VEGETATION DYNAMICS OF A LIVE OAK-JUNIPER SAVANNA: AN ISOTOPIC ASSESSMENT

THESIS

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ABSTRACT

Vegetation of the Edwards Plateau of central Texas prior to European settlement (i.e., 150–200 years ago) is not well characterized. Anecdotal accounts suggest grasslands and plateau live oak savannas (*Quercus virginiana* var. *fusiformis*) may have covered more of the region than they do at present, while the invasive woody Ashe juniper (*Juniperus ashei*) was restricted to fire-protected refugia such as steep cliffs and riparian areas. Stable carbon isotope ($\delta^{13}C$) values from vegetation and soil organic matter were measured to test for shifts in the relative contribution of C_3 and C_4 plants in grasslands, as well as to investigate the stability of live oak cluster and Ashe juniper woodland patches during the last several hundred years at an upland savanna parkland in the eastern Edwards Plateau. In addition, soil organic carbon, soil nitrogen, and stable nitrogen isotopes ($\delta^{15}N$) were measured to investigate differences between the woody patches and grasslands in nutrient storage and cycling. The $\delta^{13}C$ profiles beneath grassland sites changed significantly with depth and averaged -20.8 ± 1.3‰ (in surface litter) to -13.2 ± 0.1‰ (in soil at 20–30 cm depth), indicating that a significant decline in the relative importance of C_4 species has occurred in these grasslands over the last several hundred years. In addition, grassland $\delta^{13}C$ profiles were distinct at all depths from profiles beneath discrete oak clusters, which averaged -26.7 ± 0.2 and -16.7 ± 0.3‰, for surface litter and soil between 20–30 cm, respectively. With depth, soil $\delta^{13}C$ values from sites within an Ashe juniper woodland converged with those of an adjacent savanna, except for values

from the most interior site which was situated near a steep, north-facing cliff. Soil organic carbon and total nitrogen values were greatest at live oak clusters, intermediate within the juniper woodland, and lowest in grasslands. The results suggest: (1) though the grassland vegetation currently contains many C_3 species known to increase under grazing pressure, the relative importance of C_4 species was much greater in these grasslands several hundred years ago, and likely declined due to grazing, (2) discrete live oaks have been present in these savannas > 300 years, (3) the contribution from C_4 and/or CAM species was greater at live oak sites in the past, possibly reflecting a more grassy understory which has since been replaced by woody shrubs, (4) Ashe juniper occurred in sites associated with steep cliffs and/or drainages prior to European settlement, but the density and range of this invasive species has increased in recent times, and (5) encroachment of woody plants on Freeman Ranch serves to increase soil carbon and nitrogen sequestration.

INTRODUCTION

Vegetation change is a well-documented phenomenon in many savanna ecosystems throughout the world. Perhaps one of the most significant recent changes in savanna vegetation worldwide has been an increase in woody plant cover at the expense of grass (herbaceous) cover (Archer 1994). This increase has likely been caused by some combination of factors including natural climate and ecological fluctuations, as well as human influenced factors such as alterations of the natural fire regime, domestic grazing, and perhaps global increases in atmospheric CO_2 (Smeins 1976, Archer et al. 1995, Fuhlendorf et al. 1996, Polley et al. 1996).

The Edwards Plateau of central Texas is an area of approximately 93,000 km^2 (24 million acres), much of which is vegetated by live oak-juniper savanna parklands and woodlands (McMahan et al. 1984). Historical accounts (e.g., Olmstead 1857) suggest that the current vegetation of the Edwards Plateau has been greatly altered from its pre-European settlement state (i.e., 150–200 years ago). Specifically, the native grasslands are thought to have experienced periods of intense grazing by domestic livestock in the recent past which has caused: (1) a decrease in grazing-sensitive C_4 tallgrasses and midgrasses; and (2) a concurrent increase in grazing-resistant C_4 and C_3 shortgrasses and C_3 forbs (Smeins and Merrill 1988, Boutton et al. 1993). This alteration of community composition has, in turn, led to an overall reduction in herbaceous productivity

(Fuhlendorf and Smeins 1997). Associated with this degradation in grasslands has been an increase in the abundance of unpalatable woody (C_3) plants such as Ashe juniper (*Juniperus ashei*) (Fuhlendorf and Smeins 1997). As in other savanna ecosystems, this shift towards greater woody plant dominance may be the most significant alteration of the area's vegetation.

In many present-day landscapes throughout the Edwards Plateau, Ashe juniper is the dominant woody species and is increasing in abundance (Smeins and Merrill 1988, Van Auken 1993). Juniper woodlands are long-term components of eastern Edwards Plateau vegetation (Krueger 1976, Bryant and Shafer 1977, Weniger 1984, Smeins et al. 1997). However, because junipers are effectively suppressed by fire (which was thought to have been more frequent in this region before European settlement), this species may have been historically restricted to fire-protected refugia such as rocky outcrops, steep cliffs, or along drainages (Olmstead 1857, Foster 1917, Goyne 1991, Smeins 1980, Fonteyn et al. 1988, Fuhlendorf et al. 1996).

