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Stable Isotopes in Ecosystem Science: Structure, Function and Dynamics of a Subtropical Savanna†

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Stable isotopes are often utilized as intrinsic tracers to study the effects of human land uses on the structural and functional characteristics of ecosystems. Here, we illustrate how stable isotopes of H, C, and O have been utilized to document changes in ecosystem structure and function using a case study from a subtropical savanna ecosystem. Specifically, we demonstrate that: (1) $\delta^{13}C$ values of soil organic carbon record a vegetation change in this ecosystem from C_4 grassland to C_3 woodland during the past 40–120 years, and (2) $\delta^{2}H$ and $\delta^{18}O$ of plant and soil water reveal changes in ecosystem hydrology that accompanied this grassland-to-woodland transition. In the Rio Grande Plains of North America, $\delta^{13}C$ values of plants and soils indicate that areas now dominated by C_3 subtropical thorn woodland were once C_4 grasslands. $\delta^{13}C$ values of current organic matter inputs from wooded landscape elements in this region are characteristic of C_3 plants (−28 to −25‰), while those of the associated soil organic carbon are higher and range from −20 to −15‰. Approximately 50–90% of soil carbon beneath the present C_3 woodlands is derived from C_4 grasses. A strong memory of the C_4 grasslands that once dominated this region is retained by $\delta^{13}C$ values of organic carbon associated with fine and coarse clay fractions. When $\delta^{13}C$ values are evaluated in conjunction with ^{14}C measurements of that same soil carbon, it appears that grassland-to-woodland conversion occurred largely within the past 40–120 years, coincident with the intensification of livestock grazing and reductions in fire frequency. These conclusions substantiate those based on demographic characteristics of the dominant tree species, historical aerial photography, and accounts of early settlers and explorers. Concurrent changes in soil $\delta^{13}C$ values and organic carbon content over the past 90 years also indicate that wooded landscape elements are behaving as sinks for atmospheric CO_2 by sequestering carbon derived from both the previous C_4 grassland and the present C_3 woody vegetation. Present day woodlands have hydrologic characteristics fundamentally different from those of the original grasslands. Compared to plants in remnant grasslands, tree and shrub species in the woodlands are rooted more deeply and have significantly greater root biomass and density than grasslands. $\delta^{18}O$ and $\delta^{2}H$ values of plant and soil water confirm that grassland species acquire soil water primarily from the upper 0.5 m of the soil profile. In contrast, trees and shrubs utilize soil water from throughout the upper 4 m of the profile. Thus, soil water that formerly may have infiltrated beyond the reach of the grassland roots and contributed to local groundwater recharge or other hydrologic fluxes may now be captured and transpired by the recently formed woodland plant communities. The natural abundances of stable isotopes revealed fundamental information regarding the impacts of human land use activities on the structure and function of this subtropical savanna. Stable isotopes provided direct, spatially explicit evidence for dramatic changes in ecosystem physiognomy and demonstrated some functional consequences for the hydrologic cycle. Furthermore, grassland-to-woodland conversion has been geographically extensive in the worlds' drylands, suggesting that these ecosystem-level changes in vegetation structure, carbon cycling, and hydrology may have implications for regional/global biogeochemistry and climate. Copyright © 1999 John Wiley & Sons, Ltd.

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The human population is growing exponentially, and its agricultural and industrial activities are placing unprecedented stresses on natural and managed ecosystems.[1] These stresses often alter ecosystem structure (i.e., species composition) and function (i.e., productivity, biogeochemistry, hydrology) in ways that can diminish sustainability and affect future land use options.[2] In addition, anthropogenic changes in ecosystem structure and function are sufficiently widespread to have consequences for atmospheric chemistry and climate on regional and global scales.[3–5] Thus, a thorough understanding of the impact of

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human activities on ecosystem structure and function is essential not only to develop approaches to resource management that will ensure the long-term productivity of our croplands, forestlands, and rangelands, but also to enhance our understanding of the function of the earth-atmosphere system. Our ability to unravel the complex interactions between human land use, ecosystem structure and function, and global biogeochemistry and climate will depend on the development of appropriate technologies and analytical techniques.

Many new and innovative analytical approaches have been applied towards understanding this complexity. Among the most powerful of these analytical techniques is isotope ratio mass spectrometry (IRMS). During the past 20 years, it has been recognized that small variations in the natural abundances of ^{2}H, ^{13}C, ^{15}N, ^{18}O, and ^{34}S in ecosystem components can be measured with high precision by IRMS, and that these variations can provide critical and novel insights into ecosystem structure and function. Natural isotopic abundances of H, C, N, O, and S are now utilized as intrinsic tracers to study the effects of human land uses on ecosystem structure (i.e., vegetation change), as well as the impact of those changes on the functional characteristics of ecosystems, such as energy flow, biogeochemistry, and hydrology.[6–11] These isotopic studies of ecosystems have contributed not only to basic ecological theory, but have also provided information with immediate practical significance for natural resource management.[12,13]

In this paper, we illustrate how stable isotopes of H, C, N, and O can be utilized to document changes in ecosystem structure and function by presenting a case study from a subtropical savanna ecosystem in North America. More specifically, we demonstrate that: (1) $\delta^{13}C$ values of soil organic carbon record a vegetation change in this ecosystem from C_4 grassland to C_3 woodland during the past 40–120 years, and (2) $\delta^{2}H$ and $\delta^{18}O$ of plant and soil water reveal changes in ecosystem hydrology that accompanied this grassland-to-woodland transition.

Reconstructing historic vegetation

Historical accounts suggest the Rio Grande Plains of southern Texas were relatively open grasslands or savannas at the time of Anglo-European settlement;[14–16] however, the present landscape in this region is subtropical thorn woodland[17] dominated by N-fixing woody legumes[18] with deep root systems.[19] This dramatic vegetation change seems to have occurred over the past 100–150 years and coincides with the intensification of livestock grazing in this region.[20] Until recently, evidence for grassland-to-woodland conversion in this region was largely based on anecdotal evidence and conjecture, much of which is contradictory.

In this region, all woody plants possess the C_3 pathway of photosynthesis ($\delta^{13}C \approx -27‰$) and nearly all grasses have the C_4 pathway ($\delta^{13}C \approx -13‰$). $\delta^{13}C$ values of soil organic carbon can reconstruct vegetation changes where C_3 and C_4 plants occur, because they integrate and record the relative contributions of plants with these constrasting photosynthetic pathways to primary productivity.[21,22] Changes in the relative proportions of C_3 and C_4 plants can be recognized as a difference between the $\delta^{13}C$ of the vegetation in the current plant community and that of the soil organic matter. This isotopic difference will be largest immediately following a vegetation change, and will decrease over time as carbon from the previous plant community decays out of the organic carbon pool and is replenished with new carbon derived from the current plant community. The isotopic discrepancy created by the vegetation change will persist for a length of time determined by the soil organic matter turnover rate. In fact, following a vegetation change ($C_3 \rightarrow C_4$ or vice versa), the rate at which the $\delta^{13}C$ value of soil organic carbon changes to approach that of the new plant community is a measure of the soil organic matter turnover rate in that system.[23,24] Here we use this methodology to provide direct, spatially explicit evidence that C_4 grasslands have in fact been replaced by C_3 woodlands in the Rio Grande Plains of southern Texas, and to demonstrate that soils in these woodlands have functioned as sinks for atmospheric carbon.

Functional consequences of vegetation change

A change from grass to woody plant domination could profoundly modify many functional attributes of Rio Grande Plains ecosystems. Hydrologic characteristics are particularly prone to alteration because of the multitude of biophysical differences between grassland vs. woodland plant cover. Among the largest and most significant differences is that woody vegetation is generally more deeply rooted than grasses and other herbaceous species characteristic of grasslands,[25,26] potentially enabling the woody species to transpire soil water from a larger proportion of the soil profile. To evaluate changes in vertical use of soil water, we measured $\delta^{2}H$ and $\delta^{18}O$ of soil water and plant xylem water in conjunction with root biomass and distribution in remnant grasslands and in wooded landscape elements. Since there is no isotope fractionation associated with uptake of soil water by roots,[27–29] the $\delta^{2}H$ and $\delta^{18}O$ values of plant xylem water represents a weighted average of all soil water acquired by all functional roots. The soil depths from which xylem water originated can therefore be estimated by comparing $\delta^{2}H$ or $\delta^{18}O$ of plant xylem water with that of soil water from different parts of the profile.[30–32]

METHODS

Study area

Research was conducted at the Texas Agricultural Experiment Station LaCopita Research Area (27° 40′ N; 98° 12′ W) located 65 km west of Corpus Christi, Texas in the eastern Rio Grande Plains. Climate is subtropical, with a mean annual temperature of 22 °C and mean annual precipitation of 715 mm. Topography consists of nearly level uplands which grade (1–3% slopes) into lower-lying drainages and playas; elevation ranges from 75–90 m. The present surface appears to be from the early Holocene, and overlies sediments from the mid-Pleistocene.[33] This site has been grazed by domestic livestock for the past century.

