Oecologia (1998) 117:504–512

**M.B. Dodd · W.K. Lauenroth · J.M. Welker**

# Differential water resource use by herbaceous and woody plant life-forms in a shortgrass steppe community

Received: 23 December 1997 / Accepted: 16 September 1998

**Abstract** We conducted a study to test the predictions of Walter's two-layer model in the shortgrass steppe of northeastern Colorado. The model suggests that grasses and woody plants use water resources from different layers of the soil profile. Four plant removal treatments were applied in the spring of 1996 within a plant community codominated by *Atriplex canescens* (a $C_4$ shrub) and *Bouteloua gracilis* (a $C_4$ grass). During the subsequent growing season, soil water content was monitored to a depth of 180 cm. In addition, stem and leaf tissue of *Atriplex*, *Bouteloua* and the streamside tree *Populus sargentii* were collected monthly during the growing seasons of 1995 and 1996 for analysis of the $\delta^{18}O$ value of plant stem water (for comparison with potential water sources) and the $\delta^{13}C$ value of leaves (as an indicator of plant water status). Selective removal of shrubs did not significantly increase water storage at any depth in the measured soil profile. Selective removal of the herbaceous understory (mainly grasses) increased water storage in the top 60 cm of the soil. Some of this water gradually percolated to lower layers, where it was utilized by the shrubs. Based on stem water $\delta^{18}O$ values, grasses were exclusively using spring and summer rain extracted from the uppermost soil layers. In contrast, trees were exclusively using groundwater, and the consistent $\delta^{13}C$ values of tree leaves over the course of the summer indicated no seasonal changes in gas exchange and therefore minimal water stress in this life-form. Based on anecdotal rooting-depth information and initial measurements of stem water $\delta^{18}O$, shrubs may have also had access to groundwater. However, their overall $\delta^{18}O$ values indicated that they mainly used water from spring and summer precipitation events, extracted from subsurface soil layers. These findings indicate that the diversity of life-forms found in this shortgrass steppe community may be a function of the spatial partitioning of soil water resources, and their differential use by grasses, shrubs, and trees. Consequently, our findings support the two-layer model in a broad sense, but indicate a relatively flexible strategy of water acquisition by shrubs.

**Key words** Plant functional types · Selective removal · Shortgrass steppe · Soil water · Stable isotopes

## Introduction

In environments where water availability is an important control in ecosystem structure, it has been suggested that the exploitation of spatially and/or temporally distinct zones of soil moisture by plants allows the coexistence of different life-forms (Noy-Meir 1973). Walter (1979) also addressed this hypothesis by proposing a simple two-layer conceptual model of soil and plant interactions. The model suggests that two generalized life-forms (shrubs and grasses) can coexist at a site where their differing rooting patterns coincide with the vertical separation of soil water resources. Distinct soil water resources can be created by climatic patterns and texture effects on deep percolation and evaporative losses (Sala et al. 1997). Differing water acquisition strategies among life-forms (or more recently 'plant functional types;' Gitay and Noble 1997) may also have a temporal element, e.g., grasses are more opportunistic, utilizing

---

M.B. Dodd (✉)[1] · W.K. Lauenroth[2]
Department of Rangeland Ecosystem Science,
Colorado State University, Fort Collins, CO 80523, USA

J.M. Welker[2]
Department of Rangeland Ecology and Watershed Management,
University of Wyoming, Laramie, WY 82071, USA

*Present addresses*:
[1] New Zealand Pastoral Agriculture Research Institute Ltd.
(AgResearch), Ruakura Research Centre, Private Bag 3123,
Hamilton, New Zealand
e-mail: doddm@agresearch.cri.nz, Fax: +64-7-8385130

[2] Natural Resource Ecology Laboratory,
Colorado State University, Fort Collins, CO 80523, USA

the short-term availability of water in upper soil layers, while shrubs rely on a deeper soil water resource that is more stable in the long term (Soriano and Sala 1983). This conceptual model is supported by evidence from studies in the Patagonian steppe (Soriano and Sala 1983; Sala et al. 1989), African savannas (Walker and Noy-Meir 1982; Knoop and Walker 1985), and California oak woodlands (Gordon et al. 1989). Our investigations of the patterns of soil water associated with soil types and plant communities (Dodd 1997; Dodd and Lauenroth 1997) indicated that the model was also applicable to the North American shortgrass steppe.

The vegetation of the shortgrass steppe region is dominated by the $C_4$ bunchgrass *Bouteloua gracilis*. One of the major reasons for the abundance of *Bouteloua* is its ability to access water that has a relatively short residence time in shallow soil layers (i.e., 4–15 cm; Sala et al. 1992). Within the grasslands, limited areas of shrub vegetation are often associated with coarse-textured soils (Lauenroth and Milchunas 1991), and trees are occasionally associated with water channels, many of these being ephemeral streams. In this study, we report the results of two experiments designed to determine whether spatial and/or temporal separation of water acquisition by grasses and woody plants occurs in this semiarid shortgrass steppe community.

In the first experiment, we tested the predictions of the resource-partitioning hypothesis in a similar manner to Sala et al. (1989), using selective vegetation removal. First, we predicted that the removal of grasses would decrease total community water acquisition and consequently increase water storage in upper layers of the soil. Additionally, we aimed to determine whether water resources freed by the grass removal could move to lower soil layers, to be utilized by shrubs. Second, we predicted that the removal of shrubs would decrease total community water acquisition and consequently increase water storage in lower soil layers.