In contrast to Ashe juniper, plateau live oaks (*Quercus virginiana* var. *fusiformis*) are thought to have been long-term components of savanna parklands in this region and possibly the only abundant woody element of the pre-settlement upland landscapes (Olmstead 1857, Buechner 1944). Today live oaks frequently occur in discrete tree-shrub clusters, which typically consist of one to several, single- or multi-stemmed live oaks and an understory dominated by shrubs, including Ashe juniper (Phillips 1999).

Overall, anecdotal accounts exist regarding the composition of the vegetation on the Edwards Plateau at the time of European settlement. However, these accounts are often biased and imprecise, and even sometimes contradictory (Smeins 1980,

Smeins et al. 1997). Consequently, the precise composition and distribution of pre-European settlement vegetation in this region remains poorly documented.

One approach that has been used to investigate long-term vegetation change and infer historic vegetation composition involves the use of stable carbon isotopes (Boutton 1996). In general, higher plants possess one of three photosynthetic pathways (i.e., C_3, C_4, or CAM) that exhibit characteristic ratios of stable carbon isotopes (i.e., ^{13}C to ^{12}C). These carbon isotope ratios, usually expressed as $\delta^{13}C$ values, typically range from ca. -32 to-22‰ (mean = -27‰) for C_3 species, while values for C_4 species range from ca. -17 to -9‰ (mean = -13‰). Species exhibiting obligate CAM photosynthesis typically have $\delta^{13}C$ values similar to C_4 plants, though facultative CAM species may range from -30 to -10‰ (Griffiths 1992). If a $\delta^{13}C$ measurement is made from a mixed C_3-C_4 community sample, the distinct isotopic differences between C_3 and C_4 species can be used to determine the relative contribution of plants from each of these two photosynthetic pathways to the net primary production of the sample (Tieszen and Boutton 1989).

Because soil organic matter (SOM) is largely derived from the decomposition of plant tissue, the $\delta^{13}C$ values of SOM resemble the $\delta^{13}C$ values of the plant communities that form it. Therefore, $\delta^{13}C$ measurements from SOM can be used to determine the relative contributions of C_3 and C_4 species to historical vegetation (Stout and Rafter 1978, Tieszen and Boutton 1989). Radiocarbon measurements indicate that the mean age of SOM typically ranges from less than 200 years old near the surface to 2000–4000 years at a depth of 1 m (Scharpenseel and Neue 1984). Thus, at sites with limited soil erosion or disturbance, organic carbon in surface soils should reflect the C_3/C_4 composition in

current and/or recent vegetation, while that of deeper soils should reflect the composition of previous communities. Comparing the $\delta^{13}C$ values of the current organic matter inputs (from vegetation and litter) with that of the soil organic matter in a profile of various depths can, therefore, be used to infer how the C_3/C_4 composition has changed over time. For example, stable carbon isotopes of vegetation and soil organic matter have previously been used in this manner to document the recent invasion of C_4-dominated grasslands by C_3 woody plants (Steuter et al. 1990, McPherson et al. 1993, Schwartz et al. 1996, Boutton et al. 1998), as well as the replacement of C_3-plant-dominated communities by C_4 plants (Hendy et al. 1972, Dzurec et al. 1985).

Some $\delta^{13}C$ studies have documented a small difference in $\delta^{13}C$ between SOM and current litter inputs, even on sites where the vegetation is known to have been compositionally stable (e.g., Nadelhoffer and Fry 1988, Balesdent et al. 1993). The exact causes of this difference (typically an enrichment with depth of +1 to 3‰) are not completely understood (Boutton 1996), though a number of possible explanations have been suggested. These explanations include shifts in the $\delta^{13}C$ of atmospheric CO_2 or, fractionation by microbial decomposers, as well as the combined effect of: (1) the addition of plant litter (which often exhibits lower $\delta^{13}C$ values than roots) to surface soils, and (2) the addition of roots to deeper soils (Boutton 1996, Biedenbender 1999).

In addition to stable carbon isotopes, stable isotopes of nitrogen (^{15}N and ^{14}N) have also proven useful in the investigation of vegetation dynamics, particularly regarding N-cycling (Högberg 1997, Martinelli et al. 1999). Specifically, $\delta^{15}N$ values tend to be higher in soil than in plant tissue, and $^{15}N/^{14}N$ ratios tend to increase with depth in the soil profile (Högberg 1997, Nadelhoffer and Fry 1994). This increase in $\delta^{15}N$ with depth may

be due to the dilution of ^{15}N in surface soils by the addition of ^{15}N-depleted litter (Nadelhoffer and Fry 1988), combined with factors such as volatilization (e.g., NH_3), leaching (e.g., NO_3^-), and/or plant fractionation during N-uptake, all of which tend to leave soil at depth enriched in ^{15}N (Högberg 1997). Ecosystems with more available N, or more open N-cycles, are thought to exhibit profiles more enriched in higher $\delta^{15}N$ values (Högberg 1997, Martinelli et al. 1999). Because the encroachment of woody plants into grasslands typically alters patterns of the storage and cycling of nitrogen (Jackson et al. 1990, Scholes and Archer 1997), it is possible that the analysis of soil $\delta^{15}N$ coupled with $\delta^{13}C$ may provide additional information regarding ecosystem level impacts of woody plant encroachment.

