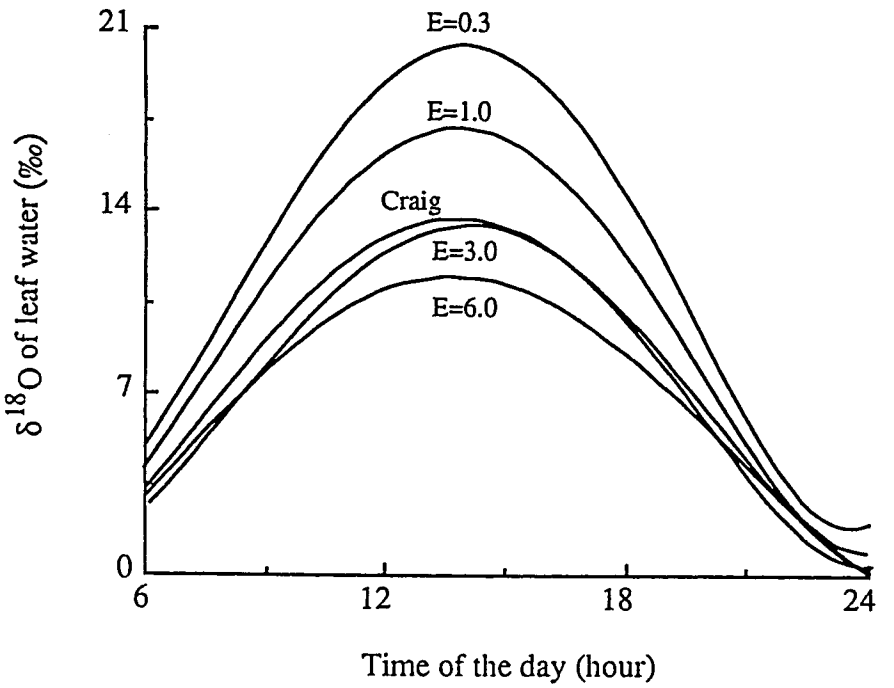

Fig.4 Diurnal variation of the $\delta^{18}O$ value of leaf water at isotopic steady state, predicted by a 3-pool-series model (see Eqs.12, 13, 15, 16). Figure legends (e.g., E=0.3) represent the highest transpiration rate during the day.

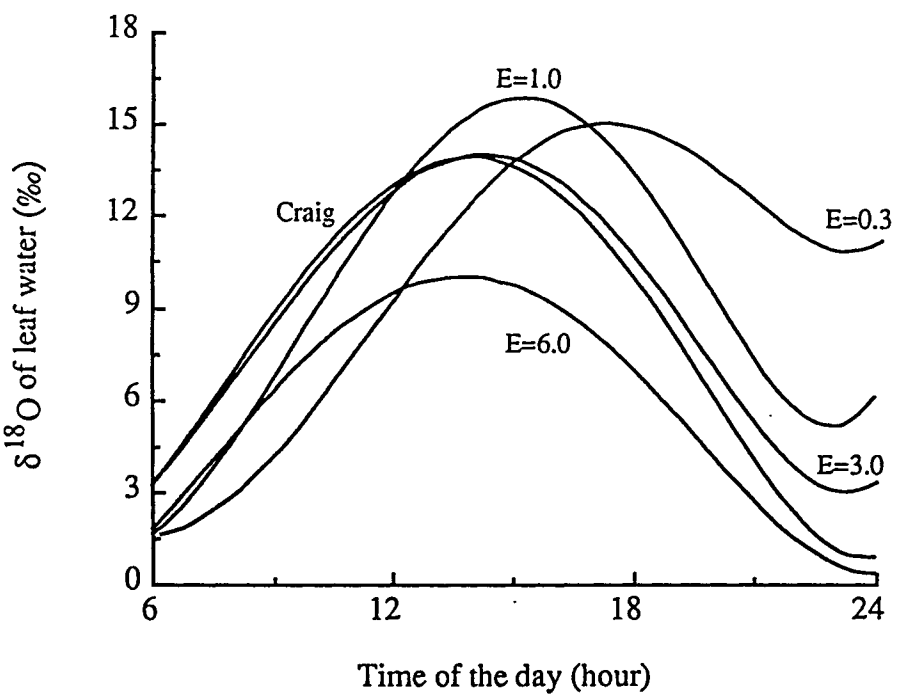

Fig.5 Diurnal approach of the $\delta^{18}O$ value of leaf water to isotopic steady state, predicted by a 3-pool-series model (see Eq.21). Figure legends (e.g., E=0.3) represent the highest transpiration rate during the day.

3.4 Fluxes of CO_2 and water between terrestrial vegetation and the atmosphere estimated from isotope measurements

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THE atmospheric budget of carbon compounds can be balanced only by invoking a significant 'missing sink' for carbon dioxide[1-3]. Identifying this sink requires a knowledge of CO_2 fluxes at global and local scales. The former can be estimated from global averages of CO_2 concentration and isotope composition[4-9]; local-scale measurements have been made by analysing individual eddies of air[10,11]. In both cases, the net CO_2 exchange is the sum of two opposing fluxes: uptake by gross primary productivity and release by respiration. Here we show that these two components can be estimated separately at the local scale from small vertical gradients in ^{13}C and ^{18}O in atmospheric CO_2 above vegetation. By also analysing the ^{18}O content of moisture in the air samples, we can estimate evapotranspiration rates, providing information on water exchange between the biosphere and atmosphere[12]. We suggest that this approach can be extended to the regional scale.

Gradients in concentrations and isotopic compositions of CO_2 and water vapour above crop fields were measured with a 12-m mast that enabled measurement of wind speed and sampling of air at several heights above the canopy. Air was sucked either through a CO_2/H_2O infrared gas analyser to determine concentration gradients or through a cold trap and a 2-l flask to obtain samples of water vapour and CO_2 for determination of isotopic gradients. The results of measurements on a sunny midday over a wheat field are shown in Fig. 1. As expected, concentrations of CO_2 decreased, and concentrations of H_2O increased, towards the vegetation because of net photosynthetic CO_2 uptake and evapotranspiration, respectively. Similarly, $\delta^{13}C$ values of the CO_2 increased towards the vegetation because of the discrimination against ^{13}C associated with photosynthetic CO_2 fixation[13], while $\delta^{18}O$ values of the CO_2 increased because of isotopic exchange with ^{18}O-enriched leaf water[7,9,14].

Variations in trace gas concentrations, such as shown in Fig. 1*a*,*f*, have been commonly used to estimate net fluxes between the land surface and the atmosphere[15,16]. This aerodynamic approach relies on the assumption that momentum, heat and mass (of, for example, CO_2 and H_2O) are transported by eddies of turbulent air and therefore behave similarly in this context. Fluxes and gradients can therefore be related by the eddy diffusivity. Although net fluxes can be more directly estimated based on co-variance of vertical wind speed and concentrations in individual eddies of air[11,17], the need for fairly large samples of air for isotopic analysis made the aerodynamic approach more appropriate for the studies reported here. The net fluxes of water vapour and CO_2 were calculated from the gradients according to:

$$J_x = -k\frac{\Delta C_x}{\Delta z} \quad (1)$$

where ΔC_x is the vertical difference in concentration C of a constituent x over the vertical distance Δz in the canopy boundary layer, and k represents the eddy diffusivity of the air calculated from wind speed profiles and surface characteristics. The results for net fluxes of CO_2 and water vapour are given in Table 1. Notably however, it is the gross fluxes that need to be considered for understanding the behaviour of the biological system and its response to environmental change.

The approach used here to partition the net CO_2 flux into its components is based on the assumption that an air sample taken at a height z above the canopy contains a mixture of air from the background atmosphere (indicated by subscript a) with air that has had CO_2 or H_2O added/removed by the surface biological system (b) according to:

$$C_z = C_a - C'_b \quad (2)$$

where a vertical gradient C_z can be converted to a flux (equation (1)). C'_b may (prime indicates a composite value) be further subdivided into respiratory and photosynthetic components of CO_2 and H_2O (discussed below). Owing to isotopic fractionations associated with biological exchange of CO_2 and H_2O, an isotopic mass balance can be approximated by:

$$C_z\delta_z = C_a\delta_a - C'_b\delta'_b \quad (3)$$

where isotopic abundance is represented in the familiar δ notation[18] (which introduces a possible error in the order of 0.01‰ when calculating δ'_b).

The mixing analysis of equations (2) and (3) can be rearranged in a useful way[19] as:

$$\delta_z = \delta'_b + M/C_z \quad (4)$$

where $M = (\delta_a - \delta'_b)C_a$ and the intercept δ'_b can be empirically obtained from a plot of δ_z versus $1/C_z$ (Fig. 1*c*, *e*). Applying this regression analysis to the atmospheric water vapour data (Fig. 1*f*–*h*) yielded an intercept (δ'_b) of −3.7‰, representing the isotopic signature of the evapotranspiration flux. This value is similar to the mean $\delta^{18}O$ value of stem water in the wheat field (-3.1 ± 0.3‰, $n = 14$) as indeed is required by the widely made assumption of isotopic steady-state of leaf water (during which the $\delta^{18}O$ values of input (stem) water, and output (transpired) water vapour are equal[4,20]). Consistency with the regression analysis was also found for the CO_2 results.

The biological flux of CO_2, with an isotopic signature δ'_b, is composed of respiratory (r) and photosynthetic (p) components that can be independently estimated. Considering their specific isotopic signatures in the context of equation (3) leads to:

$$C_z\delta_z = C_a\delta_a - C_p\delta_p + C_r\delta_r \quad (5)$$

Equation (5) can be solved for C_p and (because $C'_b = C_p - C_r$,) for C_r. Using these values, together with estimates of the eddy diffusivity, the gross photosynthetic and respiratory fluxes, J_p and J_r respectively, can now be calculated (see equation (1)) according to:

$$J_p = -k\frac{\Delta C_p}{\Delta z} = -k\frac{\Delta C'_b}{\Delta z}f_1 \quad (6)$$

and

$$J_r = -k\frac{\Delta C_r}{\Delta z} = -k\frac{\Delta C'_b}{\Delta z}f_2 \quad (7)$$

where $f_1 = (\delta'_b - \delta_r)/(\delta_p - \delta_r)$ and $f_2 = (\delta'_b - \delta_p)/(\delta_p - \delta_r)$. Notably, the δ values used in the equations above represent either $\delta^{18}O$ or $\delta^{13}C$, each dominated by different processes and yielding independent estimates of J_p and J_r.

To apply the above approach at the field scale, we characterized the isotopic signatures of the CO_2 associated with photosynthesis,

δ_p, and respiration, δ_r, based on leaf scale measurements and sampling of plant material and soil organics across each field.

For $\delta^{18}O$, the isotopic signature of the CO_2 associated with photosynthesis, δ_p^{18}, is influenced by catalysed oxygen exchange with water that occurs primarily in the chloroplast. Assuming that the $\delta^{18}O$ value of water in chloroplasts is similar to that of bulk leaf water (δ_{LW})[14], we used δ_{LW} measured across the field (9.9 ± 0.8‰, $n = 14$ for the wheat field reported in Fig. 1) to estimate the $\delta^{18}O$ value of CO_2 in equilibrium with chloroplast water[21] before CO_2 assimilation in photosynthesis (δ_{ch}). This value was then used to estimate δ_p^{18} according to[9]:

$$\delta_p^{18} = (1 + \varepsilon)\delta_a - \varepsilon\delta_{ch} - \bar{a}_1 \quad (8)$$

where $\bar{a}_1$, the diffusional fractionation, was estimated to be 8.0‰ and $\varepsilon = C_{ch}/(C_a - C_{ch})$, calculated based on leaf scale gas exchange measurements of photosynthesis and stomatal conductance[13] across the fields. Alternatively, the $\delta^{13}C$ value of leaf organics which is influenced by ε can also provide a time-integrated record of this parameter[13]. Notably, estimates of δ_p^{18} were consistent with δ_b^{18} values calculated from the regression analysis discussed above (Table 1).

The $\delta^{18}O$ value of the respiratory CO_2 is expected to reflect isotopic equilibrium with soil and stem water (which are isotopically similar) and modifications due to diffusion into the atmosphere[22], and was calculated according to:

$$\delta_r^{18} = (\delta_s + \varepsilon_{eq}) - \bar{a}_2 \quad (9)$$

where δ_s represents the $\delta^{18}O$ of soil and stem water (directly-measured), $\varepsilon_{eq} = (\alpha - 1)10^3$, where α is the temperature-sensitive CO_2–H_2O equilibrium fractionation[21], and $\bar{a}_2$, the diffusional fractionation, was taken as the maximum value for molecular diffusion, 8.8‰, found useful for measurements in a grassland ecosystem[22]. The results are shown in Table 1. More experimental work is needed to reduce uncertainties with respect to possible variations in both $\bar{a}_1$ and $\bar{a}_2$.

Estimates of δ_r^{13} were based on the $\delta^{13}C$ value of plant organics sampled across the field (Table 1) which was, as expected in these mostly monoculture fields, very similar to that of total soil organics sampled at 40 cm depth at the centre of the fields. Significantly, these long-term mean $\delta^{13}C$ values were distinct from the short-term, midday δ_p^{13} values that were calculated based on leaf scale measurements carried out concurrently with the air sampling. As noted above for δ_p^{18}, estimates of δ_p^{13} were consistent with δ_b^{13} values obtained from the regression analyses (Table 1). Note that δ_p^{13} and consequently δ_b^{13} values were ~10‰ higher in the corn field than in wheat, as would be expected above C_4 plants. The much smaller differences in δ_p^{18} between the wheat and corn fields reflected small differences in leaf water enrichment and stomatal conductance (not shown).

Using the estimates of the isotopic signatures reported in Table 1, combined with the concentration measurements and concurrently estimated eddy diffusivity (0.194 $m^2 s^{-1}$ for the wheat field reported in Fig. 1), the fluxes J_p and J_r for each of the crop fields studied here could be calculated according to equations (6) and (7) (Table 1). Rates of net CO_2 uptake by the crop fields were consistent with commonly observed rates[23,24], and partitioning of the net CO_2 flux yielded rates of respiration comparable to those made by either extrapolation from night-time or soil-profile measurements[22,25]. The isotopic approach showed that in wheat, a decrease in net CO_2 uptake at the end of the growing season (from 44.2 to 20.6 μmol $m^{-2} s^{-1}$) reflected a proportionally greater decrease in respiration (71%) than in photosynthesis (56%), possibly due to soil drying near the surface.

Interestingly, combining estimates of water vapour flux, $J_{N(H_2O)}$, with those for the gross photosynthetic flux, J_p (Table 1), can provide a gross-primary-productivity-related estimate of the

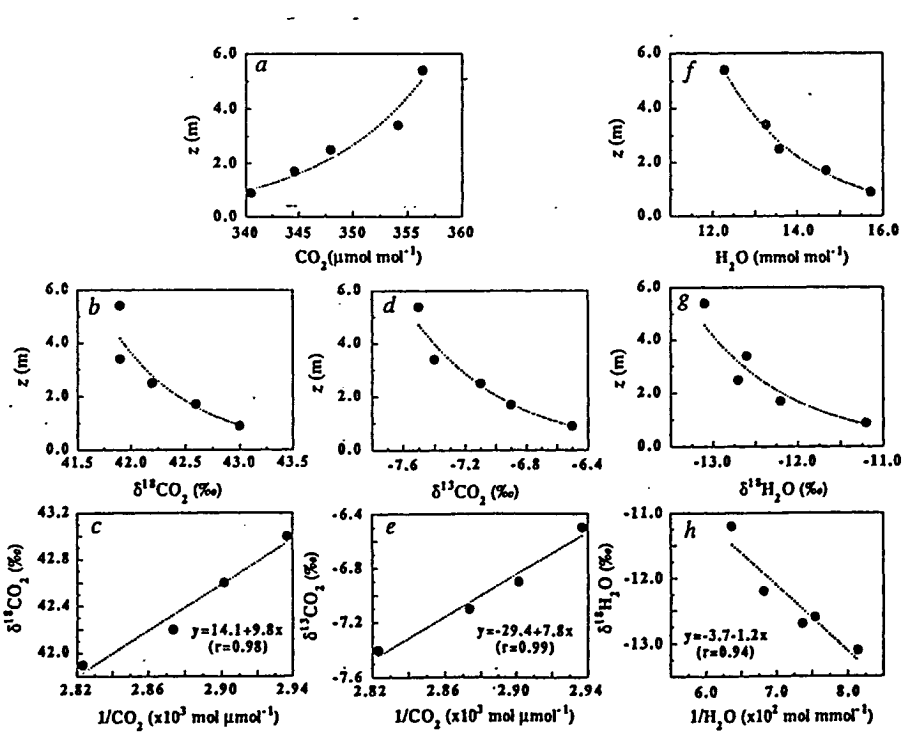

FIG. 1 Vertical gradients in concentration and isotopic composition of CO_2 and water vapour in the atmosphere above a wheat field (500 m long in the wind direction) in central Israel during midday. *a*, *f*, Spectrometrically determined CO_2 and water vapour concentrations; *b*, *d*, oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotopic compositions of air samples taken at the same heights; *g*, $\delta^{18}O$ values of the moisture stripped off the air samples during collection; *c*, *e*, *h*, plots of the isotopic data versus the reciprocal of concentration fitted with a line from which the intercept (*a*) was obtained. A 12-m mast was used to suck air samples (at a rate of 250 ml min^{-1}, and for 30 min per sample) through plastic tubes from 4–5 heights (*z*) within the canopy boundary layer and from the background air. Air from the different levels was simultaneously fed through a CO_2/H_2O infrared gas analyser (Li-Cor 6262, rotating between levels, integrating measurements over 30 min for each), or through cryogenic traps (−80 °C) collecting the moisture and 2-l glass flasks pre-filled with nitrogen for storage of air samples. Wind speed at 3–4 levels was concurrently measured with cup anemometers. In the laboratory, water samples were distilled under vacuum from the traps, and CO_2 was cryogenically separated from the air samples for mass-spectrometric analysis (Finnigan MAT 250). CO_2 samples were directly analysed while 2 μl subsamples of water were first pyrolysed and converted to CO_2 for the isotopic analysis[30]. Precisions were ±1 μmol mol^{-1} for CO_2 and ±0.1 mmol mol^{-1} for H_2O, ±0.05‰ for ^{18}O and ^{13}C in CO_2 and 0.1‰ for water. $\delta = [(R_{sample}/R_{standard}) - 1]$ per mil, where R is $^{18}O/^{16}O$ or $^{13}C/^{12}C$, and the standard is VSMOW for ^{18}O and VPDB for ^{13}C.

TABLE 1 Estimated field–atmosphere fluxes

	J_N (mol m^{-2} s^{-1})		^{18}O (‰)			^{13}C (‰)			J_p (μmol m^{-2} s^{-1})		J_r (μmol m^{-2} s^{-1})	
	CO_2 (×10^6)	H_2O (×10^3)	δ'_p	δ_b	δ_r	δ'_b	δ_p	δ_r	^{18}O	^{13}C	^{18}O	^{13}C
Wheat 1	44.2	7.7	14.12	16.01	29.40	−29.36	−29.00	−27.40	50.4	54.1	−6.2	−9.9
Wheat 2	20.6	4.8	16.06	17.14	29.20	−28.47	–	−27.40	22.4	–	−1.8	–
Cotton	35.4	13.8	19.30	20.68	29.50	−25.91	−25.87	−25.40	40.9	37.8	−5.5	−2.4
Corn	27.6	7.5	17.59	18.88	30.40	−19.97	−15.67	–	30.7	–	−3.1	–

Estimated fluxes of CO_2 and water vapour exchanged between different crop fields and the atmosphere during midday. Net CO_2 and water vapour fluxes (J_N) were estimated from concentration gradients (see Fig. 1) and eddy diffusivity (estimated from wind speed profiles, measured with cup anemometers, and surface characteristics). Net CO_2 fluxes were partitioned to photosynthetic CO_2 uptake (J_p) and respiratory CO_2 release (J_r) by using analysis of the isotopic gradients in the canopy boundary layer to estimate δ'_b (Fig. 1), and estimates of the isotopic signatures of the CO_2 taken up in photosynthesis (δ_p) and released in respiration (δ_r). Estimates of δ_p and δ_r were based on analysis of water and organic matter from leaves, stems and soil samples collected across the fields concurrently with the air samples. Wheat 1 was measured on 20 February 1994 during peak activity; wheat 2 on 23 March at the end of the growing season.

vegetation water use efficiency ($J_p/J_{N(H_2O)}$). Independent estimates of $J_{N(H_2O)}$, say from energy balance[15] or by incorporation of eddy correlation techniques[17], may help in extracting J_p. $J_{N(H_2O)}$ itself could also be evaluated by using isotopic values as surrogates for concentrations[12]. In this case gradients and fluxes could be estimated based on $C_w = C_a(\delta_a - \delta_s)/(\delta_z - \delta_s)$, assuming $\delta'_b = \delta_s$ and the latter obtained from stem water (where C_w substitutes for C_b when considering water). Consistency between estimates obtained in this way and those reported in Table 1 provided another check on the isotopic mass balance approach employed here (not shown).

The intermediate (field) scale chosen here allowed us to establish the quantitative relationships between isotopic variations in the atmosphere and specific biospheric processes, which are uncertain at the global scale and unrealistic in the laboratory. Expansion of such measurements to scales which are spatially and/or temporally larger may be challenging. Larger, heterogeneous systems will be increasingly difficult to characterize in terms of surface isotopic signatures, and will need appropriately scaled sampling strategies. Important prerequisites for the application of the isotopic approach are the existence of the differences between δ_r and δ_p and between δ_b and δ_a. Differences between δ_r and δ_p in ^{13}C may be small in established ecosystems where the $\delta^{13}C$ values of decomposing and newly fixed organic matter are very similar (although seasonal variations may help in this respect). In contrast, considerable differences in ^{18}O between δ_p and δ_r can be generally expected because of the large ^{18}O enrichment in leaf water[26]. This may provide a significant advantage for ^{18}O over ^{13}C as tracer for partitioning leaf and soil CO_2 exchange (an advantage that may be restricted in some locations where geographical variations in the $\delta^{18}O$ values of environmental water[26] impose similar δ_b^{18} and δ_a^{18} values). Research is clearly needed to further reduce uncertainties in estimating δ_r and δ_p. Specifically, isotopic fractionations associated with diffusion and CO_2–H_2O equilibrium of soil CO_2, as well as the isotopic signature of chloroplast water, are not well characterized at present. The specific ^{18}O signature of chloroplast water has, however, been addressed in two recent laboratory studies[9,14]. In practice, it is often useful to rely on a hydrological model that predicts the $\delta^{18}O$ value of water undergoing evaporation based on ambient humidity and its $\delta^{18}O$ value[27,28]. It was encouraging to find that transpiration from the crop fields canopies appeared to be at isotopic steady-state as required by the model, and that the evaporation model yielded δ_{LW} values that were generally similar to measured values when the observed gradients within the canopy boundary layer (for example, Fig. 1*f*,*g*) were considered. However, this approach does not fully account at present for variations in humidity and its $\delta^{18}O$ values within the canopy boundary layer, or for physiological effects such as those observed when different plant species growing at the same site have very different δ_{LW} values[26].