Stable isotope methods offer another means to identify the water sources utilized by individual plants. Where there is seasonal variation in the isotopic abundance ratios of precipitation (Dansgaard 1964), the natural abundance of hydrogen and oxygen isotopes in water can be used as a 'signature' of potential plant water sources. In recent years, a growing number of studies have used the D/H and $^{18}O/^{16}O$ ratios of water samples to determine the water sources of particular plant species (White et al. 1985; Ehleringer et al. 1991; Flanagan et al. 1992; Le Roux et al. 1995; Welker et al. 1995). The isotopic abundance ratios of water extracted from plants can be compared with those of potential source waters, e.g., concurrent precipitation, groundwater stored in aquifers, and free water in lakes and streams on the surface. Whole-plant water relations can also be examined by the analysis of carbon isotope abundance values in leaf tissue (Farquhar et al. 1989). Plants that experience water stress typically close their stomata to minimize water loss by transpiration, thereby reducing the ratio of internal to ambient $CO_2$ ($c_i/c_a$). Under conditions of reduced $c_i/c_a$, plants do not discriminate against $^{13}C$ to as great a degree during photosynthesis, and therefore plant tissue becomes relatively enriched in $^{13}C$ (Farquhar et al. 1989; Ehleringer et al. 1992). The implication of this for water source studies is that plants using plentiful and reliable water resources (e.g., groundwater) should exhibit only minimal changes in isotopic abundance ratios during the growing season, whereas plants using depletable water resources (e.g., soil water in upper layers) are likely to show an enrichment in $^{13}C$ as drought conditions progress (Smith and Osmond 1987; Smedley et al. 1991; Mole et al. 1994).

In the second experiment reported here, we used both these stable isotope techniques to examine: (a) whether different oxygen isotope ratios of water occurred in the major potential sources of water at the site; (b) whether these values provided evidence of differential utilization of these sources by the three plant functional types identified (grasses, shrubs, and trees); (c) whether there was any indication of changes in water source utilization by the plants during the growing season, and (d) whether the carbon isotope ratios of leaf tissue showed evidence of changes in $c_i/c_a$, symptomatic of changes in leaf gas exchange and shifts in plant water status.

We anticipated that a combination of our two approaches (selective vegetation removal and isotopic studies) would provide a reliable test of Walter's two-layer water resource model and would elucidate the nature of competitive interactions among important plant functional types in the shortgrass steppe.

## Methods and materials

### Study site location

The study was conducted at the Central Plains Experimental Range (CPER), located in the shortgrass steppe of northeastern Colorado (40°51′N, 104°43′W, elevation 1650 m). The experimental site was a mixed shrubland/grassland area adjacent to an ephemeral stream bed (Owl Creek). The soil type at this site is an Ascalon-Vona loamy sand (Ustollic Haplargid; Crabb 1982). The vegetation of this area is characterized by a high population density ($0.43 \pm 0.01$ shrubs $m^{-2}$) of *Atriplex canescens* (Pursh.) Nutt. (four-wing saltbush), and a herbaceous understory dominated by two grasses – the $C_4$ perennial bunchgrass *Bouteloua gracilis* (H.B.K.) Lag. ex Griffiths (blue grama) and the $C_3$ winter annual *Bromus tectorum* L. (cheatgrass). A small number of plains cottonwood trees (*Populus sargentii* Dode) were also present on the banks of the stream channel.

Two potential water sources were identified at this site. Mean annual precipitation at the CPER is 321 mm (Lauenroth and Sala 1992), the majority of which falls during the warm season (Sala et al. 1992). Significant groundwater aquifers occur throughout the study area, as indicated by the presence of local wells: an 18-m well approximately 2 km southeast of the site and adjacent to the creek (well A), and a 26-m well approximately 1 km north of the site (well B).

### Selective vegetation removal

In the early spring of 1995, we marked 20 circular plots of 2 m radius containing three shrubs in a triangular arrangement. At the center of each plot (equidistant from the three shrub bases) we extracted a 45-mm-diameter soil core in 30-cm sections to a depth of 2 m and installed a capped aluminum tube in the hole. We then took soil moisture measurements at 30-cm-depth intervals in the access tube using a Troxler 105 A neutron moisture probe (Troxler Electronic Laboratories, Research Triangle Park, N.C.). Subsamples were taken from the soil sections for analysis of soil water content (SWC) and texture. Gravimetric SWC data (% by weight) were obtained by oven-drying soils at 55°C for 24 h. Soil texture (sand, silt and clay %) was determined using the hydrometer method (Bouyoucos 1962). The pattern of soil texture with depth was characterized by very coarse textured loamy sands (80–90% sand) that extended to a depth of 120 cm, over sandy loams ($<70\%$ sand) in the subsoil layers between 150–210 cm.

The 20 plots were assigned to four removal treatment groups: VSG = all plants left intact; V_G = shrubs removed, understory (grasses, forbs, etc., i.e., the herbaceous component) left intact; VS_ = understory species removed, shrubs left intact; V__ = all vegetation removed. A randomized block design was chosen, because of topographic variation at the site. The removal treatments were applied on 19 April 1996. For the V_G and V__ treatments, we removed all the *Atriplex* individuals within a 2-m radius of the access tube by severing the stem at ground level with a hand saw. Although it is possible that the roots of adjacent shrubs intruded into the shrub removal plots, we were confident that this would not affect the zone of soil water measurement by the probe (less than a 30-cm radius from the access tube), based on previous measurements of the radial extent of typical shrub root systems at this location (40–50 cm; Lee and Lauenroth 1994). For the VS_ and V__ treatments, we killed all the understory species within a 2-m radius of the access tube by spraying with Roundup®, leaving the plants as standing dead. We avoided spray damage to the *Atriplex* plants in the VS_ treatment by temporarily covering the shrubs with polythene bags, and observed no damage to the shrubs after treatment application. During the subsequent growing season, we regularly removed regrowth of both herbaceous and shrub components by cutting or spraying as necessary.

## Soil water measurements

Throughout the 1996 growing season, we took SWC measurements from each plot, using the neutron probe, at depths of 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 cm. We took the first measurements on 9 May, before the onset of significant spring precipitation, and subsequently at 4- to 5-day intervals from 12 June to 12 August, and at 10- to 11-day intervals from 12 August to 11 October.

The gravimetric SWC data that we obtained when the access tubes were installed were used to calibrate the neutron moisture probe. We ensured that the range in SWC observed at that time reflected the range in SWC typically observable during an entire season (2–30%). The best-fit least-squares regression relationship between gravimetric SWC ($\theta_g$) and the neutron probe counts (expressed as NPC, the soil probe count divided by the shield count) had an exponential form (Eq. 1). Separate regression equations calculated for sandy and loamy soils did not give an improved calibration on the basis of $r^2$ comparisons.

$$\theta_g = e^{(-3.94+2.69\times \mathrm{NPC})} \tag{1}$$

where $r^2 = 0.82$, $n = 60$, and $P \leq 0.01$.