Prosopis glandulosa Torr. var. *glandulosa* (honey mesquite) is the dominant plant in all wooded landscape elements. Sandy loam soils of the uplands (Typic and Pachic Argiustolls) are characterized by a two-phase vegetation pattern consisting of clusters of C_3 woody vegetation (discrete phase) embedded within a matrix of C_4 grasses and C_3 herbaceous dicots (continuous phase).[34] Formation of woody plant clusters is initiated when grassland areas are colonized by the N-fixing tree legume, *P. glandulosa*, which subsequently facilitates recruitment of other woody plant

species beneath its canopy.[35] Where a subsurface argillic horizon (i.e. a clay-rich layer) is present, woody clusters are spaced widely and consist of a single *P. glandulosa* tree with up to 15 understory tree/shrub species. Where the argillic horizon is absent, woody clusters appear to expand laterally and fuse to form groves.[20] Low-lying intermittent drainages have clay loam soils (Pachic Argiustolls) and are characterized by continuous-canopy C_3 woodlands dominated by *P. glandulosa*. These drainage woodlands appear to have originated via the same successional processes currently underway in the uplands, and their plant species composition is similar to that of upland woody clusters and groves. In some areas, these low-lying woodlands appear to be moving up-slope, and these are referred to as transitional woodlands. Additional details regarding the plant communities and soils at this site have been published.[20,36]

Soil collection for stable carbon isotope analyses of vegetation change

Herbaceous patches between shrub clusters (hereafter denoted as 'grasslands'), mature discrete clusters (argillic horizon present) and groves (argillic horizon absent) of uplands, and transitional and drainage woodlands of lowlands, were sampled on two landscapes. Each landscape consisted of a hillslope gradient extending from the crown of sandy loam uplands to the bottom of clay loam intermittent drainages. In each landscape, soil cores (5×150 cm) were collected from grassland (n = 6), discrete cluster (n = 6), grove (n = 6), transitional woodland (n = 9), and drainage woodland (n = 9) patches in May 1991. For each patch type within each landscape, one core was subjected to soil characterization, one was utilized for radiocarbon dating of soil organic carbon, and one was used for isolation of particle size separates for $\delta^{13}C$; all other cores were utilized for $\delta^{13}C$ analyses of roots and whole-soil organic carbon. In wooded landscape elements, cores were taken within 1 m of the bole of large *P. glandulosa* trees. In grasslands, cores were taken in areas that were at least 10 m from the nearest woody vegetation. Prior to extracting each core, all litter within a 0.5×0.5 m area centered over the core location was collected. In addition, live foliage from the dominant plant species within each major vegetation type was collected. Litter and live foliage samples were dried at 60 °C, pulverized, and saved for isotopic analysis.

In April 1994, additional soil samples were collected in upland grasslands and groves (n = 40–50 for each patch type). In each grove, 3 soil cores (2.5×10 cm) were taken beneath the largest *P. glandulosa* tree in each stand and the 3 samples from each depth increment were pooled. The approximate age of each grove was determined by counting annual growth rings in a basal stem cross-section of the largest *P. glandulosa* tree in the grove.[37] Soil cores were taken in an identical manner from grasslands located adjacent to the groves.

Preparation of roots and soils for stable carbon isotope analyses

Soil cores collected in May 1991 were divided into 6 depth increments (0–15, 15–30, 30–60, 60–90, 90–120, and 120–150 cm) and coarse roots removed manually. Soil samples were then dried at 60 °C, ground to pass a 2 mm screen, and fine roots removed by flotation in saturated NaCl solutions (density = 1.2 g cm^{-3}).[38,39] Fine and coarse roots were pooled, treated with 1 N HCl to remove carbonates, dried, weighed, pulverized, and saved for isotopic analysis.

Root-free soils were treated with 1 N HCl at 25 °C for 3 days to remove carbonate carbon, washed to neutrality with distilled water, dried, and pulverized. Controlled studies revealed no effect of acid treatment on $\delta^{13}C$ of bulk soil organic matter.[40] Soils treated in this manner contain largely the 'heavy fraction' of soil organic carbon, consisting of humified, organomineral-complexed organic matter.[41]

Soil particle size separates (>50 μm, sand; 50–5 μm, coarse silt; 5–2 μm, fine silt; 2–0.2 μm, coarse clay; and <0.2 μm, fine clay) were isolated from one soil core in each patch type by sieving and sedimentation,[42] and carbonates were removed from the separates with 1 N HCl as described above. Particle size separates were then dried, pulverized, and saved for isotopic analysis.

Supplemental soil samples collected from grasslands and groves in April 1994 were passed through a 2 mm screen to remove coarse roots, dried, and pulverized.

Stable carbon isotope analyses

Plant foliage, litter, roots, and organic matter in whole soils and particle size separates were combusted to CO_2 at 900 °C for 2 h and then 650 °C for 2 h in the presence of CuO and Cu in sealed quartz tubes.[43] The CO_2 was isolated and purified by cryogenic distillation, and its isotopic composition determined on a Micromass-903 (VG Isogas, Middlewich, UK) dual inlet, triple collector isotope ratio mass spectrometer. Grassland and grove soils collected in 1994 were weighed into silver capsules (5×9 mm), treated with 10 N HCl, and dried prior to determination of carbon concentration[44] and carbon isotope composition on a Delta Plus/Carlo Erba EA-1108 continuous flow isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). Carbon isotope ratios are presented in δ-notation:

$$\delta^{13}C(‰) = [(R_{SAMPLE} - R_{STD})/R_{STD}] \times 10^3 \quad (1)$$

where R is the $^{13}C/^{12}C$ ratio of the sample or the standard gas. Results are reported relative to the international Vienna Pre Dee Belemite (V-PDB) standard by calibration with NBS-19.[45,46] Overall precision (machine error plus sample preparation error) was <0.2‰ for both techniques.

The fraction of carbon derived from C_4 sources (F_{C4}) in herbaceous biomass, litter, roots, and soil organic matter was estimated by the mass balance equation:

$$F_{C4} = (\delta_{SAMPLE} - \delta_{C3})/(\delta_{C4} - \delta_{C3}) \quad (2)$$

where δ_{SAMPLE} is the $\delta^{13}C$ value of the whole sample (biomass, litter, roots, or soil organic matter), δ_{C4} is the average $\delta^{13}C$ value of the C_4 components of the sample, and δ_{C3} is the average $\delta^{13}C$ value of the C_3 components. The fraction of carbon derived from C_3 plant sources would then be $1 - F_{C4}$.

Radiocarbon content and mean residence time of soil organic matter

Two soil cores per patch type (one from each landscape) were divided into 0–15, 15–30, 30–60, and 90–120 cm depth intervals and analyzed for natural ^{14}C content at the University of Arizona Laboratory of Isotope Geochemistry. Roots and particulate organic debris were removed by sieving and flotation, and carbonates were removed by treatment with HCl prior to ^{14}C analyses. Thus, ^{14}C

Table 1. $\delta^{13}C$ values (‰ vs. VPDB) of leaf tissue from savanna plant species at the La Copita Research Area in southern Texas, USA. Reprinted from Boutton *et al.*[36] with permission from Elsevier Science B.V.

	$\delta^{13}C$ (‰)	
	Mean	S.E.†
C_3 Woody Plants:		
Celtis pallida Torr.	−26.8	0.3
Condalia hookeri M.C. Johnst.	−27.3	0.4
Diospyros texana Scheele	−25.6	0.3
Mahonia trifoliolata (Moric.) Fedde	−27.2	0.7
Prosopis glandulosa Torr. var. *glandulosa*	−25.1	0.5
Zanthoxyplum fagara (L.) Sarg.	−29.1	0.3
Mean for C_3 woody plants	−26.9	0.6
C_3 Forbs:		
Amblyolepis setigera DC	−28.7	—
Ambrosia confertiflora DC.	−31.0	—
Aphanostephus riddellii T. & G.	−31.6	—
Commelina sp.	−27.7	—
Gaillardia pulchella Foug.	−29.0	—
Helenium microcephalum DC.	−29.6	—
Lepidium sp.	−26.2	—
Melampodium cinereum DC.	−30.5	—
Oxalis sp.	−30.8	—
Parthenium confertum Gray	−29.7	—
Parthenium hysterophorus L.	−27.6	—
Plantago sp.	−28.7	—
Verbesina encelioides (Cav.) Benth. & Hook. *ex* Gray	−32.0	—
Wedelia hispida H.B.K.	−28.8	—
Mean for C_3 forbs	−29.4	0.4
C_4 Forbs:		
Froelichia gracilis (Hook.) Moq.	−16.5	—
C_4 Grasses:		
Bouteloua hirsuta Lag.	−14.1	0.1
Bouteloua trifida Thurb.	−15.0	0.0
Chloris cucullata Bisch.	−13.8	0.3
Chloris pluriflora (Fourn.) Clayton	−14.5	0.1
Panicum hallii var. *filipes* (Scribn.) Waller	−14.4	0.1
Paspalum pubiflorum Rupr. & Fourn.	−13.1	0.1
Setaria texana Emery	−12.4	0.1
Tridens albescens (Vasey) Woot. & Standl.	−15.0	0.2
Mean for C_4 grasses	−14.0	0.3
CAM Plants:		
Opuntia leptocaulis DC.	−15.8	3.1
Opuntia lindheimeri Engelm.	−15.4	0.7
Mean for CAM plants	−15.6	0.2

† Standard error.