The overall objective of this study was to use stable isotopes of carbon and nitrogen from soils and vegetation to investigate historical vegetation changes in a live oak-juniper savanna in the eastern Edwards Plateau of Texas. Specifically, the following hypotheses were tested: (1) the relative contribution from C_4 species to the herbaceous community of these upland grasslands has decreased over the last several hundred years, (2) live oak clusters represent long-term, stable woody patches in these upland savanna parklands, (3) Ashe juniper woodlands were historically associated with steep cliffs or drainages, but the boundaries of these woody patches with grasslands have been dynamic over time, and (4) relative to grasslands, live oak clusters and Ashe juniper woodlands represent sites of increased soil carbon and nitrogen storage and altered patterns of nitrogen cycling.

METHODS

Study site

Studies were conducted at the Southwest Texas State University (SWT) Freeman Ranch (Fig. 1), which is a 1,701 ha (4,204 acre) tract located in the Balcones Canyonlands subregion on the eastern edge of the Edwards Plateau. The area averages 83.0 cm (32.7 in) of precipitation a year (National Climatic Data Center, *personal communication*), though drought and high-intensity rainfalls are common. Mean annual temperature for nearby San Marcos (ca. 8 km east of Freeman Ranch) is approximately 20°C.

The soils at the Freeman Ranch have been classified in the Rumple-Comfort association and the Comfort-Rock outcrop complex (USDA 1984). Both of these soil types consist of shallow, well-drained Argiustolls that range in texture from cherty or stony clay loam to cherty or stony clay. Depth to indurated Cretaceous limestone may range from 0–100 cm in these soil types (USDA 1984), though field observations indicated that soil depth in much of this study area was ≤ 30 cm.

The Freeman ranch grasslands are thought to have been continuously grazed by cattle and sheep since the early- to mid-1800s. Although the current stocking rates are relatively low (1 animal unit/38 acres; Brian Davis, *personal communication*), the

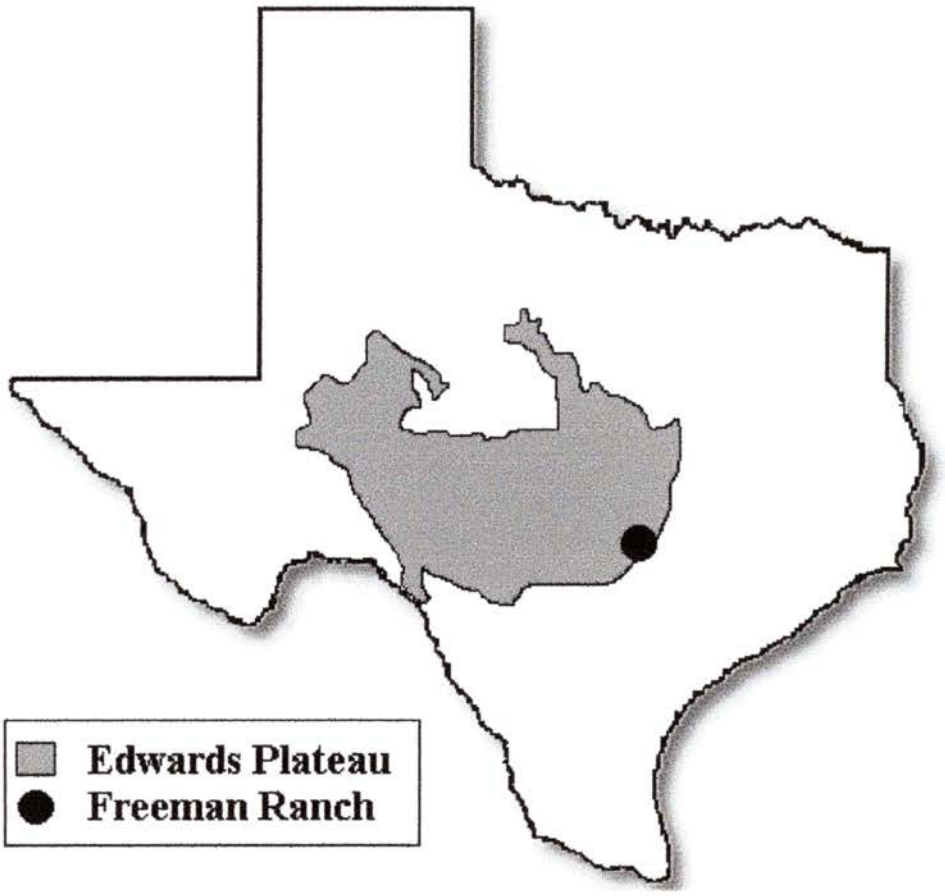


Figure 1. Location of the Southwest Texas State University Freeman Ranch on the Edwards Plateau of Texas.

composition of the grassland vegetation is suggestive of heavy grazing pressures in the past. The potential or climax vegetation at this site is thought to be a live oak savanna (USDA 1984). The grassland community of these savannas should be dominated by C_4 tallgrass-prairie grasses such as little bluestem (*Schizachyrium scoparium*), Indiangrass (*Sorghastrum nutans*), and big bluestem (*Andropogon gerardii*). Barnes et al. (2000), however, found the grasslands at the Freeman Ranch to be currently co-dominated by the C_3 grass, Texas wintergrass (*Nassella leucotricha*), and the C_4 shortgrass, Texas grama (*Bouteloua rigidiseta*). Both of these species tend to increase in grazed rangelands of this region (Launchbaugh 1955, USDA 1984, Boutton 1996). Other contemporary species in these grasslands include C_3 forbs such as prairie croton (*Croton monanthogynus*) and broomsnakeweed (*Gutierrezia texana*), and the C_4 grass King Ranch bluestem (*Bothriochloa ischaemum*).