We pooled the soil water data from individual-layer measurements into three major profile sections (0–60, 60–120 and 120–180 cm) each of which represented data averaged over four measured layers. This grouping was based on observed similarities in patterns of soil water dynamics in adjacent layers during the growing season.

## Statistical analysis of soil water data

Gravimetric SWC data obtained for each date were statistically analyzed in a randomized block design using the general linear model procedure of SAS®. These analyses indicated a large amount of variation in SWC among plots within the treatment groups, and therefore we used the initial ('preseason') SWC measured on 9 May as a covariate in the analyses. Adjusted least-squares mean data are presented in the figures.

## Plant and water sample collection for isotope analysis

We collected tissue samples from *Bouteloua, Atriplex*, and *Populus* plants at approximately monthly intervals during the growing seasons of 1995 and 1996. Four replicate samples of both stem and leaf, each from a separate plant, were collected from areas of undisturbed vegetation on 5 June, 30 June, 27 July, and 30 August 1995, and on 14 May, 14 June, 15 July, and 14 August 1996. The plant stem tissue (for oxygen isotope analysis) consisted of previous years' woody tissue from the tree and shrub, and crown tissue from the grass. The current years' leaf tissue attached to the stems was removed for carbon isotope analysis, and to prevent potential alteration of its isotopic composition due to continued gas exchange activity (Dawson and Ehleringer 1993). All samples were placed into plastic vials with sealed caps and stored in a freezer prior to water extraction and isotopic analysis.

Two weeks prior to the monthly collections of the plant samples, we set up three rain gauges to collect precipitation water. Oil was placed in the gauges to prevent evaporation of the collected water, and likely isotopic enrichment of the remaining water (Craig 1961; Ehleringer and Dawson 1992). On each of the sample dates we also collected 40 ml of water from well A. Samples from well B were collected during 1996 only.

## Water sample extraction and analysis

Water was extracted from plant stem tissue using a vacuum distillation extraction line (Ehleringer et al. 1991). We took 0.2-ml subsamples of the extracted water and transferred them to 1.0-ml glass vials, thoroughly aspirated the vials with $CO_2$ in a glove bag and equilibrated each batch for 10 h at 40°C. The isotopic composition of the $CO_2$ in the headspace was measured using a multiprep sampler connected to a dual-inlet VG-Optima mass spectrometer (Epstein and Mayeda 1953; Ehleringer and Osmond 1989).

The standardized means of expressing isotopic composition is in 'delta' units ($\delta$), which is the ratio of the molar abundance ratios ($^{18}O/^{16}O$)of the sample ($R_{sam}$) and a standard ($R_{std}$), expressed on a ‰ ('per mil') basis (Eq. 2).

$$\delta = \left(\frac{R_{sam}}{R_{std}} - 1\right) \times 1000 \tag{2}$$

The usual primary standard for oxygen is V-SMOW (Vienna standard mean ocean water), although the $\delta$-values of the samples and three secondary standards were calculated using a linear calibration of the mass spectrometer against three primary standards of known $\delta$-values: V-SMOW, GISP, and SLAP (Gonfiantini 1978). The three secondary standards were deionized tap water (DI), 'cooked' deionized water (DI boiled for 12 h), and snowmelt collected from Cameron Pass, Colorado; all three were used in each batch of samples. We calculated the $\delta^{18}O$ values of the water samples relative to V-SMOW by adjusting for any shift in the secondary standard occurring in separate batches, and applying the original linear calibration (Eq. 3)

$$\text{Actual } \delta^{18}O = 0.9901 \times \text{observed } \delta^{18}O - 6.243 \tag{3}$$

where $r^2 = 0.999$.

The use of the calibration equation accounts for the fractionation coefficient of the $H_2O$-$CO_2$ equilibrium, and the contribution of O from the tank $CO_2$ used to aspirate the samples, which can be considered negligible, based on a 125:1 ratio of O in $H_2O$ versus $CO_2$ in the vials.

For the analysis of leaf tissue $\delta^{13}C$ values, we ground the leaf samples in a ball mill to pass a 40-mesh screen, and took subsamples (< 0.01 g) for incineration in a Carlo-Erba NA 1500 Series 2 nitrogen analyzer attached to a VG-Optima mass spectrometer. The mass spectrometer was initially calibrated against the NBS 22 primary standard, although the sample δ-values are expressed relative to the usual primary standard for carbon, Pee Dee Belemnite (PDB; Craig 1957). The secondary standard included in the calibration and used in each batch of samples was vacuum pump oil, with a nominal $\delta^{13}C$ value of − 27.00 ± 0.20 relative to PDB.

### Statistical analysis of isotope data

We analyzed the isotopic abundance data with the general linear model procedure of SAS, using a simple factorial design incorporating collection date (four separate months) and sample source (three plant species, precipitation, and two groundwater sources) as the two factors for $\delta^{18}O$ data, and a one-way ANOVA design comparing dates for the $\delta^{13}C$ data.

## Results

### Soil water dynamics

In the top 60 cm of the soil in all treatments, SWC increased sharply through May and June, fluctuated through June and July, and decreased steadily throughout the remainder of the season, with a slight increase in late September (Fig. 1a). The patterns of SWC among the four treatments diverged into two pairs early on in the season. At no time during the season were there significant differences in SWC between the VS_ and V__ treatments or between the VSG and V_G treatments ($P > 0.05$). The differences in SWC between the two pairs of treatments were significant from 17 June to July 1 and from 24 July to 11 October ($P \leq 0.05$). However, the key difference between the two pairs of treatments was the early season increase, which was much greater in the VS_ and V__ treatments (2.8 and 2.6%, respectively) compared to the VSG and V_G treatments (1.7 and 1.9%, respectively). The resultant difference in SWC between the two pairs of treatments (approximately 1%) was maintained throughout the rest of the season.