The usefulness of including isotopic measurements of CO_2 at the global scale has already been noted[4–9], and complementing these studies with local-scale measurements will undoubtedly improve our understanding of the net CO_2 exchange between the biosphere and the atmosphere. But it may prove essential to evaluate the gross fluxes of photosynthesis and respiration in order to gain insights into ecosystem response to environmental changes (such as variations in temperature or atmospheric CO_2 concentrations[29]). It is in this context that the isotopic approach, linked with existing regional-scale flux measurements relying on analysis of concentration alone, may have its greatest potential. □

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3.5 Isotopic interactions between vegetation and the atmosphere: Evapotranspiration from crop fields and desert agroforest

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Abstract

Evapotranspiration (ET) is a major components of the water and energy balance on regional and global scales. Several meteorological techniques (e.g., energy balance Bowen ratio, eddy correlation, aerodynamic, etc.) have been used for estimating ET fluxes, which are time averaged quantities computed from measurements taken at one or several points in space. Even though they are relatively representative of a large underlying area, uncertainties still remain in ET estimation above natural systems (e.g. Morton, 1994). To simplify field instrumentation, constrain estimates and provide additional insights, a stable isotope method was tested. Mid-day ET from fields of winter wheat, maize and cotton were estimated during different growing periods throughout 1994, and diurnal ET measurements were made above a field of sunflower and above a desert agroforest system in 1995. The isotope approach is based on water vapor interactions between vegetation and the background atmosphere within the canopy boundary layer (CBL) on the stable oxygen isotope concentration ($\delta^{18}O$) of the ambient moisture. Gradients of both water vapor concentration and of the $\delta^{18}O$ value of the water vapor in the CBL could be measured under normal meteorological conditions. Computation of latent heat flux could be carried out from isotopic measurements of plant stem water and of the atmospheric moisture content (inside and outside the CBL) and from the knowledge of the specific humidity of the ambient atmosphere. ET estimates varied between 2.1 to 14.3 mmol m^{-2} s^{-1}, which were comparable to those obtained from Bowen ratio method (6.6 to 13.4 mmol m^{-2} s^{-1}), or direct concentration measurements (4.8 to 13.8 mmol m^{-2} s^{-1}).

Introduction

Plants are the major transducers of water and energy between the terrestrial biosphere and the atmosphere. Quantitative knowledge of evapotranspiration is of great interest in many different disciplines (Brutsaert, 1982). On small scales, e.g. agriculture, ET estimation is essential for the precise management of intensive irrigation (Mahrer and Rytwo, 1991); while on large scales it is a key component for the hydrological cycle and may also reflect the biomass productivity and water use efficiency of vegetation (Magaritz et al., 1990; Loaiciga et al., 1996). Conventionally, two different approaches were independently used by physiologists and meteorologists to estimate transpiration (Jarvis and McNaughton, 1986). One is based on stomatal conductance emphasizing the important role of plants in the regulation of transpiration (Burrows and Milthorpe, 1976); while the second one is based on determining the energy consumed in evaporating water from a certain area (Brutsaert and Sugita, 1992). Transpiration rates obtained from single leaf measurements are usually not representative of the plant or canopy as a whole. As a result of plant and soil variability and heterogeneity of water application, relatively large variability between replicates is found when transpiration is determined on single plants in the field. In order to overcome such variability, particularly noticeable in forest stands, a large number of measurements is required. Micrometeorological methods enable us to compute relatively realistic ET estimations (Morton, 1994). Eddy correlation and Bowen ratio are two commonly used techniques to estimate total water loss (ET: evaporation from soil + transpiration from plants) at the canopy scale. Both approaches have however some drawbacks. The eddy correlation method, is limited by the frequency response of the instruments. The Bowen ratio is proportional to the ratio of the difference in temperature and tin vapor pressure between instruments located at two levels above the evaporating surface. The Bowen ratio technique is based on the assumptions that the eddy diffusivities for sensible heat and water vapor are equal or their ratio is constant over a wide range of stability conditions. Common to both techniques is the difficulty of performing routine measurements due to the high cost of instruments needed to obtain precise measurements of temperature and specific humidity profiles, or vertical velocity within the CBL. An additional alternative method that would simplify field instrumentation and constrain ET estimates would seem to be welcome.

While accurate estimation of the transpiration flux from vegetation is difficult, it was recently suggested that it could be improved by the use of stable isotopes of water by focusing on the isotopic interaction of different water vapor sources (Bariac et al., 1989; Walker and Brunel, 1990; Simpson and Herczeg, 1991; Brunel et al., 1992). As air moves above the surfaces of transpiring leaves, water vapor together with heat would be added to the air flow. The influence of the $\delta^{18}O$ value ($\delta^{18}O$ (‰)= ($R_{sample}/R_{standard}$ - 1)·10^3, where, R is $^{18}O/^{16}O$ ratio, the standard is Vienna Standard Mean Ocean Water (VSMOW)) of plant transpiring water would then be

superimposed on the $\delta^{18}O$ signature of the ambient atmospheric moisture within the CBL (Figure 1). Therefore, the $\delta^{18}O$ value of the atmospheric moisture above the vegetation must contain information on the size of the transpiration flux.

The objective of this study was to test the isotope approach by comparing the ET estimates obtained by the isotope method and by Bowen ratio and concentration gradients above different vegetation.

Theoretical Considerations

Evapotranspiration effects on isotopic composition of air moisture

Understanding stable isotope interactions between vegetation and the regional atmosphere relies mainly on quantitative knowledge of the $\delta^{18}O$ values of the moisture transduced by soil and plants (White and Gedzelman, 1984). Since direct measurement of the $\delta^{18}O$ value of transpired water from the canopy is difficult to obtain, it becomes necessary that the factors controlling $\delta^{18}O$ signature of the leaf water are well understood. During the transpiration process, the isotopic composition of the leaf water becomes enriched due to equilibrium and kinetic effects (Craig and Gordon, 1965; Dongmann et al., 1974; Farris and Strain, 1978; Flanagan et al., 1991). Equilibrium isotope fractionation happens during phase transition at the interface between liquid water and gaseous vapor and is caused by the differences in the saturated vapor pressures of water with different isotopes. For example, the water containing the lighter isotope has a greater saturated vapor pressure than that containing the heavier one and therefore moves faster (Majoube, 1971). Kinetic isotope fractionation occurs above the surface of liquid water and is caused by different molecular diffusion rates of the isotopes (Merlivat, 1978).

The changes in $\delta^{18}O$ value of an evaporating water pool have been studied in detail (e.g., Craig and Gordon, 1965; Gat, 1981). The evaporated water is depleted in heavy isotopes relative to the source water, and therefore the residual water becomes enriched in the heavy isotopes. Traditionally, leaves have been considered as thin, well-mixed water pools (Dongmann et al., 1974). According to the Craig and Gordon (1965) model, the $\delta^{18}O$ of the evaporation flux (δ_E) is calculated as:

$$\delta_E = \frac{\alpha^* \cdot \delta_L - h \cdot \delta_a - \varepsilon_{eq} - (1-h) \cdot \varepsilon_k}{(1-h) + (1-h) \cdot \varepsilon_k / 1000} \approx \frac{\delta_L - h \cdot \delta_a - \varepsilon_{eq} - (1-h) \cdot \varepsilon_k}{1-h} \qquad (1)$$

where, subscripts *E, L* and *a* stand for transpired water, leaf water and ambient air respectively; α^* is the equilibrium fractionation factor, $\varepsilon_{eq} = (\alpha^* - 1)10^3$ (9.4 ‰ at 25°C; Majoube 1971); ε_k is the kinetic fractionation factor (it depends on the molecular diffusion rate and is influenced by

the aerodynamic nature of the boundary condition (Merlivat, 1978)); and h is relative humidity of the ambient air at the leaf surface temperature.

Generally, in plants with a high transpiration rate, the leaf water is expected to reach isotopic steady state shortly after a change in the relative humidity of the ambient air (Walker et al., 1989; Wang and Yakir, 1995). At isotopic steady state δ_E should be identical to the $\delta^{18}O$ value of the source water (δ_S). The difference between δ_E and δ_S can be used as an indicator of the approach to isotopic steady state of the leaf water. As a result, $\delta^{18}O$ value of the leaf water at isotopic steady state (δ_L) can be calculated by using δ_S as the isotopic composition of the transpired water.

Under controlled environment conditions, the observed isotopic composition of bulk leaf water is often less enriched than that predicted by the model (Eq. 1) for the evaporative sites within leaves (Flanagan & Ehleringer, 1991; Yakir et al, 1989). Much of the difference between the modeled and observed leaf water isotopic compositions was proposed to result from gradients within the leaf caused by a shifting balance between the bulk flow of unfractionated water into the leaf (convection) and the back diffusion of water with heavy isotopes away from the evaporating sites (Flanagan et al, 1991; Farquhar & Lloyd, 1993). The extent of the difference between modeled and observed leaf water isotopic compositions has been shown to be positively related to leaf transpiration rate (White 1988; Walker et al. 1989; Flanagan et al. 1991).

Energy Balance - Bowen Ratio (EBBR)

A widely used technique for estimating evapotranspiration is the energy balance - Bowen ratio (EBBR) technique, which is based on the combination of the surface energy balance and the Bowen ratio (Thom, 1975; Brutsaert, 1982; Brunel et al., 1992):

$$R_n + H + LE + G + J = 0 \qquad (2)$$

where R_n is the net radiation, H is the sensible heat flux to the atmosphere, L is the latent heat flux of evapotranspiration, E is the rate of evapotranspiration, G is the change in heat storage in the ground, J is the change in heat stored in the volume occupied by the canopy (the energy used in the photosynthesis process has been neglected); and the Bowen ratio (b) which is defined as the ratio of H to LE, and based on the assumption that the eddy diffusivities for sensible heat and water vapor are equal or their ratio constant over a wide range of stability conditions. Terms of H and LE can be estimated from the temperature (T) and the water vapor (q) profiles as follows:

$$H = \frac{\rho c_p (T_2 - T_1)}{r} \qquad (3a)$$

$$E = \frac{\rho(q_2 - q_1)}{r} \tag{3b}$$

$$\beta = \frac{H}{LE} = \frac{\rho c_p}{L\rho} \cdot \frac{T_2 - T_1}{q_2 - q_1} = \gamma \cdot \frac{dT}{dq} \tag{3c}$$

where ρ is the density of air; C_p is the specific heat for constant pressure; r is the aerodynamic resistance between the canopy surface and height z (Brutsaert, 1982); dT is the vertical gradient of dry bulb air temperature, dq is vertical gradient of the specific humidity, and γ is the psychrometric constant (=0.066 kPa K^{-1}, Brutsaert, 1982). Combining Eqs. 2 and 3 we get:

$$LE = \frac{R_n - (G + J + \varepsilon A)}{1 + \beta} \tag{4}$$

All of the quantities on the right side of Eq. 4 can be measured directly (see micrometeorological measurements below).

The rate of evapotranspiration can also be more directly estimated by the eddy correlation method, which requires fast response instrumentation (such as sonic anemometer, Krypton absorption hydrometer and bead-thermistor thermometers) in order to obtain high precision measurements of air temperature and specific humidity (i.e., measurements of high frequency fluctuations of as small as 10 Hz of the interested parameters). The eddy correlation method also requires the averaging period as short as possible to allow the measurements within the stationary time series, but long enough period to cover the slowest turbulent fluctuations as well (Brutsaert, 1982; Mahrer and Rytwo, 1991).

Isotope approach to estimating ET

In order to quantitatively resolve the isotopic mass balance of a mixed air sample above vegetation we must know the $\delta^{18}O$ value of the moisture of the incoming air mass, and also that of the transpiration flux. The former can be determined by collecting air moisture sample outside the CBL, while the latter is an estimate based primarily on the assumption that the transpiration from the leaves is at isotopic steady state (Yakir, 1992; Yakir and Wang, 1996). Under these conditions the $\delta^{18}O$ values of the transpired water should be identical to that of the source water (soil water or plant stem water), which in turn can be measured directly (IAEA, 1990).

By aerodynamic approach, it is assumed that mass transfer of any quantity J (e.g., heat, H_2O or CO_2) within the constant stress layer above land surface can be represented by the product of a turbulent transfer coefficient with the gradient of concentration of the quantity in question, which usually takes the following form (e.g., see *Nobel,* 1991):

$$J = k_m \cdot \frac{\partial C}{\partial z} \tag{5}$$

where, k_m is the eddy diffusivity ($m^2\ s^{-1}$); C is the mean concentration of the quantity in question in a desired time period (mol m^{-3}); and z is the height (m).

The ET flux from the soil and vegetation (E_b) can be calculated by using Eq. 5, provided the concentration gradient of water vapor at any two levels above the vegetation is known.

Based on the mass balance of atmospheric moisture at a height z within the CBL, we have:

$$q_z = q_a + q_b \tag{6}$$

where q is specific humidity (mmol H_2O mol^{-1} air), subscript a stands for ambient atmosphere, b for terrestrial biosphere. Since q_a is relatively constant, gradient in q_z (Δq_z) should be equal to gradient in q_b (Δq_b).

Considering mass balance for the ^{18}O stable isotope in water vapor above the canopy and introducing the "δ" notation into the formula, we obtain:

$$q_z \cdot \delta_z = q_a \cdot \delta_a + q_b \cdot \delta_b \tag{7}$$

where δ_b is the isotopic signature of the water vapor contributed from both the plants and the soils. Note that there will be a small error in the order of 0.01‰ when calculating δ_b (Hayes, 1982).

Substituting $q_b = q_z - q_a$ from Eq. 6 into Eq. 7 and rearranging the equation, we can express the specific humidity at level z_1 in terms of the isotopic compositions of water from different sources:

$$q_{z1} = \frac{\delta_b - \delta_a}{\delta_b - \delta_{z1}} \cdot q_a \tag{8}$$

Similarly, q_{z2} can be obtained by substituting δ_{z2} from another level z_2 within the CBL for δ_{z1} in Eq. 8. It shows that, based solely on a single measurement of the specific humidity of the ambient air (q_a) outside the CBL together with four $\delta^{18}O$ measurements of water samples (one from the ambient background atmosphere (δ_a), two from the canopy boundary layer (δ_{z1}, δ_{z2}), and one from the stem water (δ_b)), it is possible to estimate the ET flux based on Eq. 5.

The specific humidity of the ambient air outside the CBL and the $\delta^{18}O$ values of air moisture at two different levels (δ_{z1}, δ_{z2}) could be directly measured, but the δ_b value is quite difficult to obtain. One way to obtain the δ_b value is to assume leaf water and soil water are both at isotopic steady state, as a result, the $\delta^{18}O$ value of plant transpired water is identical to that of the source (soil) water, since there is no isotopic fractionation as water moves from the soil through the plant stem, that is, $\delta_s = \delta_p$. Since soil water is also assumed to be at steady state, the $\delta^{18}O$ value of the soil evaporated water is identical to δ_s. Therefore, the isotopic signal of the water vapor contributed from both plants and soils is equal to that of the plant stem water or the soil water, e.g., $\delta_b = \delta_s = \delta_p$. Another way to obtain the δ_b value is to use an intercept approach which will be discussed later.

After the specific humidity gradient Δq_z (or Δq_b) is reconstructed based on the isotope measurements within the CBL, the ET flux (E_b) can be calculated according to:

$$E_b = -\overline{K}_m \cdot \frac{\Delta q_z}{\Delta z} = -\overline{K}_m \cdot \frac{\Delta q_b}{\Delta z} = -(\overline{K}_m \cdot \frac{q_a}{\Delta z}) \cdot f \qquad (9)$$

where $f = (\delta_b - \delta_a)[1/(\delta_b - \delta_{z2}) - 1/(\delta_b - \delta_{z1})]$; $\overline{K}_m$ is the average of eddy diffusivity in layer Δz (from *z1* to *z2*), and computed from:

$$K_m = k \cdot u_* \cdot (z - d) \qquad (10)$$

where k is the von Karman constant (0.41, dimensionless; Thom, 1975); d is the zero plane displacement height (m), which is taken here as 0.67 of average canopy height (Brutsaert, 1982); and u_* is friction velocity (m s^{-1}), can be calculated under neutral conditions (which prevailed during isotope sampling periods) as (Thom, 1975):

$$u_* = k \cdot (u_2 - u_1) / \cdot \ln(\frac{z_2 - d}{z_1 - d}) \qquad (11)$$

where $u(z)$ is the wind velocity measured at height z (m s^{-1}).

One should notice that Eq. 8 differs from the one developed by Brunel et al. (1992) in that the equation developed here requires only a single measurement of specific humidity of the ambient atmosphere outside the CBL (q_a), which is relatively stable and easy to specify compared to the specific humidity within the CBL.

Potential for partitioning ET flux

The water vapor contributed from the terrestrial biosphere is composed of plant transpiration and soil evaporation:

$$q_b = q_p + q_s \tag{12}$$

where q_p and q_s are specific humidities contributed from plants and soils, respectively.

Analogous to Eq. 7 by incorporating the individual isotopic signatures into Eq. 12, the mass balance equation for the stable isotopes from the terrestrial moisture contribution is:

$$q_b \cdot \delta_b = q_p \cdot \delta_p + q_s \cdot \delta_s \tag{13}$$

where δ_p and δ_s are the isotopic values of the water vapor contributed from plants and soils, respectively.

Substituting ($q_s = q_b - q_p$) or ($q_p = q_b - q_s$) from Eq. 12 into Eq. 13 and rearranging the equation, we obtain :

$$q_p = f_1 \cdot q_b \tag{14}$$

$$q_s = f_2 \cdot q_b \tag{15}$$

where $f_1 = (\delta_b - \delta_s) / (\delta_p - \delta_s)$, and $f_2 = (\delta_p - \delta_b) / (\delta_p - \delta_s)$.

Analogous to Eq. 9, fluxes of plant transpiration (E_T) and soil evaporation (E_S) can now be calculated according to:

$$E_T = -\overline{K} \cdot \frac{\Delta q_p}{\Delta z} = f_1 \left(-\overline{K} \cdot \frac{\Delta q_b}{\Delta z}\right) = f_1 \cdot E_b \tag{16}$$

and

$$E_s = -\overline{K} \cdot \frac{\Delta q_s}{\Delta z} = f_2 \left(-\overline{K} \cdot \frac{\Delta q_b}{\Delta z}\right) = f_2 \cdot E_b \tag{17}$$

It is notable that the ratio of plant transpiration over soil evaporation is proportional to the ratio of f_1/f_2, that is, proportional to the ratio of $(\delta_b - \delta_s) / (\delta_p - \delta_b)$.

The prerequisite to partitioning the total ET flux into plant transpiration and soil evaporation depends on the existence of isotopic difference between the plant transpired water and the soil evaporated water, and on the knowledge of the δ_b value. The δ_p value may be different from the δ_s value if the leaf water or soil water is not at isotopic steady state; or even

though they are at the isotopic steady state, there may be a time delay in approaching the steady state between δ_p and δ_s values; or the plant may uptake different source water than the surrounding prevalent soil water. It has been observed that plant stem water (δ_p) could have different isotopic values from the surrounding soil water (White, 1989; Ziegler, 1989; Dawson & Ehleringer, 1991; Yakir & Yechieli, 1995), which demonstrates the potential of this approach (Eqs. 14 &15). In the case $\delta_p = \delta_s$, the above equations (Eqs. 14-17) are no longer useful.

Regardless of the isotopic steady state, δ_b values can be obtained by mixing analysis of Eqs. 6 & 7, the mixing equation is similar to the one developed by Keeling (1961):

$$\delta_z = \delta_b + M / q_z \tag{18}$$

where $M = (\delta_a - \delta_b) \cdot q_a$.

Notably, Eq. 18 links the δ_z value of H_2O at any height z with its specific humidity in the ambient atmosphere. If δ_z is plotted against $1/q_z$ over profiles of isotopes and specific humidity above the canopy, the resulting intercept of the mixing line should be the δ_b value, i.e., the isotopic signature of the water vapor contributed from both the plants and the soils.

Materials and Methods

Field Description: Mid-day measurements were carried out above fields of wheat, maize and cotton during 1994 in Kibbutz Givat Brener, 30 km south of Tel Aviv; while during 1995 diurnal measurements were carried out above a sunflower field in Kibbutz Brurim and above a small *Acacia saligna* plantation in the Negev. The wheat was about 0.8 m high during the sampling period between February and March; and received most of its water from winter precipitation. The maize canopy height averaged 3.4 m in June and the cotton canopy height averaged 1.3 m in August (both crops were irrigated). Sunflower was about 1.5m tall and acacia about 5.7m . The dimensions of the fields were as follows: 550 x 300 m for wheat, 600 x 300 m for maize, 900 x 200 m for cotton, 1500 x 600 m for sunflower, and 300 x 60 m for acacia. A portable tower was always positioned in the fields downwind but some 20 m from the edge of the field. Canopy boundary layer depth, (ca 1% of the fetch length), was about 3 - 4 m in the crop fields and about 1-2 m in the acacia plot for the prevailing wind direction (NW). Mid-day sampling was carried out during peak activity periods of the vegetation (usually from 12:00 pm to 15:00 pm of the local time). Except for the day in March which was partly cloudy, the rest were clear days. During the sampling periods, the ambient air temperature was about 20°C for wheat, and about 30°C for maize and cotton; while the relative humidity was close to 75% for wheat, 40% for maize and 70% for cotton. The ambient conditions for diurnal measurements in the sunflower

and acacia fields were plotted in Figure 2. The annual mean $\delta^{18}O$ value of air moisture in Israel is about -11‰, while the $\delta^{18}O$ value of the mean ground water is about -5‰ (*IAEA*, 1990).

Sample Collection: Two sets of air samples were collected simultaneously. For the first set, air was sucked through a computerized valve system and plastic tubes (Bev-a-Line IV Tubing, I.D. 3 mm, Cole-Parmer Ins. Company, Illinois, USA) by a mechanical diaphragm pump. The tubes were tested for very low water adsorption. From the valve system, air from each sample position was alternatively directed into a CO_2/H_2O infra red gas analyzer (IRGA, LiCor-6262, Lincoln, Nebraska, USA) for one minute per position (10 seconds flushing followed by 10 H_2O concentration measurements every 5 seconds) over 30 minutes. All of the data measured by IRGA were recorded by a data logger (21X, Campbell Scientific Inc., Utah, USA). For the second set, air was identically sucked but through a cryogenic trap, filled with glass beads of 2-3 mm in diameter to increase surface area for sufficient trapping, at -80 °C (with a mixture of the dry ice plus acetone) and at a slow flow rate of 200-250 mL min^{-1}. Trapping efficiency in this case was above 99%, tested with the on-line IRGA. The sampling system made it possible to collect ambient air samples at any desired height inside the CBL and another one outside the CBL for reference. In these studies, we collected air moisture samples from four heights (from ground, 0.9, 1.7, 2.5, and 3.4 m for wheat; 4.0, 4.7, 5.8 and 7.0 m for maize; 2.0, 3.0, 4.2, and 5.7 m for cotton; 2.2, 3.1, 4.0 and 5.0 m for sunflower) within the CBL in each field, and another reference sample 2 m above the top level in each field. For the acacia plot, three levels of sampling (6.0, 6.6, and 8.4 m) were above the canopy, while the other two levels (0.3 and 1.3 m) were within the canopy. Each set of sampling was carried out for only 30 min (as a minimum necessary to average short time fluctuations) in the field, and water traps were sealed and transferred to the laboratory afterwards. All of the air moisture samples were cryogenically extracted out of the traps using a vacuum distillation apparatus within 24 hours after sampling, and then each sample was sealed inside a 50 mL Pyrex capillary with a micro torch flame (Blazer, Piezo micro torch, Japan) for storage. During the same period for air sampling, leaf and stem samples were collected from various positions across the field and saved in test tubes (Vacutainer, Rutherford, New Jersey, U.S.A.) which were closed tightly and kept cold in a dry ice box in the field or in a freezer in the lab to prevent any further loss of water until further isotopic assessments.

