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Partitioning evapotranspiration fluxes from a Colorado grassland using stable isotopes: Seasonal variations and ecosystem implications of elevated atmospheric CO_2

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Abstract

The stable isotopic composition of soil water is controlled by precipitation inputs, antecedent conditions, and evaporative losses. Because transpiration does not fractionate soil water isotopes, the relative proportions of evaporation and transpiration can be estimated using a simple isotopic mass balance approach. At our site in the shortgrass steppe in semi-arid northeastern Colorado, $\delta^{18}O$ values of soil water were almost always more enriched than those of precipitation inputs, owing to evaporative losses. The proportion of water lost by evaporation (E/ET) during the growing season ranged from nil to about 40% (to >90% in the dormant season), and was related to the timing of precipitation inputs. The sum of transpiration plus evaporation losses estimated by isotopic mass balance were similar to actual evapotranspiration measured from a nearby Bowen ratio system. We also investigated the evapotranspiration response of this mixed C_3/C_4 grassland to doubled atmospheric [CO_2] using Open-Top Chambers (OTC). Elevated atmospheric [CO_2] led to increased soil-water conservation via reduced stomatal conductance, despite greater biomass growth. We used a non-invasive method to measure the $\delta^{18}O$ of soil CO_2 as a proxy for soil water, after establishing a strong relationship between $\delta^{18}O$ of soil CO_2 from non-chambered control (NC) plots and $\delta^{18}O$ of soil–water from an adjacent area of native grassland. Soil–CO_2 $\delta^{18}O$ values showed significant treatment effects, particularly during a dry summer: values in ambient chambers (AC) were more enriched than in NC and elevated chamber (EC) plots. During the dry growing season of 2000, transpiration from the EC treatment was higher than from AC and lower than from NC treatments, but during 2001, transpiration was similar on all three treatments. Slightly higher evaporation rates from AC than either EC or NC treatments in 2000 may have resulted from increased convection across the soil surface from the OTC blowers, combined with lower biomass and litter cover on the AC treatment. Transpiration-use efficiency, or the amount of above-ground biomass produced per mm water transpired, was always greatest on EC and lowest on NC treatments.

Introduction

Land-cover change and climate change have the potential to alter ecosystem physiology and biosphere-atmosphere interaction through changes in the partitioning of energy, water and carbon fluxes among ecosystem components. Predictions of future mid-continental drying associated with global warming may increase precipitation variability and the likelihood of drought conditions (Kattenberg et al., 1996). In the semi-arid western United States, water is a key limiting factor to plant growth, and precipitation variability at daily to interannual timescales is likely to be the main control over variations in evapotranspiration (ET) rates (Reynolds et al., 2000). Although potential

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evapotranspiration (PET) has been successfully measured and modeled for at least half a century (e.g., Penman, 1948; Thornthwaite, 1948), techniques to measure actual ET (AET) rates at the landscape scale have only been developed over the last decade or so (e.g., Meyers, 2001; Wilson et al., 1999). Partitioning of the total ET flux into its components of evaporation and transpiration at the landscape scale has been modeled (Jackson et al., 1998; Paruelo et al, 2000; Reynolds et al., 2000), but rarely measured, because of difficulties in scaling up of leaf-level conductance measurements to the canopy.

Measuring transpiration from a single leaf is easy and precise (e.g. Pearcy et al., 1989), however, scaling such measurements to larger canopy, ecosystem, or regional scales is complex, and more difficult measurements are required (Jarvis and McNaughton, 1986; McNaughton and Jarvis, 1991; Wilson et al., 1999). In the current study, we applied an alternative approach, based on stable isotopes, to partition ET into its two components. Evaporation, or the loss of water from soil to air via a change of phase, results in the fractionation of soil water isotopes. Consequently, soil-evaporation alters both the soil-water content and soil-water isotopic composition (e.g., Allison and Barnes, 1983). In contrast, transpiration, which is the loss of water through stomata and cuticle, does not fractionate soil-water isotopes. Although evaporation from stomata on leaves does cause enrichment of leaf water, the volume of the leaf water pool is small, and it generally approaches isotopic steady state with environmental conditions (i.e., temperature, humidity, air vapor isotopic composition). Thus, at steady state, the isotopic composition of the outgoing flux, or transpired vapor, is the same as that of the incoming flux, or stem water (which is derived from soil water) (e.g., Allison et al., 1985; Bariac et al., 1991; Dawson, 1993; White et al., 1985; Zimmerman et al., 1967).

The direct effect of elevated atmospheric [CO_2] on most terrestrial ecosystems is enhanced growth, resulting from increased C assimilation rates (e.g., Morgan et al., 2001; Owensby et al., 1999). Indirect effects of elevated [CO_2], primarily related to N and water cycling, can contribute substantially to the uncertainties of terrestrial ecosystem response to climate change. In grasslands, improved soil–plant–water relations and increased water-use efficiency of plants under elevated [CO_2] may be partly responsible for increases in growth (Morgan et al., 2001; Owensby et al., 1999; Volk et al., 2001). Modeling work has shown that elevated [CO_2] contributes to reduced transpiration and increased soil water recharge, but that other factors (plant productivity, amount and timing of rainfall) also contribute to ecosystem-scale hydrological responses (Jackson et al., 1998). Here we attempt to address the partitioning of evapotranspiration into its components of soil-evaporation and leaf-transpiration under native and elevated [CO_2] conditions in the semi-arid shortgrass steppe. The aims of this study were to (1) examine the influence of precipitation variations on the separate fluxes of evaporation and transpiration in undisturbed shortgrass steppe, and (2) evaluate at the canopy scale whether the soil-water feedback we observe under elevated [CO_2] is attributable to reduced transpiration rates.

Methods

Field methods

The experiment was conducted in the shortgrass steppe region of northeastern Colorado, at the USDA-ARS Central Plains Experimental Range (CPER; 40° 40′ N, 104° 45′ W). Three main study areas were used, all on native grassland: the Open-Top Chamber (OTC) elevated [CO_2] experimental site, a nearby 'Native' undisturbed site, and a Bowen ratio (BR) tower site about 4 km distant from OTC and Native sites. At the study region, the long-term mean annual precipitation is 320 mm, and mean annual temperature averages 15.6 °C in summer and 0.6 °C in winter (Lauenroth and Milchunas, 1991). The most abundant species were the C_4 grass, *Bouteloua gracilis* (H.B.K) Lag. (blue grama), and the C_3 grasses *Stipa comata* Trin and Rupr. (needle-and-thread grass) and *Pascopyrum smithii* (Rydb.) A. Love (western wheatgrass).