In the middle section of the soil profile (60–120 cm), SWC increased only slightly during May and June in all treatments, and for the rest of the season declined steadily in the VSG treatment (Fig. 1b). From late June through early July, SWC increased by 1.3% in the VS_ treatment to peak at 5.8% by 15 July, and increased by 2.7% in the V__ treatment to peak at 7.4% by 15 July. The differences in SWC between these two treatments and the VSG control were significant from 26 June to 29 July ($P \leq 0.05$), and the difference in SWC between these two grass removal treatments was significant from 15 July to 11 October ($P \leq 0.05$). The apparent increase in SWC in the V_G treatment during early July was only 0.6%, and the SWC in this treatment was never significantly greater than that of the VSG treatment ($P > 0.05$). After 15 July, all treatments showed a steady decrease in SWC throughout the remainder of the season. By October, only in the V__ treatment was SWC still greater than preseason levels.

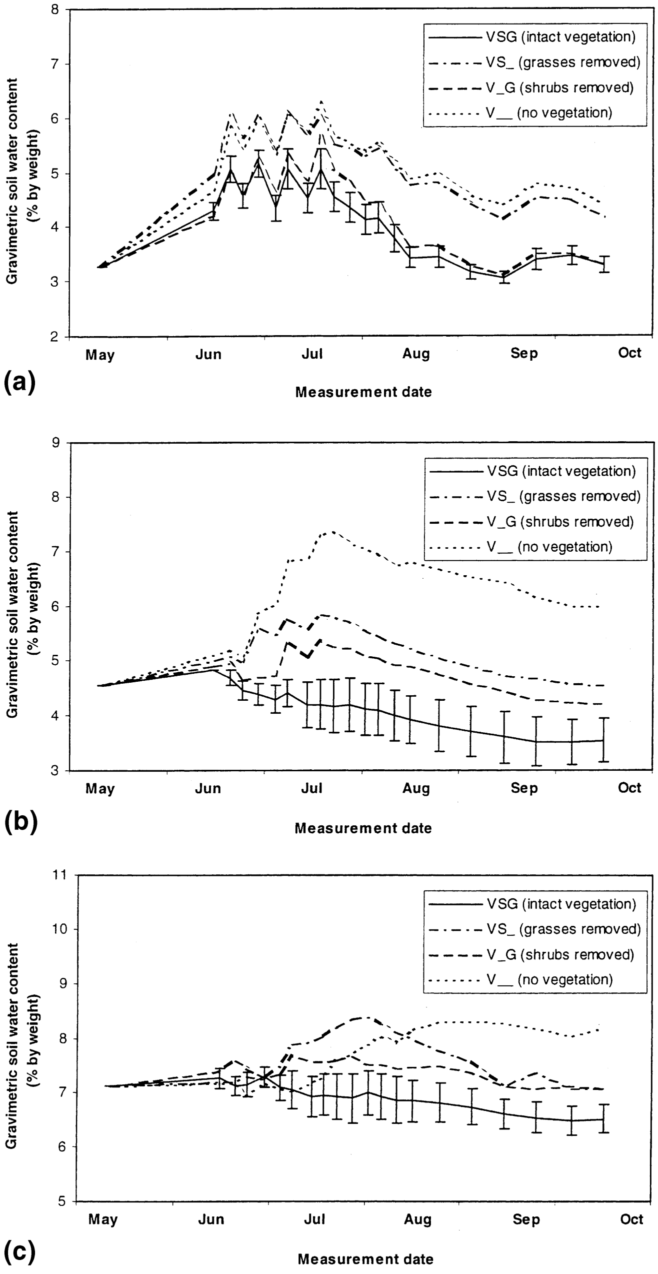

**Fig. 1** Gravimetric soil water content (%) during the 1996 growing season in three depth increments, in response to four selective removal treatments (*bars* SEs of all treatments). **a** 0–60 cm. **b** 60–120 cm. **c** 120–180 cm

In the lowest 60 cm of the profile (120–180 cm), SWC increased very little during May and June, and declined very gradually in the VSG treatment throughout the rest of the season (Fig. 1c). In both the VSG and V_G treatments, the seasonal range in measured SWC values was less than 0.6%. However, SWC did increase markedly in both the VS_ and V__ treatments. In the VS_ treatment, this increase of 1.3% began in late June and peaked at 8.4% in late July. In the V__ treatment, the increase was 1.2%, but began slightly later (early July), and peaked at 8.4% in late August. The difference in SWC between the VS_ treatment and the VSG control was significant from 24 July to 12 August ($P \leq 0.05$), and the difference in SWC between the V__ treatment and the VSG control was significant from 31 August to 11 October ($P \leq 0.05$). The water gained in the first half of the season in the VS_ treatment was subsequently lost during August and September, so that by October, SWC was back to preseason levels. By contrast, in the V__ treatment, there was no observable late-season decline in SWC, such that by 11 October, SWC was still approximately 1% above preseason levels.

## Oxygen isotope ratios

The $\delta^{18}$O values of the well water samples were relatively consistent during both seasons. Values for well A ranged between −10.6 and −10.7‰ during 1995 and between −11.0 and −11.5‰ during 1996, and for well B between −10.7 and −11.3‰ during 1996 (Fig. 2). In contrast, the $\delta^{18}$O values of precipitation water varied greatly within seasons, ranging between −1.3 and −9.3‰ during 1995, and between −5.6 and −10.9‰ during 1996 (Fig. 2).

Water extracted from *Bouteloua* plants consistently had the highest $\delta^{18}$O values of all plants, between −3.6 and −6.2‰ during 1995, and between −0.3 and −3.3‰ during 1996 (Fig. 2). There was a general tendency for the $\delta^{18}$O values of *Bouteloua* stem water to increase through May, June, and July, then decrease slightly in August, in both years. The $\delta^{18}$O values of water extracted from both *Atriplex* and *Populus* were consistently significantly lower ($P \leq 0.01$) than those of *Bouteloua* (Fig. 2). During 1995, the $\delta^{18}$O values for *Atriplex* were between −9.6 and −10.3‰ and showed no apparent seasonal trend, while the $\delta^{18}$O values for *Populus* were between −10.3 and −11.3‰ and only decreased slightly in August. There were no significant differences between the $\delta^{18}$O values for *Atriplex* and *Populus* for any month except August ($P > 0.05$). During 1996, the $\delta^{18}$O values for *Atriplex* were consistently higher than they had been during the previous year, between −6.3 and −8.7‰ (Fig. 2). However, the $\delta^{18}$O values for *Populus* were similar to the previous year, between −10.1 and −11.4‰, and decreased slightly as the season progressed. Consequently, the $\delta^{18}$O values of *Atriplex* were significantly greater than those of *Populus* in both July and August, and in addition the overall seasonal mean $\delta^{18}$O value for *Atriplex* was significantly greater than that of *Populus* ($P \leq 0.05$).