Started in June 1995, leaf and stem samples of acacia plantation were collected, between 9:30-10:00 am in the Negev, for isotopic analysis of their water. The sampling was done once a week till October 1995. The temperature, relative humidity and the ET fluxes of the plantation during the sampling period were determined by the conventional EBBR method.

Sample Preparation for Isotopic Analysis: Traditionally, water samples for isotopic analysis were prepared in a way that they were first equilibrated with lab standard CO_2 of known isotopic composition overnight (at least for 24 hours) at 25 °C, and then the CO_2 sample was separated from the water cryogenically (Epstein and Mayeda, 1953). This method is not only time consuming, but needs a lot of water (at least 0.3 mL) in order to get good precision. Such large amount of an air moisture sample requires either high flow or long period of pumping. While high flow pumping usually results in fractionated vapor sample which prohibits isotope approach from any flux estimation, sampling over a long period will lose temporal changes in the rate of evapotranspiration. In order to apply low flow pumping over a short period which produces only a small amount of water, we used a method similar to that described by Santrock and Hayes (1987). By using a Carlo-Erba Elemental Analyzer (Model 1108, Erba Instruments, Inc., Italy), a CO_2 sample is produced by combusting directly a water sample of as little as 0.8 μL with pure carbon, and then the CO_2 sample is ready for $\delta^{18}O$ analysis in a mass spectrometer (Finnigan MAT 250). This analytical instrument had some disadvantage, such as blank and memory effects due to the characteristic of the reactor tube and contamination by the previous sample. In order to improve the analytical ability, following modifications were made: (a) a ceramic combustion tube instead of a quartz tube was used to avoid major blank effect; (b) an efficient mixing of 5% CCl_4 in heptane was done in order to remove the memory effect. We are now able to use the analyzer to convert a micro-size water sample to a CO_2 sample for isotopic analysis of the $\delta^{18}O$ value of the water sample. After analyzing the converted CO_2 by the mass spectrometer, it showed that we could obtain a repeated value several times by running each set of 10 samples of the same water. The reliability of this method was tested several times from 1993 to 1994 by running a series of standard water samples (i.e., SMOW, GISP and SLAP samples from IAEA; UCLA from the U.S.A; and WIS from Weizmann Institute of Science) with known $\delta^{18}O$ values. During the calibration, the size of the sample was varied from 0.8 μL to 2 μL. Triplicate were taken for each sample. The last two runs from each triplicate were analyzed by the mass spectrometer, and the average of those $\delta^{18}O$ values was used as the final machine value. Both calibrations in 1993 and in 1994 showed an excellent correlation between machine measured $\delta^{18}O$ values and the known values relative to the international standard value of SMOW. The correlation coefficients in both cases were 0.99998. External precision for the $\delta^{18}O$ analysis of the micro-size samples was 0.1‰ in our field studies.

Micrometeorological Measurements: Measurements were carried out simultaneously as the air samples collected. A polyethylene shielded Q7 net radiometer, (Radiation & Energy Balance Systems Inc., Seattle, USA) was installed on the tower at a height of 2-3 m above canopy tops. Two sets of soil heat flux plates (REBS), were buried at a depth of 5 cm in the soil. One of the

heat flux plates was always placed in the row and the other one in between two rows. The temperature change of the upper 5 cm soil layer was measured by a pair of copper-constant (T-type) thermocouples placed 2.5 cm above each of the heat flux plates. The soil water content in the upper 5 cm layer was measured by gravimetric method and the corresponding bulk density of the soil was determined. During 1994 a fixed psychrometer system was used, while during 1995 the Bowen system consisted of two psychrometers which changed their position once every 5 minutes, was set up on the tower so that the lower psychrometer was positioned at a height of 0.5-1 m above the canopy. The dry bulb and wet bulb temperatures were measured using T-type thermocouples. During 1995 the sensible heat flux (H) was additionally estimated using a vertical one dimensional CA27 eddy correlation system (Campbell Scientific Inc., USA) composed of a fine wire thermocouple and a pair of sonic anemometers. The eddy correlation system was installed above the canopy at an intermediate height (between the uppermost and lowest psychrometer). The temperature change of the vegetation was monitored by four pairs of T type thermocouples inserted into the trunks (or stems) and stuck on the leaves. The heat storage in the vegetation was calculated by the power average of the trunk and leaf. The photosynthetic energy storage was excluded in this study. The signals of all the above instruments were scanned and recorded using two micro data loggers (21X, Campbell Scientific Inc., USA). All the signals were scanned once every 5 seconds and averaged every 30 minutes except the eddy correlation system which was scanned at a rate of 5 Hz and averaged every 30 minutes. The computed Bowen ratios ranged from 0.1 to 0.8 in these studies. The friction velocity was computed for each integration period from wind profiles measurement with two cup anemometers (Met One Instruments Inc., USA) and the corresponding eddy diffusivity inside the CBL was obtained.

Results

The averages of the measured $\delta^{18}O$ values of leaf water (δ_m) for mid-day and from diurnal measurements in the acacia experiments are listed in Table 1, together with corresponding predicted $\delta^{18}O$ values of leaf water (Eq. 1, using either lower or upper canopy boundary layer conditions, ε_k was 27.5‰ for mid-day and 25‰ for diurnal mean). The isotopic composition of stem water (δ_s) was relatively stable over time and ranged between -3.2‰ and -2.9‰ throughout the field experiments, except for δ_s value of the maize (Table 1). The predicted δ_c values were in most cases higher than the observed δ_m values when using the upper boundary level, but could be lower than δ_m values when using ambient conditions from the lower boundary level (e.g., on March 23, 1994 in Table 1). The δ_m values for experiments in February and August had quite good agreements with δ_c values using the lower boundary conditions (± 1.0‰). There existed a

large discrepancy (ca. 10‰) between the δ_c value and the δ_m value for the maize leaf water. For diurnal measurements of leaf water in acacia plantation under extremely arid condition, the boundary effect on δ_c prediction was within ± 0.5‰ (Table 1). By adjusting the ε_k value between 25‰ and 28‰, most of the predicted δ_c values of leaf water were in good agreement with those measured between 8:00 and 14:00 o'clock, but there existed large discrepancy between δ_m and δ_c values in either late evening or early morning (Figure 3). At mid-night (e.g., 1:00 am in Fig. 3), the δ_m values could be about +10‰ higher than the predicted δ_c values, but in late evening (e.g., 20:00 pm), the δ_m values could be about -3‰ lower than the predicted δ_c values.

Seasonal variations in measured $\delta^{18}O$ values of both leaf and stem water of the acacia plantation are shown in Figure 4. From June to October 1995, there were small variations in the $\delta^{18}O$ values of the stem water (ca. +5‰), but there existed large variations in the $\delta^{18}O$ values of the leaf water (ca. +15‰).

Small gradients in both concentrations of atmospheric water vapor (Figure 5) and their $\delta^{18}O$ values (Figure 6) above the canopy were clearly detected in both mid-day and diurnal measurements. For mid-day sampling, the smallest gradient in water vapor concentration was around 1 mmol H_2O mol^{-1} air in the maize field (Fig. 5d), while the largest gradient was nearly 4 mmol H_2O mol^{-1} air in the wheat field (Fig. 5b); as for $\delta^{18}O$ values of air moisture, the smallest gradient was about 0.5‰ in the cotton field (Fig. 6e), while the largest gradient was nearly 2‰ in the wheat field (Fig. 6a, b & c). Most of the atmospheric moisture data and their $\delta^{18}O$ data could be fitted with exponential curves in their vertical profile. Such gradients in concentrations of the atmospheric water vapor and their $\delta^{18}O$ values were formed mainly within the CBL, while the concentration and isotopic data from the reference points remained almost the same as those points next to them (Figs. 5 & 6) except for the data in March (Fig. 6c).

For the diurnal measurements, the changes in the $\delta^{18}O$ values of air moisture at different levels with time were shown in Figure 7. The $\delta^{18}O$ value of air moisture at the lowest level (e.g., 6.0 m for acacia in Fig. 7a &b; 2.2 m for sunflower in Fig. 7c) above the canopy had usually the highest enrichment, during the active photosynthetic period in the morning, than those in other, higher levels. Within the canopy in the acacia plantation in the Negev, there was a similar trend of high enrichment in the $\delta^{18}O$ value at a lower level of 0.3 m relative to the higher level of 1.3 m (Fig. 7a &b). Shortly after sunrise, say, from 6 to 7 am, the air moisture at the lowest level above the canopy had a rise in the $\delta^{18}O$ value, followed by a drop in the $\delta^{18}O$ value near mid-day (acacia) or early afternoon (Sunflower), and then slowly increasing again towards the late evening. The isotopic differences between different levels above the canopy were larger in the morning during active photosynthetic period, and were diminished in the late evening. There were large variations in the $\delta^{18}O$ values of the air moisture in the acacia field from morning to the evening on May 11 (as large as 6‰ at the top, reference level, Fig. 7a). The $\delta^{18}O$ values of

the air moisture were more enriched on June 17 than on May 11 in the acacia plantation (Fig. 7a & b). In the sunflower field, the $\delta^{18}O$ values of air moisture were relatively stable from mid-night to early in the morning (-12.5‰, in Fig. 7c). The highest measured $\delta^{18}O$ enrichment in air moisture was observed at 9:30 am at the lowest level above the canopy (-9.5‰, Fig. 7c). The isotopic profile within CBL was then fluctuating between the mid-night profile and the profile that occurred at 9:30 am, except for one lower point observed at 14:25 pm, probably due to the wind conditions. The wind condition was usually gentle in the morning and became turbulent in the afternoon, and then reversed to gentle conditions again in the evening.

Estimation of evapotranspiration rates was carried out by three methods for mid-day measurements simultaneously. The first is the EBBR (Brutsaert, 1982); the second is the aerodynamic method based on water concentration gradient measurements; and the third is the isotope approach based mainly on Eq. 8 by using the isotopic signatures to reconstruct atmospheric water vapor gradient.

The last two approaches were also applied in the diurnal measurements. Results of mid-day evapotranspiration by different methods are listed in Table 2. Evapotranspiration estimates (E_b) by the isotope approach varied from 2.1 to 14.3 mmol m^{-2} s^{-1} in different crop fields, while ET ranged from 6.6 to 13.4 mmol m^{-2} s^{-1} by the Bowen ratio technique or from 4.8 to 13.8 mmol m^{-2} s^{-1} by aerodynamic measurements. The results showed that the calculated water fluxes based on the isotopic approach gave comparable results to those obtained by either the Bowen ratio technique or the aerodynamic method. Although four level measurements were made in each test within the CBL, the comparable results were obtained only from measurements at the lower and upper boundary levels. The variability of the meteorological and isotopic parameters between the lower and upper boundary levels was the lowest compared to that from any other two level combinations.

For diurnal measurements, rates of evapotranspiration from both a single leaf scale (Fig. 8a, c & e), measured by a portable infra-red gas exchange analyzer, and from the field scale (Fig. 8b, d & f) by aerodynamic concentration method are shown in Fig. 8. Daily evapotranspiration rates in the acacia field in the Negev in May ranged from 0 to 5 mmol m^{-2} s^{-1} by a single leaf measurements, compared to 0 to 9 mmol m^{-2} s^{-1} by the concentration method; while in June, the maximum ET dropped to 3.3 mmol m^{-2} s^{-1} by single leaf measurements, compared to about 6 mmol m^{-2} s^{-1} by the direct concentration method. For sunflower field, the maximum rate of ET was about 5 mmol m^{-2} s^{-1} by the single leaf measurements, while by the direct concentration method the maximum rate of ET was about 8 mmol m^{-2} s^{-1}. The results from the isotopic approach is given in Table 3, together with corresponding measurements by the concentration method. There were two comparable pairs of ET measurements in the acacia field in The Negev in May (10:30 am, 13:30 pm, in Table 3), and two pairs in the same field in June (8:30 am, 10:00

pm, in Table 3); while in the sunflower field, there were four comparable pairs of ET measurements (0:00 am, 6:30 am, 11:00 am and 20:30 pm, in Table 3). The maximum ET was, estimated by the isotopic approach, about 8.8 or 3.0 mmol m^{-2} s^{-1} between 10:00 to 11:00 in acacia field in May or June, and 12.8 mmol m^{-2} s^{-1} in the sunflower field at 11:00 am (Table 3).

Seasonal changes (from June to October 1995) in the ET fluxes from the acacia plantation were shown in Fig. 9a. The ET fluxes were estimated to be about 6.5 mmol m^{-2} s^{-1} at the end of June 1995, and then decreased to about 2.5 mmol m^{-2} s^{-1} towards the end of October 1995. There was an exception in early September, when the ET estimate was extremely high at about 9 mmol m^{-2} s^{-1}. When these seasonal changes in the ET fluxes were plotted against the corresponding leaf water $\delta^{18}O$ differences between modeled and measured (Fig. 9b), a positive linear relationship was observed with a correlation coefficient R=0.78. Leaf water $\delta^{18}O$ differences between modeled and measured varied between -0.5‰ to +2.0‰

According to Eq. 18, the relationship between the atmospheric water vapor and its $\delta^{18}O$ content was plotted in a reciprocal shape in Figure 10. The correlation coefficients in Figure 6 were all above 0.85, except the data in March (Fig. 10c). The intercepts resulted from each mixing line are shown in the corresponding subplots of Fig. 10. In a simple case study in the wheat field, an estimate of δ_b was obtained (-4.2‰) using the Keeling (1961) mixing model. This estimate of the ^{18}O signature of the combined ET flux from the surface was more negative than the transpiration signature estimated from the $\delta^{18}O$ value of stem water (-2.9‰). The ^{18}O signature of the soil evaporation flux (δ_E) can be estimated for the dense wheat canopy by assuming that any evaporation occurred at a very high humidity and relatively little isotopic enrichment had taken place between irrigations. In this case, soil water should have a $\delta^{18}O$ value near that of stem water, and δ_E can be estimated by Eq. 1 (relative humidity near the soil surface, 85%; soil surface temperature, 20°C and a kinetic fractionation for mostly stagnant boundary layer of 28‰). This yielded an estimate of δ_E=-39.1‰. Using this estimate in Fig. 11, which shows the full range of relationships between δ_E and the ratio of E/ET, indicated that soil evaporation contributed 1.5-3.5% of the total ET flux in the wheat field at the times of measurements.

Discussion

The $\delta^{18}O$ values of Leaf Water

The isotopic composition of the leaf water was enriched relative to the source stem water (Table 1) due to the isotopic fractionation associated with transpiration. Evaluation of the isotopic steady state attained by the leaf water is necessary before applying the isotope approach to estimate ET fluxes. As mentioned before, the isotopic composition of the leaf water (or the

difference between δ_E and δ_s) can serve as an indicator of the isotopic steady state. Kinetic fractionation factor (ε_k) is determined by two processes (Farquhar et al., 1989), one process is for the molecular diffusion through a stagnant layer inside the stomata of the leaf, and the other is for diffusion in a laminar situation in the leaf boundary layer. The weighted average of ε_k is therefore determined by the relative contribution from the resistance (or conductance) from the two boundary layers. Under field conditions, leaf stomatal resistance can be one or two orders of magnitude larger than the leaf boundary layer resistance (Grace, 1977), provided that the size of the leaf is not so large as some of the tropical tree leaves (Grace, 1980). We assigned 5% of the leaf resistance to boundary layer, according to Wang and Yakir (1995), and the ε_k used in Eq. 1 was 27.5‰ for field crops. It was shown in Table 1 that the δ_c values were in most cases higher than the δ_m values when using ambient relative humidity data from the upper boundary levels. Similar results have been observed before with explanations of either isotopic compartmentation (Yakir et al., 1990) or continuous gradients (Flanagan et al., 1991; Farquhar & Lloyd, 1993). The gradient in specific humidity inside the CBL could result in a totally different δ_c value, for instance, a lower δ_c value would be calculated when using specific humidity data from the lower boundary levels. This implied additional possibility for the higher predicted δ_c value than the measured δ_m value: the specific humidity at or above the upper boundary level is usually used for predicting the δ_c value instead of using the specific humidity just from the bottom boundary layer above the canopy. The larger the gradient in specific humidity within the CBL in the field, the larger the difference between the δ_c and the δ_m values. Such a gradient in specific humidity inside the canopy boundary layer, however, is often being neglected and the upper boundary meteorological conditions are being used as input parameters for the calculation of δ_c values.

As for diurnal measurements in the acacia field in June 1995, effects of lower or upper boundary conditions on predicting δ_c value of leaf water are small (Table 1), probably due to its decreased low activity of production in the arid condition without sufficient water supply. By varying the kinetic fractionation factor ε_k between 25‰ and 28‰, most of the measured δ_m values of leaf water were within the predicted δ_c range between 8:00 am and 14:00 pm. Very early in the morning, however, the measured δ_m value of the acacia leaf water was about +10‰ heavier than δ_c values (e.g., 1:00 am in Fig. 3). This may indicate slow exchange rate with the ambient atmosphere and slow response to the actual humidity, as predicted by δ_c at the same period.

The measured δ_m value of leaf water is usually lower than that predicted by the Craig and Gordon model (Leaney et al., 1985; Walker et al., 1989; Yakir et al., 1994). Such a discrepancy between δ_c and δ_m values could be as high as +10‰ as shown here for maize leaf water (Table 1). This can be explained by the convection-diffusion model proposed by Farquhar & Lloyd (1993). It considers the δ_c value predicted by the Craig and Gordon model as the upper limit of

the leaf water isotopic enrichment, while the δ_m value is the result of a mixing process between δ_c and δ_s. However, it is interesting to note that δ_m values in March were higher than the δ_c values (Table 1). The same phenomenon has also been observed in the field before (Walker and Lance, 1991; Flanagan et al., 1993). These extraordinary cases were attributed at least partially to enrichment of stem and vein water, and isotopic heterogeneity of the transpiring leaf water (Yakir et al., 1994; Wang & Yakir, 1995), but other mechanisms are needed.

Comparing the δ_m values with the δ_c value calculated by using lower boundary parameters (Table 1), seems to indicate that leaf water of wheat in February and cotton in August 1994 was close to isotopic steady state. Similarly, for diurnal measurements in 1995, the data seems to indicate that leaf water was close to the isotopic steady state during the day time.

Atmospheric Water Vapor and its $\delta^{18}O$ Composition

For mid-day measurements in different field crops, the transpired water contributed from vegetation to the atmosphere was enriched in ^{18}O, and both moisture content and the $\delta^{18}O$ value increase toward the canopy surface within the CBL. Even though the gradients were very small, the results showed that they could be clearly detected (Fig. 5). The application of a modified procedure for analyzing small water vapor samples made it possible for short term, high temporal resolution measurements (cf., Wang & Yakir, 1995).

Since wind profile above the vegetation is usually in a form of exponential decay toward the canopy surface (see Eq. 11), most of the atmospheric moisture and the $\delta^{18}O$ data had also good exponential curve fit in their vertical profiles, consistent with the assumption that mass mixing process was controlled also by the eddy diffusion process.

For diurnal measurements in acacia and sunflower fields, the results indicated that the highest enrichment in $\delta^{18}O$ value of the air moisture at the lowest level above the canopy occurred in the early morning, at about 7:00 am to 8:00 am for acacia in May and June, and about 9:30 am for sunflower (Fig. 7). Following this maximum enrichment, there was a slow decrease in $\delta^{18}O$ value of the air moisture at the lowest level above the canopy, and then approaching a minimum value at about noon time. The observed phenomenon can be related to the photosynthetic activity of the vegetation. The $\delta^{18}O$ value of the source water contributed from the plants and the soil is much heavier than the ambient air moisture (IAEA, 1990). Due to the aerodynamic mixing of the isotopic signature from three sources above the canopy, the higher rate of the evapotranspiration from the plants and the soil, the higher the $\delta^{18}O$ value at the lowest level above the canopy. Similarly, if there is little or no evapotranspiration, the $\delta^{18}O$ value at the lowest level above the canopy will approach the isotopic composition of the background ambient atmosphere.

Estimation of ET fluxes

Measurements of evapotranspiration have been done intensively before (cf., Penman, 1948; Tom, 1975; Brutsaert, 1982; Javis and McNaughton, 1986; Gat and Matsui, 1991). At present, the Bowen ratio and eddy correlation techniques are the major methods used (e.g., Wofsy et al, 1993; Grace et al., 1995). To test the isotope method against the conventional methodology we employed several methods simultaneously to estimate ET fluxes from different crops fields and a desert agroforest.

For mid-day samplings, there was good agreement between E_b estimates, obtained by the isotope and EBBR techniques, from maize and cotton fields (within 5%, Table 2). In general, the isotope technique yielded comparable results to those obtained by the EBBR method, except one low estimate for wheat in March (Table 2). The decreasing trend of the wheat E_b flux from February to March has been clearly detected by both the isotope and EBBR methods. It is reasonable to have such a decrease in water consumption at the end of growing season (end of March), due to reduced photosynthetic activity of the plants and to soil drying at the surface layer. It is also notable from Table 2 that the E_b estimates obtained by the isotope method were generally lower than those obtained by the EBBR technique (by ca. 10%), but were closer to those obtained by direct concentration method.

For diurnal measurements, the isotope approach could also yield comparable estimates of ET fluxes, to those estimated by the EBBR and eddy correlation techniques, between 8:00 to 14:00, while in early morning or late evening, the isotope approach gave in some cases unrealistic ET estimates (Table 3). The flux calculation based on the isotope approach depends, not only on the $\delta^{18}O$ measurements of the air moisture, but also on wind conditions. As mentioned before, the present isotope approach is useful under neutral wind conditions. In the acacia field in the Negev, however, there were usually strong turbulent wind conditions in the afternoon and as a result, the isotope approach did not produce a reasonable ET estimate. In the late evening, the air became stagnant. Compared to direct concentration measurement, the isotopic approach developed here gave higher estimates of evapotranspiration in most cases. However, the changes in the maximum evapotranspiration estimated by the isotopic approach were from 8.8 mmol m^{-2} s^{-1} in May to 3.0 mmol m^{-2} s^{-1} in June, which is similar to those estimated by the direct concentration method (from 6.5 mmol m^{-2} s^{-1} in May to 2.5 mmol m^{-2} s^{-1} in June). The acacia plants took up water stored in the soil profile during the previous rainy season (November to February). The decrease in evapotranspiration in acacia field reflected the decrease in soil water availability in this arid system.