The 'Native' grassland plot was established to conduct detailed analysis of changes in soil water content and isotopic composition where destructive sampling could be done. Soil water samples were collected from the Native site on 10 dates between May and November, 1999; on 11 dates between February and October, 2000; and on 9 dates between April and October, 2001. Soil pits were excavated and soil samples were removed from 8 depths (1, 3, 5, 7, 10, 15, 25, and 50 cm), quickly sealed, and stored frozen in glass vials with poly-cone seals until analysis for gravimetric soil-water content and soil-water δ^{18}O.

A Bowen ration energy balance (BR) tower (023/CO_2 Bowen Ratio System, Campbell Scientific, Inc., Logan, UT, USA) was installed on native shortgrass steppe vegetation on the CPER 4 km from the Native site. It was operated through the growing season of 1999, and year-round during 2000 and 2001. The BR system determined gradients of water vapor and [CO_2] every second from air sampled at one and two meters height above the canopy, and pumped to an infra-red gas analyzer for analysis (Model LI-6262, LiCor, Inc., Lincoln, NE, USA). Temperature gradients were also obtained at the two heights from fine-wire chromel-constantan thermocouples, and along with ancillary measurements of net radiation and soil heat flux were used to calculate fluxes of CO_2, water vapor and energy in a Bowen ratio energy balance approach every 20 min with the data logger that collected the micrometerological data (21X datalogger, Campbell Scientific, Inc., Logan, UT, USA). The calculations require the assumption that eddy diffusivity was the same for heat, water vapor, and CO_2. More details and theory of operation may be found in Dugas (1993) and Dugas et al. (1999).

The OTC site was located about 80 m away from the Native site; the soil at both sites was classified as an Ustollic Camborthid, in the Remmit fine sandy loam series. Beginning in 1997, open-top chambers (OTC; 4.5-m diameter) were used to elevate atmospheric [CO_2] to twice-ambient (720±20 μmol mol^{-1}). Chambers were placed on two plots in each of three blocks (six total) from late March until mid-October of every year. Within each block, one chamber was assigned an ambient [CO_2] treatment (AC), the other an elevated [CO_2] treatment (EC). Each block also had a nonchambered plot (NC) which served as a control. On the chambered plots, air was introduced through three 1.14 m^2 inlet ports located 0.16 m above the soil surface at an exchange rate of $\sim$1.5 chamber volumes per minute (3 m s^{-1} wind equivalent). An outlet fan at the top of each chamber helped reduce chamber pressure anomalies associated with dynamic OTC techniques (e.g., Longdoz et al., 2000; Lund et al., 1999).

Destructive samples could not be collected at the OTC site, so we used soil-CO_2 δ^{18}O as a proxy for that of soil water. Beginning in 1999, soil gas samples were collected from stainless steel tubes (1/8″ o.d.) inserted at 6 depths (3, 5, 10, 15, 25 and 50 cm) in each plot of the OTC experiment. The tubes were L-shaped, with slots cut into the terminal 5 cm, and placed horizontally into excavated pits which were subsequently backfilled (Pendall et al., 2001). Before sample collection, a 6-mm diameter section of glass tubing filled with magnesium perchlorate, capped with a rubber septum, was attached with a nylon reducing union to the stainless steel tubing, and a volume of soil air equivalent to the tubing volume (10–15 cm^3) was removed with a syringe. The tubes remained sealed for a 30-min period while the diffusion gradient stabilized. Gas samples were then collected into gas-tight glass syringes which were greased with Apiezon M. Volumes of 10 cm^3 were collected at all depths, except for the 3 cm depth, where 6 cm^3 were collected to ensure that only soil air was pulled into the syringe. Samples were collected every 2–3 weeks during the growing season, and every 4–8 weeks during the winter, on the same dates that soil water samples were collected from the Native site. At the time of gas sampling, soil temperature profiles were measured at 0, 5, 10, 20, 30, 40, and 50 cm depths; soil temperature was also recorded continuously in each plot at 10-cm depth. Weekly volumetric soil moisture content (θ) was measured by neutron probe at depths of 10–40, 40–70 and 70–100 cm.

Precipitation samples were collected for isotope analysis near the OTC and Native sites on a daily (event) basis beginning in August, 1999; therefore, our ET partitioning begins at that time. Other than collecting the samples within 24 h of precipitation events, no precautions were taken to prevent evaporation. Rainfall amounts less than 2 mm were excluded from weighted isotopic average values.

Laboratory methods

Soil samples from the Native site soil pit were stored frozen until they were equilibrated directly with CO_2 for 12 h; CO_2 was analyzed by dual-inlet mass spectrometery for δ^{18}O of soil water (Miller et al., 1999). Oxygen isotope ratios (δ^{18}O) were expressed in delta notation relative to a known reference as per mille (‰) deviations in the relationship $\delta^{18}O_{sample} = 1000(R_s/R_r - 1)$ where R_s is the ^{18}O/^{16}O ratio in the sample and R_r is the ^{18}O/^{16}O ratio in the reference. Precision was determined to be better than 0.15‰ for δ^{18}O of soil-water based on replicate analyses of soils with volumetric soil-water content ranging from 1 to 25%. Isotopic values for soil water were reported with respect to V-SMOW (Coplen, 1994). Gravimetric soil-water content was determined by weighing soils before and after drying soils at 70 °C to constant weight.

Tests indicated that CO_2 standards stored in the greased syringes used for collection of the soil gas samples could be kept for up to one week without significant leakage or isotopic exchange. Gas samples were, however, analyzed within 24 h of collection as a precaution against contamination. Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-IRMS) performed isotopic measurements as follows. Soil gas samples were injected through a sample loop varying from ~7 to ~250 μL into a He carrier gas stream, which was further split before being introduced into the mass spectrometer. Sample loop size was determined based on [CO_2], which was measured by an infrared gas analyzer, LiCor LI-6251 (Lincoln, NE, USA). This allowed the peak height of the sample to be within 10% of the peak height of the standard, to ensure a linear response of the mass spectrometer (Miller et al., 1999). Precision of the GC-IRMS technique was determined to be better than 0.15‰ for $\delta^{18}O$ measurements based on replicate analyses of standard gases. $\delta^{18}O$ was reported with respect to V-PDB for soil CO_2 (Coplen, 1994).