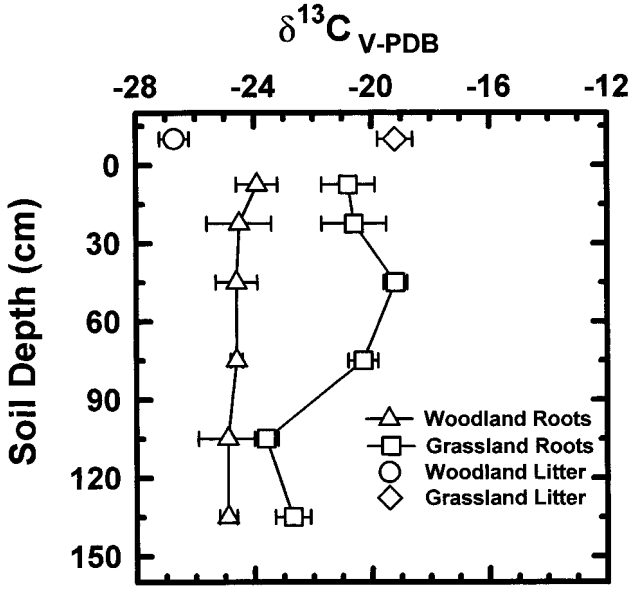

Figure 1. Mean $\delta^{13}C$ (‰ vs. VPDB) values of litter and roots from woody and herbaceous patches at the La Copita Research Area in southern Texas. $\delta^{13}C$ values for woodland roots and litter are means ± standard deviation (n = 36) for all woody patch types combined (discrete clusters, groves, and transitional and drainage woodlands). Data from Boutton *et al.*[36]

measurements were made on the same soil organic carbon fraction (humified, organomineral-complexed organic matter) as the $\delta^{13}C$ measurements. Procedures, instrumentation, and instrument performance for ^{14}C counting are described by Kalin and Long.[47] The mean residence time (MRT) of soil organic matter was estimated using a simple proportional replacement soil carbon model.[48]

Collection of plant and soil samples for δ^2H and $\delta^{18}O$ analyses

Plant and soil samples were collected from four contrasting patch types (grassland, discrete cluster, grove, and drainage woodland) in May 1995, October 1995, and May 1996 for δ^2H and $\delta^{18}O$ analyses. During each sample period, patch types were replicated twice; within each patch type, there were five replicates of each plant species. Branches >2 cm in diameter were sampled from woody plants, while crown or root tissue (near the root/shoot interface) was collected from grasses and forbs (= herbaceous dicots). Plant tissue samples were stored individually in dry toluene immediately after collection. One soil core (10 cm diameter) was taken to a depth of 5 m within each patch replicate during each sample period. Soil cores were divided into 10 cm depth increments. Each depth increment was then bisected longitudinally; half of each increment was stored in dry toluene for subsequent isotopic analysis, and the other half was sealed in a metal tin for determination of gravimetric soil water content and root biomass. Gravimetric water content was assessed by weighing soil samples before and after drying at 105 °C for 3 days. Root biomass was determined by washing the soil through a 0.5 mm screen, then drying and weighing the roots.

Extraction and isotopic analyses of plant and soil water

Water was extracted from soil and stem samples by azeotropic distillation in dry toluene.[49] This method has been shown to extract hydrologically active water, but not heat-labile or crystallization water which is isotopically fractionated and not intimately associated with active soil water.[49,50]

δ^2H determinations were made after reducing water samples to H_2 by zinc reduction[51] in Pyrex vessels at 500 °C. Zinc was obtained from the Biogeochemical Laboratories at University of Indiana (Bloomington, IN, USA). H_2 was analyzed on a Sigma 6 (CJS Sciences, Winsford, UK) dual-inlet gas isotope ratio mass spectrometer. $\delta^{18}O$ was determined after equilibration of water samples[52] with CO_2 in Vacutainers. The $\delta^{18}O$ content of the CO_2 was then determined on a Micromass-903 (VG Isogas, Middlewich, UK) dual-inlet gas isotope ratio mass spectrometer. Hydrogen and oxygen isotopic composition was expressed

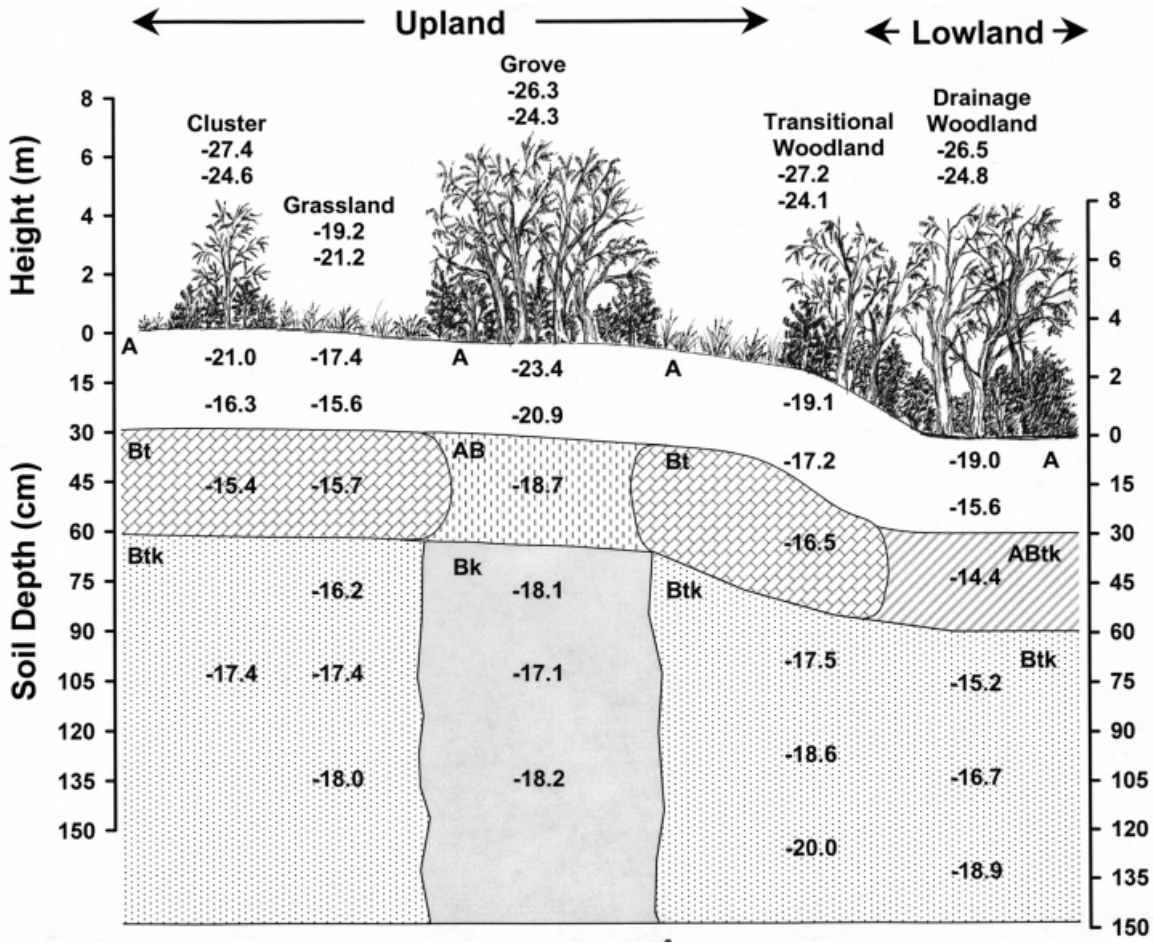

Figure 2. Horizonation and mean $\delta^{13}C$ (‰ vs. VPDB) values of whole soil organic carbon with depth and for different patch types at the LaCopita Research Area in southern Texas. Values on the left axis pertain to upland patches; those on the right axis refer to transitional and drainage woodland patches. For soil organic carbon, each point is the mean of 6 (grasslands, clusters, groves) or 12 (transitional and drainage woodlands) replicates. The first number below the name of each patch type is the mean $\delta^{13}C$ value for aboveground litter in that patch; the second number below the name is the mean $\delta^{13}C$ value (averaged across all soil depths) for roots. The standard error associated with each $\delta^{13}C$ value in this figure is less than 1.3‰. Letters within the belowground portion of the figure denote soil horizons. Note that the aboveground scale (m) differs from that belowground (cm). The slope change from upland to lowland is exaggerated. Data from Boutton *et al.*[36]

in conventional δ-notation relative to Vienna Standard Mean Ocean Water (V-SMOW):

$$\delta^2H_{V-SMOW} \text{or} \delta^{18}O_{V-SMOW}(‰) = \{(R_{SAMPLE}/R_{V-SMOW}) - 1\} \times 10^3 \quad (3)$$

where R_{SAMPLE} and $R_{V\text{-}SMOW}$ are the $^2H/^1H$ or $^{18}O/^{16}O$ ratios of the sample and V-SMOW, respectively. $\delta^2H_{V\text{-}SMOW}$ and $\delta^{18}O_{V\text{-}SMOW}$ values were normalized relative to the V-SMOW/SLAP scale.[53] Precision of duplicate analyses was <2‰ for δ^2H, and <0.4‰ for $\delta^{18}O$.

In May 1995, one replicate of each landscape element was sampled immediately before and the other replicate immediately after an isotopically distinctive 3 cm rainfall event, permitting an evaluation of changes in water uptake strategies in response to that precipitation. Rainfall utilization was estimated from a two-source linear mixing model:

$$F_R = (\delta_{SW-POST} - \delta_{SW-PRE})/(\delta_{RAIN} - \delta_{SW-PRE}) \quad (4)$$

where F_R is the fraction of stem water derived from rain, $\delta_{SW\text{-}POST}$ is the δ^2H or $\delta^{18}O$ of stem water after the rain, $\delta_{SW\text{-}PRE}$ is the δ^2H or $\delta^{18}O$ of stem water before the rain, and δ_{RAIN} is the δ^2H (−0.4‰) or $\delta^{18}O$ (−1.0‰) of the rain.

RESULTS

$\delta^{13}C$ of vegetation and soil organic carbon and its implications for ecosystem dynamics

All woody plants and forbs (except *Froelichia gracilis*) had $\delta^{13}C$ values characteristic of C_3 plants (Table 1). $\delta^{13}C$ values of woody plants averaged -26.9 ± 0.6‰, while those of the forbs (herbaceous dicots) averaged -29.4 ± 0.4‰. All grasses were C_4 species, with a mean $\delta^{13}C$ value of -14.0 ± 0.3‰. $\delta^{13}C$ values of the CAM cacti, *Opuntia leptocaulis* and *O. lindheimeri*, averaged -15.6 ± 0.2‰, similar to those of the C_4 species.