The $\delta^{18}O$ values of leaf water in relation to ET fluxes

Farquhar & Lloyd (1993) convection diffusion model indicates that plant transpiration rate regulates the degree of discrepancy between predicted and measured values of leaf water. Here we tested these relationships in the acacia forest over about 60 days. There were large seasonal variations in the measured $\delta^{18}O$ values of leaf water, as compared to small seasonal variations in the measured $\delta^{18}O$ values of stem water (Fig. 4). This is because the $\delta^{18}O$ values of leaf water, all collected at around 10:00 am in the morning, are more subject to instant changes in the meteorological condition than those of the stem water. The acacia plants might uptake their water supply from 6 m below the soil surface (data not show). From June to October 1995, it was changing from relatively hot and dry weather to relative warm and more humid weather; as a result, there was a seasonal declining in the measured $\delta^{18}O$ values of leaf water, which was consistent to that predicted. During the same period, the soil water reservoir dried up and therefore the $\delta^{18}O$ values of the stem water was getting a little enriched due to evaporation effect (Fig. 4). The decrease in soil water supply from June to October could also be demonstrated by the decrease in the ET fluxes during the same period (Fig. 9a). The plot in Fig. 9b indicated that there was a linear correlation between ET and the $\delta^{18}O$ difference between modeled and predicted values; the larger the ET flux, the bigger the leaf water $\delta^{18}O$ difference (δ_c-δ_m). Therefore, leaf water $\delta^{18}O$ difference between predicted and measured values could be used as an indicator, in the acacia forest in the Negev, of changes in the ET fluxes over the seasonal cycle (Fig. 9b).

Partitioning ET fluxes

It is of great interest to separate plant transpiration from soil evaporation for a better understanding the global hydrological cycle (Gat and Matsui, 1991; Massman, 1992). Previous efforts have been made to separate plant transpiration from soil evaporation indicated that this is complex (e.g., Massman, 1992). Here we show that the present isotope technique has the potential to partition the total ET flux into its plant and soil fractions. As shown before, this partitioning process depends largely on the isotopic difference between plant and soil evaporation flux.

If plants use water resources different from the soil water near the surface (Ehleringer and Dawson, 1992; Yakir and Yechieli, 1995), δ_p value (leaf water) will be different from the δ_s value (soil water). High precision measurements of specific humidity and its $\delta^{18}O$ values within the CBL could provide a reliable intercept from the plot of specific humidity data versus the corresponding $\delta^{18}O$ values (Fig. 10). As discussed before, this intercept represents the $\delta^{18}O$ signature of a mixture of δ_s and δ_p values. We could then use Eqs. 16 & 17 for estimating the ET components contributed from plants and soil.

As mentioned above, using the Keeling (1961) mixing model, an estimate of δ_b was obtained (-4.2‰). This estimate of the ^{18}O signature of the combined ET flux from the surface was more negative than the transpiration signature estimated from the $\delta^{18}O$ value of stem water (-2.9‰, assuming isotopic steady state for leaf transpiration). This was likely to reflect the contribution of soil evaporation of highly depleted water. The ^{18}O signature of the soil evaporation flux was estimated for the dense wheat canopy by assuming that any evaporation occurred at a very high humidity and relatively little isotopic enrichment had taken place between irrigations. In this case, soil water should have a $\delta^{18}O$ value near that of stem water, and δ_E can be estimated by Eq. 1. This yields an estimate of δ_E=-39.1‰. Using this estimate in Fig. 11, which shows the full range of relationships between δ_E and the ratio of E/ET, indicated that soil evaporation contributed 1.5-3.5% of the total ET flux in the wheat field at the times of measurements. Fig. 11 also indicates that for simplified systems such as the wheat field, estimates of E/ET are relatively robust and are not very sensitive to estimates of soil δ_E values. This is not the case when soils are drying and approach isotopic steady state. Note that the above discussion is used for illustrative purposes, using a simplified field system and considering soil water as a simple water body. Much uncertainty still remains, however, in estimating soil δ_E values during soil drying (c.f. Mathieu & Bariac, 1996a, b).

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Table 1. Comparison of the midday $\delta^{18}O$ values of leaf water predicted ($\delta^{18}O_c$) with those measured ($\delta^{18}O_{LW}$) from total leaf water of the plants. The calculated $\delta^{18}O$ values were based on meteorological data from either lower or upper boundary layer of the crops, and kinetic fractionation factor (ε_k) was taken as either 25 or 28‰. The $\delta^{18}O$ values of the source (stem) water ($\delta^{18}O_s$) were also listed.

Fields of	Date	$\delta^{18}O_s$ (‰)	$\delta^{18}O_{LW}$ mean±s.d. (‰)	$\delta^{18}O_c$ (‰) Lower boundary		Upper boundary	
				ε_k=25	ε_k=28	ε_k=25	ε_k=28
Wheat	18/02/94	-2.9	+10.5±0.6 (n=4)	+9.2	+10.2	+11.3	+12.6
Wheat	20/02/94	-3.1	+9.6±2.7 (n=4)	+9.3	+10.3	+13.7	+15.1
Wheat	23/03/94	-3.2	+9.7±0.7 (n=6)	+6.2	+7.0	+8.7	+9.7
Maize	29/06/94	-2.0	+11.1±1.0 (n=8)	+18.8	+20.6	+19.0	+20.8
Cotton	11/08/94	-2.9	+6.5±1.1 (n=10)	+7.0	+7.6	+8.3	+9.1
Cotton	12/08/94	-2.9	+6.5±1.0 (n=6)	+5.5	+6.0	+6.5	+7.1
Acacia	16/06/95	-3.1	+16.6±1.1 (n=3)	+15.7	+17.5	+16.2	+18.0
Sunflower	30/05/95	-2.6	+8.8±1.2 (n=3)	+12.7	+14.0	+13.9	+15.4

Table 2. Evapotranspiration flux (E_b) from different crop fields in Israel during 1994, based on conventional Bowen ratio (B_o), specific humidity (q) and isotopic ($\delta^{18}O$) methods, respectively. Bowen ratio method was based on pyranometer and net radiometer; specific humidity method on an infra red gas analyser and isotopic methodology described in text.

Height	Concentration		E_b (mmol m^{-2} s^{-1})		
	q	$\delta^{18}O$	B_o	q	$\delta^{18}O$
(m)	(mmol mol^{-1})	(‰)			
Wheat (18/02/94)					
0.9	13.63	-13.1			
3.4	11.88	-14.6	8.0	5.5	5.5
5.4	11.59	-14.9			
Wheat (20/02/94)					
0.9	15.71	-11.2			
3.4	13.26	-12.6	8.8	7.7	7.0
5.4	12.28	-13.1			
Wheat (23/03/94)					
0.9	15.36	-12.7			
3.4	13.43	-13.2	6.6	4.8	2.1
5.4	13.33	-14.9			
Maize (29/06/94)					
4.05	9.47	-11.0			
7.00	8.61	-12.0	8.7	7.5	8.3
9.00	8.59	-12.0			
Cotton (11/08/94)					
2.0	31.27	-8.6			
5.7	28.75	-9.1	11.6	11.5	11.5
7.7	28.70	-9.1			
Cotton (12/08/94)					
2.0	34.13	-8.7			
5.7	31.03	-9.3	13.4	13.8	14.3
7.7	31.03	-9.3			

Table 3. Estimation of evapotranspiration rates by the isotopic approach($\delta^{18}O$), in comparison with direct concentration method ([H_2O]). Diurnal isotope measurements were done in an Acacia Saligna plantation on both May 11 and June 17, and in a sunflower field on May 30, 1995. Negative flux indicates water condensation downward.

Time (Date)	H_2O (mmol m^{-2} s^{-1})											
	[H_2O]	$\delta^{18}O$	[H_2O]	$\delta^{18}O$	[H_2O]	$\delta^{18}O$	[H_2O]	$\delta^{18}O$	[H_2O]	$\delta^{18}O$	[H_2O]	$\delta^{18}O$
Time	22:30pm		6:30am		10:30am		13:30pm		18:00pm		21:30pm	
(May 11, 95)	0.0	134.0	0.6	54.9	3.5	8.8	6.5	3.9	2.9	160.6	-1.1	2.2
Time	21:30pm		6:30am		8:30am		10:00am		12:00am		19:30pm	
(June 17, 95)	0.7	130.9	0.7	-32.2	2.5	3.0	0.4	2.8	4.8	27.3	1.3	6.6
Time	0:00am		6:30am		9:00am		11:00am		17:30pm		20:30pm	
(May 30, 95)	-0.3	0.4	0.3	3.0	-3.5	12.3	7.1	12.8	0.3	-21.9	-0.2	2.9

Figure Captions

Figure 2. Measurements of photon flux density, temperature and relative humidity at individual leaf level were made with a portable photosynthetic instrument (ADC) in *Acacia saligna* desert agroforest system in the Negev Desert on June 17 (a, b & c), and in a sunflower field on May 30 (d, e & f), in 1995.

Figure 3. Diurnal $\delta^{18}O$ measurements of acacia leaf water (filled circle) and stem water (filled diamond) in the Negev Desert on June 17, 1995, in comparison with those predicted by the Craig and Gordon model (solid line for ε_k=28‰, while dotted line for ε_k=25‰).

Figure 4. Seasonal variations in the $\delta^{18}O$ values of leaf (filled circles) and stem (open squares) water, sampled in the Negev Desert between June to October, 1995. The best fit linear lines are given for each data set.

Figure 5. Mid-day measurements of atmospheric water vapor profile within the canopy boundary layer in different crop fields in 1994. Sampling date was a. Feb. 18; b. Feb. 20; c. Mach 23; d. June 29; e. Aug. 11; f. Aug. 12. Data in February and March were from winter wheat, in June from maize and in August from cotton fields. Dotted lines are exponential best fit lines.

Figure 6. Mid-day measurements of the $\delta^{18}O$ values of the atmospheric water vapor profile within the canopy boundary layer in different crop fields in 1994. Sampling date was a. Feb. 18; b. Feb. 20; c. Mach 23; d. June 29; e. Aug. 11; f. Aug. 12. Data in February and March were from winter wheat, in June from maize and in August from cotton fields. Dotted lines are exponential best fit lines.

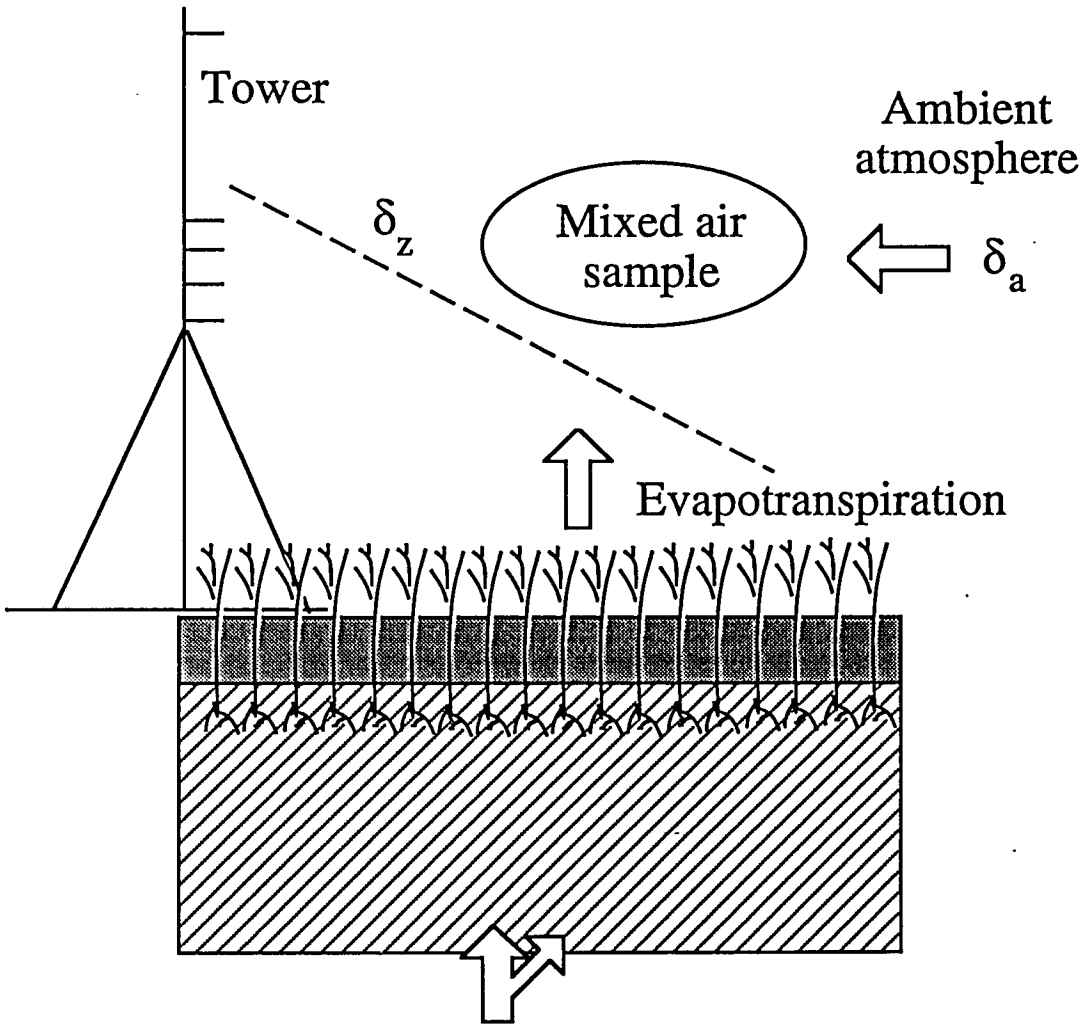
Figure 7. Isotopic profiles of the $\delta^{18}O$ value of the air moisture at different times of the day in acacia agroforest (a. May 11; b. June 17, 1995) and in a sunflower field (c. May 30, 1995).

Figure 8. Estimation of evapotranspiration rates from either a single leaf level by a portable IRGA instrument (a, c and e) or from the field scale by direct concentration method (b, d and f), in acacia agroforest in May (a & b), June (c & d), and in sunflower field (e & f).

Figure 9. a. Seasonal changes in the rate of evapotranspiration (ET) measured by energy balance and Bowen ratio method, when leaf and stem water samples were also collected (see Fig. 3). The best fit linear equation is shown, with correlation coefficient. b. Relationship between the rate of evapotranspiration (ET) (as in Fig. 9a) and leaf water $\delta^{18}O$-difference between modeled (δ_c) and measured (δ_m).

Figure 10. Relationship between $\delta^{18}O$ of atmospheric water vapor and specific humidities at the same heights within the canopy boundary layer measured in mid-days in different crop fields in 1994. Sampling date was a. Feb. 18; b. Feb. 20; c. Mach 23; d. June 29; e. Aug. 11; f. Aug. 12. Data in February and March were from winter wheat, in June from maize and in August from cotton fields. Dotted lines are best fit lines (y=a+b/x).

Figure 11. Relationships between the ratio of soil evaporation (E) over total evapotranspiration (E+T) and estimates of the $\delta^{18}O$ values of the soil evaporation flux. Wheat 1 and 2 represent two measurement dates (Feb. 18, 20, 1994). $\delta^{18}O$ values of soil evaporation, and ET fluxes were calculated according to Eqs. 1 and 16 &17, respectively.



$$q_z\,\delta_z = q_b\,\delta_b + (q_z - q_a)\,\delta_a$$

Figure 1. Conceptual scheme of the isotopic approach, where δ stands for $\delta^{18}O$ of water vapor, q for specific humidity; refer to the text for detailed discussion.

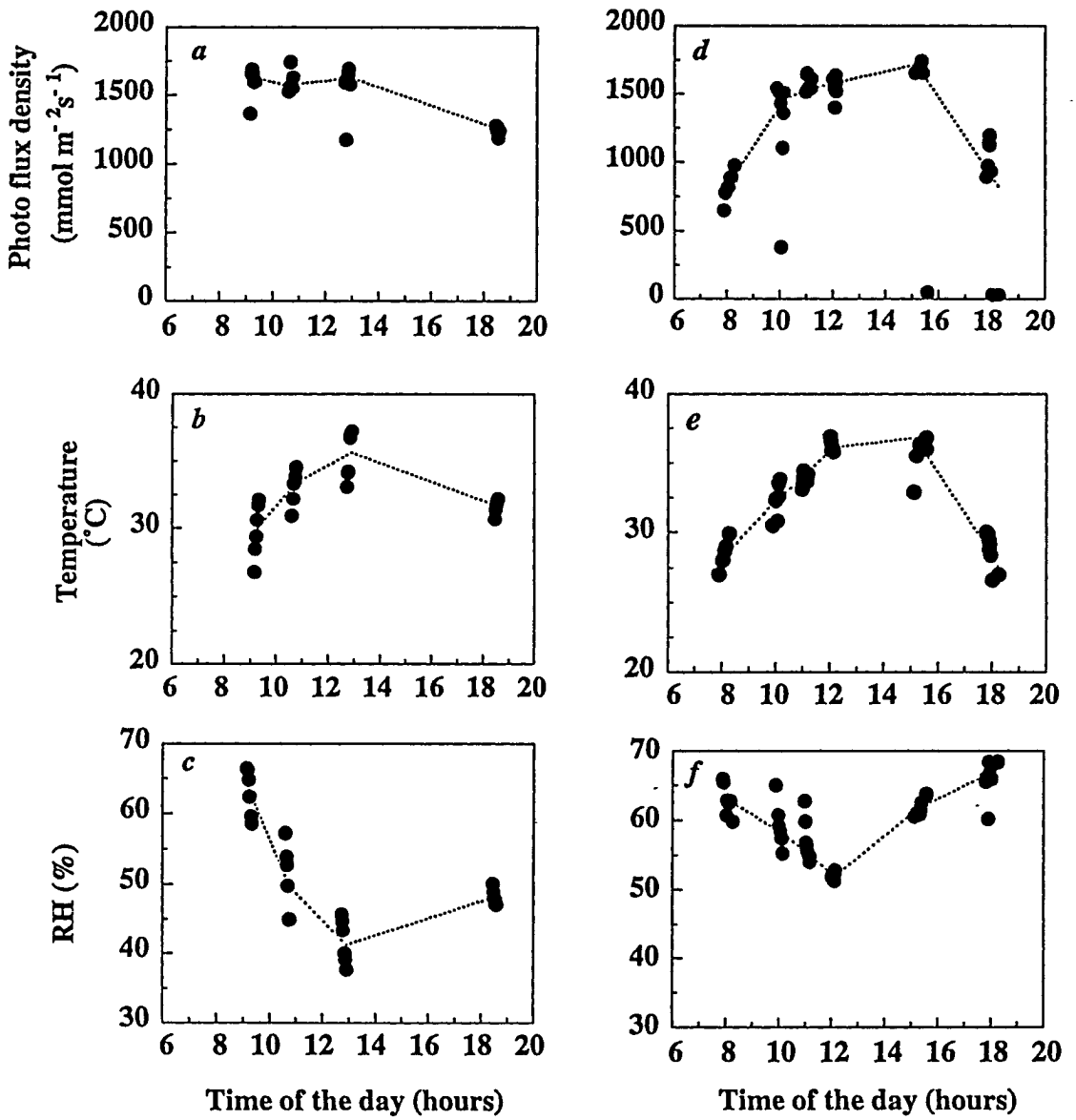
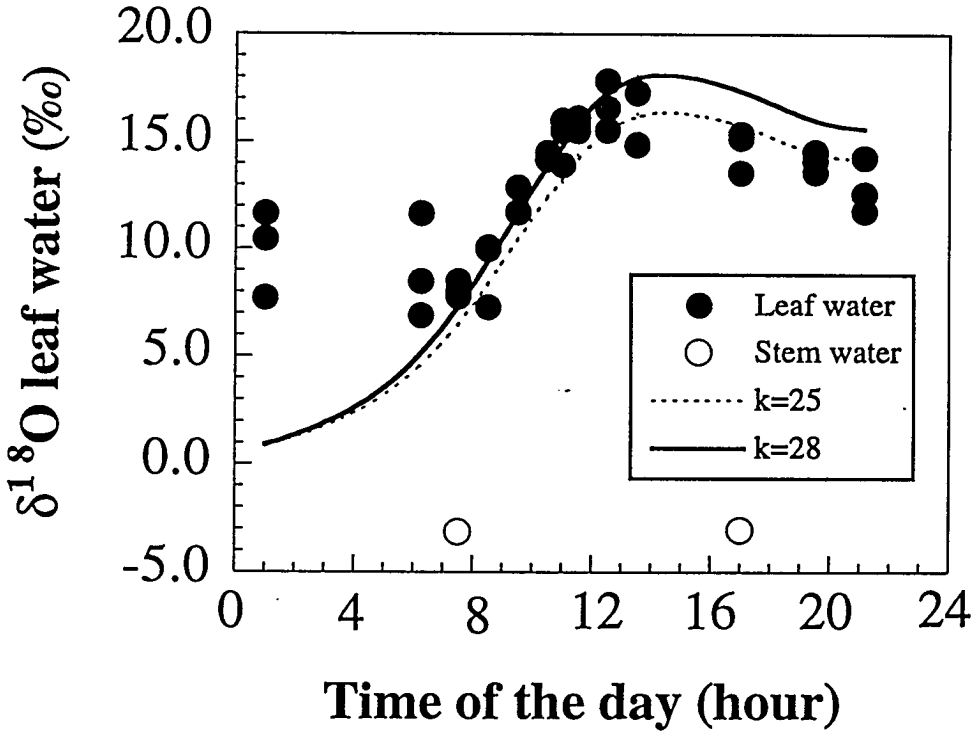


Photo flux density (mmol m-2s-1)
Temperature (°C)
RH (%)
Time of the day (hours)
Time of the day (hours)
a
b
c
d
e
f

Fig. 2


20.0
15.0
10.0
5.0
0.0
-5.0
0
4
8
12
16
20
24
δ18O leaf water (‰)
Time of the day (hour)
Leaf water
Stem water
k=25
k=28