ET partitioning model

We used a simple isotope mass balance model to determine the fractions of water lost through soil-evaporation and leaf-transpiration (Hsieh et al., 1998). The $\delta^{18}O$ values and amount of both precipitation and soil-water are used to calculate ET partitioning as follows:

$$\chi_i\delta_i + \chi_r\delta_r = \chi_f\delta_f + \chi_v\delta_v + \chi_t\delta_t \tag{1}$$

$$m_i + m_r = m_f + m_v + m_t, \tag{2}$$

where m represents the mass of water per cm^3 of soil and δ represents the $\delta^{18}O$ value of each component. Subscripts $j = i, r, f, v,$ or t represent water fraction in the initial soil, rainfall, final soil, vapor, and transpired fractions, respectively; $\chi_j = m_j/m_{total}$. Inputs to equations (1) and (2) in the model were: precipitation amount, $\delta^{18}O$ of precipitation, depth weighted volumetric soil-water content (determined by integration over 0–50 cm depth using the commercial graphic program, Kaleidagraph) at initial and final times, and initial and final amount weighted soil-water $\delta^{18}O$ (determined by multiplying the isotopic composition by water content of each layer and dividing by the depth weighted water content). Each sampling date is the final sampling date for the prior interval, and the initial date for the following interval.

The isotope mass balance model assumes that $\delta^{18}O_v$ is in equilibrium with $\delta^{18}O_f$, so we therefore used the temperature-dependent equilibrium fractionation factor of Majoube (1971) based on the soil temperature at 5-cm depth. This fractionation factor makes the vapor phase about 9.5‰ depleted in ^{18}O compared with the liquid phase at 25 °C. This phase-change fractionation is the main source of the ^{18}O enrichment observed in evaporating soil water profiles. The model also assumes that $\delta^{18}O_t$ can be represented as the amount-weighted average of $\delta^{18}O_i$ and $\delta^{18}O_r$, and that precipitation occurs as a pulse immediately after initial sampling, with evapotranspiration occurring up to final sampling (Hsieh et al., 1998). We solved for χ_v and χ_t, the evaporation and transpiration fractions, and then determined fluxes by multiplying the fractions by the change in soil water content between sampling dates.

From the Native grassland site, we demonstrated that equilibrium exists between soil $C^{18}O^{16}O$ and soil $H_2{}^{18}O$. Thus, we used depth weighted soil-CO_2 $\delta^{18}O$ as a proxy for soil-water $\delta^{18}O$ inside the OTC, where destructive harvesting of soil was not possible. We multiplied the 10–40 cm neutron probe measurements by 95% as an estimate of average water content in the top 50 cm (based on detailed observations in the nearby soil pit).

Statistical analysis

Repeated measures analysis of variance (ANOVAR) was used to evaluate differences in soil CO_2 $\delta^{18}O$ values among [CO_2] treatments, with soil depth and year as secondary effects. The repeated measure was date of sampling. Because we found significant [CO_2] by year interactions, we tested each year separately. ANOVAR was also used to evaluate significance of differences in soil water content and soil temperature in the OTC experiment. Paired *T*-tests were used to evaluate differences in evaporation and transpiration rates under elevated [CO_2].

Results

Dynamics of native grassland soil water content and isotopic composition

Precipitation at the study site contrasted strongly between 1999, 2000 and 2001 (557 mm, 311 mm and 348 mm, respectively; Figure 1). Soil water content in the Native plot averaged between 7 and 15% by volume over the nearly 3-year study period, but on individual sampling dates the range varied from 2 to

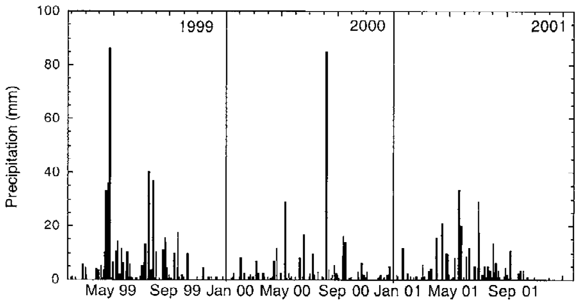

Figure 1. Precipitation amount (April, 1999, to October, 2001) at Central Plains Experimental Range field station, about 200 M distance from Open-Top Chamber and Native grassland sites.

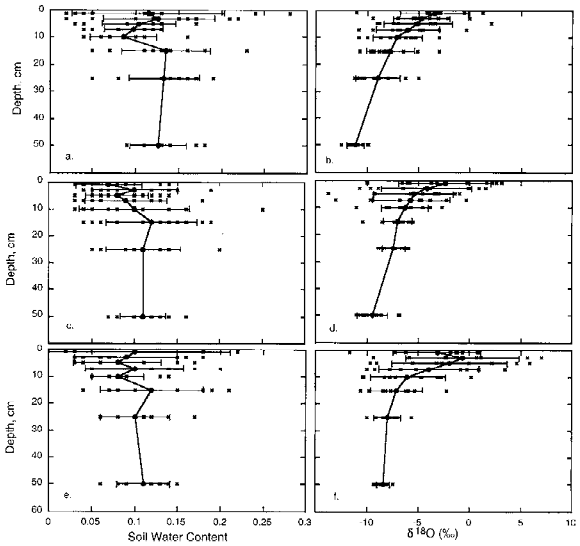

Figure 2. Soil water content and isotopic composition at the Native grassland site. Squares and solid lines, average for each season; error bars, standard deviation for each season; crosses, values for each sampling date. (a), (c) and (e) show soil water content for 1999, 2000 and 2001; (b), (d) and (f) show δ^{18}O values for 1999, 2000 and 2001, respectively.