$\delta^{13}C$ values of litter and roots in upland and lowland woody patches were characteristic of C_3 plants. The mean $\delta^{13}C$ value of bulk litter samples averaged across all wooded landscape elements (clusters, groves, transition woodlands, and drainage woodlands) was -26.7 ± 0.5‰. (Fig. 1). Roots from these same woodlands ranged from -23.9 ± 0.7‰ at 0–15 cm to -24.9 ± 0.3‰ at 120–150 cm. In contrast, the $\delta^{13}C$ of grassland litter (-19.2 ± 0.6‰) reflected the mixed C_3 forb-C_4 grass composition of that landscape element (Fig. 1). If C_4 grasses are −14.0‰ and

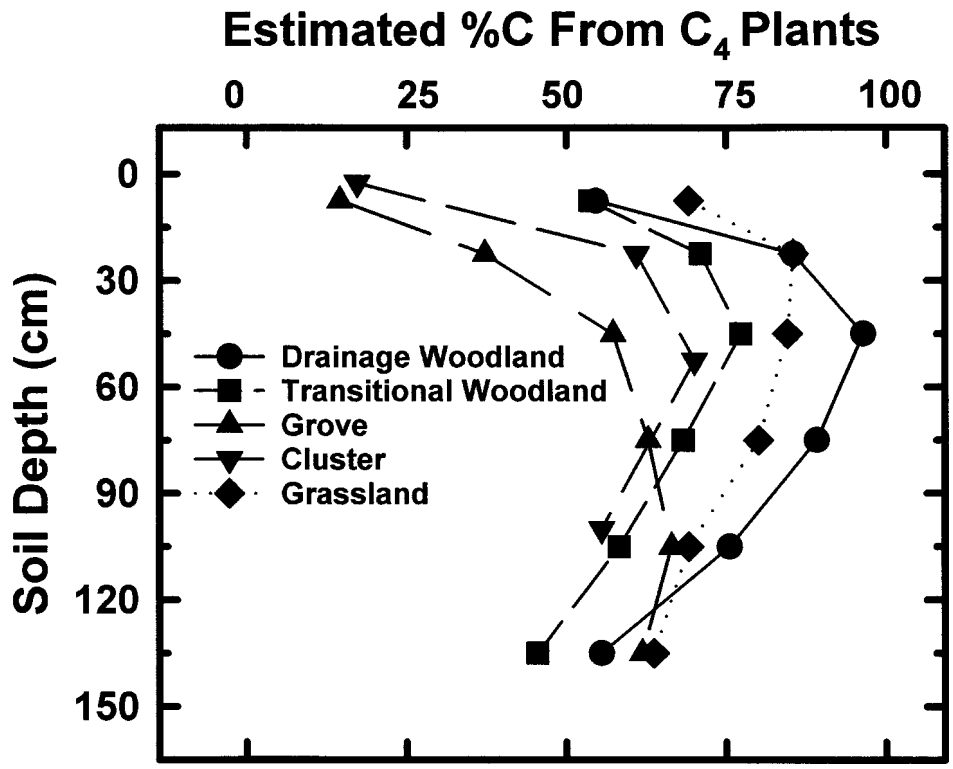

Figure 3. Percentage of total soil organic carbon derived from C_4 plants in different patch types at the LaCopita Research Area in southern Texas. Values were estimated by mass balance (Eqn. 2) based on the $\delta^{13}C$ of soil organic carbon (Fig. 2), and using $\delta^{13}C$ values of $-14‰$ for C_4 plants and $-25‰$ for C_3 plants. Data from Boutton *et al.*[36]

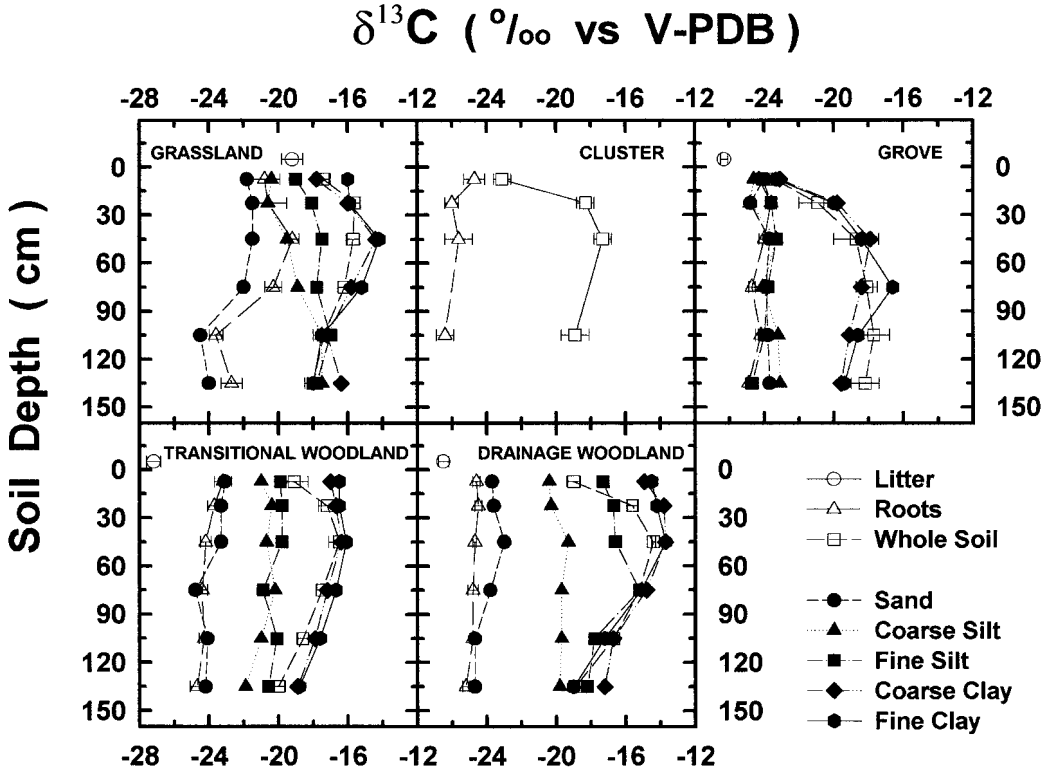

Figure 4. $\delta^{13}C$ (‰ vs. VPDB) values of organic carbon in soil particle size fractions in relation to those for litter, roots, and whole soils in landscape elements at LaCopita Research Area in southern Texas. Particle size fractionation was conducted on one core per landscape unit; hence, values for soil fractions are based on a single analysis. For litter, roots, and whole soils, each point is a mean ± standard error (n = 6 for grasslands, clusters, and groves; n = 12 for transitional and drainage woodlands). Reprinted from Boutton *et al.*[36] with permission from Elsevier Science B.V.

C_3 forbs are −29.4‰ (Table 1), then approximately 66% of the litter is from C_4 grasses according to mass balance (Eqn. 2). $\delta^{13}C$ values of roots in the upper 90 cm of the grassland soil profile were near −20‰, while those from 90–150 cm were near −23‰ (Fig. 1).

There was no significant difference in the isotopic composition of soil organic carbon within a given patch type between the two landscapes. Therefore, data for patch types were pooled across landscapes. $\delta^{13}C$ values of organic carbon in whole soils in the grassland patches were significantly higher than those of the current organic matter inputs (roots and litter), ranging from approximately −18‰ at the surface to −16‰ in the middle of the profile (Fig. 2). $\delta^{13}C$ values of whole soils in the wooded landscape elements were generally much higher (1–10‰) than those of the associated roots and litter. Whole-soil $\delta^{13}C$ values in wooded patches were lowest near the soil surface (−23 to −19‰), and increased to maximum values (−17 to −15‰) between 15–45 cm. With the exception of groves, $\delta^{13}C$ values became more negative below 45 cm, and approached values similar to those of the surface soils. In groves, whole-soil $\delta^{13}C$ values increased gradually from −24‰ in the surface soil to −18‰ below 75 cm. $\delta^{13}C$ values of whole soils in drainage and transitional woodlands were usually within about 1–2‰ of those from the remnant grasslands throughout the soil profile. In contrast, $\delta^{13}C$ values of soils in the upland clusters and groves were significantly lower (by 2–5‰ above 90 cm) than those in the adjacent upland grasslands (Fig. 2).

The proportion of soil organic carbon derived from C_4 plants in each landscape element was estimated from whole-soil $\delta^{13}C$ values by mass balance (Eqn. 2). Based on $\delta^{13}C$ values of litter and roots in wooded areas (Fig. 1), a value of −25‰ was employed as an average for C_3-derived carbon. $\delta^{13}C$ values of C_4 roots and litter were not measured in this study; however, live foliage from C_4 grass species had a mean $\delta^{13}C$ value of −14‰ (Table 1), which we used as the average value for C_4-derived carbon. Based on these assumptions, the proportion of soil organic carbon derived from C_4 sources was greatest in drainage woodlands (55–95%), grasslands (63–86%), and transitional woodlands (45–76%), and lowest in groves (12–65%) and clusters (18–70%) (Fig. 3). For all landscape elements, the greatest proportion of C_4-derived soil organic carbon was located between 15–90 cm in the soil profile.

$\delta^{13}C$ values of organic carbon associated with different particle size fractions isolated from the same soil sample differed by as much as 10‰ (Fig. 4). Within every landscape element, organic carbon associated with the sand fraction had the lowest $\delta^{13}C$ values, and was generally within 1–2‰ of the values for roots and litter. In contrast, fine and coarse clay had higher $\delta^{13}C$ values than all other particle size fractions, and were generally within 1–2‰ of the values for whole soils. $\delta^{13}C$ values for fine and coarse silt were usually intermediate between those of the sand and clay fractions.