## Carbon isotope ratios

The $\delta^{13}$C values of leaf samples collected from *Bouteloua* ranged between −14.4 and −15.1‰ during 1995, and between −14.5 and −15.5‰ during 1996 (Table 1). In both years, the $\delta^{13}$C values tended to increase through May, June, and July, and decrease again in August. The $\delta^{13}$C values of leaf samples collected in July were significantly greater than those collected in May, both in 1995 ($P \leq 0.10$) and 1996 ($P \leq 0.05$). The $\delta^{13}$C values of *Atriplex* leaves were slightly greater than those of *Bouteloua* leaves, between −14.0 and −14.5‰ during 1995, and between −13.7 and −14.4‰ during 1996 (Table 1). A similar seasonal pattern was observed in the leaf samples collected from *Atriplex*, although the difference between $\delta^{13}$C values in July and May was only significant in 1996 ($P \leq 0.05$). The $\delta^{13}$C values of *Pop-*

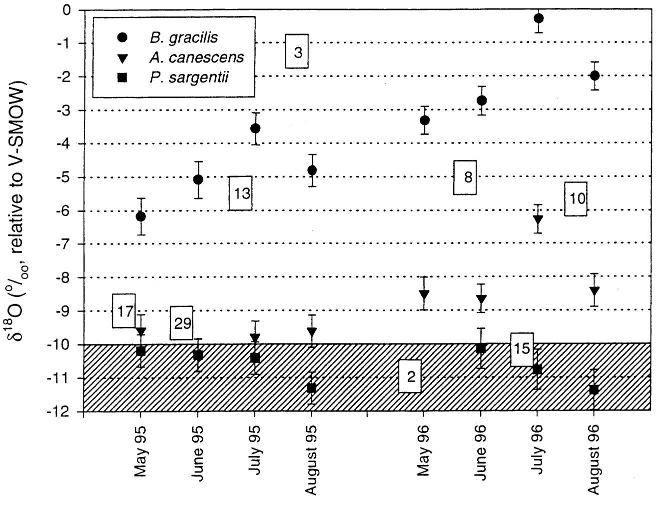

**Fig. 2** $\delta^{18}$O values of plant and source water samples collected during 1995 and 1996 (*bars* SEs). The *shaded portion* represents the range of all well water samples (including SEs), and the *clear boxes* represent precipitation events sampled, with event sizes in mm (*box heights* SEs)

**Table 1** $\delta^{13}$C values (‰, relative to PDB) of leaf tissue sampled from three plant functional types during two successive growing seasons (different letters within columns indicate significant differences between sample dates)

| | 1995 | | | 1996 | | |
|---|---|---|---|---|---|---|
| | *Bouteloua gracilis* | *Atriplex canescens* | *Populus sargentii* | *Bouteloua gracilis* | *Atriplex canescens* | *Populus sargentii* |
| May | − 15.1 a | − 14.5 a | − 27.4 a | − 15.5 a | − 14.4 a | − 29.2 a |
| June | − 15.0 a | − 14.3 a | − 27.9 a | − 14.8 ab | − 14.1 ab | − 28.8 a |
| July | − 14.4 a | − 14.0 a | − 28.1 a | − 14.5 b | − 13.7 b | − 28.8 a |
| August | − 14.9 a | − 14.1 a | − 28.4 a | − 15.3 ab | − 14.3 ab | − 29.1 a |

*ulus* leaves were typical of $C_3$ species, between −27.4 and −28.4‰ during 1995, and between −28.8 and −29.2‰ during 1996 (Table 1). There were no significant differences in leaf $\delta^{13}C$ values for *Populus* leaves between months in either year ($P > 0.05$).

## Discussion

The selective removal of grasses had an immediate impact on SWC in the top 60 cm of soil (Fig. 1a). In both treatments where the grasses were removed, SWC increased markedly relative to the intact vegetation. In the treatment where only the shrubs were removed, there was no impact on SWC, and comparing the shrub + grass removal treatment with the grass-only removal treatment there appeared to be no additional impact on SWC of shrub removal. This distinction into two pairs of treatments indicates that the herbaceous component of the community dominates soil water dynamics in this zone. A similar result was reported by Sala et al. (1989), where soil water potentials at depths of 15, 30 and 60 cm were greater when grasses were removed relative to the control, but also did not increase significantly when shrubs were removed.

This finding is consistent with expectations of water acquisition by *Bouteloua*. The roots of *Bouteloua* are generally confined to the upper 90 cm of the soil at this location, with approximately 80% of root length above 60 cm (Lee and Lauenroth 1994). This gives *Bouteloua* a greater competitive advantage in exploiting water available in surface soil layers through its ability to respond to the small rainfall events characteristic of the shortgrass steppe region (Sala and Lauenroth 1982). In fact, the dominant zone of soil water acquisition by *Bouteloua* probably includes only the top 15 cm. Data on soil water potential changes under *Bouteloua* during a drying cycle showed that water is acquired exclusively from the upper 15 cm during the early stages of drought, and that the soil water potential at 60 cm did not change significantly until 39 days of drought (Sala et al. 1981).

This understanding of the soil water use patterns of *Bouteloua* enables us to interpret the apparently poor relationship between the $\delta^{18}O$ values of plant water samples and meteoric water samples seen in this study (Fig. 2). Water entering the soil is expected to become enriched in $^{18}O$ as a result of water loss to evaporation. In this experiment, the $\delta^{18}O$ value of *Bouteloua* stem water was generally more enriched than that of precipitation. Given the limited depth of soil water acquisition activity in *Bouteloua* plants, it is clear that they used exclusively soil water derived from a meteoric source. Therefore, we can use the $\delta^{18}O$ value of *Bouteloua* as a indicator of the $\delta^{18}O$ of soil water in the upper 15 cm.