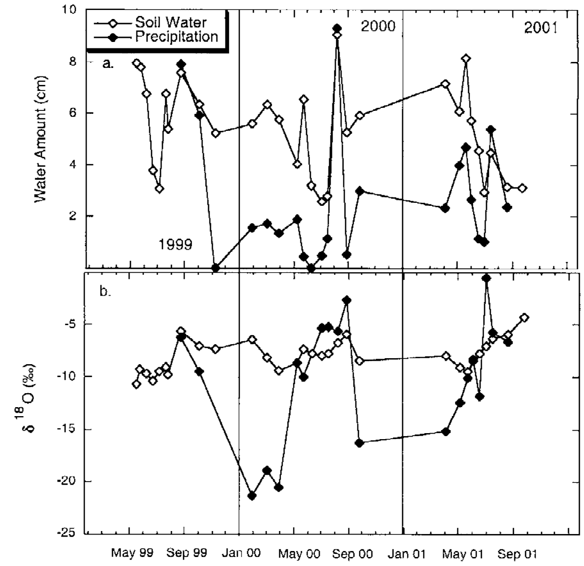

Figure 3. Inputs for isotope mass balance model for Native grassland site. (a) Time series (May, 1999 to October, 2001) of precipitation amount and depth weighted-soil water content the 50-cm profile. (b). Time series of δ^{18}O values of precipitation and amount weighted-soil water in the 50-cm profile.

30% (Figure 2a, c, e). Temporal variations in depth-weighted soil water content for each sampling date, and the precipitation falling during the period preceding the sampling date, are shown in Figure 3a. Rainfall in April and May of 1999 was above average, and led to high soil moisture contents in early summer. Soil moisture decreased rapidly in June and July, until monsoon rains re-wetted the profile in August and September. Very little precipitation fell from November of 1999 to August of 2000, when a 9-cm rainfall event recharged the profile. Soil moisture and precipitation amount were closer to average in 2001, but as very little rain fell after May, soils gradually dried.

The average δ^{18}O value of soil water decreased with depth in 1999 and 2000 (Figure 2b, d), as has been observed in evaporation profiles (Allison and Barnes, 1983). In 2001, the highest average δ^{18}O value was at a depth of 3 cm rather than at the surface (1 cm), most likely because of vapor equilibration with moisture in the top cm (Figure 2f). Average values were slightly higher at most depths in 2000 than in 1999 or 2001, although some individual dates in 2001 had δ^{18}O values of +5 to +7‰(August – October), demonstrating significant evaporative enrichment (Figure 2b, d, f). Depth weighted soil water δ^{18}O values were less variable than precipitation inputs (Figure 3b). Soil water was more enriched than precipitation values during the winters and early growing seasons, and similar to precipitation values following summer rainfall events (Figure 3b). Isotopically depleted precipitation inputs (ca. –20‰) in January – April, 2000, had little influence on δ^{18}O of soil water, because the amounts were low, and precipitation occurred as snow, which did not infiltrate much before sublimating or blowing away. The increasing trend of amount weighted soil water δ^{18}O during summer of 2001 was mainly attributed to a drying trend, but small inputs of relatively enriched rainfall also contributed to the effect (Figure 3a, b).

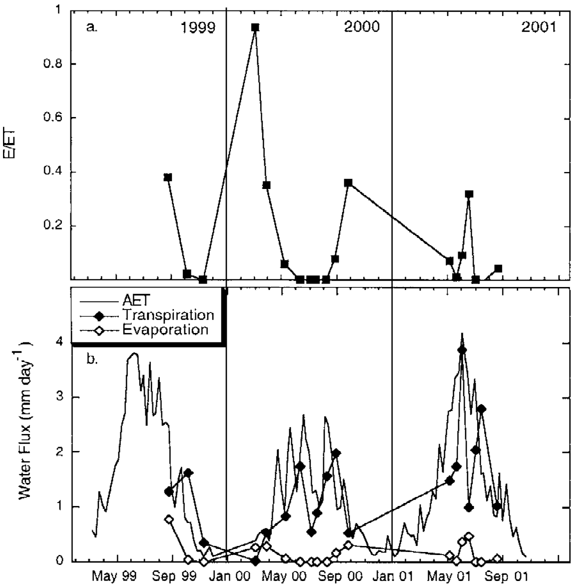

Figure 4. (a) Ratio of evaporation to evapotranspiration estimated from isotope mass balance model (August, 1999, to October, 2001) at the Native shortgrass steppe site. (b) Actual evapotranspiration (AET) at the Bowen ratio tower compared to transpiration and evaporation at the Native site estimated from the isotope mass balance model (1999 – 2001).

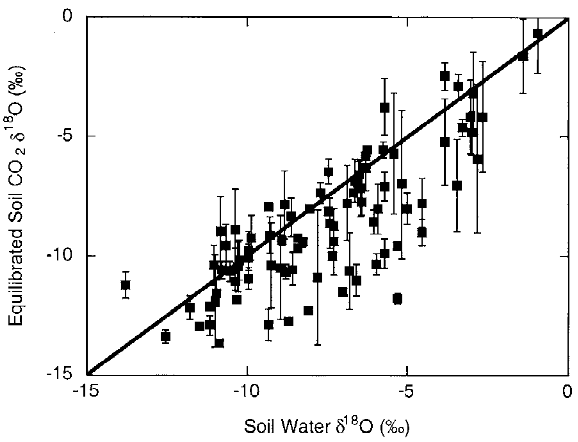

Figure 5. Equilibrated Soil-CO_2 $\delta^{18}O$ – water-$\delta^{18}O$ correlation for 1999 and 2000. Measured soil-CO_2 $\delta^{18}O$ at depths 5, 10, 15, 25, and 50 cm were adjusted to the soil–water $\delta^{18}O$ measurements at the same depths using measured temperature profiles. The soil-water measurements were obtained from the Native grassland site located 80 M from the non-chambered control plots from which the soil-CO_2 $\delta^{18}O$ measurements were obtained. Error bars on soil CO_2 represent standard deviation of the mean (n=3), and on soil H_2O they show analytical error.

Evapotranspiration partitioning in native grassland

We applied the isotope mass balance model (Equations 1 and 2; Hsieh et al., 1998) to the soil water data from the Native grassland site. The amount and isotopic composition of precipitation and soil water (amount weighted profile averages), as shown in Figure 3, were used as inputs to the model. We calculated the proportion of evaporation to total loss by evapotranspiration (E/ET) over 2 years (Figure 4a). E/ET was close to nil during the growing season, except for periods in midsummer of 1999 and 2001, when drying soils reduced plant activity, but enough moisture was available for evaporation. During the summer of 2000, rainfall was scarce enough that E/ET remained near nil.