Fig, 3

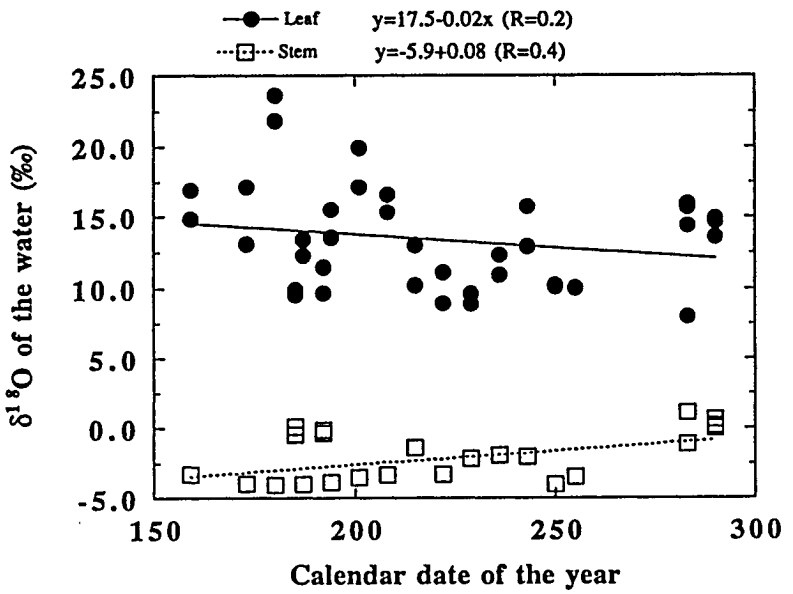

Leaf
y=17.5-0.02x (R=0.2)
Stem
y=-5.9+0.08 (R=0.4)
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
δ18O of the water (‰)
150
200
250
300
Calendar date of the year

Fig. 4

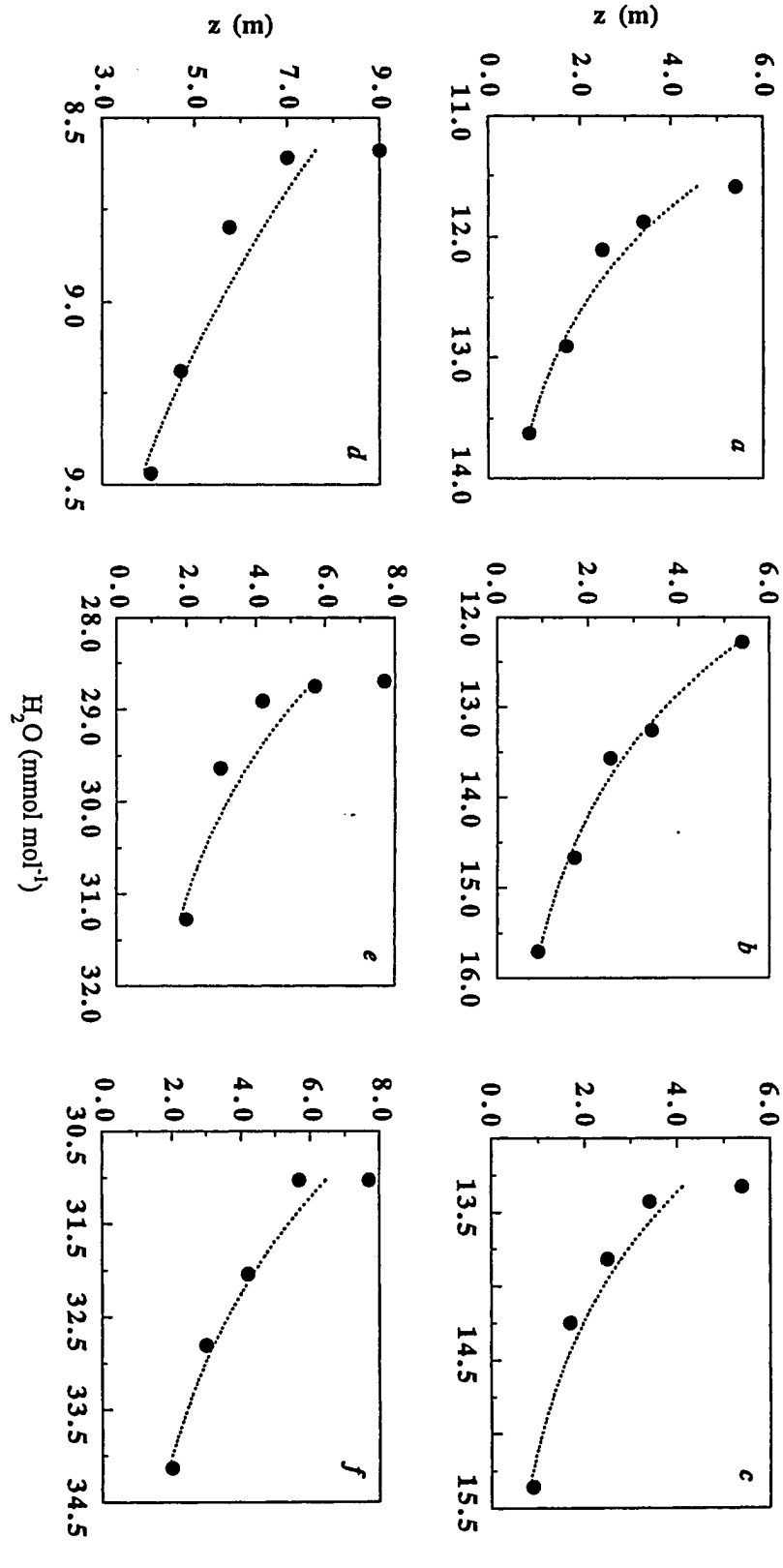
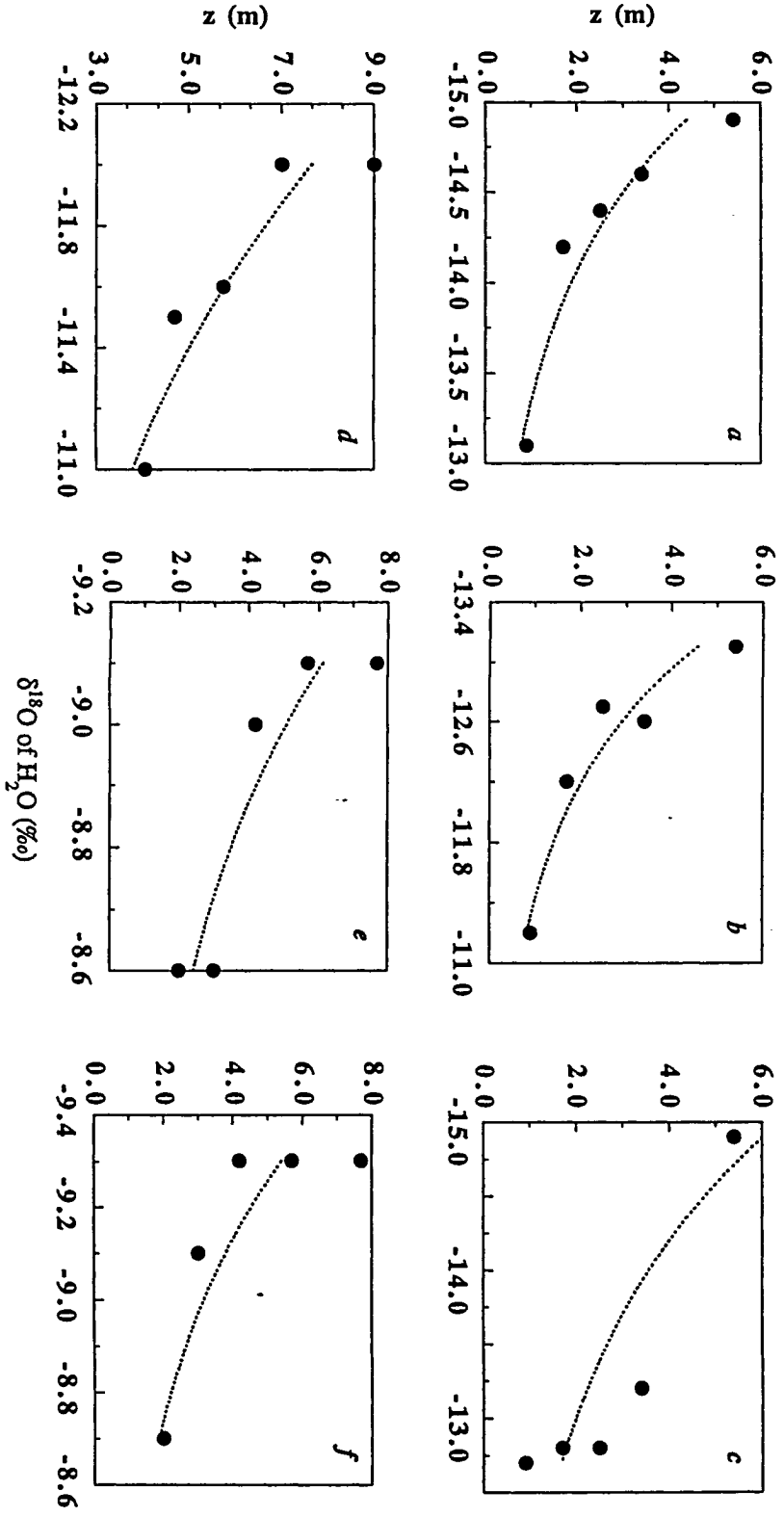
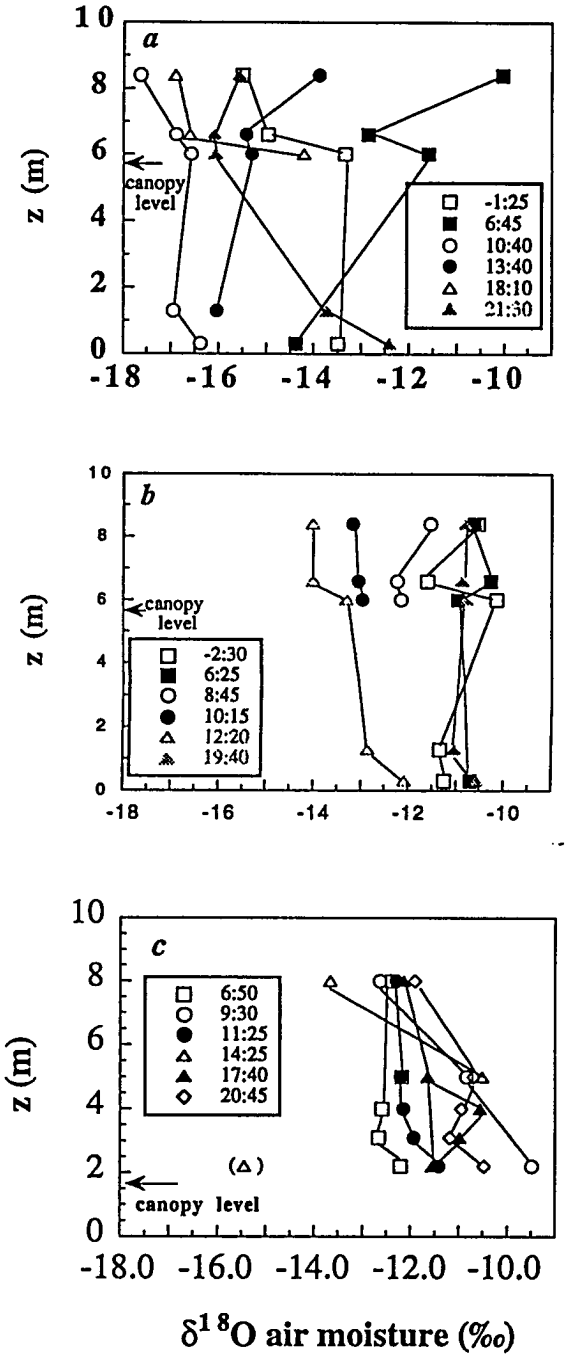


Fig. 5



Fig, 6


a
z (m)
canopy level
-1:25
6:45
10:40
13:40
18:10
21:50
b
canopy level
-2:30
6:25
8:45
10:15
12:20
19:40
c
6:50
9:30
11:25
14:25
17:40
20:45
(△)
canopy level
-18.0 -16.0 -14.0 -12.0 -10.0
δ18O air moisture (‰)

Fig, 7

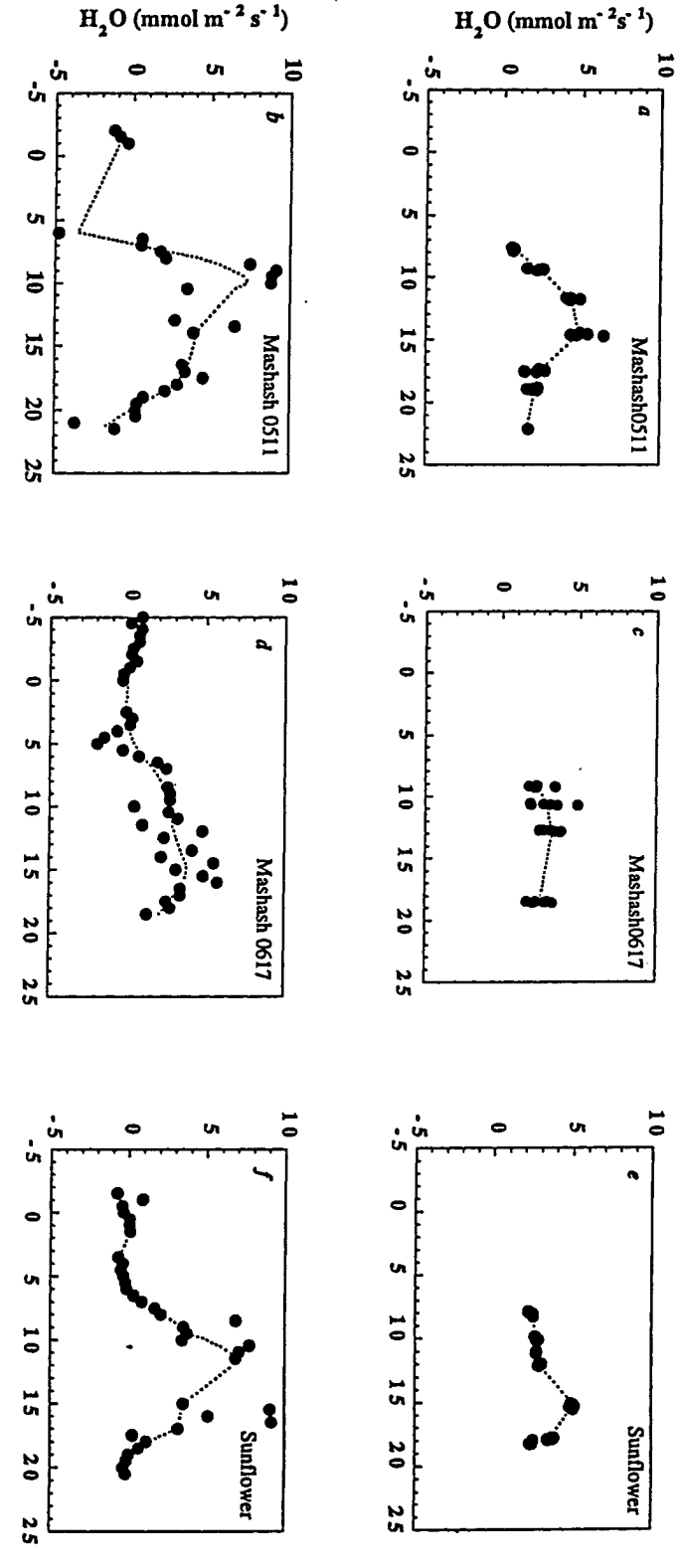

H2O (mmol m-2s-1)
a
Mashash0511
b
Mashash 0511
c
Mashash0617
d
Mashash 0617
e
Sunflower
f
Sunflower
Time of the day (Hours)

Fig. 8

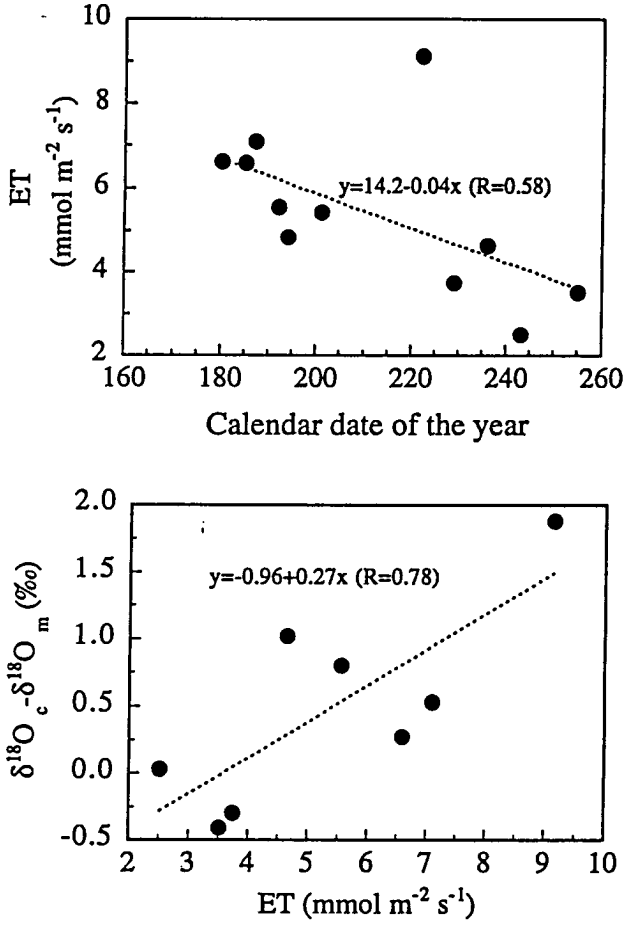

ET (mmol m-2 s-1)
y=14.2-0.04x (R=0.58)
Calendar date of the year
δ18Oc-δ18Om (‰)
y=-0.96+0.27x (R=0.78)
ET (mmol m-2 s-1)

Fig. 9

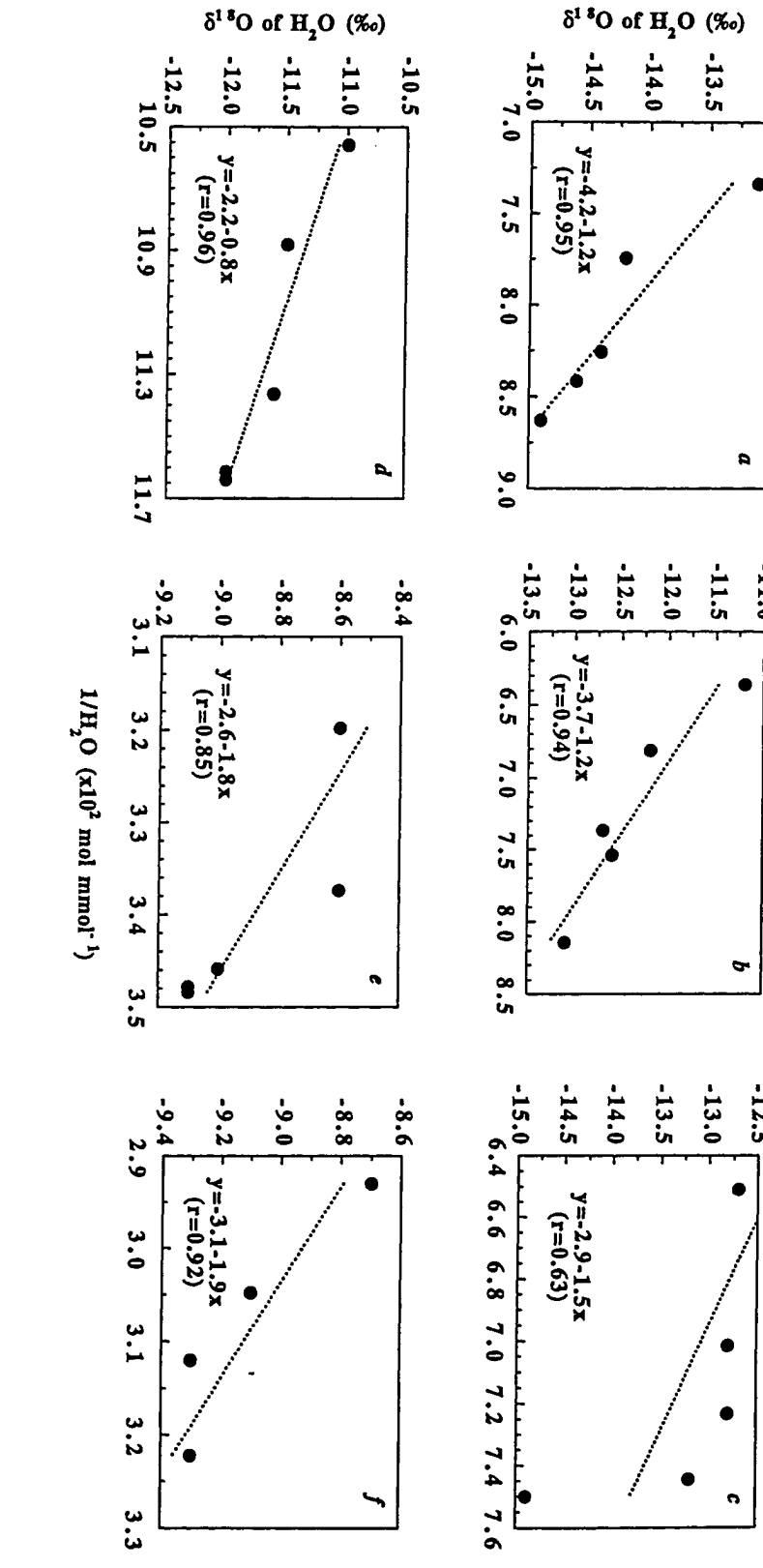

δ18O of H2O (‰)
y=-4.2-1.2x
(r=0.95)
a
δ18O of H2O (‰)
y=-3.7-1.2x
(r=0.94)
b
δ18O of H2O (‰)
y=-2.9-1.5x
(r=0.63)
c
δ18O of H2O (‰)
y=-2.2-0.8x
(r=0.96)
d
δ18O of H2O (‰)
y=-2.6-1.8x
(r=0.85)
e
δ18O of H2O (‰)
y=-3.1-1.9x
(r=0.92)
f
1/H2O (x10² mol mmol⁻¹)

Fig. 10

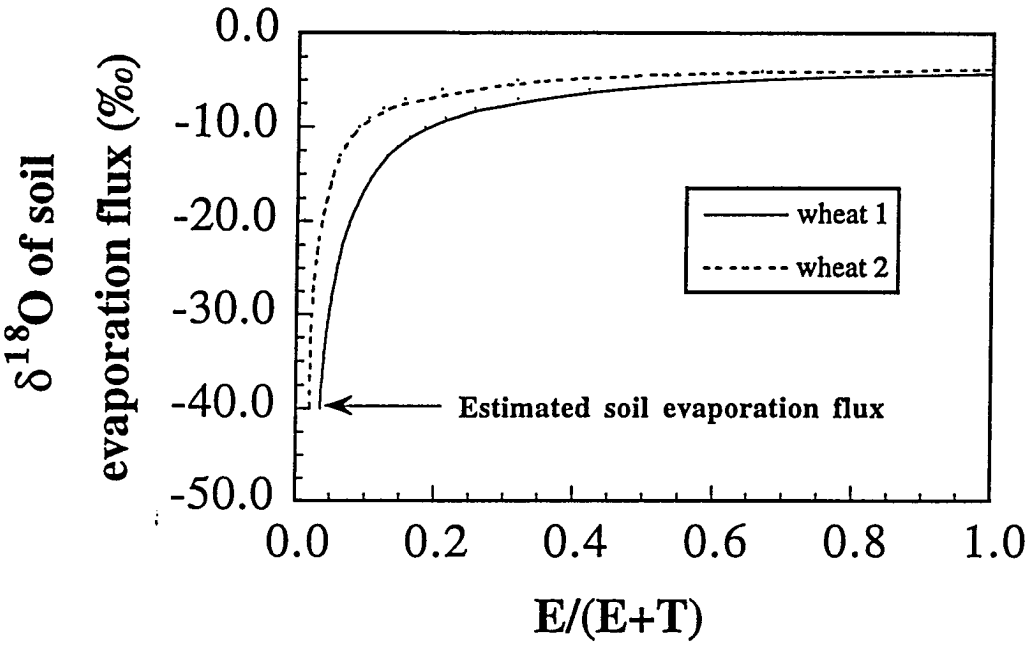

0.0
-10.0
-20.0
-30.0
-40.0
-50.0
δ18O of soil evaporation flux (‰)
wheat 1
wheat 2
Estimated soil evaporation flux
0.0
0.2
0.4
0.6
0.8
1.0
E/(E+T)

Fig. 11

4. Summary, Discussion and Conclusions

Environmental conditions influence oxygen isotopic composition (^{18}O) of leaf water of terrestrial plants which, in turn, influence the ^{18}O signatures of atmospheric CO_2, H_2O, O_2 and of plant organic matter (Dongmann et al., 1974; Epstein et al., 1977; Francey & Tans, 1987; Berry, 1992; Brunel et al., 1992; Farquhar et al., 1993; Bender et al., 1994; Ciais et al., 1997; Yakir, 1992). The $\delta^{18}O$ value of the leaf water has been increasingly used in various biogeochemical models that investigate the exchange of CO_2 , H_2O and O_2 between the land surface and the atmosphere (e.g. Francey & Tans, 1987; Berry, 1992; Brunel et al., 1992; Farquhar et al., 1993; Bender et al., 1994; Ciais et al., 1997). The incorporation of ^{18}O isotope with other conventional techniques provides useful information and increases our understanding of ecosystem response to environmental changes. In addition, the incorporation of ^{18}O measurements is useful in addressing questions associated with the partitioning of net fluxes of CO_2, O_2 and water between photosynthetic uptake and respiratory release (for CO_2, e.g. Ciais et al., 1997), or between land and ocean productivities (for O_2, e.g. Bender et al., 1994), or between soil evaporation and plant transpiration (see above). Quantitative analysis of the $\delta^{18}O$ value of the leaf water is important for all of these cases.