In contrast to the contemporary grassland community, where many C_3 and C_4 species are found, nearly all the species currently occurring within live oak clusters exhibit the C_3 photosynthetic pathway. These include the live oak and all the understory shrubs and vines, as well as most of the herbaceous understory (*personal observation*). The current dominant species in the woody understory community of these clusters are Ashe juniper, hackberry (*Celtis laevigata*), cedar elm (*Ulmus crassifolia*), Texas persimmon (*Diospyros texana*), elbowbush (*Forestiera pubescens*), and agarito (*Berberis trifoliata*) (Phillips 1999). Common herbaceous understory species (Barnes et al. 2000) of these oak clusters include Texas wintergrass and cedar sedge (*Carex planostachys*). Some epiphytic CAM species, however, also occur with the oaks, such as ballmoss (*Tillandsia*

recurvata), Spanish moss (*Tillandsia usneoides*), and several species of cacti (*Opuntia* spp.) that live on the lower tree branches or in the understory.

Field sampling

To test hypotheses 1 and 2 (i.e., stability of upland grassland and live oak cluster vegetation), six separate upland savanna parkland locations (n = 6) were selected for sampling (Figs. 2 and 3). Each sampling location occurred within a separate fenced pasture and was represented by two habitat patches: a discrete live oak cluster (typically containing a single large live oak (Table 1)) and an adjacent grassland. These sampling locations were also selected, in part, because they were relatively flat (slope < 4°), and occurred at least 100 m from major drainages. Four of the six replicate sampling locations were in pastures that are currently grazed by livestock, while two were located within 5-year old livestock grazing exclosures.

All sampling in the upland grasslands and live oak clusters occurred in July 1999. To assess the $\delta^{13}C$ of the current vegetation community, current organic matter inputs were collected and pooled from three 0.5 × 0.5 m plots within each patch type (oak cluster and grassland) at each sampling location. These current organic matter samples included surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material. Hereafter these current organic matter samples are referred to as "litter". Samples from the live oak cluster patches were taken well-within the edge of the oak canopy (1–3 m from the oak trunk). Grassland samples were taken from adjacent herbaceous interspaces located 10–30 m from the edge of the

Figure 2. Digital color IR aerial image of Freeman Ranch taken in 1995, showing the distribution of upland live oak and grassland sampling locations and the site of the Ashe juniper woodland. Each white circle represents a sampling location which contained a live oak cluster and an open grassland sampling site. The number within the circle indicates the Freeman Ranch pasture in which the sampling location is found. Pasture locations 1 and 8 are within 5-year grazing exclosures, while the others are currently grazed. The Ashe juniper woodland sample site is indicated by a green star, and located in pasture 9.