We estimated average daily evaporation and transpiration fluxes for each time interval by multiplying the fraction of each flux by the total moisture for that interval (Figure 4b). Transpiration was much greater than evaporation except during early spring and late fall, when plants were less active. In 2000 and 2001, transpiration had a bimodal distribution, with peaks in early and late summer and lower values during the midsummer dry period. Although these two years received similar annual rainfall, rates in 2001 were roughly double those of 2000 because of the timing of precipitation. The dry spring of 2000 reduced biomass growth, and the late August rainfall was not utilized by the shortgrass community. By contrast, the much wetter spring of 2001 favored growth, and hence higher transpiration rates, throughout the growing season. In the middle of the 2001 growing season, enough moisture was available in upper soil layers for both evaporation and transpiration to occur.

The sum of evaporation and transpiration estimated using the isotope mass balance approach compares well with actual evapotranspiration (AET) measured from the Bowen ratio system located 4 km away (Figure 4b). Although more detail is apparent in the AET measurements, the isotope based estimates successfully captured intra- and inter-seasonal variations. Because the isotope method is labor intensive, measurements were made infrequently, but it offers the potential to estimate the evaporation and transpiration fluxes separately. Thus, it may be best suited for use in conjunction with more continuous measurements.

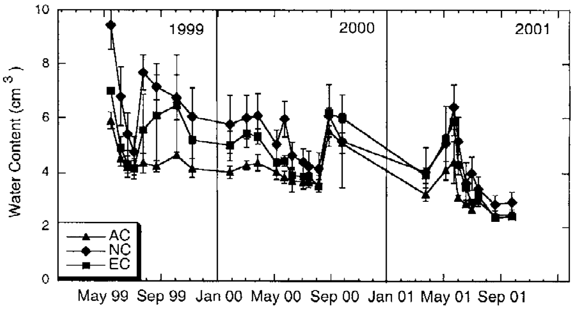

Figure 6. Soil water content as measured by neutron probe, total volume of water in 10–40 cm depth, at Open-Top Chamber experiment (May, 1999, to October, 2001). AC, ambient chambers; EC, elevated [CO_2] chambers; NC, non-chamber control plots. Error bars show standard deviation of the mean.

Evaluation of $\delta^{18}O$ equilibrium between soil-CO_2 and soil-water

The OTC experimental design precluded destructive collection of soil-water samples for measurement of δ^{18}O, but our permanently installed soil gas probes provided a detailed record of δ^{18}O in soil CO_2. Several studies have previously shown equilibrium between δ^{18}O of soil-CO_2 and that of soil water (Hesterberg and Siegenthaler, 1991; Stern et al., 2001; Tang and Feng, 2001). This occurs because the rate of equilibration is at least as rapid as the rate of diffusion out of the soil, at least up to about 5-cm depth (Miller et al., 1999). Nonetheless, we tested this assumption to verify CO_2-H_2O isotopic equilibration for these soils. Soil temperature measurements were used to calculate soil-water δ^{18}O values (on the V-SMOW scale; Coplen, 1994) from our soil-CO_2 δ^{18}O measurements (V-PDB scale; Coplen, 1994) at 3, 5, 10, 15, 25, and 50-cm depths. We refer to these calculated values as 'equilibrated soil-CO_2 δ^{18}O.' Equilibrated soil-CO_2 δ^{18}O was strongly correlated with soil-water δ^{18}O values measured in the soil pit near the chambers (Figure 5; y = –2.4 ±0.5 + 0.8±0.07 x; R^2 = 0.64, $P <$ 0.0001). We used equilibrated soil-CO_2 δ^{18}O values as an approximation of soil water δ^{18}O, but because the intercept was –2.4‰and the slope was less than 1, the equilibrated values were systematically lower than soil water values. We therefore used a conservative approach to estimating evaporation rates in the OTC experiment.

Effects of elevated [CO_2] and open-top chambers on the shortgrass steppe

Within the Open-Top Chamber plots there were significant [CO_2], year, and [CO_2] × year differences in soil water content (Figure 6). Overall, 2001 was the driest year ($P<0.0001$), AC treatment soils were drier than EC soils, and EC soils were drier than NC soils ($P<0.0001$) for the three years. Low water content in the AC treatment carried through the unchambered winter periods (Figure 6). Aboveground biomass production was highest under elevated [CO_2] and lowest in the nonchambered plots, and was generally lower in 2000 than 2001 owing to a lack of spring rainfall (Table 1). Soil temperatures at a depth of 10 cm were ~1 °C higher in the chambered plots than in the NC plot, except during the unchambered winter period (data not shown). During the growing season of 1999, this difference was not significant, but during the drier growing seasons of 2000 and 2001, chambered treatments were significantly warmer than unchambered plots ($P<0.001$ for both years).

Soil-CO_2 $\delta^{18}O$ and evapotranspiration partitioning under elevated [CO_2]

Seasonally averaged soil-CO_2 δ^{18}O values for each year are shown in Figure 7a, b, c (non-growing season data omitted for clarity). Average δ^{18}O values decreased with depth in all treatments and all years. In 1999, the sampling dates used were only from July to October, whereas in 2000 and 2001, dates from April to October were included. As a result, average δ^{18}O values in 1999 were somewhat higher than in 2000 or 2001 (Figure 7a, b, c). Soil CO_2 δ^{18}O values showed significant effects of [CO_2] treatment, depth, and time, but the significance varied by year (Table 2). Depth in the soil and sampling date within each year always showed significant effects on soil CO_2 δ^{18}O values. All depths were significantly different from one another except 3 and 5 cm-depths, 10 and 15 cm-depths, 25 and 50 cm-depths in 2000 and 15 and 50 cm-depths in 2001 ($P<0.05$). [CO_2] treatment effects were significant only during the 2000 season, when soil CO_2 δ^{18}O was significantly enriched in AC plots relative to EC and NC plots ($P<0.0001$; Table 2, Figure 7).