$\delta^{13}C$ values of organic matter in the upper 10 cm of soils in upland groves decreased exponentially with increasing grove age (Fig. 5(a)). Values decreased from −19‰ prior to woody plant establishment to −23‰ in groves that were approximately 40–50 years old, then changed little between 50–90 years. Mass balance calculations revealed that even after 90 years of woody plant succession, approximately 47% of the soil carbon (0–10 cm) in groves is from the original C_4 grassland (Fig. 5(b)). When these proportions

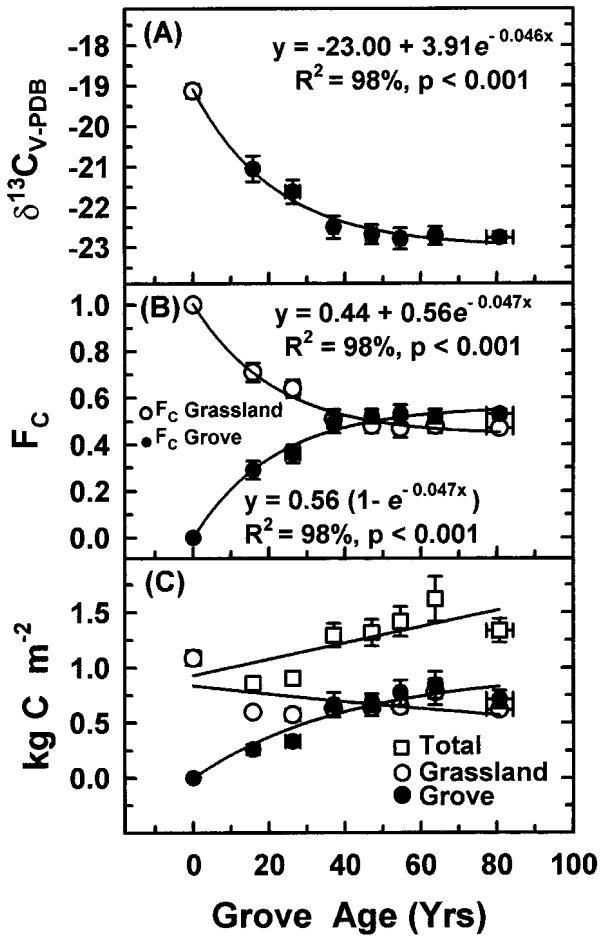

Figure 5. (a) $\delta^{13}C$ (‰ vs. VPDB) values of soil organic carbon (0–10 cm) as a function of upland grove age (as determined by dendrochronology). The hollow circle at time 0 is the $\delta^{13}C$ value (mean ± SE, n = 40) of soil carbon in the remnant grasslands. Each solid circle is the mean of a 10 yr age increment comprised of approximately 5–8 replicates. (b) The fraction of soil carbon (F_c) in the top 10 cm of the profile derived from the original grassland (○) or the present grove (●) vegetation. F_c was calculated by mass balance using −19‰ as the $\delta^{13}C$ of the original grassland and −26‰ as the $\delta^{13}C$ of grove carbon. (c) Soil organic carbon (kg C m^{-2}, 0–10 cm) derived from original grassland (○) and grove (●) sources, and their total (□).

are applied to the actual mass of soil carbon, it appears that carbon derived from the original grassland decreased from 1.09 to 0.67 kg C m^{-2} during 90 years of grove development (Fig. 5(c)). Most of that decrease in grassland-derived carbon occurred within the first 20 years following the establishment of woody plants. Conversely, 0.78 kg C m^{-2} derived from C_3 woody plants accumulated in the soil during that same time interval. Hence, the development of groves on sites that were once grassland resulted in a 33% increase in soil carbon storage from 1.09 to 1.45 kg C m^{-2} (Fig. 5(c)).

The mean residence times (MRTs) of soil organic carbon derived from ^{14}C measurements increased linearly with depth in the profile. MRTs ranged from 34–118 years in the 0–15 cm depth interval to 1370–2930 years in the 90–120 cm interval (Fig. 6). For the 0–15 cm depth interval, MRTs tended to be lower in upland groves and drainage woodlands (≈50 years) than in upland grasslands and transitional woodlands (≈75 years). Below 15 cm, MRTs

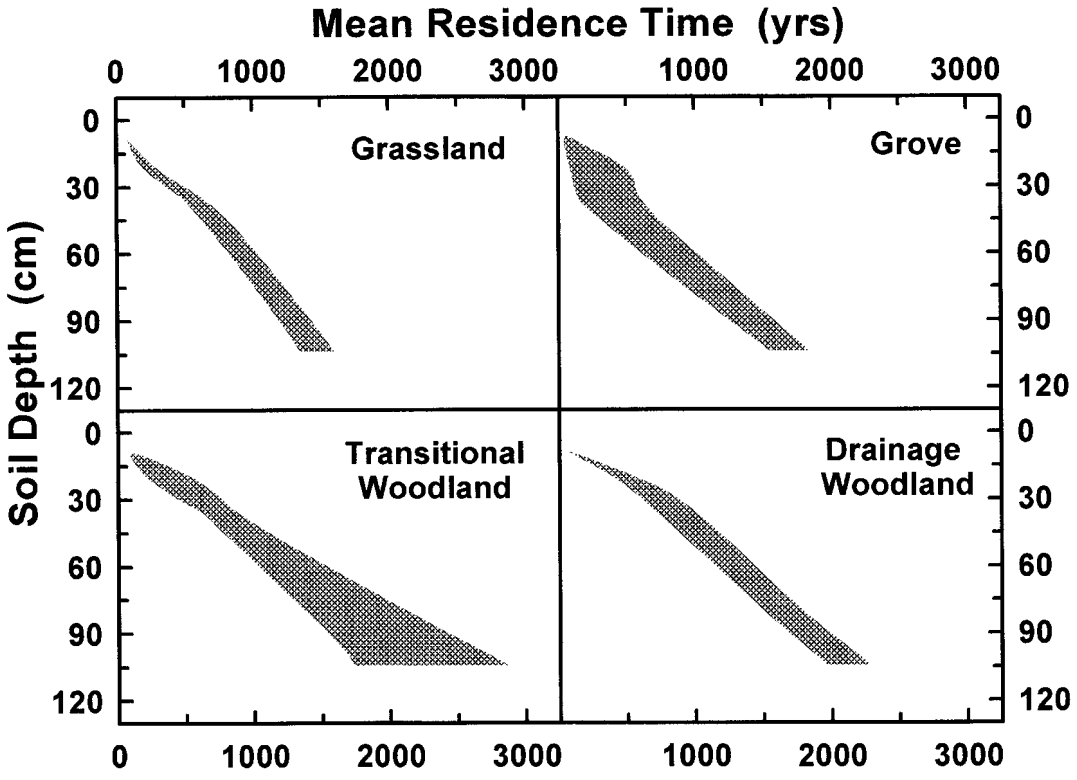

Figure 6. Mean residence times (MRTs) of soil organic carbon in different patch types at the La Copita Research Area in southern Texas. MRTs were model-estimated from the natural ^{14}C content in soil organic carbon. Shaded areas represent the range of values computed by the model when analytical error associated with ^{14}C measurements was considered. Data from Boutton *et al.*[36]

in upland grasslands and groves were generally lower than those from transitional and drainage woodlands (Fig. 6).

δ^2H – $\delta^{18}O$ of the plant-soil-water system and changes in the hydrologic cycle

Root biomass was significantly greater throughout the soil profile in all woody patches compared with remnant grassland patches. For example, root biomass in surface soil layers in groves was generally >1.0 kg m^{-2}, while comparable soil depths in grasslands contained less than half that amount (Fig. 7). In addition, wooded landscape elements had significant root biomass to depths below 5 m, while grasslands had almost no root mass below 1.5 m (Fig. 7).

δ^2H values of stem water from individual plant species ranged from −36 to +10‰ when averaged across sample dates and landscape elements (Fig. 8). The low variation associated with these mean values is noteworthy considering the spatial and temporal ranges they encompass. When these species are examined by functional groups, it is evident that the grasses (−9‰) and cactus (+10‰) had the highest stem water δ^2H values (Fig. 9). In contrast, evergreen trees/shrubs (−27‰), deciduous non-N-fixing trees/shrubs (−29‰), and deciduous N-fixing trees/shrubs (−32‰) had significantly lower values.

δ^2H values of soil water decreased exponentially from +11‰ in the top 10 cm of the profile to −36‰ at a depth of 5 m (Fig. 9). When δ^2H values of plant functional groups are examined in relation to those of the soil water, it appears

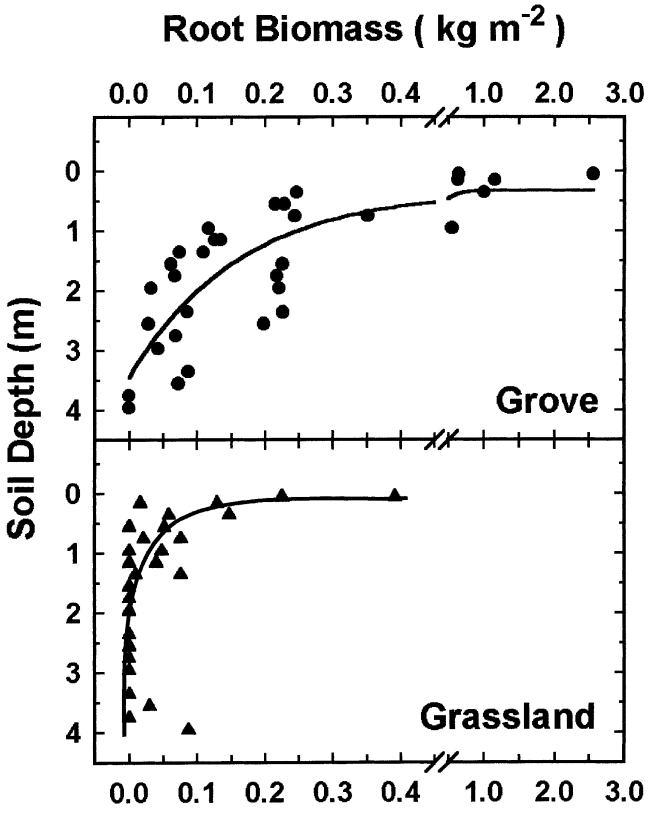

Figure 7. Distribution of root biomass with respect to soil depth in grove (top) and grassland (bottom) patches. Note the change in the scale of the x-axis at 0.5 kg m^{-2}.