The ^{18}O content of leaf water is, however, not well characterized at present. There have been several models for predicting the $\delta^{18}O$ value of leaf water, but all of the models rely on the assumption that isotopic steady state is attained in the leaf water (Dongmann et al., 1974; Allison et al., 1985; Yakir et al., 1990; Farquhar et al., 1993). In nature, however, there were large discrepancies between observed $\delta^{18}O$ values of bulk leaf water and those predicted by the model (White, 1989; Walker et al., 1989; Yakir et al, 1990, 1994; Flanagan & Ehleringer, 1991).

To test the assumption of isotopic steady state in leaf water, temporal and spatial variations in ^{18}O content of leaf water were studied in detail in the first part of the present research (section 3.1). Changes in the $\delta^{18}O$ values of leaf transpiration water (δ_T) were monitored over time in different plant species in a non-destructive way, and the results indicated that leaves approach isotopic steady state in two stages. The first stage takes 10 to 35 minutes (with a rate of change of about 3.3‰ h^{-1}), while in the second stage further approach to isotopic steady state is asymptotic (with a rate of change of about 0.4‰ h^{-1}), and in low transpiration leaves can last for many hours. From mass balance calculations, the significance of the variations in δ_T was evaluated by reconstructing the corresponding changes in the $\delta^{18}O$ value of sunflower leaf water (Fig. 1b in section 3.1). The results indicate that, although the sunflower leaf had a typical enrichment during the early part of the cycle, the overall pattern and extent of enrichment were markedly different from any of the predicted ones (Fig. 1b in section 3.1). Even when transpiring

leaves were maintained under constant conditions in the laboratory for at least three hours, strict isotopic steady state conditions of leaf water (equality of the $\delta^{18}O$ values in the input and transpired water) were rarely attained in a variety of plant species. In addition, large spatial isotopic heterogeneity was maintained even when leaves were at or near isotopic steady state. An underlying pattern in this isotopic heterogeneity is often discerned with increasing $\delta^{18}O$ values from base to tip, and from center to edges of leaves. It is furthermore shown that tissue water along these spatial isotopic gradients, as well as the average leaf water, can have the $\delta^{18}O$ values both lower and higher than predicted by the conventional Craig and Gordon model (1965). The results indicate that the attainment of isotopic steady state in leaf water can not be *apriori* assumed at any given time during the diurnal cycle in relative humidity. However, it is important to note that under field conditions changes in humidity are gradual and the $\delta^{18}O$ values of leaf and transpiration water may fluctuate closer to the expected values than under laboratory conditions. Similarly, potential effects of patchy stomatal closure due to humidity changes may be less under field conditions. To further improve the prediction, the results suggest that it may be necessary to include more species-specific physiological parameters in future models for leaf water $\delta^{18}O$ values.

In order to acquire a global perspective on climate-independent variability in the $\delta^{18}O$ value of leaf water, an isotopic survey was conducted in the second part of the present research (section 3.2), in a collection of 90 plant species from all continents grown under the same climatic conditions in the Jerusalem Botanical Garden. Large, non-climatic variations among different plant species were observed in $\delta^{18}O$ values of plant water and organic matter. Variations of about 9‰ were observed in the $\delta^{18}O$ values of stem water (δ_S), and of about 14‰ in the mid-day $\delta^{18}O$ enrichment of bulk leaf water ($\delta_{LW} - \delta_S$). Differences between $\delta^{18}O$ values predicted by a conventional evaporation model and measured ($\delta_M - \delta_{LW}$) ranged between -3.3‰ and +11.8‰. Leaf water is known to represent an isotopically heterogeneous system (Yakir et al 1989; Walker & Lance, 1991; Luo & Sternberg, 1992; Bariac et al., 1994), only part of which participate in labeling atmospheric CO_2 and O_2, i.e. the leaf water fraction contained within the chloroplasts (δ_{ch}). The leaf chloroplast is where oxygen is evolved from water during photosynthesis, and where, predominantly, the enzyme carbonic anhydrase (CA) facilitates the isotopic exchange of oxygen between water and CO_2 (cf. Yakir et al., 1994). To further address the uncertainty of leaf water $\delta^{18}O$ signature responsible for ^{18}O exchange with that in atmospheric CO_2, on-line measurements of ^{18}O discrimination against $C^{18}O^{16}O$ were conducted in leaves of 10 selected plants under both low light regime (ca. 500 μmol photos $m^{-2}\,s^{-1}$) in the lab and natural light irradiance (ca. 1650 μmol photos $m^{-2}\,s^{-1}$) in the field. It was shown in section 3.2 that estimates of δ_{ch} are quite sensitive to four parameters used in its calculation, namely, the CO_2 concentration in the chloroplast (c_c), the biochemical discrimination factor (b),

the discrimination factor associated with photorespiration (f) and the kinetic discrimination factor (b). The biochemical discrimination of Rubisco was determined as 29‰ (Guy et al., 1987; including in a green tissue), but assumed sometimes to effectively be lower (~27‰) due to activity of other, low discrimination, carboxylases in the same tissue (Farquhar & Lloyd, 1993). Since little information is available on the discrimination associated with photorespiration, a value of f=7 (Rooney, 1988) is often adopted, but it may vary with plant species and can, at least in some cases, be close to zero (Gillon & Griffiths, personal communication). While ^{13}C discrimination provides an estimate of c_c (Evans et al., 1986), it may not be the appropriate parameter for ^{18}O measurements. This is because CA activity and the rapid oxygen exchange between CO_2 and water cancels out any ^{18}O gradients due to diffusion within the chloroplast (any CA activity outside the chloroplast membrane could do the same for cytoplasm). Since chloroplast resistance to diffusion can be a significant component of the liquid phase diffusion pathway (Evans & von Caemmerer, 1996; Nobel, 1991), some intermediate value, c_{cs}, between c_i (CO_2 concentration in the intracellular spaces) and c_c may be more appropriate. Uncertainties associated with c_{cs} also contribute to variations in $\bar{a}$, which for $C^{16}O^{18}O$ has a maximum value of 8.8‰ (Hesterberg & Siegenthaler, 1991). The effective value, however, can be significantly lower (e.g. 7.4‰, Farquhar & Lloyd, 1993) mainly because of small discrimination (~0.8‰) in the liquid phase. Therefore, $\bar{a}$ is influenced by the extent of the liquid phase resistance that is considered. The best estimate of δ_{ch} were obtained based on b=29; f=4; $\bar{a}$=7.7; $\rho = 0$ and $c_{cs}=(c_i+c_c)/2$ (Fig. 4b, in section 3.2), and assuming that δ_M is generally the upper limit for leaf water enrichment. Although much uncertainty is still involved in these estimates, the results indicated the reconstructed δ_{ch} values can significantly deviate from δ_M in species with high leaf peclet number (p). Since variations in p among plant species clearly reflected an intrinsic plant characteristics (Fig. 3f, in section 3.2), the results indicated that while a good prediction of δ_{ch} may be achieved by Eq. 1 for some species, considering specific species-specific characteristics are likely to improve such predictions for other species as well. It was indicated by statistical analyses that the $\delta^{18}O$ values of bulk leaf water significantly correlated with $\delta^{18}O$ values of leaf cellulose (directly) and with instantaneous water use efficiency (A/E, inversely). Differences in isotopic characteristics among conventionally defined vegetation types were not significant, except for conifers that significantly differed from shrubs in $\delta^{18}O$ and $\delta^{13}C$ values of cellulose and in their peclet numbers, and from deciduous woodland species in their $\delta^{18}O$ and $\delta^{13}C$ values of cellulose. This section of the research provides not only detailed background information on physiological parameters of the divergent plant species, but new insights to the contribution of plant-specific characteristics to the observed variation in the ^{18}O signature of leaf water and related parameters.

In the third part of the present research (section 3.3), a two-dimensional model was developed, expanding on the Farquhar & Lloyd (1993) one-dimensional advection-diffusion model, for predicting the $\delta^{18}O$ value of bulk leaf water. The model, which is dependent on leaf transpiration rate, considers a leaf as a series of evaporating pools with the $\delta^{18}O$ values continuously enriched along the series (Gat & Bowser, 1991). A simplified 3-pool-series model was tested to predict $\delta^{18}O$ enrichment in leaf water in response to diurnal variations in environmental parameters in a cotton filed. Taking into account advection and diffusion effects from both horizontal and vertical directions, the model predicted that a plant with low transpiration rate will have greater isotopic enrichment in its leaf water than that predicted by the Craig & Gordon model; in contrast, a plant with high transpiration rate might have lower isotopic composition in its leaf water compared to that predicted by the Craig & Gordon model; the predictions hold true irrespective of isotopic steady state. The maximum leaf water $\delta^{18}O$ enrichment, when using the Craig & Gordon model, is always achieved at minimum relative humidity of the ambient air. In the present modeling, however, a delay was predicted in the timing of the maximum leaf water $\delta^{18}O$ enrichment during the day relative to the minimum relative humidity of the ambient air, which is consistent with previous observations. This model described well the temporal and spatial $\delta^{18}O$ variations of leaf water under natural conditions.

To improve the understanding of gas exchange processes between terrestrial vegetation and the atmosphere, an isotope method was tested and applied together with the conventional meteorological technique in the fourth part of the present research (section 3.4). Forecasts of the likely climatic effects of anthropogenic emissions of carbon dioxide rely on an accurate knowledge of its various natural sources and sinks. The exchange of CO_2 between the atmosphere and the biosphere consists of two opposing fluxes: uptake of CO_2 by photosynthesis, and its release by respiration. It was demonstrated that these two opposing fluxes can be estimated separately at a local scale, by measuring small vertical gradients in $\delta^{13}C$ and $\delta^{18}O$ values in the atmospheric CO_2 above vegetation. Notably, the field scale chosen here allowed us to establish the quantitative relationships between isotopic variations in the atmosphere and specific biospheric processes, too uncertain at the global scale and unrealistic in the laboratory. Larger systems may pose additional challenges such as due to heterogeneity of the vegetation and will require larger numbers of plant and soil samples to characterize it. Simple sampling procedures and rapidly increasing automation in isotopic analyses will help overcome these difficulties. Uncertainties associated with estimating δ_r (for respiration) and δ_p (for photosynthesis) can be greatly reduced by direct sampling of soil CO_2 (for δ_r), or of CO_2 exchanged by individual leaves in the field (for δ_p). It is encouraging to note though that a systematic approach to estimating δ_r and δ_p produced consistent results for three different crops. The precision in estimating δ_r and δ_p notwithstanding, the existence of significant differences

between them is critical. Such differences, beyond the analytical uncertainty, were clearly observed in this study with mean differences between δ_p and δ_r (for the results presented in Table 1 in section 3.4) of 11.4‰ for $\delta^{18}O$ and 1.0‰ for $\delta^{13}C$. Though they may vary with time and location due primarily to changes in ^{18}O enrichment of leaf water (inversely correlated with relative humidity) or in the isotopic relationships between plants and soil organic matter, these differences may provide a significant advantage for ^{18}O over ^{13}C as tracer for partitioning leaf and soil CO_2 exchange. Research is clearly needed to further reduce uncertainties associated with estimating δ_r and δ_p. Specifically, isotopic fractionations associated with diffusion and CO_2–H_2O equilibrium of soil CO_2, as well as the isotopic signature of chloroplast water, are not well characterized at present. The usefulness of including isotopic measurements of CO_2 at the global scale has already been noted (Farquhar et al., 1993; Tans et al., 1993; Ciais et al., 1995; Francey et al., 1995). The isotope methods used here should prove useful in the future in investigating climate feedback arising from the responses of ecosystems to environmental changes.

In the last part of the present research (section 3.5), the isotopic interaction between terrestrial vegetation and the atmosphere associated with evapotranspiration (ET) was evaluated above different vegetation. The water exchange process was studied at different time scales, including mid-day, diurnal and seasonal. Mid-day ET from fields of winter wheat, maize and cotton were estimated during different growing periods throughout 1994, and diurnal ET measurements were made above sunflower and above a desert agroforestry system in 1995. The isotope approach was based on the effects of water vapor interactions between vegetation and the atmosphere within the canopy boundary layer (CBL). Gradients of both water vapor concentrations and the $\delta^{18}O$ value of the water vapor could be clearly detected under normal meteorological conditions within the CBL. Such gradients in specific humidity inside the CBL could also help improve prediction of $\delta^{18}O$ values of leaf water (δ_c).Generally lower δ_c values were calculated when using specific humidity data from the lower boundary levels. This provided additional explanation for the higher predicted δ_c values than the measured δ_m which are usually reported. Larger gradients in specific humidity within the CBL in the field, resulted with larger difference between the δ_c and the δ_m values. Such gradients in specific humidity in the canopy boundary layers, are often neglected and the upper boundary, regional, meteorological conditions are being used. Computation of the ET flux could be carried out from isotopic measurements of plant stem water and of the atmospheric moisture content (inside and outside the CBL) and from the knowledge of the specific humidity of the background ambient atmosphere. For mid-day experiments, the ET flux estimates by the isotope method were comparable to those obtained by the conventional meteorological techniques, including energy balance and Bowen ratio method, as well as eddy correlation method. For diurnal measurements, the isotope approach could also yield comparable estimates of ET fluxes during the day, but not

in the early morning or late evening. This is because the flux calculation by the isotope approach depends also on wind conditions.

Similar to the partitioning of the CO_2 flux, the inherent advantage of the isotope method in partitioning the ET flux into evaporation and transpiration was examined. Taking measurements in the wheat field as an example, the results indicated that soil evaporation contributed 1.5-3.5% of the total ET flux in the wheat field at the times of measurements. It is important to emphasize, however, that the above results were obtained by using a simplified field system and considering soil water as a simple water body. Much uncertainty still remains, however, in estimating $\delta^{18}O$ values of soil evaporating water during soil drying (Mathieu & Bariac, 1996).

In the course of the present research, a method was developed for $\delta^{18}O$ analysis of a micro-size (ca. 1 μL) water sample with an external precision of ±0.1‰ (Appendix A1), which makes it possible for short term, high temporal and spatial resolution measurements of leaf and atmospheric water samples.

In conclusion, the present study provided a basis using ^{18}O measurements for evaluating plant responses to environmental change at the local field scale, and the isotope technique has the potential to be scaled up to regional or even global ecosystem studies.

A. Appendix

A1. Methodology

A1.1 Analysis of the $\delta^{18}O$ value of a micro size water sample

The conventional water--CO_2 equilibrium approach was used in the past for small water samples (Kishima & Sakai, 1980), but is labor intensive and requires corrections for equilibrium and evaporation isotope effects in the equilibration vessels. Small water samples can also be analyzed by the Guanidine Hydrochloride method in which the oxygen of water is quantitatively converted to CO_2 for the isotopic analysis (Dugan et al., 1985). This method, and a few others (Gat & Gonfiantini, 1981; Speakman et al., 1990), have not been widely applied, probably because of their complexity and the relatively low sample output. Here we present a simple and rapid technique for the analysis of 0.5~2 μL water samples carried out on-line with an isotope ratio mass spectrometer. This method is based on the original approach of Santrock and Hayes (1987).

An elemental analyzer (e.g. EA1108, Carlo Erba Instruments, Inc., Italy) is used to pyrolyze (1090°C) 0.5-2 μL of water samples (the same method is used also for pyrolizing about 1 mg of organic matter) on a Nickelised carbon (Elemental Micro-analysis Limited, Devon, UK) column to quantitatively produce carbon monoxide. The CO sample was focused on a packed GC column (9 mm, molecular sieve 5A, 80/100 mesh, 70°C) and was carried, on-line, by the He carrier gas (120 mL min^{-1}) through an open split into an isotope ratio mass spectrometer fitted with an on-line port (e.g. Optima, Micromass, UK). The 30/28 mass ratio of the sample and a reference CO gas (injected between samples from the reference-bellow of the mass spectrometer's dual inlet) were used for determination of the $\delta^{18}O$ values of the samples. Because mass 28 used for the analysis of CO is the same as that for N_2, care must be taken to avoid air leaks in the system. The pyrolysis is also sensitive to both blank and memory effects (Santrock & Hayes, 1987). The major blank effect was due to oxygen interactions with glass (e.g. the reaction tube, glass chips and glass wool). This was minimized by replacing the conventional quartz reaction tube with one made of ceramic (e.g. Carlo Erba, Italy; Bolt Technical, Texas). Similarly, any quartz chips required for the column packing were replaced with ceramic chips (prepared by sacrifying a reaction column), and quartz wool was replaced with silver wool for packing purposes (Nickle wool used in the conventional application was also eliminated). The memory effects due to interactions of oxygen with the carbon in the reaction tube, was minimized by doping the helium carrier stream with 5% CCl_4 in heptane, reducing by half the quantity of the carbon reactant, as compared to conventional packing for oxygen analysis, and by "flushing" the system with a "blank" sample prior to sample analysis. Doping was achieved by placing a glass capsule (ca 10 mm long, 5 mm O.D. with a 1 mm hole on its side) filled to about half with the doping solution inside ca. 200 mm long 13 mm

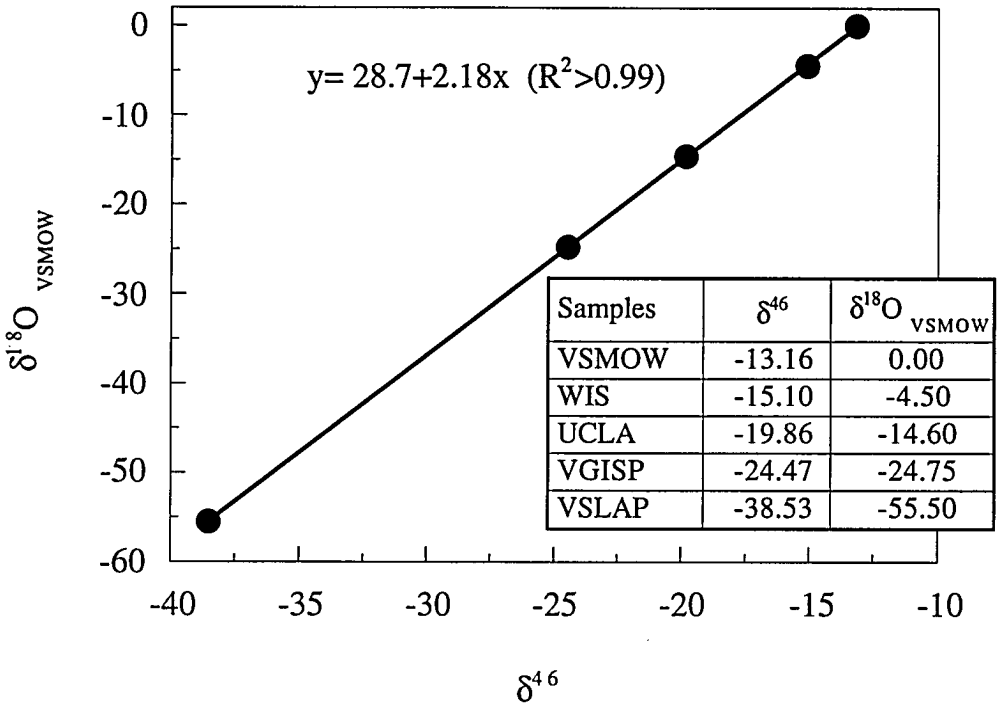
O.D. glass tube that was fitted on the carrier gas line. A packed reaction column used in the above analysis was 46 cm long and filled, in going from bottom to top, with 3 cm quartz wool (positioned outside the furnace), 18 cm ceramic chips, 1 cm silver wool, 2 cm Nickelised Carbon (substituted in some cases by spectrographic graphite). The top of the carbon reagent was positioned at the center of the furnace. The speed of the analysis was about 4 min. per sample.

Handling of water samples was critical for the analysis and two approaches have been used. The first employed the standard multi-sample carousel of the EA. In this case ca. 1 μL water sample was placed with a gas-tight syringe (Hamilton Co., Reno, Nevada, USA) in a silver capsule and dropped directly into the first sample position with no waiting time for the EA analysis. Silver capsules were made hydrophobic by annealing at 400°C and slow cooling under vacuum. This allowed the placement of a single ca. 1 μL droplet onto the bottom of the capsule and sealing the capsule without "smearing" the water sample. Such arrangement avoided evaporative loss for the time needed for the transfer and initiation of the pyrolysis (tested as a weight drift<0.01 mg per minute, compared with about 10 sec for sample handling). The second approach employed an injector provided by the manufacturer of the analyzer (Carlo Erba Inst., Italy) that replaces the standard multi-sample carousel. This injector allowed conventional GC mode operation by injection of the small water sample directly into the reactor through a septa. A gas tight syringe with a long hypodermal needle was used to facilitate the delivery of the sample to the center of the furnace.