Using the simple mass balance model of Hsieh et al. (1998) we investigated the partitioning of evapotranspiration in detail for the growing seasons of 2000 and 2001. As expected, transpiration rates were always much higher than evaporation rates; and rates

Table 1. Seasonal transpiration flux and transpiration-use efficiency (TUE) at the OTC site compared with TUE at the nearby Native grassland site. AC, ambient chambers, EC, elevated [CO_2] chambers, NC, nonchambered plots

Site	2000			2001		
	Biomass ($g\ m^{-2}$)	Transpiration (mm)	TUE ($g\ m^{-2}\ mm^{-1}$)	Biomass ($g\ m^{-2}$)	Transpiration (mm)	TUE ($g\ m^{-2}\ mm^{-1}$)
AC	76.7	199	0.39	102.3	226	0.45
EC	128.4	235	0.55	136.7	228	0.60
NC	51.2	269	0.19	67.0	227	0.30
Native Grassland	51.2	211	0.24	67.0	272	0.25

Table 2. Repeated measures analysis of variance results for $\delta^{18}O$ of soil CO_2 in Ambient Chambers, Elevated Chambers, and Non-Chambered plots for the years 1999, 2000, and 2001. DF, degrees of freedom; *P*-value, level of significance

Effect	1999		2000		2001	
	DF	*P*-value	DF	*P*-value	DF	*P*-value
[CO_2] Treatment	2	0.2334	2	<0.0001	2	0.5453
Depth	5	<0.0001	5	<0.0001	4	<0.0001
[CO_2]*Depth	10	0.2481	10	0.1087	8	0.252
Repeated Measure						
Date	2	<0.0001	6	<0.0001	6	<0.0001
Date*[CO_2]	4	0.2548	12	<0.0001	12	0.0883
Date*Depth	10	0.0011	30	<0.0001	24	<0.0001
Date*[CO_2] *Depth	20	0.0348	60	0.5098	48	0.0146

Table 3. Growing season (May – October) average values of transpiration and evaporation rates, and the ratio of evaporation to evapotranspiration (E/ET), for 2000 and 2001, estimated from the isotope mass balance method. AC, Ambient [CO_2] chambers, EC, elevated [CO_2] chambers, NC, non-chambered control plots. Seasonal standard deviations in parentheses. Within a column, values followed by the same letter are not different (paired *t*-tests, $P<0.1$)

Site	2000			2001		
	Transpiration Rate (mm d^{-1})	Evaporation Rate (mm d^{-1})	E/ET	Transpiration rate(mm d^{-1})	Evaporation Rate(mm d^{-1})	E/ET
AC	1.13 (1.78)a	0.33 (.32)a	0.44 (.29)a	1.67 (1.39)a	0.24 (.18)a	0.19 (.22)a
EC	1.28 (1.88)b	0.25 (.32)b	0.31 (.29)b	1.71 (1.34)a	0.32 (.23)a	0.20 (.17)a
NC	1.38 (1.76)c	0.22 (.24)b	0.25 (.20)c	1.66 (1.27)a	0.51 (.35)b	0.25 (.17)a
Native grassland	1.15 (0.60)	0.07 (0.11)	0.07 (.13)	1.99 (1.03)	0.14 (0.19)	0.07 (.11)

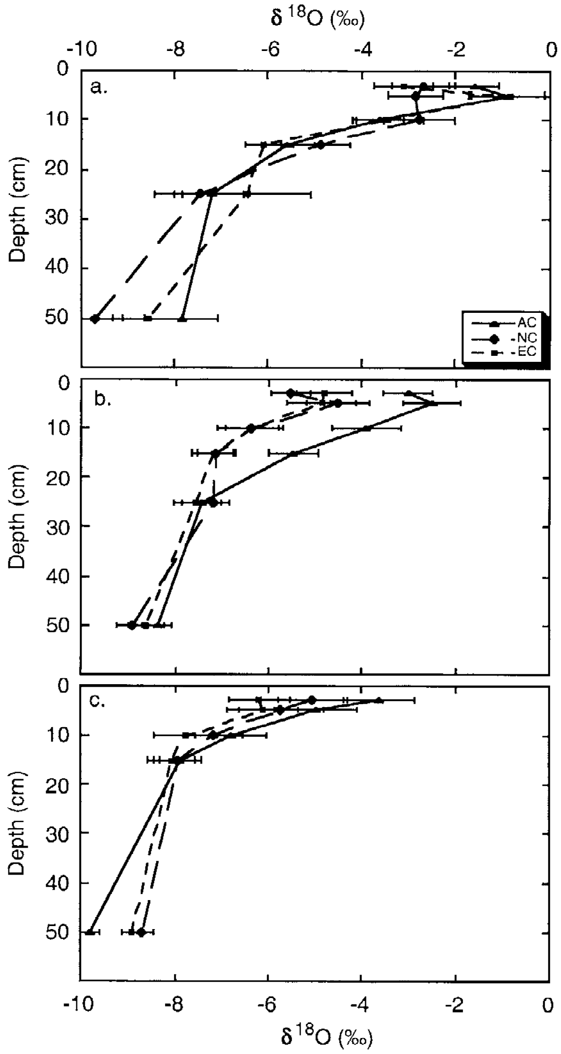

Figure 7. Average δ^{18}O values of soil water at Open-Top Chamber experiment for (a), 1999; (b), 2000; (c), 2001. Growing season data was included; winter data was excluded for clarity. Error bars show growing season standard deviations (n=3 for 1999, 6 for 2000 and 2001). AC, ambient chambers; EC, elevated [CO_2] chambers; NC, non-chamber control plots.