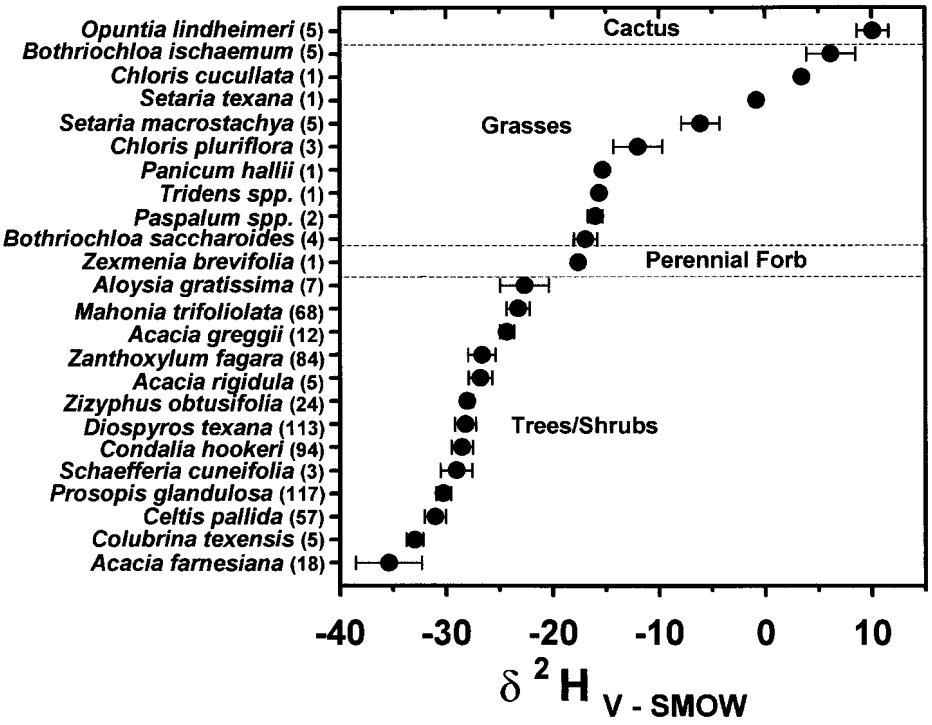

Figure 8. δ^2H values (‰ vs. V-SMOW) of stem water from individual plant species in a savanna ecosystem. Numbers in parentheses following species names indicate number of observations. Each point is the mean of observations acquired from three sample periods and four landscape elements. All species with δ^2H values greater than -20‰ are grasses except for *Opuntia lindheimeri* (cactus). All species with δ^2H values less than -20‰ are trees or shrubs except for *Zexmenia brevifolia* (forb).

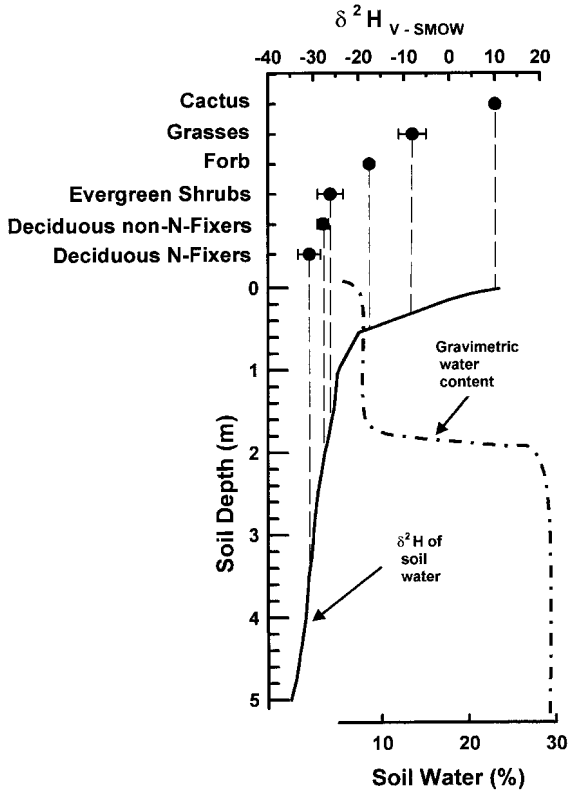

Figure 9. δ^2H values (‰ vs. V-SMOW) of stem water from plant functional groups in relation to δ^2H values of soil water and soil gravimetric water content. δ^2H values of plant and soil water and gravimetric water content are averaged across three sample periods and four landscape elements. See Fig. 8 for list of species comprising the various functional groups.

that plant groups characteristic of remnant grasslands (grasses, forbs, and cactus) obtain their water primarily from the upper 1 m of the profile. However, those groups characteristic of the wooded landscape elements (evergreen shrubs, deciduous non-N-fixing trees/shrubs, and deciduous N-fixing trees/shrubs) appear to obtain soil water from approximately 1–4 m in the profile.

Gravimetric soil water content was $<10\%$ in the upper 1.7 m of the soil profile (Fig. 9). Below that depth, soil water content increased abruptly to 29% and remained relatively constant at that value from 2–5 m in the profile. δ^2H values of plant and soil water indicate that trees and shrubs of wooded landscape elements have access to water in this zone of high gravimetric water content, while the grasses do not.

Immediately after sampling plant and soil water in May 1995, the study area received 3 cm of rainfall with a $\delta^2\text{H} = -0.4$‰ and a $\delta^{18}\text{O} = -1.0$‰. Plants and soils were resampled 2 days after this moisture input. Gravimetric soil water content in the upper 1 m of the profile increased from approximately 3–4% before the rainfall to 6–7% after the rainfall, with the largest increases occurring in the top 30 cm of the soil (Fig. 10). δ^2H and δ^{18}O values of soil water also increased in most depth intervals throughout the upper 1 m of the profile in response to this rainfall event.

The six dominant woody plant species in groves had significantly higher δ^2H and δ^{18}O values of stem water after the rainfall; on average, δ^2H values of stem water increased by $+16$‰, while δ^{18}O values increased by $+3$‰ (Fig. 10). When the isotopic composition of plant stem water is compared with that of soil water, it is evident that these woody species were acquiring soil water primarily from 1–3 m in the profile prior to the rain. After the rainfall,

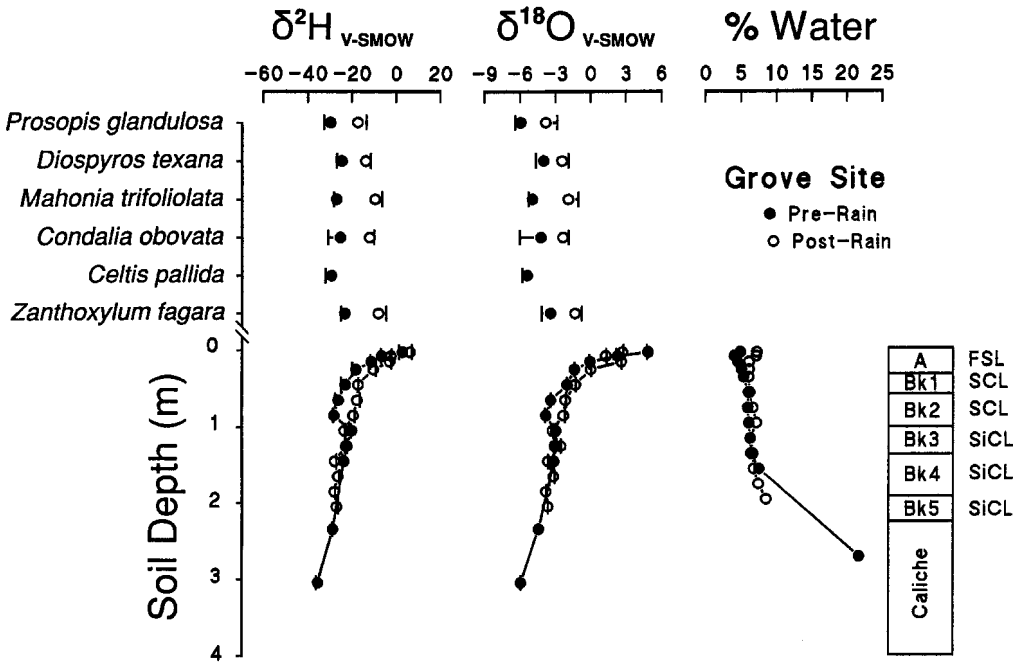

Figure 10. δ^2H and δ^{18}O values (‰ vs. V-SMOW) of plant stem water in relation to those of soil water in groves during May 1995. Gravimetric water content, horizonation, and texture (F = fine, S = sand, Si = silt, C = clay, L = loam) of soil are also shown. Data shown as solid circles were collected immediately prior to a 3 cm rainfall event (δ^2H = −0.4‰; δ^{18}O = −1.0‰), while those shown as hollow circles were collected immediately after that rainfall. All species listed are woody.

all but one of these same species were acquiring soil water from $\leq$1 m in the profile. The exception to this was *Prosopis glandulosa*, which acquired soil water from 2–3 m before the rainfall, and 1–2 m after the rain (Fig. 10). Mass balance calculations (Eqn. 4) suggested that, after the rainfall event, approximately 20–47% of plant stem water was derived from the rain water (Table 2). *Zanthoxylum fagara* (47%) and *Mahonia trifoliolata* (45%) had the highest percentages of rain in their xylem water, while *Prosopis glandulosa* (20%) and *Diospyros texana* (21%) had the lowest.

DISCUSSION

δ^{13}C values of plants and soils from the LaCopita site show that C_3 herbaceous dicots have increased in upland grasslands and that woodlands, groves, and discrete shrub clusters dominated exclusively by C_3 vegetation now occupy sites once dominated by C_4 grasses. ^{14}C ages indicate that soil organic matter derived from C_3 plants is dominated by carbon with MRTs of 40–120 years, substantiating other lines of evidence (tree rings,[36] historical aerial photos,[35] and historical accounts[15]) which indicate that these vegetation changes occurred recently. Concurrent changes in soil δ^{13}C and organic carbon content over the past 90 years demonstrate that this shift in vegetation structure has resulted in significant carbon sequestration. δ^{18}O and δ^2H values of plant and soil water further indicate that grassland species acquire soil water primarily from the upper 1 m of the soil profile, whereas the woody species which have displaced them utilize soil water from throughout the upper 4 m of the profile. These historical changes in vegetation structure have therefore substantively altered the carbon and hydrologic cycles.