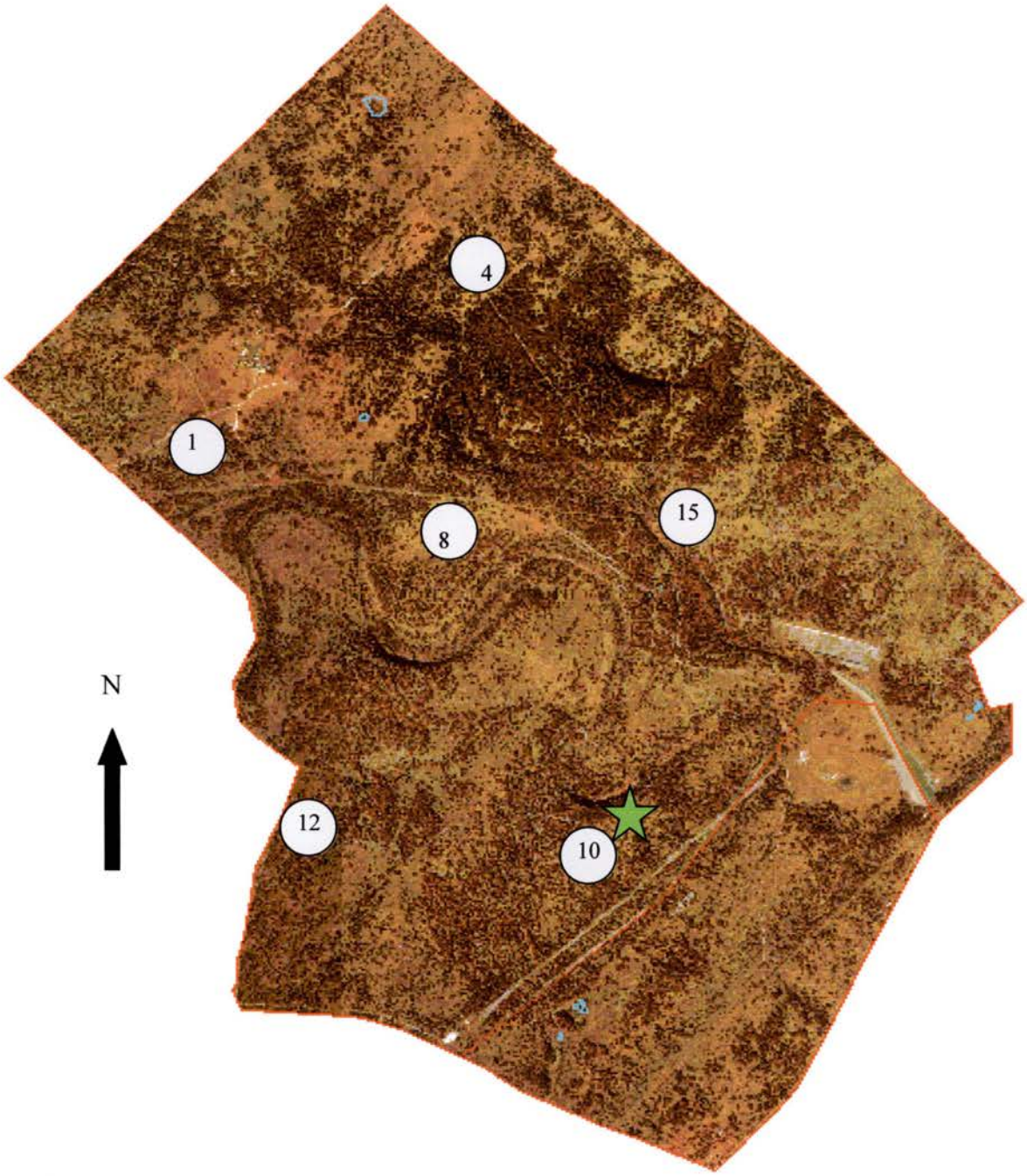

4
1
8
15
N
12
10

Figure 3. An upland live oak savanna sampling location at the Southwest Texas State Freeman Ranch (within pasture 8). Each sampling location included a discrete live oak cluster and a nearby open grassland.

Table 1. Basal diameters (m) of live oaks (*Quercus virginiana* var. *fusiformis*) in the live oak clusters sampled on the Freeman Ranch, July 1999. Pasture locations are shown in Figure 2. *In addition to a live stem, this live oak cluster contained an attached dead stump (basal diam. = 0.56 m).

Pasture	basal diameter (m)
1	1.21
4	1.27
8	1.87
10	0.77*
12	1.78
15	1.33
Mean ± SE	**1.4 ± 0.2**

corresponding oak cluster. Following the removal of litter, soil samples were obtained from each of the three plots with a soil sampling tube (5 × 30 cm soil cores). If rocky soils prevented the use of a soil sampling tube (as was the case at most sites), samples were obtained by hand, by first excavating soil pits, and then removing soil from the side walls. The rocky substrate prevented the collection of soil below 30 cm at most sites. Consequently, soil cores or pit samples were collected from the following four depth increments: 0–5, 5–10, 10–20, and 20–30 cm. Within each habitat patch (grassland or live oak cluster), the three samples for litter and each soil depth were then pooled to obtain one representative sample of each for the sampling locations (i.e., replicates). Also, in 3 of the 6 replicates, an additional surface soil sample (0–20 cm) was collected for soil texture and nutrient characterization (described later).

Seasonal shifts in the relative biomass contribution from C_3 and C_4 plants are known to occur in other mixed prairie grasslands (Ode et al. 1980). Therefore, since the current organic matter inputs were collected from Freeman Ranch grasslands during one month of the year (July), it was necessary to determine if these samples were representative of the overall annual contribution of C_3 and C_4 species to these grasslands. For this test, two 20-m transects located approximately 200 m apart were established within a 5-year old grazing exclosure (Pasture 1, Fig. 1). Five 1.0 × 1.0 m plots were established at 5-m intervals along each transect (i.e., a total of 10 total plots were sampled). Each of these plots were then divided into four 0.5 × 0.5 m sub-plots. On four sampling dates (once every three months from July 30, 1999 to April 30, 2000), standing live and dead biomass was clipped and collected from one of the four sub-plots (n = 10), such that different sub-plots were harvested at each sampling time. These vegetation

samples were then separated into C_3 graminoids (grasses and sedges), C_3 forbs, or C_4 grasses, oven-dried at 80°C for 48 hrs and weighed. These three components of each sample were then re-combined and processed for $\delta^{13}C$ analysis (as described later).