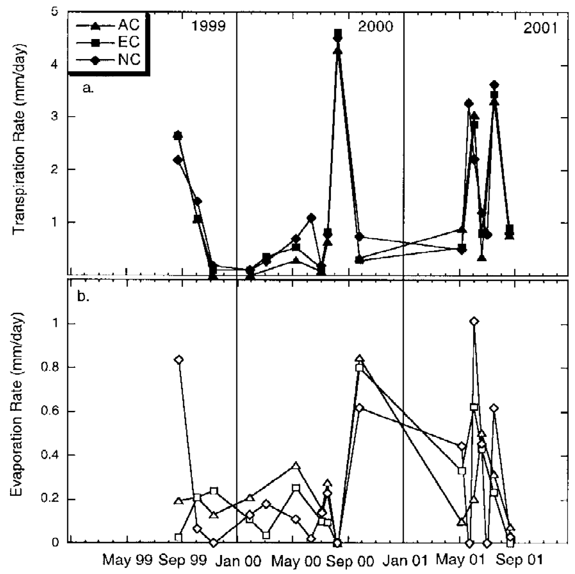

Figure 8. (a), Transpiration rate; and (b), Evaporation rate, from Open-Top chamber experiment from August, 1999, to October, 2001. AC, ambient chambers; EC, elevated [CO_2] chambers; NC, non-chamber control plots. Note difference in scales in parts a and b.

were higher in 2001 than in 2000 (Figure 8, note scale difference in parts a and b). Average transpiration rates were lowest in the AC and highest in the NC treatments of the OTC experiment in 2000 (P<0.1), but were similar among treatments in 2001 (Table 3). Evaporation rate from AC plots was higher than from NC or EC plots in 2000, but was higher from NC than AC or EC plots in 2001 (P<0.1). The fraction of evaporation (E/ET) was highest from AC and lowest from NC plots in 2000, but no differences were observed in 2001 (Table 3). Transpiration rates estimated from the Native grassland site were of similar magnitude to those in the OTC experiment for both years, but evaporation rates and E/ET were lower than in the OTC experiment (Table 3).

We estimated growing season transpirational water use by integrating the area under the curve in Figure 8, and calculated transpiration use efficiency (TUE; aboveground biomass produced per mm water transpired in a growing season; Table 1). Our use of TUE distinguishes water used by plants, whereas water-use efficiency (WUE) includes both evaporation and transpiration losses. TUE was higher in 2001 than in 2000 for all treatments, reflecting the importance of spring moisture to growth in this ecosystem. Total transpiration on EC plots was similar to that on AC plots in 2000 and 2001. TUE appeared to be highest under elevated [CO_2], lowest in non-chambered control plots, and intermediate in ambient chambers (Table 1). In the Native grassland plot, total transpiration was 211 mm in 2000 and 272 mm in 2001. Assuming that the Native plot had similar aboveground biomass yield as the nearby NC plots, TUE was about 0.25 g m^{-2} mm^{-1} in both years, similar to TUE values on the NC plots (Table 1).

Discussion

Soil water isotopes and water fluxes on the native shortgrass steppe

Soil water content and isotopic composition at the native grassland site changed dynamically over the 2 1/2-year-long study period, especially in the upper 15 cm of the soil profile. The sandy loam texture of the soil, with a field capacity of about 18%, contributed to the wide seasonal variations. Evaporative enrichment of soil water of up to 15‰ occurred in the upper 10 cm, relative to the base of the soil profile (50-cm depth). In a semi-arid region of Hawaii, maximum enrichment of ^{18}O was only 8‰from the base to top of the profile, and reflects the higher clay content and water holding capacity of that soil (Hsieh et al., 1998). At our Native grassland plot, the average $\delta^{18}O$ value at 50-cm depth was about –10‰, the same as that of groundwater from a nearby well (12-m depth), sampled on 3 occasions in 1998–1999 (Pendall, unpublished data). The isotopic composition of groundwater in shallow aquifers was representative of annual average rainfall inputs at other sites in the southwestern US (Pendall, 1997).

Temporal variations in $\delta^{18}O$ of soil water were damped compared to those of precipitation, owing to mixing of inputs with antecedent moisture which had experienced evaporation. Soil water isotopic composition, especially above about 15-cm depth, only reflected that of precipitation during short intervals immediately following rainfall events. This has implications for global-scale modeling of the $\delta^{18}O$ values of atmospheric [CO_2], because $\delta^{18}O$ of soil water exerts a strong control over that of respired CO_2, and these models assume that soil water is isotopically identical to precipitation (e.g., Ciais et al., 1997a, b; Farquhar et al., 1993; Peylin et al., 1999). In general, $\delta^{18}O$ of soil water was higher than precipitation in winter, and slightly lower than precipitation in summer.

Water loss at our Native grassland site occurred primarily by transpiration, but E/ET varied from nil to over 90% on different sampling dates. Seasonal transpiration was 68 and 78% of annual precipitation in 2000 and 2001, respectively. Transpiration losses were 32% of mean annual precipitation in the Chihuahuan desert of New Mexico (Reynolds et al., 2000) and 38% in the winter-moist Patagonian steppe of Argentina (Paruelo et al., 2000). The shortgrass steppe ecosystem apparently utilizes water more efficiently than the desert grasslands of southern New Mexico, or than the C3 grasslands in temperate South America.

The seasonality of precipitation in the two different years strongly affected the transpiration flux, and to a lesser degree, the evaporation flux. Total precipitation in 2000 and 2001 was similar, but spring of 2000 was dry, and spring of 2001 was relatively moist. In 2000, water was severely limiting to plant growth, and lower average transpiration rates ($\sim$1 mm d^{-1}) were attributed to low biomass growth, and to the fact that a significant portion of the year's rain fell near the end of the growing season. In 2001, ample spring rains promoted biomass growth, and transpiration rates were double those of 2000 ($\sim$2 mm d^{-1}). In June, 2001, enough moisture remained in the upper soil layers that some evaporation occurred ($\sim$0.5 mm d^{-1}), but during the remainder of the progressively drying 2001 growing season, no evaporation occurred.

Soil water isotopes and water fluxes under elevated [CO_2]

In this study, care was taken to ensure that all nine treatment plots received rainfall inputs that were as similar as possible. However, we observed that soil water content (θ) in the AC plot was significantly lower than NC and EC plots. The most significant effect of elevated [CO_2] on the hydrologic budget in water limited ecosystems is likely to be an increase in soil water storage (Jackson et al., 1998). Soil temperature was slightly higher in the chambered treatments than in nonchambered controls, a typical result of OTC experiments where increases of 1–3 °C in air temperature are common (van Oijen et al., 1999). Warmer temperatures would promote evaporation, and partly account for the lower θ observed in AC relative to NC soils. In addition, the continuous air movement (3 m s^{-1}, 24 h d^{-1}) created by the blowers probably increased evaporation in chambered compared to nonchambered plots. The effect of elevated [CO_2] on θ in this experiment is therefore a combination of the chamber effect and any ecosystem-level effects caused by elevated [CO_2]. Wetter soils in the EC plot relative to the AC plot are most likely a result of improved soil-water conservation as a result of reduced stomatal conductance under elevated CO_2 (Bunce, 2001; Morgan et al., 2001). Modeling results showed that increased soil water recharge was an important effect of elevated [CO_2] in an annual grassland in California (Jackson et al., 1998). More detailed explanations for improved soil-water conservation were explored using the isotope partitioning model.