The results were reported here for analyses of 0.5-2 μL water samples of some international water standards obtained from the IAEA in Vienna (VSMOW, VGISP, VSLAP), and laboratory standards, UCLA and WIS (local tap water). The $\delta^{18}O$ (VSMOW) values assigned to the lab standards were based on long term mean values obtained by the conventional H_2O-CO_2 equilibration method. The results showed that external precision for the various analyses ranged between 0.06 and 0.20‰ (Table A1), with the later currently used as the stated precision for the new method (both for water and organic samples). Calibrating the $\delta^{18}O$ values against the international standard (Fig. A1) showed a good linear correlation (R^2>0.99) and a slope of the best fit line close to the predicted value of 2.

Table A1. Results of the analyses of 0.5-2 μL water samples by the on-line micro-analysis method described in the text. VSMOW, VGISP, VSLAP are the IAEA international Vienna water standards. WIS and UCLA, are laboratory standard repeatedly analyzed versus the IAEA standards using the conventional CO_2 equilibration method. One blank sample was run prior to each set of standards; δ^{46} denotes the uncalibrated "machine" $\delta^{18}O$ value.

Samples	δ^{46}	Mean ± s.d.	Samples	δ^{46}	Mean ± s.d.
1. March 23, 1993			4. December 30, 1993 (with doping)		
VSMOW2	-13.76		WIS3C	-11.06	
VSMOW3	-13.60		WIS3D	-11.41	
VSMOW4	-13.67	-13.68±0.08	WIS3E	-11.24	-11.24±0.17
WIS7	-15.53		VSMOW3B	-9.89	
WIS8	-15.76		VSMOW3C	-9.97	
WIS9	-15.81	-15.70±0.15	VSMOW3D	-10.02	-9.96±0.07
2. March 28, 1993			VGISP3B	-18.28	
VSMOW12	-13.80		VGISP3C	-18.50	
VSMOW13	-13.72		VGISP3D	-18.44	-18.41±0.12
VSMOW14	-13.85	-13.79±0.07	5. October 30, 1994		
VGISP8	-25.57		VSMOW1B	-10.70	
VGISP9	-25.52		VSMOW1C	-10.96	
VGISP10	-25.66	-25.58±0.07	VSMOW1D	-10.56	-10.74±0.20
3. April 7, 1993			VGISP1B	-21.88	
VSMOW18	-12.79		VGISP1C	-21.85	
VSMOW19	-13.19		VGISP1D	-21.57	-21.77±0.17
VSMOW20	-13.03	-13.01±0.20	VSLAP1B	-34.75	
UCLA2	-19.71		VSLAP1C	-34.85	
UCLA3	-19.93		VSLAP1D	-35.01	-34.87±0.13
UCLA4	-20.00	-19.88±0.15			
VSLAP2	-38.33				
VSLAP3	-38.44				
VSLAP4	-38.35	-38.37±0.06			



Samples	δ^{46}	$\delta^{18}O_{VSMOW}$
VSMOW	-13.16	0.00
WIS	-15.10	-4.50
UCLA	-19.86	-14.60
VGISP	-24.47	-24.75
VSLAP	-38.53	-55.50

Figure A1. The $\delta^{18}O$ calibration curve for IAEA Vienna standard water samples (VSMOW, VGISP and VSLAP) and laboratory standard water (UCLA and WIS) based on the on-line micro-analysis method described in the text using 0.5-2 μL water samples. δ^{46} donates uncalibrated machine values, $\delta^{18}O_{VVSMOW}$ indicates $\delta^{18}O$ values calibrated on the VSMOW scale.

A1.2 Equilibrium of H_2O samples with laboratory standard CO_2

1. The line is under high vacuum without leak;
2. Fill each vial with a water sample (0.5 ml or 0.3 ml), close the vial but not pumping;
3. Freeze the water sample from the bottom of the vial, one by one, to pump the air away;
4. After they've done (all of the air being pumped away), wait for 30 min. to allow for the water samples warm up to the room temperature;
5. Close the main valve, open CO_2 flask to introduce it to the line, record the pressure before;
6. Open the valve to the samples, record the pressure after, wait for 1 min., then close the valve of the vial;
7. Collect a background CO_2 sample in an ampoule, number it;
8. Recollecting CO_2 sample source remaining in the line, back to CO_2 flask;
9. Disconnect the vials and clean the grease, put all of the samples in the water bath, at 25 °C, for 24 hours, to allow full equilibrium between CO_2 and H_2O;

10. Record the gauge pressure reading for the ambient atmosphere at the end.

A1.3 Extraction of leaf cellulose

1. Grind a sample to fine power;
2. Transfer sample into a beaker 100 ~ 300 ml;
3. Add H_2O and boil for 1 hour;
4. Remove and cool to ~ 70 °C;
5. Add 1 ml Acetic acid, and 1 gram $NaClO_2$ (Oxidizing agent as power) react for 1 hour at ~ 70 °C. Repeat 4 ~ 6 times until the cellulose is a clean white powder;
6. Decant. Wash cellulose with water 3 times decanting after each time;
7. Soak in 17% NaOH solution for 45 min.;
8. Wash with water 3 times;
9. Add 10% Acetic acid and wait for 15 min.;
10. Rinse and wash with H_2O to remove acetic acid 3 times;
11. Let sit in water (distilled 3 times) overnight;
12. Filter the next day;
13. Dry the samples in lyophilizer.

A1.4 Calibration of the H_2O span in LiCor-6262 and ADC instruments

The calibration was made with a Dew Point Generator from the Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot. Procedures are described below.

1. Set dew point temperature to be 15°C. After equilibrating, it reads 14.95°C. Then connect the outlet hose to the sample inlet of the LiCOr. Due to mass conservation, the same amount of water molecules should go into LiCor and give the same dew point temperature, if the optical bench and interconnecting hose were maintained above the dew point temperature to prevent any condensation.

2. The LiCor reads dew point temperature (Fun 38) 18.6°C, using water span to correct it to the same dew point temperature, say, 14.95°C. The corresponding specific humidity is 16.96 mmol mol^{-1}.

3. In the same way, we can connect the outlet hose from the Generator to the inlet tubing of the leaf chamber of the ADC (with yellow band). Before connecting, close the leaf chamber. Turn throttle of H_2O to totally "–", to filter all of the water from the reference side. After equilibrating, the RH in/out from the ADC should be balanced with each other, and near zero.

4. Connecting the outlet hose from the Generator to the inlet tubing of the leaf chamber of the ADC, with leaf chamber closed. Then based on the same idea, the dew point temperature

should be the same. We read RH = 56%. Based on the pre-set dew point, we calculate the theoretical relative humidity is 57.2%. It is within the precision of ± 1% in RH measurement.

A1.5 Collecting H_2O vapor and CO_2 samples simultaneously

The setup of the system are shown in Fig. A2 (for 1994) and Fig. A3 (for 1995).

1. Before the sampling, make sure that all of the traps and flasks are totally dry;
2. If taps and flasks are in vacuum, fill them up with dry nitrogen from the balloon;
3. Pump all of the 5 pools of Bev-A-Line before connecting them to the traps;
4. Use (dry ice + Acetone) to cool down all of the traps before starting to trap water moisture;
5. With the pump on, open two stopcocks of one flask at the same time, then one flask after another to open all of the other flasks; it is within 7 seconds;
6. After all of the flasks are opened, open water traps in the same way, one trap at one time; it is usually within 15 seconds;
7. After 30 minutes of sampling, close two stopcocks of one flask at the same time, then one flask after another to open all of the other flasks; it is within 7 seconds;
8. After all of the flasks are closed, close water traps in the same way, one trap at one time; it is usually within 15 seconds.

A1.6 Transferring an air CO_2 sample from a flask to an ampoule

In order to get good replicates of an air sample, following practice should be taken into account (please refer to Fig. A4):

1. Pump the whole line; use flame to heat the ampoule, and traps No. 1 and No. 2, if necessary;
2. Put dewers filled with liquid nitrogen to traps No. 1 and No. 2, just to one half of the traps. Wait for 5 minutes to let the traps cool down;
3. Open the upper stopcock of the flask, very slowly, the maximum opening should be limited to certain degree, which can be monitored by a Varian meter. The maximum reading of the Varian meter is about $6.5 \times 10^{+2}$ torr for gauge No. 8. When the vacuum reading goes down, one can open the upper stopcock further, but the reading of the Varian meter should not go up, in any case, to a full scale of 7.6×10^{2} torr;
4. Usually, the air flask can be vacuated to 1.0×10^{-3} torr in one hour. Then, close the upper stopcock of the air flask. Close also stopcocks No. 1 and No. 2;
5. Warm trap No. 2 to freeze all the CO_2 trapped into trap No. 1. The vacuum reading in this step is usually below 1.0×10^{-2} torr, sometimes even better to 1.0×10^{-3} torr depending on the sample. Pump the line again, if the reading is above 1.0×10^{-3} torr;

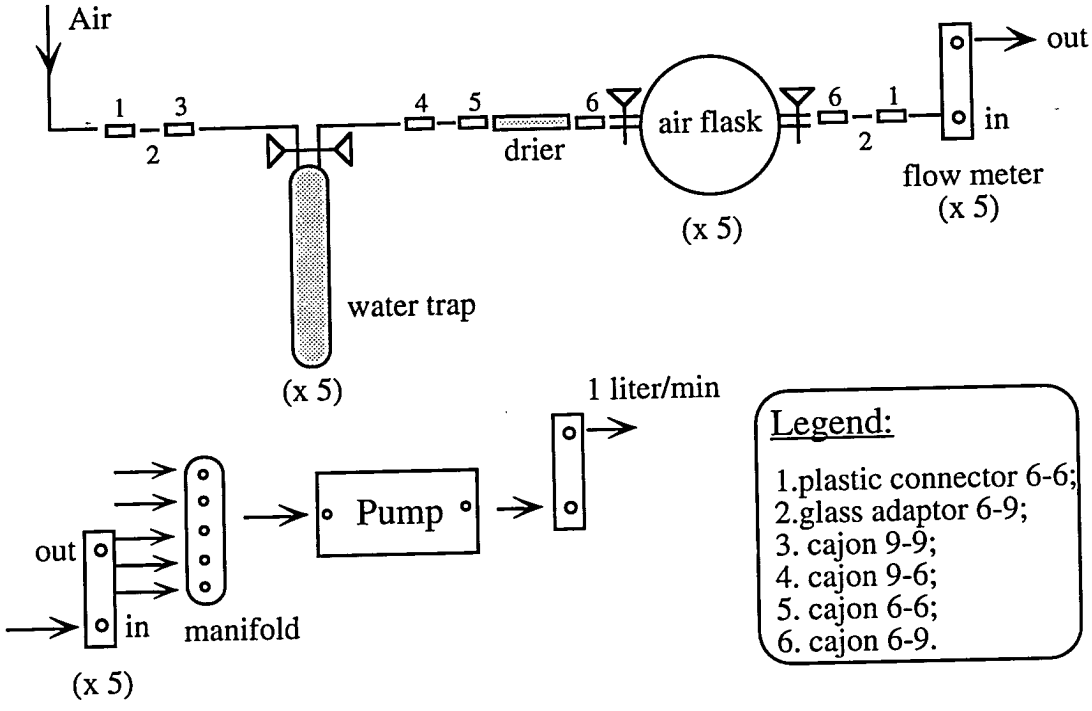
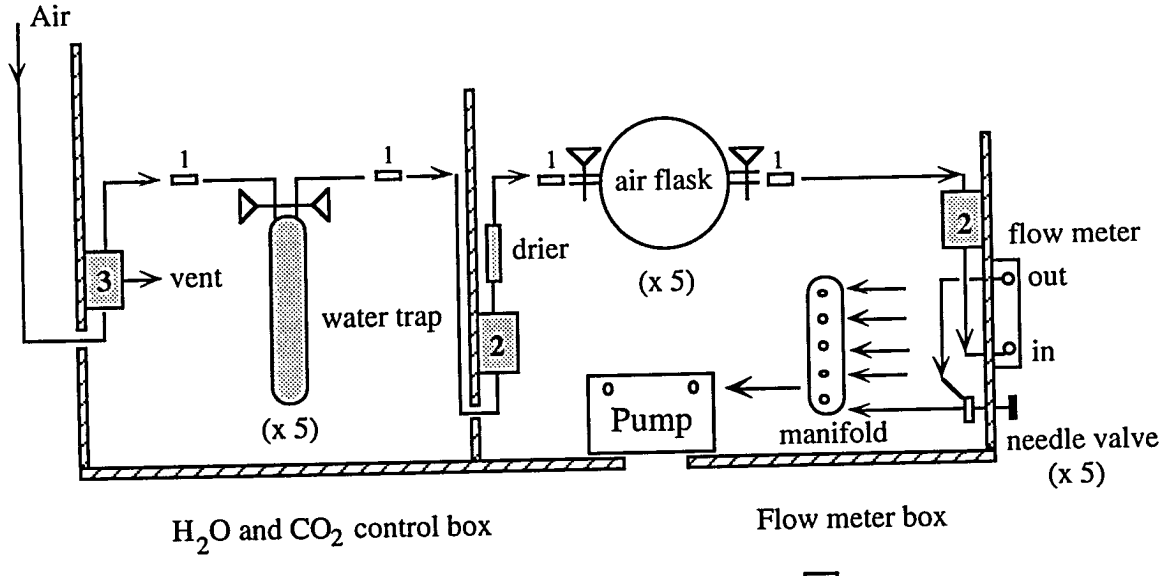


Figure A2. A setup for collecting CO_2 and H_2O vapor samples in fields in 1994.



Legend: 1 for cajon connector 6-9; 2 for 2-way solenoid; 3 for 3-way solenoid.

Figure A3. A setup for collecting CO_2 and H_2O vapor samples in fields in 1995.

6. Replace the dewer filled with liquid nitrogen from trap No. 1, with another dewer filled with (dry ice+acetone) at the same time, to make sure that the CO_2 transferred to the ampoule is totally dry. The vacuum in this step is usually read below $5.0x10^{-3}$ torr;
7. After taking off liquid nitrogen from trap No. 1, use liquid nitrogen to freeze CO_2 at the bottom of the ampoule. Wait until the vacuum reading keeps stable. Pump the whole line and close the ampoule with flame at the end.

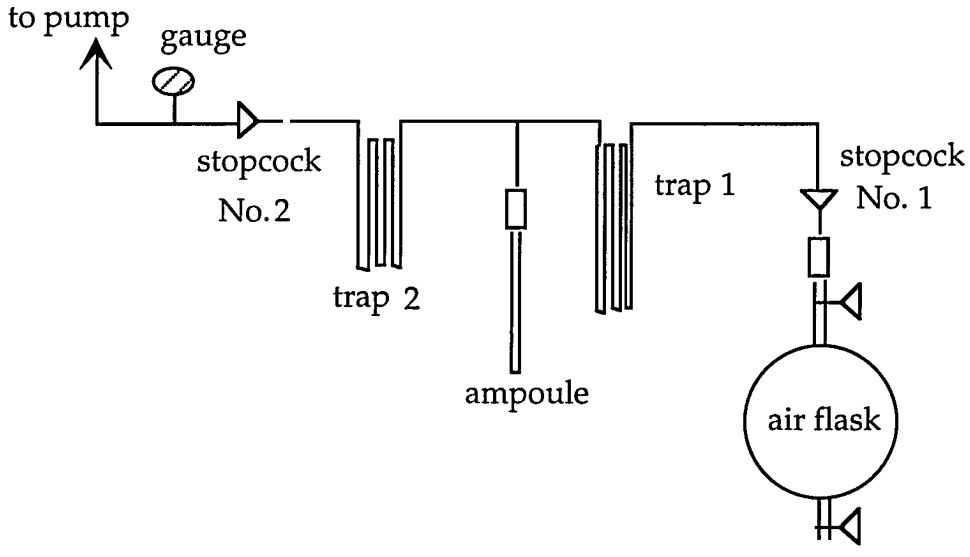


Figure A4. Diagram of the lab system for transferring an CO_2 sample out of a flask.

A2. Setups used in laboratory and field experiments

A2.1 Setup used for gas-exchange studies in laboratory

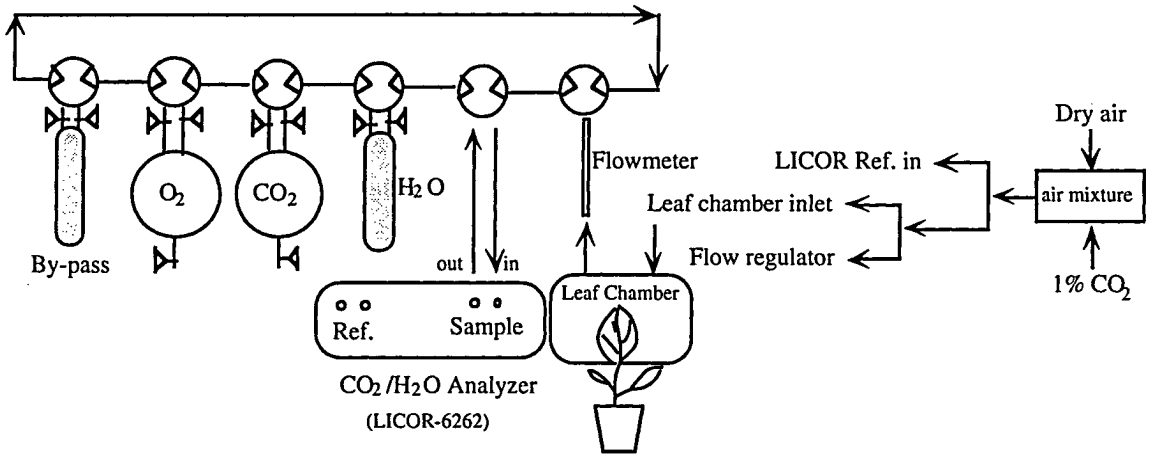


Figure A5. Sketchmap of a setup for the study of environmental influence on the $\delta^{18}O$ values of leaf water, as reported in Wang & Yakir (1995).

A2.2 Setup used for CO_2 and H_2O measurements in the fields

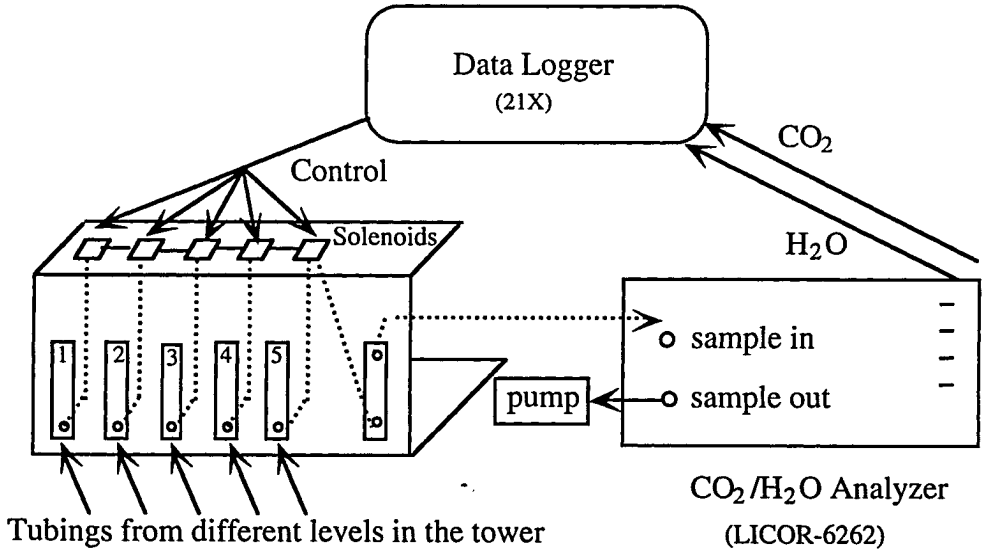


Figure A6. Sketchmap of a setup for simultaneous measuring of air CO_2 and H_2O vapor in the fields, as reported in Yakir & Wang (1996).

A3. Reproducibility of isotopic analysis of air CO_2 samples

Table A2. Isotopic results of air CO_2 samples collected from an identical source outside the laboratory, by sucking the air through groups of a cold water trap and an air flask, controlled by a needle valve and monitored by a flow meter, according to Fig. A2.