Field observations coupled with analysis of aerial photographs taken in the 1950s suggested that true "old-growth" Ashe juniper stands were rare at the Freeman Ranch. Consequently, all sampling to test hypothesis 3 (i.e., juniper woodland stability) was confined to a single woodland (Figs. 4 and 5). This woodland was located at the top of a steep, north-facing cliff that bordered the major drainage of Freeman Ranch (i.e., Sink Creek). The junipers near the cliff edge were large (two individuals had basal diameters > 1.0 m), "tree-like", and widely-spaced. This juniper stand graded into a mixed juniper-oak woodland approximately 30 m southeast of the cliff edge and then terminated at a fence line (with an unknown history) ca. 100 m from the cliff. On the other side of the fence was a more open savanna containing isolated tree/shrub clusters.

To address the stability of this juniper-dominated woodland and the woodland/savanna boundary, five 50-m transects were established at this site, with each transect running approximately parallel to the cliff edge (Fig. 5). Transect one (T1) was laid deep within the woodland (20 m from the cliff edge). Transect two (T2) was established about 80 m from the cliff edge, but still within the woodland. Transect three (T3) was laid on the boundary between the juniper woodland and the open savanna. Transects four (T4) and 5 (T5) were established within the savanna, at distances of 20 and 80 m from the woodland/savanna boundary, respectively. Litter and soil samples were collected from three composite sample points, located 20 m apart on each transect

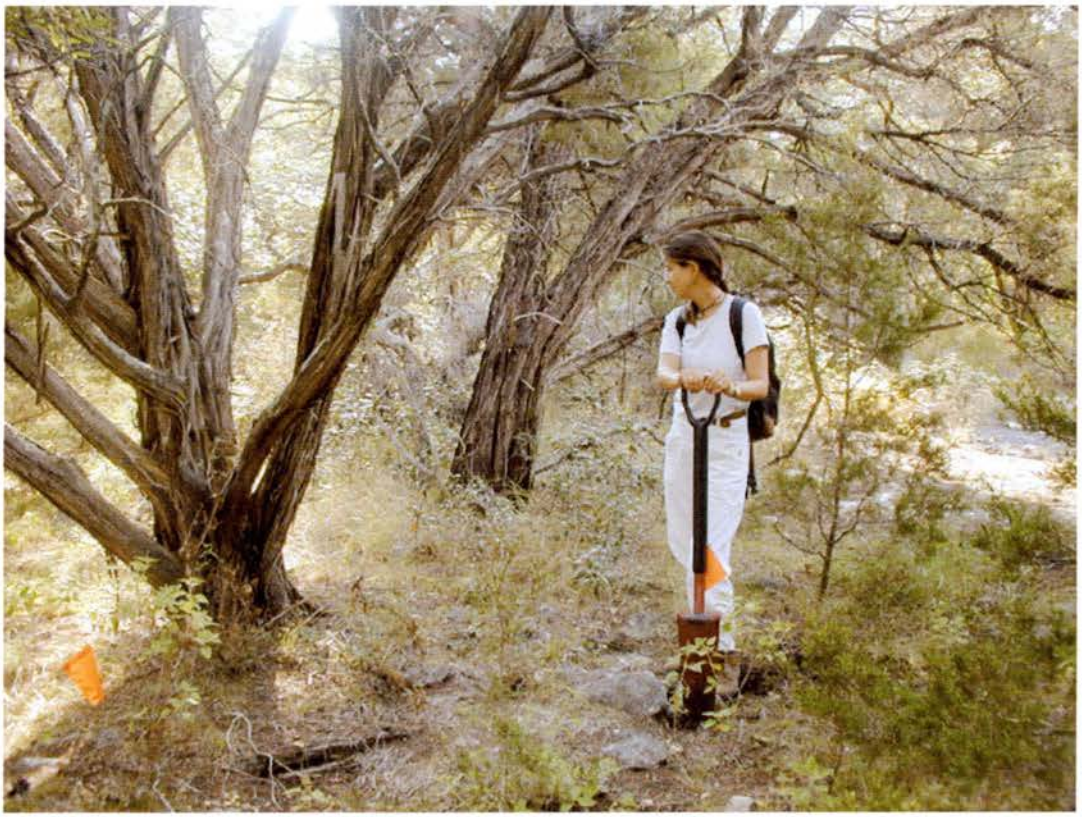
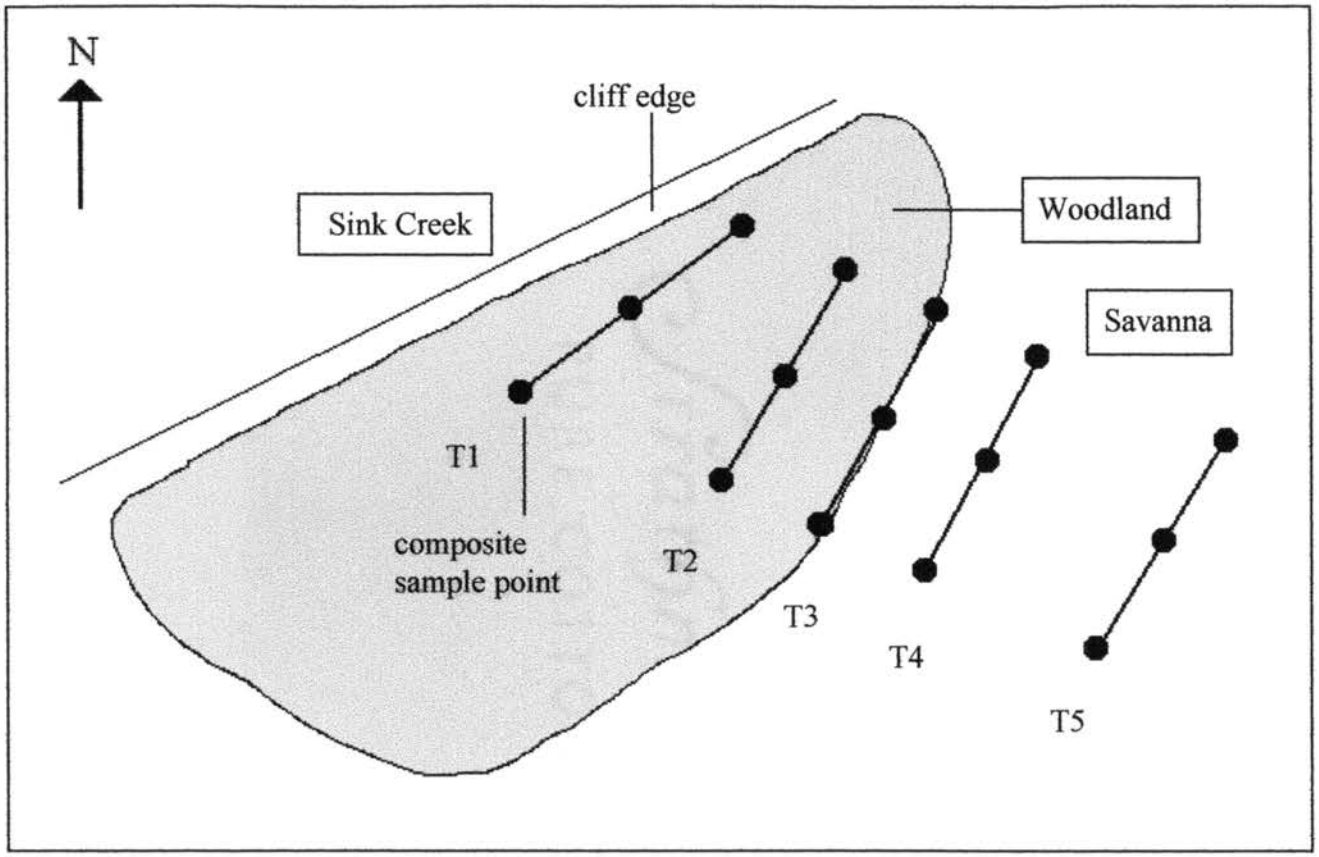