During the 2000 growing season, $\delta^{18}O$ of soil CO_2 was significantly higher in AC than EC or NC plots, suggesting that evaporation rates were highest from AC soils. Apparently, the presence of chambers altered pathways of water loss, at least during a dry year. Evaporation rates and E/ET were lower in EC than AC plots, possibly because greater biomass contributed to a deeper litter layer and less bare ground. In 2000, average transpiration rates and total loss by transpiration were greater in elevated [CO_2] than in ambient chambers, because total biomass was greater, and more water was available in soils for transpiration than in the drier AC treatment. In 1999 and 2001, $\delta^{18}O$ values varied by depth, but were similar on all treatments, suggesting that water losses were following similar pathways in all treatments. However, significant interactions among [CO_2] treatment, sampling date and depth were observed in 1999 and 2001, suggesting that on certain dates or depths, a chamber and/or [CO_2] treatment effect was important. Simulations by Jackson et al. (1998) also indicated that interannual and seasonal variability in rainfall amount was a key factor in controlling the response of grassland ecosystem water fluxes to elevated [CO_2].

Transpiration use efficiency was highest under elevated [CO_2] and lowest in non-chamber control plots in both 2000 and 2001, suggesting that both a [CO_2] effect and a chamber effect contributed to more efficient use of water. Biomass production on AC plots was consistently greater than on NC plots, despite drier and warmer soils inside the chambers. It is possible that the warmer, chambered soils stimulated N mineralization, and that this greater N availability was responsible for the enhanced biomass growth and higher TUE that we observed in AC plots. Additionally, the presence of chambers may have lengthened the growing season slightly by increasing the rate of soil warming in spring.

In this study over a mixed C_3/C_4 grassland we have not attempted to distinguish between C_3/C_4 competition but as Morgan et al. (2001) and Bunce (2001) discussed, the amount of reduced stomatal conductance and hence leaf-transpiration as a result of elevated [CO_2] varies by species (and photosynthetic pathway); hence, the partitioning of ET could significantly change over time as plant communities evolve under elevated [CO_2]. Thus, monitoring species composition and competition with time in response to [CO_2] may be important in future studies of water loss pathways.

Limitations of the isotope partitioning approach

The sum of evaporation plus transpiration estimated using the isotope mass balance model was very similar to actual evapotranspiration measurements from a nearby Bowen ratio system, suggesting that our approach is robust. However, several assumptions may contribute to erroneous results in the isotope mass balance model. Our use of equilibrated soil-CO_2 $\delta^{18}O$ as a proxy for soil-water $\delta^{18}O$ in the OTC plots is an approximation that may underestimate the influence of evaporation. The equilibrated soil-CO_2 $\delta^{18}O$ values were lower than the soil-water $\delta^{18}O$ because near the top of the soil profile (above 5-cm depth), diffusion occurs more rapidly than equilibration, and the soil CO_2 carries a $\delta^{18}O$ value that is influenced by deeper soil water that is depleted in ^{18}O.

The most significant simplification of the isotope model comes from treating all inputs as a single recharge event, followed by continuous losses. In about 10% of the cases, arithmetic solutions to the model equations resulted in negative values, as observed by Hsieh et al. (1998). These results were omitted from the averages. The $\delta^{18}O$ value assigned to transpiration, δ_t, was the amount weighted average of precipitation inputs and soil water, but if frequent recharge events partially reset the soil water profile, this δ_t value would not be accurate. We also assumed that transpiration occurred at steady state, but non-steady state conditions are likely to occur, especially during dry or exceptionally humid periods (Harwood et al., 1998; Pendall, 1997). The assumption of vapor-precipitation equilibrium was probably valid, as evidenced by a study in the southwestern USA (Pendall, 1997). Direct measurements of vapor and of the transpiration stream would reduce dependence on these assumptions, but resources to perform these measurements were unavailable for this study. Despite these limitations, the isotopic approach has merit, and can provide additional insight into pathways of water loss from natural and managed ecosystems.

Summary and conclusions

Over the period from May 1999 to October 2001, we investigated the dynamics of soil water isotopes and water cycling in a mixed C_3/C_4 grassland in the western Great Plains region of the USA An isotope mass-balance model used to estimate evaporation and transpiration compared well with actual evapotranspiration measured from a nearby Bowen ratio tower. Transpiration use efficiency was $\sim$0.25 g m^{-2} mm^{-1} on native grassland, with 68–78% of annual precipitation used in transpiration. Elevated [CO_2] had the effect of increasing soil-water conservation as has been previously found (e.g., Morgan et al., 2001; Volk et al., 2000). The isotope mass balance approach suggested that reduced evaporation was mainly responsible for greater soil water content under elevated [CO_2]. Although transpiration use efficiency was much higher under elevated than ambient [CO_2], total water transpired in the elevated [CO_2] treatment was at least as much as in ambient chambers, owing to higher biomass (and thus leaf area) under elevated [CO_2]. Slight reductions in soil-evaporation in the EC treatment may have been caused by its more densely vegetated surface restricting vapor diffusion flux.

Additional field [CO_2] enrichment studies such as this, combined with modeling and process-level studies are required to further improve our understanding of global-δ^{18}O and ecosystem response to changing environmental forcing factors. More accurate estimates of soil water δ^{18}O values, based on empirical relationships between precipitation, climate and soil texture, would improve global-scale C cycle models that use inversion techniques to estimate gross fluxes of photosynthesis and respiration (e.g., Riley et al., 2002). Advances in measuring the isotopic composition of small atmospheric water vapor samples, together with co-measurement of net fluxes (from gradient or eddy correlation techniques), will enable ET to be partitioned between its gross components over large areas (Brunel et al., 1992; Moreiea et al., 1997; Wang and Yakir, 2000), and thus improve our understanding of surface-atmosphere water and energy exchange.

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