Sample	$\delta^{13}C$ (‰)	precision	$\delta^{18}O$ (‰) *	precision
Group 1				
CO_2-21	19.265	0.006	23.629	0.013
CO_2-22	19.644	0.006	23.969	-0.001
CO_2-23	19.566	0.013	23.797	-0.001
CO_2-24	19.510	0.009	23.673	0.009
CO_2-25	19.612	0.009	23.681	0.009
Mean±s.d.	19.519±0.15		23.749±0.14	
Group 2				
CO_2-26	19.417	-0.001	25.030	0.013
CO_2-27	19.573	0.006	25.238	0.027
CO_2-28	19.599	-0.001	25.339	0.016
CO_2-29	19.603	0.018	25.294	0.022
CO_2-30	19.596	0.006	25.262	0.009
Mean±s.d.	19.558±0.08		25.233±0.12	
Group 3				
CO_2-31	18.103	0.013	23.001	-0.001
CO_2-32	18.158	0.009	23.096	-0.001
CO_2-33	18.148	0.006	23.142	-0.001
CO_2-34	18.190	0.006	23.042	0.016
CO_2-35	18.041	-0.001	23.040	0.009
Mean±s.d.	18.128±0.06		23.064±0.06	

A4. Original data obtained from the general survey in the Botanical Garden in Jerusalem

General botanical survey of different plant species on July 18-24, 1995
Irrigation water=-4.8; air moisture=-9.69
Isotopic composition of the leaf water

Species number	Species names	Sections	Stem water SMOW	Measured leaf SMOW	Temp.	Equili.	RH	Stomatal condu.	Kinetic factor	Craig (new K)	Craig - measured	ΔL/ΔE Farquhar	Peclet number	(1-exp(-p))/p	Mixing pathlength (cm)	Final E mmol m-2 s-1	Photo. A micro mol	A/E (WUE) (x 1000)	V/E hour	Ci/Ca from ADC	C-13 Cellulose	Oxy-18 Cellulose	Morpho. type	Avishai grouping
1	*Banksia integrifolia*	Australian	-4.4	12.2	34.9	8.61	0.44	0.57	26.00	16.50	4.30	0.79	0.50	0.79	4.29	1.72	4.02	2.34	4.11	0.62	-23.4	35.1	1b	8
2	*Eucalyptus woodwardii*	vegetation	-4.6	8.4	34.7	8.63	0.42	1.13	24.75	16.24	7.84	0.62	1.06	0.62	8.46	1.85	5.93	3.21	12.01	0.58	-24.4	28.7	1a	8
3	*Melaleuca nesophila*		-3.9	12.2	37.5	8.42	0.41	0.39	26.53	17.92	5.72	0.74	0.65	0.74	6.66	1.44	2.36	1.64	26.21	1.22	-22.6	33.1	3a	1
5	*Acacia turner*		-4.6	14.7	36.9	8.47	0.40	0.58	25.98	17.48	2.78	0.87	0.29	0.87	2.71	1.58	4.43	2.80	18.91	0.50	-26.3	27.8	3a	8
6	*Callistemon phoeniceus*		-3.9	9.5	37.5	8.42	0.42	0.43	26.41	17.53	8.03	0.63	1.00	0.63	8.53	1.73	11.25	6.50	12.16	0.68	-23.6	31.4	1c	1
7	*Calocedrus decurrens*	N. America	-5.7	13.8	38.4	8.36	0.35	0.15	27.37	19.14	5.34	0.79	0.50	0.79	6.31	1.17	1.60	1.37	70.57	0.62	-21.8	37.3	2b	4
8	*Pinus sabiniana*	conifer &	-6.0	11.9	38.2	8.37	0.35	0.44	26.38	18.10	6.20	0.74	0.65	0.74	6.00	1.60	4.29	2.68	67.21	0.42	-21.9	31.3	2a	4
9	*Styrax officinalis*	evergreen	-4.9	14.8	37.1	8.45	0.40	0.42	26.44	17.66	2.86	0.87	0.29	0.87	2.56	1.67	2.37	1.42	0.71	1.18	-27.3	32.8	1a	7
12	*Cupressus arizonica*		-7.5	10.9	35.4	8.58	0.36	0.47	26.29	17.25	6.35	0.74	0.65	0.74	6.76	1.42	3.55	2.50	49.84	0.93	-24.8	30.0	2b	4
13	*Acacia farnesiana*	Desert plant	-3.1	14.7	38.3	8.37	0.32	0.65	25.79	20.58	5.88	0.75	0.62	0.75	3.26	2.81	4.76	1.69	3.43	0.55	-23.7	31.5	3a	7
14	*Prosopis juliflora*		-5.8	13.4	38.5	8.35	0.33	1.06	24.88	17.91	4.51	0.81	0.45	0.81	1.40	4.76	3.81	0.80	6.44	0.65	-22.2	37.9	3b	
16	*Magnolia grandiflora*		-4.6	16.0	36.6	8.49	0.34	1.10	24.81	18.59	2.59	0.89	0.24	0.89	1.85	1.92	0.19	0.10	1.33	0.47	-21.8	39.8	1a	8
17	*Acer negundo*		-4.3	13.6	35.8	8.55	0.36	1.31	24.44	18.01	4.41	0.80	0.47	0.80	3.23	2.15	5.61	2.61	0.73	0.63	-27.0	37.3	1a	5
18	*Fraxinus pennsylvanica*		-5.3	11.7	35.5	8.57	0.37	3.06	22.56	15.88	4.18	0.80	0.47	0.80	2.31	3.00	7.65	2.55	0.92	0.60	-23.5	34.0	1a	5
19	*Sequoia sempervirens*	Calif. Woodl.	-3.9	20.2	36.8	8.47	0.36	0.57	26.00	18.99	-1.21	1.05	0.00	1.00	0.00	2.19	3.91	1.79	7.90	0.57	-22.9	37.4	3b	4
20	*Ceanothus arboreus*		-4.8	9.3	37.4	8.43	0.37	1.35	24.37	17.07	7.77	0.64	0.98	0.64	3.86	3.75	10.52	2.81	0.31	0.41	-23.8	29.9	1a	1
21	*Pleigynium timoriense*	Mediterran	-5.0	11.0	33.1	8.75	0.42	0.52	26.14	17.01	6.01	0.73	0.68	0.73	6.39	1.57	5.42	3.45	0.88	0.50	-22.9	30.8	1b	1
22	*Caesalpinia palmeri*	N. Africa	-5.0	9.1	34.1	8.67	0.42	0.64	25.82	16.73	7.63	0.65	0.95	0.65	8.93	1.57	10.85	6.91	0.88	0.12	-23.5	33.3	1b	3
23	*Amygdalvs communis*		-6.0	10.6	33.7	8.70	0.42	0.55	26.06	16.30	5.70	0.74	0.65	0.74	3.46	2.77	9.69	3.50	0.43	0.39	-23.7	28.3	1b	7
24	*Cordiline australis*		-5.0	6.1	34.4	8.65	0.39	0.78	25.47	17.24	11.14	0.50	1.60	0.50	8.41	2.81	8.44	3.00	2.17	0.47	-24.6	28.3	1b	6
25	*Cercis siliquastrum*		-5.4	9.7	34.8	8.62	0.39	1.97	23.53	15.91	6.21	0.71	0.74	0.71	5.55	1.97	7.99	4.06	1.30	0.47	-23.6	32.5	1a	7
26	*Populus angulata*	Deciduous	-4.7	11.9	34.7	8.63	0.40	0.99	25.02	16.88	4.98	0.77	0.56	0.77	4.95	1.67	4.59	2.75	1.06	0.59	-23.0	30.4	1a	5
27	*Platanus orientalis*	forest	-4.8	15.6	35.0	8.61	0.40	0.58	25.98	17.50	1.90	0.91	0.20	0.91	1.82	1.62	3.76	2.32	1.94	0.58	-20.5	32.8	1a	7
28	*Diospyros virginiana*		-5.1	15.0	35.5	8.57	0.37	0.21	27.14	18.96	3.96	0.84	0.35	0.84	5.81	0.89	1.10	1.24	1.99	0.69	-22.2	31.1	1a	5
29	*Betula papyrifera var suberidata*		-4.2	11.5	36.4	8.50	0.35	1.22	24.59	18.26	6.76	0.70	0.76	0.70	2.84	3.95	8.06	2.04	0.70	0.57	-23.4	28.9	1b	5
30	*Umbellularia californica*	Calif. Woodl.	-4.1	12.3	40.5	8.25	0.30	0.76	25.52	20.21	7.91	0.67	0.88	0.67	3.16	4.11	4.26	1.04	0.62	0.64	-28.1	35.2	1b	1
31	*Salvia leucophylla*		-4.3	10.3	37.2	8.45	0.32	1.47	24.19	18.95	8.65	0.63	0.99	0.63	1.99	7.34	16.15	2.20	0.56	0.45	-25.9	26.1	3b	1
32	*Aesculus californica*		-4.2	10.4	35.3	8.58	0.34	1.03	24.94	18.94	8.54	0.63	0.99	0.63	4.58	3.19	5.82	1.82	1.29	0.61	-24.7	31.8	1b	2
33	*Fraxinus velutina*		-4.7	10.7	34.4	8.65	0.34	1.74	23.81	18.04	7.34	0.68	0.84	0.68	3.56	3.48	9.66	2.78	1.41	0.52	-26.1	33.0	1b	7
34	*Olea africana*	American	-4.1	13.4	35.2	8.59	0.34	1.45	24.22	18.44	5.04	0.78	0.53	0.78	3.06	2.56	6.93	2.71	12.67	0.53	-25.0	29.7	1b	8
35	*Podocarpus latifolius*	montane forest	-4.3	15.9	35.4	8.58	0.34	0.23	27.07	20.45	4.55	0.82	0.40	0.82	5.52	1.07	1.37	1.28	35.83	0.68	-21.8	32.8	1b	4
36	*Dais cotinifolia*	S. African	-3.8	13.6	35.4	8.58	0.35	0.87	25.27	19.32	5.72	0.75	0.62	0.75	4.22	2.17	4.47	2.06	0.63	0.59	-25.1	31.0	1a	2
37	*Podocarpus falcatus*	montane forest	-4.4	17.0	35.8	8.55	0.34	0.17	27.29	20.38	3.38	0.86	0.31	0.86	5.38	0.85	1.28	1.51	0.93	0.62	-24.0	37.0	1b	4
38	*Rhus leptodictya*		-3.0	13.0	35.3	8.58	0.34	6.51	21.12	17.27	4.27	0.79	0.50	0.79	0.36	20.26	34.73	1.71	0.05	0.63	-25.9	28.5	3b	1
39	*Cussonia paniculata*		-0.9	12.3	36.3	8.51	0.32	0.06	27.74	23.54	11.24	0.54	1.38	0.54	63.67	0.32	0.32	1.00	6.76	0.66	-22.3	31.0	1a	3
40	*Celtis africana*		-2.3	17.7	36.5	8.50	0.33	0.47	26.29	21.33	3.63	0.85	0.34	0.85	2.56	1.96	2.70	1.38	14.04	0.64	-21.4	32.3	1a	7
41	*eshel (Hebrew name)*	Austral.	-5.6	9.0	28.0	9.14	0.50	0.45	26.35	14.81	5.81	0.71	0.72	0.71	11.81	0.90	3.60	4.00	4.37	0.54	-24.7	33.4	1a	3
42	*Acacia pendula*	trop. vegeta.	-5.8	11.2	30.9	8.92	0.43	0.28	26.89	16.82	5.62	0.75	0.61	0.75	9.28	0.97	2.92	3.01	3.24	0.53	-25.0	35.9	1b	8
43	*Paulownia tomentosa*		-5.1	11.5	32.4	8.80	0.41	0.63	25.84	17.20	5.70	0.74	0.64	0.74	6.61	1.43	3.60	2.52	1.79	0.60	-22.9	32.3	1a	7
44	*Ailanthus glandulosa*	Turanian	-4.8	8.7	32.5	8.79	0.40	2.66	22.86	15.89	7.19	0.65	0.95	0.65	4.30	3.26	9.77	3.00	0.60	0.60	-26.9	26.8	1a	7
45	*Malus cerasifera*	section	-4.6	10.3	32.5	8.79	0.39	2.62	22.90	16.19	5.89	0.72	0.70	0.72	2.32	4.45	15.83	3.56	0.71	0.49	-26.1	28.4	1a	7
46	*Ficus carica*		-4.7	11.0	32.3	8.81	0.40	1.16	24.70	16.85	5.85	0.73	0.68	0.73	5.02	2.00	3.54	1.77	1.92	0.72	-21.1	29.3	1a	7
47	*Lycium barbarum*	Turanian	-4.4	11.3	32.9	8.76	0.38	1.65	23.93	17.23	5.93	0.73	0.68	0.73	3.14	3.20	8.72	2.73	2.61	0.57	-24.9	28.2	3b	2
48	*Rhamnus dolichophylla*	desert	-3.8	10.6	33.1	8.75	0.38	1.57	24.04	17.48	6.88	0.68	0.84	0.68	3.39	3.66	8.37	2.29	0.70	0.60	-24.8	28.2	3b	2
49	*Tamarix nilotica*		-5.2	8.7	33.2	8.82	0.38	0.49	26.23	18.33	9.63	0.59	1.18	0.59	16.59	1.05	3.08	2.93	21.91	0.55	-25.6	32.3	2b	3
50	*Malus orientalis*		-4.8	10.2	32.7	8.78	0.39	1.49	24.16	16.75	6.55	0.70	0.77	0.70	2.98	3.81	15.89	4.17	0.72	0.36	-23.1	34.0	1b	7
51	*Pistacia vera*	Central Asian	-3.2	10.0	31.8	8.85	0.41	1.57	24.04	17.22	7.22	0.65	0.95	0.65	4.29	3.27	11.38	3.48	1.68	0.50	-24.0	29.2	1a	7
52	*to right of apple tree (observer fa*	section	-4.9	7.2	32.7	8.78	0.38	2.78	22.77	16.21	9.01	0.57	1.25	0.57	5.86	3.15	11.70	3.71	1.37	0.51	-25.9	35.1	1a	
53	*Crataegus songarica*		-3.7	10.3	33.0	8.76	0.38	1.13	24.75	18.12	7.82	0.64	0.98	0.64	5.48	2.64	6.04	2.29	1.12	0.62	-25.2	27.3	1b	7
54	*Juniperus excelsa*		-5.0	11.1	33.7	8.70	0.35	0.58	25.98	18.93	7.83	0.67	0.88	0.67	5.62	2.31	5.20	2.25	13.87	0.53	-21.1	33.3	2b	4
55	*Halimodendron halodendron*		-4.0	11.7	32.9	8.76	0.36	0.86	25.29	19.05	7.35	0.68	0.84	0.68	4.58	2.71	7.50	2.77	7.91	0.50	-22.9	31.0	3b	3
56	*Anagyris foetida*	Steppe forest	-3.9	14.1	33.7	8.70	0.35	1.54	24.08	18.47	4.37	0.80	0.47	0.80	1.36	5.12	10.49	2.05	0.42	0.59	-25.6	28.0	1b	2
57	*Prunus divaricata*		-4.5	13.6	34.4	8.65	0.34	0.71	25.64	19.49	5.89	0.75	0.61	0.75	3.90	2.31	5.52	2.39	1.79	0.53	-22.4	26.1	3b	7

General botanical survey of different species on July 18, 1995

Irrigation water=-4.8; air moisture=-9.69

Isotopic composition of the leaf water

Species number	Species names	Sections	Stem water SMOW	Measured leaf SMOW	Temp.	Equili.	RH	Stomatal condu.	Kinetic factor	Craig (new K)	Craig - measured	ΔL/ΔE Farquhar	Peclet number	(1-exp(-p))/p	Mixing pathlength (cm)	Final E mmol m-2 s-1	Photo. A micro mol	A/E (WUE) (x 1000)	V/E hour	Ci/Ca from ADC	C-13 Cellulose	Oxy-18 Cellulose	Morpho. type	Avishai grouping
58	*Quercus pedunculiflora*	Mediteranean	-4.6	14.1	34.6	8.64	0.34	1.17	24.68	18.54	4.44	0.81	0.45	0.81	2.02	3.29	7.75	2.36	0.78	0.55	-25.3	28.5	1a	7
59	*Punica granatum*	section	-3.4	14.9	33.9	8.69	0.35	0.58	25.98	20.08	5.18	0.78	0.52	0.78	3.52	2.18	5.38	2.47	1.98	0.49	-23.9	30.4	1b	2
60	*Quercus boissierii*		-5.3	12.8	34.3	8.66	0.33	1.26	24.52	18.34	5.54	0.77	0.54	0.77	1.85	4.31	11.85	2.75	0.27	0.45	-26.0	27.0	1b	7
61	*Celtis tournefortii*		-4.6	12.0	34.4	8.65	0.33	1.75	23.80	18.28	6.28	0.73	0.67	0.73	3.56	2.78	8.41	3.03	0.28	0.51	-23.0	31.8	1a	7
62	*Lygos monosperma*	Canary Island	-3.2	17.5	34.3	8.66	0.34	0.82	25.38	20.10	2.60	0.89	0.24	0.89	1.08	3.28	9.42	2.87	11.75	0.42	-24.3	39.1	2a	2
63	*Bupleurum fruticosum*	scrub	-2.6	15.0	33.9	8.69	0.34	1.69	23.88	19.47	4.47	0.80	0.47	0.80	0.88	7.88	14.63	1.86	0.92	0.60	-23.1	32.4	1c	1
64	*Phoenix canariensis*		-1.9	17.6	35.0	8.61	0.33	0.32	26.76	22.15	4.55	0.81	0.45	0.81	4.40	1.51	2.06	1.36	3.78	0.66	-24.1	32.1	1b	2
65	*Typha angustata*		2.2	16.8	35.1	8.60	0.36	1.63	23.96	21.67	4.87	0.75	0.60	0.75	1.74	5.08	13.07	2.57	1.70	0.49	-27.2	34.4	1b	9
66	*Scyrpus*		3.3	28.0	35.4	8.58	0.31	0.47	26.29	25.86	-2.14	1.10	0.00	1.00	0.00	3.05	5.86	1.92	1.74	0.50	-10.8	33.6	1c	9
67	*Jasminum odaratissimium*	Mediteranean	-2.9	16.4	36.5	8.50	0.30	0.70	25.67	21.70	5.30	0.78	0.52	0.78	1.68	4.57	0.70	0.15	0.22	0.86	-23.4	27.4	3b	1
68	*Laurus nobilis*	section	-3.7	13.6	36.5	8.50	0.29	0.70	25.67	21.20	7.60	0.70	0.77	0.70	3.27	3.48	4.18	1.20	0.62	0.65	-28.2	36.2	1b	1
69	*Pinus canariensis*		-4.2	10.0	36.1	8.53	0.29	0.33	26.73	21.80	11.80	0.55	1.34	0.55	12.93	1.53	2.51	1.64	45.23	0.55	-23.0	29.8	2a	4
70	*Myrsine africana*		-4.1	13.3	36.9	8.47	0.31	0.23	27.07	21.24	7.94	0.69	0.79	0.69	8.77	1.33	1.64	1.23	1.03	0.64	-24.4	24.1	3a	1
71	*Viburnum opulus*	European	-1.3	14.3	30.7	8.93	0.46	0.97	25.06	17.28	2.98	0.84	0.36	0.84	3.80	1.40	5.16	3.69	1.54	0.57	-26.3	25.6	1a	1
72	*Plantago lanceolata*	section	-3.2	16.4	32.3	8.81	0.47	0.74	25.57	16.19	-0.21	1.01	0.00	1.00	0.00	1.48	3.51	2.37	19.92	0.66	-25.2	25.6	2a	6
73	*Saponaria officinalis*		-2.9	12.7	33.0	8.76	0.46	1.53	24.10	15.80	3.10	0.83	0.38	0.83	1.22	4.60	12.66	2.75	1.97	0.55	-25.5	29.6	1b	6
74	*Lonicera xylosteum*		-2.7	12.4	33.7	8.70	0.46	0.39	26.53	17.02	4.62	0.77	0.54	0.77	5.91	1.35	5.31	3.93	1.89	0.37	-24.7	28.6	1a	2
75	*Cynosorus cristatus*		-2.0	13.2	33.6	8.71	0.46	1.30	24.45	16.45	3.25	0.82	0.40	0.82	1.51	3.90	7.79	2.00	0.35	0.64	-23.6	27.3	1b	
76	*Ulmus glabra*		-2.7	12.0	33.7	8.70	0.41	1.15	24.71	17.60	5.60	0.72	0.70	0.72	5.02	2.06	6.74	3.27	0.86	0.52	-22.5	26.1	1a	7
77	*Rhamnus cathartica*		-1.6	15.6	33.2	8.74	0.38	0.41	26.47	20.32	4.72	0.79	0.48	0.79	4.35	1.63	3.86	2.37	2.05	0.53	-21.2	34.0	1b	2
78	*Tilia platyphylla*		-2.8	12.7	34.2	8.67	0.38	2.02	23.48	17.77	5.07	0.75	0.60	0.75	3.92	2.26	5.53	2.45	0.96	0.64	-22.0	31.7	1a	7
79	*Myosotis laxa*		-3.9	11.6	34.5	8.64	0.38	1.66	23.92	17.31	5.71	0.73	0.66	0.73	2.43	4.01	10.93	2.73	1.23	0.52	-25.8	29.4	1b	6
80	*Cornus sanguinea*		-4.1	11.8	34.7	8.63	0.39	2.03	23.47	16.54	4.74	0.77	0.55	0.77	2.21	3.67	12.44	3.39	1.55	0.47	-23.5	34.4	1a	2
81	*Cedrus Libani*		-3.6	14.8	34.2	8.67	0.38	0.87	25.27	18.27	3.47	0.84	0.35	0.84	1.65	3.13	1.04	0.33	3.89	0.86	-23.6	30.4	2b	4
82	*Magnolia grandiflora*		-3.9	14.0	34.4	8.65	0.42	0.41	26.47	17.84	3.84	0.82	0.40	0.82	5.47	1.08	2.66	2.46	7.28	0.55	-22.2	40.1	1a	8
83	*Ceratonia siliqua*		-4.9	11.2	34.2	8.67	0.38	1.19	24.64	17.15	5.95	0.73	0.66	0.73	2.60	3.75	5.29	1.41	0.68	0.70	-26.2	27.6	1a	8
84	*Pistacia lentiscus*	Mediteranean	-3.7	13.1	33.4	8.73	0.41	1.17	24.68	17.15	4.05	0.81	0.44	0.81	2.31	2.81	8.79	3.13	1.40	0.48	-25.2	23.2	3b	1
85	*Olea europea var silvestris*	section	-3.4	12.7	33.9	8.69	0.39	0.94	25.12	18.05	5.35	0.75	0.60	0.75	2.70	3.28	6.10	1.86	1.32	0.64	-22.6	24.9	1b	8
86	*Pistacia atlantica*		-3.6	12.3	33.7	8.70	0.38	2.70	22.83	16.97	4.67	0.77	0.55	0.77	0.80	10.13	16.88	1.67	0.23	0.65	-24.4	28.0	3b	7
87	*Artemisia arborescens*		-2.4	13.2	34.1	8.67	0.39	0.38	26.56	19.77	6.57	0.70	0.75	0.70	6.55	1.69	4.13	2.44	8.96	0.51	-24.3	32.3	3a	1
88	*Spartium junceum*		-3.8	19.6	34.7	8.63	0.37	1.19	24.64	18.12	-1.48	1.07	0.00	1.00	0.00	4.74	11.25	2.37	10.53	0.51	-22.5	41.3	2a	2
89	*Liquidambar syraciflua*		-3.9	16.0	34.5	8.64	0.38	1.02	24.96	17.99	1.99	0.91	0.20	0.91	1.37	2.15	5.61	2.61	1.55	0.55	-24.0	32.9	1a	5
90	*Araucaria excelsa*		-3.1	17.8	34.7	8.63	0.36	0.50	26.20	19.82	2.02	0.91	0.20	0.91	1.18	2.50	3.75	1.50	33.66	0.64	-23.5	37.8	2b	4
91	*Metasequoia glyptostroboides*		-3.8	17.5	34.9	8.61	0.39	0.57	26.00	18.56	1.06	0.95	0.10	0.95	0.82	1.79	3.82	2.13	26.25	0.57	-23.5	31.4	2b	7
92	*Paulownia tomentosa*		-4.1	12.3	35.2	8.59	0.36	1.00	25.00	18.37	6.07	0.73	0.66	0.73	5.41	1.80	3.43	1.91	2.08	0.66	-24.3	28.6	1a	7
93	*Nerium var nanum*		-4.1	12.5	34.1	8.67	0.38	1.44	24.23	17.63	5.13	0.76	0.58	0.76	1.89	4.53	9.88	2.18	2.04	0.56	-24.1	26.6	1b	1
94	*Vitis vitifera*		-3.4	12.6	34.0	8.68	0.37	0.50	26.20	19.34	6.74	0.70	0.75	0.70	7.97	1.39	2.55	1.83	2.97	0.64	-23.5	38.7	1a	

Note

Morpho. types:

1a: long, wide
1b: long, thin
1c: long, broad;
2a: short needle
2b: short needle
3a: tiny leaf
3b:small leaf

Avishai grouping:

1.evergreen shrub
2.deciduous shrub
3.arid shrub
4.conifer
5.deciduous broad leaf, cool
6. grassland
7.deciduous tree, woodland
8. evergreen woodland
9. perennial herb,

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שיו-פנג וונג

תכולת O^{18} של מי העלה ושל פחמן דו חמצני ואדי מים העוברים חילוף איזוטופי ע״י העלים

The ^{18}O content of leaf water and of CO_2 and water vapor exchanged by leaves

April 1997

ניסן, תשנ״ז