Figure 4. A composite sample point within the Ashe juniper woodland on the Southwest Texas State University Freeman Ranch.

Figure 5. Representation of the Ashe juniper woodland sampling location at the Southwest Texas State Freeman Ranch. The drawing illustrates the five sampling transects (T1-T5) that ran approximately parallel to the cliff edge. For each composite sample site, three sub-plots of litter and three sub-plots of soil were collected and pooled. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous material. Distances from T1 to T2 and T4 to T5 were 60 m, while the distance from T2 to T4 was approximately 40 m. The distance between composite sample sites (along transects) = 20 m. Drawing is not to scale. The location of this woodland is also illustrated on the aerial photograph in Figure 2.


N
cliff edge
Sink Creek
Woodland
Savanna
T1
composite
sample point
T2
T3
T4
T5

(n = 3; Fig. 5). As in the oak and grassland habitats, each composite sample consisted of three sub-samples that had been collected and pooled. The first of the three sub-samples was obtained from the center of the composite sampling point, and the other two were obtained from 5 m on either side (along the transect) of the first sub-sample. In addition, separate surface soil samples (0–20 cm) were collected from the three sample points along T1 (the site containing the largest junipers), for soil texture and nutrient characterization. Samples for soil texture and nutrient analysis from all three habitat types (live oak cluster, grassland, and juniper woodland) were air dried and sent to the Texas Agricultural Extension Service - Soil Testing Laboratory at Texas A&M University, College Station, Texas, for analysis.

Preparation of samples for element and isotope analyses

Prior to isotopic analysis, plant and soil samples were oven-dried at 80°C for 48 hrs. Dried plant tissue samples were then ground and homogenized in a commercial blender until able to pass through a 0.25 mm sieve. This finely-ground material was then re-mixed by hand for 2 minutes and weighed into tin capsules for $\delta^{13}C$, $\delta^{15}N$, %C, and %N measurements.

For dried soil, large rocks and roots were first removed by hand. Soil samples were then hand-ground with a mortar and pestle until they could pass through a 0.25 mm sieve. Soil samples were then re-mixed by hand for 2 minutes. From each soil sample, a representative sub-sample was weighed into silver capsules and treated with 3N HCl until no reaction occurred (Midwood and Boutton 1998). This sub-sample was used to

measure % organic carbon content. A second sub-sample was weighed into tin capsules to measure % total N, % total C, and $\delta^{15}N$ of total N. This sub-sample was not subjected to acid treatment. Finally, a third sub-sample was used for $\delta^{13}C$ measurements of older, more resistant, humified forms of soil organic carbon. This sub-sample was stirred vigorously in a high-density, saturated NaCl solution (density = 1.2 g/cm^3) which caused the fine roots, leaf litter, and other undecomposed organic debris (i.e., the "light fraction", Wolf et al. 1994) to float to the surface. This flotation was repeated a minimum of 5 times (i.e., until virtually all particulate organ debris had been removed), whereupon the remaining soil was washed with distilled water to remove the NaCl (Wolf et al. 1994). Soil from these sub-samples was then weighed into silver capsules and treated with 3N HCl.