The results of the removal study indicated that the additional water made available in the upper layer (when the understory was removed) penetrated to lower layers. The data show a gradual increase in SWC in the 60–120 cm section during late June and early July (Fig. 1b), and in the 120–180 cm section during July and early August (Fig. 1c). Water movement through the profile probably occurred via relatively slow unsaturated flow processes (Hanks and Ashcroft 1980). The result of this flow was the creation of a subsoil water resource, normally only observed in very wet years at this site (Dodd and Lauenroth 1997).

There were important differences between the two grass removal treatments in both lower sections. Where the shrub component remained (VS_), the initial increase in SWC during the late spring and early summer in the 60–120 cm section was not as marked as where the shrub component was also removed (V__). In the 120–180 cm section, there was a late-season decline in the extra soil water resource created by the grass only removal treatment, but where the shrub component had also been removed (V__), this water resource remained for the rest of the season. These observations provide evidence that shrubs may be using the water resource freed by the removal of grasses over a wide range of soil depths. This finding is similar to the observations of Fernandez and Caldwell (1975) that the root growth activity of *A. confertifolia* progresses from upper to lower soil layers during the growing season.

However, this evidence of uptake by shrubs from the lower layers conflicts somewhat with the more direct evidence provided by the comparison of the intact-vegetation control and shrub only removal treatment. Although spring soil water accumulation in both the 60–120 cm and 120–180 cm sections appeared to be slightly greater in the shrub only removal treatment compared with the intact-vegetation control (Fig. 1b,c), this difference was not significant. This finding did not support the prediction that removal of shrubs would increase water storage in lower layers. Similarly, the removal of shrubs in the Sala et al. (1989) study did not result in a significant increase in soil water potential in those layers measured (5, 15, 30, and 60 cm) and it was suggested that shrubs were utilizing water from still deeper layers. This may be true of the present location as well, given our observations of large *Atriplex* roots extending below 5 m. Deep root systems of *Atriplex* have been noted by authors elsewhere (Goodin 1985, cited in Adair et al. 1992), and *Atriplex* is known to be a facultative phreatophyte on some sites (Le Houérou 1992). The lack of any significant increases in SWC as a result of shrub removal might also be attributable to the inability of our measurement technique to detect small changes in SWC over the large soil volume exploited by shrub roots (Lee and Lauenroth 1994).

The observation that the $\delta^{18}O$ value of water extracted from *Atriplex* was closer to that of groundwater than precipitation (particularly during the 1995 season) would also appear to support the hypothesis that the shrubs were largely utilizing groundwater. However, the $\delta^{18}O$ values of *Atriplex* were generally less negative than those of *Populus*, and the seasonal pattern of shrub $\delta^{18}O$ measurements in 1996 corresponded with those of *Bouteloua*.

The $\delta^{18}O$ values of *Atriplex* stem water were most similar to groundwater during 1995, indicating that if groundwater was being used, proportionally more groundwater relative to meteoric water was used in that year. This is surprising given that the spring of 1995 was the second wettest on record, and soil water availability was extremely high during that season. In addition, during the drier year (1996), the increase in $\delta^{18}O$ values during the season would suggest that less groundwater was being used as drought stress increased – an unlikely strategy for a plant with access to reliable groundwater resources.

That there are potentially two soil water resources (surface and subsoil), as implied by the removal study and our previous data on soil water storage through the profile at this site (Dodd and Lauenroth 1997), offers an alternative explanation. Shrubs may have used mainly subsoil water of meteoric origin derived from low-temperature, high-intensity storms early in the growing season. The $\delta^{18}O$ values of these precipitation events were not significantly different from those of the shrub stem water in May and June 1995 (Fig. 2). The consistency of the $\delta^{18}O$ values of shrub stem water during 1995 could be explained by the high level of spring rainfall in that year, which would have penetrated deep into the loamy sands at the site, and remained throughout the rest of the season. It is unlikely that this water would be affected by evaporative losses, which are limited to the top 20 cm in this soil type (Wythers 1996) – hence the consistency of the $\delta^{18}O$ values.

Soil water data for the site from the Shortgrass Steppe Long-Term Ecological Research database showed that SWC at a depth of 120 cm was extremely high at the beginning of the 1995 growing season ($>20\%$), and remained well above the level of soil moisture that might be expected in a year of 'typical' precipitation inputs (5–8%). This information supports the idea that there was a reliable subsoil water resource below the grass rooting zone throughout the 1995 growing season. By contrast, in 1996, spring precipitation was relatively low, and the subsoil water resource not as favorable ($<7\%$ at 120 cm). The greater values of $\delta^{18}O$ in *Atriplex* stem water during this year may reflect the need for this species to use a greater proportion of recent precipitation.

The $\delta^{13}C$ data also provide support for the latter scenario. The relationship between $c_i/c_a$ and carbon isotope discrimination ($\Delta$) for $C_4$ species is dependant upon the level of bundle sheath leakiness ($\phi$), which determines the extent to which discrimination by Rubisco is expressed (Farquhar et al. 1989). At $\phi > 0.32$, the relationship is positive (as it is for $C_3$ species), and at $\phi < 0.32$, the relationship is negative, while for $\phi \approx 0.32$, $\Delta$ is constant at 4.4 and unaffected by $c_i/c_a$ (Sandquist and Ehleringer 1995). The nature of the relationship for the two $C_4$ species in this study can therefore be inferred from the $\Delta$-values calculated from measured $\delta^{13}C$ values using the following equation (Sandquist and Ehleringer 1995):