Table 2. Rainfall utilization (%) by woody plants in upland groves based on δ^2H and δ^{18}O values of stem water and a simple two-source linear mixing model (Eqn 4). Values represent the proportion of stem water derived from the rainfall event. End members were the isotopic composition of (1) pre-rain plant stem water, and (2) the rainfall. Rainfall utilization was then calculated from the post-rain plant stem water values

	% Rain Utilization		
Plant species	Mean	S.E.†	N
Zanthoxylum fagara	47	12	4
Mahonia trifoliolata	45	8	4
Condalia obovata	29	8	4
Diospyros texana	21	4	4
Prosopis glandulosa	20	7	4

† Standard error.

Change in ecosystem structure: increased abundance of forbs in remnant grasslands

δ^{13}C values of whole-soil carbon in the grasslands (−18 to −16‰) were near those for C_4 grasses in the study area (−14‰), and comparable to those for soil organic carbon in other C_4 grasslands and savannas throughout the world (−18 to −14‰).[54–56] Mass balance calculations (Eqn. 2) show that approximately 75–90% of grassland soil organic carbon was derived from C_4 plants, confirming that soil carbon in that portion of the landscape was inherited from C_4-dominated grassland. However, δ^{13}C values of aboveground biomass and roots averaged −20‰, implying that only about 60% of organic matter production in the present grassland was attributable to C_4 grasses and the remainder from C_3 forbs. δ^{13}C values of soil organic carbon

throughout the grassland soil profile (−18 to −16‰) were significantly higher than those of the above- and below-ground biomass, indicating that the relative productivity of the C_4 grass component was greater in the past. Therefore, it appears that the vegetation history of the present-day grasslands has been one of decreased C_4 grass production and increased C_3 forb production.

Change in ecosystem structure: encroachment of C_3 woodlands into C_4 grasslands

In all wooded landscape elements, the $\delta^{13}C$ values of live foliage (−29 to −25‰), surface litter (−28 to −26‰), and roots (−25 to −24‰) confirmed that carbon inputs are completely C_3-dominated at present. Nonetheless, $\delta^{13}C$ values of soil organic carbon indicate clearly that a substantial proportion of organic carbon in those soils was derived from C_4 plants (Figs 2 and 3). In fact, most of the whole-soil $\delta^{13}C$ values were between −20 and −14‰, suggesting that approximately 50–95% of soil carbon in wooded areas was C_4 in origin. These data provide direct and spatially explicit evidence that areas now dominated by C_3 woody plants once supported plant communities dominated by C_4 grasses.

A particularly strong memory of the C_4 grasslands that once occupied these sites was evident in the $\delta^{13}C$ values of organic carbon associated with the fine and coarse clay fractions, which ranged from −18 to −14‰ across all sites and soil depths. The average $\delta^{13}C$ value of the clay fraction in all wooded landscape elements between 15–90 cm in the profile was −16‰, typical of soil organic carbon in C_4-dominated grasslands throughout the world.[56] These results indicate that organic carbon associated with the fine and coarse clay fractions was derived almost exclusively from C_4 plants, and that little carbon from the present C_3 woodland has been incorporated into this particle size fraction. These findings are consistent with other studies demonstrating slow turnover of clay-associated organic carbon.[41,54,57,58] Organic carbon associated with the clay fraction is comprised principally of aliphatic hydrocarbons,[59–62] which are known to be nearly inert with respect to carbon turnover.[63]

In contrast to the clay fraction, $\delta^{13}C$ values of sand-associated organic carbon in wooded landscape elements were similar to those of the current organic matter inputs (roots and litter), indicating that this fraction was comprised almost exclusively of carbon from the present C_3 plant cover and that carbon turnover in this particle size fraction has been rapid. Mass spectrometric analyses of sand-associated organic carbon have revealed that this fraction is almost identical biochemically to recent plant residues,[64] and the labile nature of this carbon pool has been well documented.[24,41,54,58,65]

Detailed sampling of surficial (0–10 cm) soils revealed that the isotopic composition of soil organic carbon in grove-dominated portions of the landscape was highly correlated with stand age estimated from counts of annual growth rings in *Prosopis glandulosa*. $\delta^{13}C$ values of soil organic carbon and their rate of change with respect to stand age confirm that groves are relatively recent components of the landscape, and provide insights into soil organic carbon dynamics. The initiation and development of groves results in a decrease in the mass of soil carbon derived from the original grassland from 1.09 to 0.67 kg C m^{-2} within the first 30 years of grove development. Martin *et al.*[54] also found that approximately 50% of soil carbon (0–10 cm) derived from C_4 grassland was lost within 25 years of C_3 woodland development in a western Africa savanna. From 30 to 90 years after the initiation of grove development, the mass of soil carbon derived from the original grassland remains relatively constant at approximately 0.67 kg C m^{-2}, suggesting that this residual grassland carbon is comprised of biochemical and/or physical fractions highly resistant to further decomposition. However, 90 years of grove development simultaneously results in the accumulation of approximately 0.78 kg C m^{-2} derived from the C_3 woody vegetation. The net result is a 33% increase in soil organic carbon storage (0–10 cm) from 1.09 kg C m^{-2} at the onset of grove development to 1.45 kg C m^{-2} after 90 years of woody plant production. Soil organic carbon storage has increased even more dramatically in the fine-textured soils of clusters (60%) and drainage woodlands (250%) over the same time interval.[66] Increased soil carbon storage beneath woody components of savannas is well documented,[67–70] and suggests that the increase in woody plant abundance observed in many grassland and savanna regions throughout the world[20] may have implications for the global carbon cycle.[3]

Change in ecosystem structure: estimating the chronology

The approximate timing of the initiation and development of C_3 woody patches in uplands and lowlands, as well as increased C_3 forb abundance in the upland grasslands, can be estimated from the $\delta^{13}C$ and ^{14}C values of soil organic carbon. $\delta^{13}C$ values of soil organic carbon indicate that the isotopic influence of C_3 trees and shrubs in woodlands, and increased C_3 forb abundance in grasslands, is evident primarily in the 0–15 cm depth increment. Measurements of ^{14}C indicate that soil organic carbon in that depth interval was dominated by post-1960s bomb carbon,[36] and the MRTs of that carbon ranged from 34–118 years (Fig. 6). Therefore, the development of wooded landscape elements, as well as the increase in forb abundance in grasslands, has occurred largely over the past 118 years. This inference for the temporal aspect of grassland-to-woodland conversion is well supported by stem age determinations on the dominant tree species (*Prosopis glandulosa*), which indicate maximum tree ages ranging from 44 years in clusters to 109 years in groves.[36]

Change in ecosystem structure: probable causes

Increased woody plant abundance, and increased forb abundance in remnant grasslands, have probably occurred as a result of domestic livestock grazing which began in the 1800s in this region.[16,71] Long-term, heavy livestock grazing has adverse effects on both the above- and below-ground productivity of the most preferred and palatable grasses,[72] and ephemeral or less palatable forbs then become relatively larger components of the herbaceous biomass.[73] Eventually, above- and below-ground biomass may decline, and gaps forming in the herbaceous layer can then be exploited by woody plants, which arrive as seed dispersed via livestock.[20] The establishment of woody species is facilitated because herbaceous interference and fire frequency and intensity have been reduced by defoliation. This grazing-driven grassland-to-woodland

succession may be reinforced by changes in ecosystem hydrology that modify the vertical distribution and abundance of soil water.

Grazing-driven changes in ecosystem structure mediated via the hydrologic cycle

Soil moisture is generally regarded as one of the most important determinants of tree/grass ratios in savannas, and Walter[74] suggested that the coexistence and relative importance of grasses vs. woody plants is controlled largely by differential use of soil water. His 'two-layer hypothesis' states that grass root systems and water acquisition are concentrated in the upper portions of the soil profile, whereas woody plant root systems and water acquisition occur at greater depths in the profile. Thus, the soil has two functionally distinct layers: (1) a surface layer in which grasses, with shallow, dense, fibrous root systems, retain and have first access to water entering from the soil surface; and (2) a deeper layer accessible primarily to the more deeply rooted woody plants. Because grasses and woody species both have roots in the surface layer, the two lifeforms may compete for water there; however, grass roots are absent, rare or less functional in the deeper layer, conferring preferential access to that water to deeply rooted woody plants. Grasses may, therefore, be expected to dominate systems where there is little recharge of deeper soils. Grasses and woody plants may coexist in a stable equilibrium where the quantity of water reaching the deeper soil layer is adequate to support the presence of trees/shrubs, but inadequate to permit the development of a continuous tree/shrub canopy that would shade-out the grass layer.[75,76] In other cases, deep stores of soil moisture may exist (e.g. in winter rainfall systems), but periodic fire may prevent woody plants from exploiting this untapped resource.

The recent woody plant encroachment at the LaCopita site may simply reflect a relaxation of the historic fire regime which resulted when large numbers and high concentrations of grazing livestock removed the fine fuels (grasses) needed to carry fire. Removal of this constraint would then afford woody plants the opportunity to reach deep stores of soil moisture, increase in density and stature, and eventually displace grasses. Alternatively, a stable equilibrium between grasses and widely scattered woody plants at the LaCopita Research Area may have been disrupted by grazing, which often reduces the transpirational surface area and root biomass of the grass layer. A reduction in the ability of the grass layer to acquire and transpire water from upper soil horizons may then have enabled more water to infiltrate deeper into the profile where it would be available to deeply rooted woody plants. As this supply of deeper water increased, woody plants could then increase in size and density at the expense of the grass layer. Reductions in herbaceous biomass and continuity associated with heavy grazing would concomitantly decrease fire frequency, further promoting expansion and development of the woody component.