$$\Delta = [\delta_a - \delta_p]/[1 + \delta_p] \tag{4}$$

where $\delta_a =$ the carbon isotope ratio of $CO_2$ in the atmosphere (−8‰) and $\delta_p$ is the carbon isotope ratio of the plant. The values for *Bouteloua* were between 6.5 and 7.6‰, and for *Atriplex* between 5.8 and 6.6‰. All values were greater than 4.4‰, indicating that the relationship between $\Delta$ and $c_i/c_a$ is positive in both species (see Fig. 3 in Sandquist and Ehleringer 1995). Therefore, we can expect decreases in $c_i/c_a$ (as would result from water stress) to lead to decreases in $\Delta$ and less negative values of $\delta^{13}C$. We did observe a significant increase in the values of $\delta^{13}C$ in leaf tissue of both grasses and shrubs from May to July 1996 (Table 1) of 1.0 and 0.7‰, respectively, and these lie within a range that can be explained solely by changes in $c_i/c_a$, given the typical limits of $c_i/c_a$ for $C_4$ plants (0.2–0.4; Pearcy and Ehleringer 1984). These data indicate that the shrubs had similar changes in gas exchange to the grasses over the course of the 1996 season, suggesting that they were experiencing similar levels of water stress. However, it is worth noting that the leaf carbon isotope data are not conclusive, since factors other than the link between water stress and $c_i/c_a$ may affect the carbon isotope ratio of $C_4$ plants. A small change in $\phi$ could produce the $\leq$1.0‰ increase in $\Delta$ seen in this study (Sandquist and Ehleringer 1995). Alternatively, the increase of ~ 8°C in ambient temperatures likely to occur between May and July at this location (Lauenroth and Milchunas 1991) could increase the fractionation associated with the $CO_2 - HCO_3^-$ equilibrium by ~ 1.0‰ (Mook et al. 1974).

The observation that stem water extracted from *Populus* had a relatively stable $\delta^{18}O$ value, which was similar to the groundwater accessed by local wells, indicates that this is the dominant water source for this species. The irregular nature of stream flow in Owl Creek suggests that surface water is not a reliable water source for the cottonwoods. Other studies of riparian vegetation have shown that mature trees growing alongside even perennial streams use groundwater rather than stream water (Dawson and Ehleringer 1991). The lack of a significant shift in the $\delta^{13}C$ value of the *Populus* leaf tissue during either year suggests that these trees have seasonally consistent patterns of gas exchange and water relations, which supports our conclusion that they have access to a reliable water source (groundwater) which is sufficient to meet their moisture demands.

In this study, we have found that the coexistence of multiple life-forms in this community can be explained by differing plant water acquisition strategies. *Bouteloua*, a $C_4$ grass, uses spring and summer rain as its dominant water resource, extracted from upper soil layers. Conversely, *Populus*, a riparian $C_3$ tree, relies on groundwater, and exhibits very consistent seasonal patterns of gas exchange. *Atriplex*, a $C_4$ shrub, with roots distributed both near the surface and at depths down to at least 2 m (Lee and Lauenroth 1994), appears to utilize both soil water stored deep in the profile (from recharge during the spring) and summer rainfall (in years when spring rainfall is low and soil water recharge is poor).

Overall, our findings support the general predictions arising out of Walter's two-layer model: grasses and woody plants mainly use different water resources that occur in different layers of the soil profile. As such, this is consistent with the ecological principle that coexistence of species requires differential resource use. However, our results demonstrate that the model is an oversimplification for a number of reasons: there is overlap between 'layers' (with shrub roots present and active in the surface soil layers) and the model does not account for interactions between layers (e.g., the influence of grass roots extends beyond the layers in which they grow). Finally, both the model and our experimental manipulations to test it are deliberately expressed in terms of a simple community of two plant functional types. In real plant communities of the shortgrass steppe that are comprised of many species other than *Bouteloua* and *Atriplex*, we would expect to find other species exploiting different moisture resources. For example, plains prickly pear (*Opuntia polyacantha* Haw.), which occurs extensively throughout the shortgrass steppe, maintains a very superficial fine-root network in the surface soil layers, enabling it to exploit small (< 5 mm) rainfall events (Dougherty et al. 1996). The conceptual expansion of the two-layer/two life-form model to a three-layer/three life-form model and beyond may be more appropriate for complex communities.

**Acknowledgements** This research was supported by the National Science Foundation, Shortgrass Steppe Long-Term Ecological Research Project (BSR-9011659), and the Colorado State University Agricultural Experiment Station (1-57661). The CPER is administered by the Great Plains Systems Research Unit of the United States Department of Agriculture-Agricultural Research Service. Facilities and instrumentation for the mass spectrometer were supported by the National Science Foundation, Atmospheric Sciences Program (BSR-9413903). For the duration of these studies, M.B.D. was on paid study leave from AgResearch New Zealand Limited. We wish to thank Carolyn Dodd, Howard Epstein, Adam Fleener, and Mark Lindquist for assistance in the field, Karrin Alstad and Sigrid Resh for assistance in the use of the vacuum distillation line, Ben Petersen for glassware construction, Dan Reuss for supervision of the mass spectrometer analysis, Philip Chapman of the CSU Statistics Department for advice on appropriate data analyses, and two anonymous reviewers for helpful comments on the manuscript.

## References

Adair LS, Andrews DL, Cairney J, Funkhouser EA, Newton RA, Aldon EF (1992) Characterizing gene responses to drought stress in fourwing saltbush [*Atriplex canescens* (Pursh.) Nutt.]. J Range Manage 45:454–461

Bouyoucos GJ (1962) Hydrometer method improved for making particle size analysis of soils. Agron J 54:464–465

Crabb JA (1982) Soil survey of Weld County, Colorado, northern part. United States Department of Agriculture, Soil Conservation Service, Washington D.C.

Craig H (1957) Isotopic standards for carbon and oxygen and correlation factors for mass spectroscopic analysis of carbon dioxide. Geochim Cosmochim Acta 42:59–62

Craig H (1961) Isotopic variations in meteoric water. Science 133:1702–1703

Dansgaard W (1964) Stable isotopes in precipitation. Tellus 16:436–486

Dawson TE, Ehleringer JR (1991) Streamside trees that do not use stream water: evidence from hydrogen isotope ratios. Nature 350:335–337

Dawson TE, Ehleringer JR (1993) Isotopic enrichment of water in the "woody" tissues of plants: implications for plant water source, water uptake, and other studies which use the stable isotope composition of cellulose. Geochim Cosmochim Acta 57:3487–3492

Dodd MB (1997) The control of vegetation structure by soil texture effects in the North American shortgrass steppe. PhD thesis, Department of Rangeland Ecosystem Science, Colorado State University, Fort Collins