This scenario has been substantiated by simulation models[75,77–79] and field studies.[3,76,80,81] Therefore, grassland-to-woodland succession in savannas may be driven by grazing-induced changes in the distribution and abundance of soil water, and the differential use of that soil water by grasses vs. woody plants. While this model of tree-grass dynamics is overly simplistic,[82–85] it nonetheless provides a framework for evaluating hydrologic mechanisms that potentially influence vegetation dynamics in savanna ecosystems.

Ecosystem structure and hydrologic function: root distribution patterns

The distribution of root biomass in woody patches and remnant grasslands provides some support for aspects of the two-layer hypothesis. Root biomass in grasslands is confined almost exclusively to the upper 1.5 m of the profile, with the majority of roots occurring in the upper 30 cm of the soil profile. Seedlings of the dominant woody plant (*P. glandulosa*) readily establish in grassland patches, owing to their ability to extend a tap root below the soil depths effectively exploited by grasses within one growing season.[86] Other established woody species in groves (Fig. 7) and other wooded landscape elements at this site[19,36,87,88] have greater maximum rooting depths and are more deeply rooted than the grasses. Indeed, we have recovered roots of woody species at depths >10 m, consistent with observations in other savannas and woodlands worldwide.[25,26] Although root distributions do not necessarily correlate with zones of water uptake,[30,89] these root distribution patterns suggest that woody species have potential access to soil water located beyond the reach of grass root systems.

Ecosystem structure and hydrologic function: δ^2H-δ^{18}O evidence for plant water use

δ^2H and δ^{18}O values of soil water decreased exponentially with increasing depth in the soil profile as a result of evaporative enrichment. This enrichment occurs due to both an equilibrium fractionation associated with small differences in chemical potentials of isotopic species, and a kinetic fractionation associated with differences in rates of vapor diffusion of isotopic species through the atmospheric boundary layer.[90] This exponential decrease in δ^2H and δ^{18}O values with soil depth is typical of soil in arid and semiarid regions.[91,92] Furthermore, these differences in the isotopic composition of soil water make it possible to evaluate patterns of plant water acquisition with respect to soil depth since there is no isotopic fractionation during water uptake by roots.[27–29]

Plant xylem water from dominant grass species in the remnant grasslands had relatively high δ^2H values, ranging from approximately -20 to $+5$‰. These values for xylem water were comparable with those for soil water in the upper 0.5 m of the profile, indicating that this is where grasses acquire the majority of their soil water. These results are in agreement with prior studies at this site[86] and other savanna sites[93–98] which indicate that water uptake by savanna grasses is confined largely to the upper 0.5–1.0 m of the soil.

All trees and shrubs appeared to be using soil water from greater depths than grasses or forbs (Figs 8 and 9). Evergreen shrubs had the highest δ^2H values (-26‰), suggesting dependence on soil water located at approximately 1–2 m. In contrast, the deciduous N-fixers were the functional group with the lowest δ^2H values (-32‰), implying that they were acquiring soil water from >3 m. A similar difference in soil water use characteristics between evergreen and deciduous tree/shrub species has been documented in a Mediterranean macchia ecosystem.[99] The deep-rooting habit of the dominant species, *Prosopis glandulosa*, is well described at this site[19,88] and elsewhere throughout its range,[100–102] but the rooting patterns of the

other N-fixers (two *Acacia* species) are unknown. Our results suggest their functional roots are located at depths comparable to those of *Prosopis*.

The potential for water resource partitioning between individual species within wooded landscape elements appears considerable. *Prosopis glandulosa* is the dominant overstory species and has >47% of its root density and biomass at depths >0.4 m, while *Zanthoxylum fagara* is the dominant understory species and has >68% of its root density and biomass at depths <0.4 m.[19,88] The functional significance of these contrasting root distribution patterns are confirmed in this study (Figs 8 and 9) and are consistent with inferences derived from seasonal patterns of xylem water potential observed for these species.[20] *Prosopis glandulosa* had a mean xylem water δ^2H value of −32‰, indicating that it acquired soil water from approximately 2–3 m in the profile. In contrast, *Zanthoxylum fagara* had a mean xylem water δ^2H value of −26‰, indicating that it acquired soil water from approximately 1–2 m in the profile. Although these data are suggestive of resource partitioning, selective removal experiments indicate that resource extraction by shallow-rooted understory shrubs has a significant negative impact on *P. glandulosa* growth, seed production, and survival,[103] whereas removal of the deeply rooted overstory *P. glandulosa* had little impact on the understory species.[104] The hypothesis that shallow-rooted understory shrubs limit deep percolation of water to zones where *P. glandulosa* roots are concentrated is not supported by our data for soil moisture content and isotopic composition (Fig. 9). Suppression of the overstory tree by the understory shrubs may, therefore, reflect competition for nutrients rather than water.

The soil depth at which most woody plant species appear to be acquiring their water coincides almost precisely with a region of elevated gravimetric water content. At approximately 1.7 m, soil water content increases from <10 to nearly 30%. Although δ^2H values of plant xylem water indicate that several species utilize this water, the extent to which this water below 1.7 m is plant-available has not been quantified. Soil texture above and below this transition is comparable (silty clay loam, Fig. 10), suggesting there are no major changes in texture that might render this deeper water less available than water located in the upper, drier portions of the profile. Furthermore, the ability of deeply rooted woody species (especially *Prosopis*) to maintain favorable water relations even during the hottest, driest portion of the growing season[20] suggests that the soil water below 1.7 m is a significant resource available to plants capable of extending and maintaining functional roots to these depths. An evaluation of δ^2H values of plant and soil water reveals that the grasses, forbs, and cactus that characterize the remnant grasslands are apparently unable to utilize this deep soil water.

Further insights regarding root function in wooded landscape elements were obtained by sampling before and after a small (3 cm) rainfall event. Before the rain, woody species in upland groves utilized soil water from depths of 1–3 m; after the rain, these same species acquired soil water from shallower (<1 to 2 m) depths. Mass balance calculations indicated that, after the rainfall, approximately half of the xylem water in shallow-rooted species (*Zanthoxylum fagara* and *Mahonia trifoliolata*)[88] was derived from that rainfall event. In contrast, only 20–30% of the xylem water in more deeply rooted species (*Prosopis glandulosa, Diospyros texana*, and *Condalia obovata*) was derived from this rainfall. These differences between woody species clearly reflect a combination of both architectural and functional differences between their root systems. *Zanthoxylum* and *Mahonia* demonstrated considerable flexibility in adjusting depth of water uptake to capitalize on the small rainfall event. Although *Prosopis* (and probably *Diospyros* and *Condalia*) has abundant shallow roots[19,88] and clearly did utilize some soil moisture in the upper profile derived from the rainfall, it continued to rely primarily on deeper soil moisture; hence, the mere presence of abundant root biomass and density in upper portions of the profile did not enable *Prosopis* to utilize this rainfall event more extensively. Other studies confirm that *Prosopis* is strongly dependent on deeper roots, and that its shallow roots are less functional than those of other co-occurring species.[105] Similar interspecific differences in the ability to utilize soil water from small rainfall events have been documented in desert shrub[106,107] and semi-arid woodland[83] communities.

Overall, our studies of the isotopic composition of plant and soil water, in conjunction with studies on root distributions, generally support the validity of the two-layer hypothesis for this ecosystem. Grasses, forbs, and cacti in the remnant grasslands appear to be shallow rooted and reliant on soil water located above 1 m in the profile. In contrast, woody plants have significantly greater and deeper root mass that enables them to acquire soil water deeper in the profile where it is apparently more abundant. However, significant differences in root biomass distribution and water acquisition were apparent among woody species. These differences among co-occurring woody plants provide a much richer perspective on the nature and complexity of tree-grass and tree-tree interactions. Contrasting below-ground patterns and processes, when viewed in conjunction with above-ground contrasts in structure and function, have furthered our understanding of tree-grass and tree-tree interactions in this subtropical savanna parkland and have generated some perspectives as to how and why their interactions might change with time. The replacement of relatively shallow-rooted grasses by relatively deeply rooted woody plants has clearly and substantially altered the hydrologic function of this landscape.

SUMMARY

δ^{13}C values of soil organic matter, above- and below-ground plant biomass, and litter in conjunction with radiocarbon dating and dendrochronology reveal that, in the Rio Grande Plains of southern Texas, C_4 grasslands and savannas have been largely replaced by C_3 woodlands. The presence of the C_4 grasslands that once occupied these sites was most evident in the δ^{13}C values of organic carbon associated with fine and coarse clay (−18 to −14‰), probably a consequence of slow organic carbon turnover rates in those soil fractions. When δ^{13}C values of soil organic carbon are evaluated in conjunction with radiocarbon measurements of that same carbon, it appears that this vegetation change from C_4 grassland to C_3 woodland occurred recently, probably within the last 40–120 years. Changes in soil δ^{13}C values and organic carbon content over the past 90 years indicate that wooded landscape elements are behaving as sinks for atmospheric carbon by sequestering carbon derived from both the previous grassland and the present woody vegetation.

Succession from grassland to woodland may have been driven by grazing-induced alterations in the vertical

distribution and abundance of soil water. Consequently, the present woodland has hydrologic characteristics fundamentally different from those of the original grasslands. Compared with plants in remnant grasslands, tree and shrub species in the woodlands have greater and deeper root biomass and density. $\delta^{18}O$ and $\delta^{2}H$ values of plant and soil water confirm that grassland species acquire soil water primarily from the upper 0.5 m of the soil profile, whereas trees and shrubs utilize soil water from throughout the upper 4 m. Thus, soil water that formerly may have infiltrated beyond the reach of the grassland roots and contributed to local groundwater recharge or other hydrologic fluxes may now be captured and transpired by the more deeply and extensively rooted woodland plant communities that dominate the present landscape.

Grassland-to-woodland conversion during the past century has been widely reported in the worlds' drylands. Even so, the ecological consequences of these transformations remain largely unquantified. Since 30–40% of the terrestrial surface consists of arid and semi-arid ecosystems, changes in vegetation structure, carbon cycling, and hydrology similar to those documented in this study may have important implications for regional/global biogeochemistry and climate.

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