Dodd MB, Lauenroth WK (1997) The influence of soil texture on the soil water dynamics and vegetation structure of a shortgrass steppe ecosystem. Plant Ecol 133:13–28

Dougherty RL, Lauenroth WK, Singh JS (1996) Response of a grassland cactus to frequency and size of rainfall events in a North American shortgrass steppe. J Ecol 84:177–183

Ehleringer JR, Dawson TE (1992) Water uptake by plants: perspectives from stable isotope composition. Plant Cell Environ 15:1073–1082

Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant physiological ecology: field methods and instrumentation. Chapman & Hall, New York, pp 281–300

Ehleringer JR, Phillips SL, Schuster WSF, Sandquist DR (1991) Differential utilization of summer rains by desert plants. Oecologia 88:430–434

Ehleringer JR, Phillips SL, Comstock JP (1992) Seasonal variation in the carbon isotopic composition of desert plants. Funct Ecol 6:396–404

Epstein S, Mayeda T (1953) Variations of $^{18}$O content of waters from natural sources. Geochim Cosmochim Acta 4:213–224

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Fernandez OA, Caldwell MM (1975) Phenology and dynamics of root growth of three cool semi-desert shrubs under field conditions J Ecol 63:703–714

Flanagan LB, Ehleringer JR, Marshall JD (1992) Differential uptake of summer precipitation among co-occurring trees and shrubs in a pinyon-juniper woodland. Plant Cell Environ 15:831–836

Gitay H, Noble IR (1997) What are functional types and how should we seek them? In: Smith TM, Shugart HH, Woodward FI (eds) Plant functional types. Cambridge University Press, Cambridge, UK, pp 3–19

Gonfiantini R (1978) Standards for stable isotope measurements in natural compounds. Nature 271:534–536

Gordon DR, Welker JM, Menke JW, Price KJ (1989) Competition for soil water between annual plants and blue oak (*Quercus douglasii*) seedlings. Oecologia 79:533–541

Hanks RJ, Ashcroft GL (1980) Applied soil physics. Springer, Berlin Heidelberg New York

Knoop WT, Walker BH (1985) Interactions of woody and herbaceous vegetation in a southern African savanna. J Ecol 73:235–253

Lauenroth WK, Milchunas DG (1991) Short-grass steppe. In: Coupland RT (ed) Ecosystems of the world 8A. Natural grasslands. Elsevier, Amsterdam, pp 183–226

Lauenroth WK, Sala OE (1992) Long term forage production of North American shortgrass steppe. Ecol Appl 2:397–403

Lee CA, Lauenroth WK (1994) Spatial distribution of grass and shrub root systems in the shortgrass steppe. Am Midl Nat 137:117–123

Le Houérou HN (1992) The role of saltbushes (*Atriplex* spp.) in arid land rehabilitation in the Mediterranean basin: a review. Agrofor Syst 18:107–148

Le Roux X, Bariac T, Mariotti A (1995) Spatial partitioning of the soil water resource between grass and shrub components in a West African humid savanna. Oecologia 104:147–155

Mole S, Joern A, O'Leary MH, Madhavan S (1994) Spatial and temporal variation in carbon isotope discrimination in prairie graminoids. Oecologia 97:316–321

Mook WG, Bommerson JC, Staverman WH (1974) Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. Earth Planet Sci Lett 22:169–176

Noy-Meir I (1973) Desert ecosystems: environment and producers. Annu Rev Ecol Syst. 4:25–51

Pearcy RW, Ehleringer JR (1984) Comparative ecophysiology of $C_3$ and $C_4$ plants. Plant Cell Environ 7:1–13

Sala OE, Lauenroth WK (1982) Small rainfall events: an ecological role in semiarid regions. Oecologia 53:301–304

Sala OE, Lauenroth WK, Parton WJ, Trlica MJ (1981) Water status of soil and vegetation in a shortgrass steppe. Oecologia 48:327–331

Sala OE, Golluscio RA, Lauenroth WK, Soriano A (1989) Resource partitioning between shrubs and grasses in the Patagonian steppe. Oecologia 81:501–505

Sala OE, Lauenroth WK, Parton WJ (1992) Long-term soil water dynamics in the shortgrass steppe. Ecology 73:1175–1181

Sala OE, Lauenroth WK, Golluscio RA (1997) Plant functional types in temperate semi-arid regions. In: Smith TM, Shugart HH, Woodward FI (eds) Plant functional types. Cambridge University Press, Cambridge, UK, pp 217–233

Sandquist DR, Ehleringer JR (1995) Carbon isotope discrimination in the $C_4$ shrub *Atriplex confertifolia* along a salinity gradient. Great Basin Nat 55:135–141

Smedley MP, Dawson TE, Comstock CS, Donovan LA, Sherrill DE, Cook CS, Ehleringer JR (1991) Seasonal carbon isotope discrimination in a grassland community. Oecologia 85:314–320

Smith SD, Osmond CB (1987) Stem photosynthesis in a desert ephemeral, *Eriogonum inflatum*: morphology, stomatal conductance and water-use-efficiency of field populations. Oecologia 72:533–541

Soriano A, Sala OE (1983) Ecological strategies in a Patagonian arid steppe. Vegetatio 56:9–15

Walker BH, Noy-Meir I (1982) Aspects of the stability and resilience of savanna ecosystems. In Huntley BJ, Walker BH (eds) Ecology of tropical savannas. Springer, Berlin Heidelberg New York, pp 556–590

Walter H (1979) Vegetation of the earth, 2nd edn. Springer, Berlin Heidelberg New York

Welker JM, Heaton TEH, Spiro B, Callahan TV (1995) Indirect effects of winter climate on the $\delta^{13}$C and the δD characteristics of annual growth segments in the long-lived, arctic plant *Cassiope tetragona*: a preliminary analysis. Paleoclim Res 15:105–119

White JWC, Cook ER, Lawrence JR, Broecker WS (1985) The D/H ratios of sap in trees: implications for water sources and tree ring D/H ratios. Geochim Cosmochim Acta 49:237–246

Wythers KR (1996) Bare-soil evaporation in the shortgrass steppe of northcentral Colorado. MSc thesis, Colorado State University, Fort Collins