Plant tissue and soil samples were analyzed for $\delta^{13}C$, $\delta^{15}N$, %C, and %N using a Carlo Erba EA-1108 interfaced with a Finnigan Delta Plus isotope ratio mass spectrometer operating in continuous flow mode. Precision for the $\delta^{13}C$ measurements was < 0.1‰, while that for $\delta^{15}N$ was < 0.2‰.

Due to the difficulty of obtaining enough soil from one of the upland live oak and grassland replicates, there were only 5 replicates for the $\delta^{15}N$, %N, %C, and C:N measurements. Also, although $\delta^{13}C$ measurements were made from all 5 transects at the juniper woodland, the organic C, total C, total N, and $\delta^{15}N$ measurements were made only from the three composite sample points on Transect 1.

Results are expressed as $\delta^{13}C$ and $\delta^{15}N$, where:

$\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{SAMPLE}/R_{STANDARD}) - 1] \times 1000$;

$R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$; and standards (Peterson and Fry 1987) are:

N_2 in air ($\delta^{15}N_{AIR} = 0‰$; $R_{STD} = 0.0036765$);

and C in Pee Dee belemnite ($\delta^{13}C_{PDB} = 0‰$; $R_{STD} = 0.0112372$).

Estimates of the proportion of carbon (in litter or SOM) derived from C_3, C_4, or C_4/CAM species were made using a mass balance equation:

$$\delta^{13}C = (\delta^{13}C_{C4})(\chi) + (\delta^{13}C_{C3})(1 - \chi)$$

where $\delta^{13}C$ is the $\delta^{13}C$ value of the mixed sample, $\delta^{13}C_{C4}$ is the average $\delta^{13}C$ value of known C_4 plants (and CAM components where appropriate), $\delta^{13}C_{C3}$ is the average $\delta^{13}C$ value of known C_3 species, χ is the proportion of carbon derived from C_4 (or C_4 and CAM) species, and $1 - \chi$ is the proportion of carbon derived from C_3 species. To determine $\delta^{13}C_{C4}$ and $\delta^{13}C_{C3}$, $\delta^{13}C$ measurements were made from foliar and stem tissue of all major C_4, CAM, and C_3 species of the three sampling habitats (Table 2; species nomenclature follows Jones et al. (1997)). Tissue samples were also collected from species that were thought to have dominated these habitats in the recent past (n = 3 for past or present dominant species, n = 2 for all others). For herbaceous species, each tissue sample consisted of all aboveground parts (live and dead) from at least one plant. Samples from woody plants came from a mixture of living leaves, as well as

Table 2. Stable carbon isotope ($\delta^{13}C$) values from all aboveground parts of plant species that currently dominate or were thought to historically dominate upland grasslands, upland live oak clusters, and Ashe juniper woodlands on the Freeman Ranch. N = 2 for all species, except those denoted by "*" for which n = 3. Historically dominant species denoted by "†". Nomenclature follows Jones et al. (1997). Replicates were collected in 1999 from different seasons and/or habitats to capture within species variation. ‡Lichen samples are from a mixture of the dominant fruticose, foliose, and crustose lichen species found to occur on Freeman Ranch live oaks. **_Tillandsia_ samples are from a mixture of both *Tillandsia recurvata* (C. Linnaeus) C. Linnaeus, and *Tillandsia usneoides* (C. Linnaeus) C. Linnaeus. The mean C_4 + CAM value is calculated to give equal weight between C_4 and CAM plants, and within CAM plants to give greater weight to the more abundant species (i.e., *Tillandsia*) over the less abundant *Opuntia* species. Mean C_4 + CAM = [(3 × *Tillandsia* $\delta^{13}C$) + (1 × *Opuntia* $\delta^{13}C$) + (4 × mean $\delta^{13}C$ for C_4 plants)]/8.

Life form	Species	Mean $\delta^{13}C$ (‰)	SE
C_3 woody	*Berberis trifoliata* M. Moricand	-27.6	0.7
	Celtis laevigata C. von Willdenow	-28.1	0.4
	Diospyros texana G. Scheele	-28.6	0.3
	Forestiera pubescens T. Nuttall	-26.7	0.5
	Juniperus ashei J. Buchholz*	-26.7	0.2
	Quercus virginiana P. Miller var. *fusiformis* (J. K. Small) C. Sargent*	-27.1	0.3
	Ulmus crassifolia T. Nuttall	-27.2	0.8
C_3 forb	*Ambrosia psilostachya* A. P. de Candolle	-29.4	0.4
	Croton fruticulosus G. Engelmann *ex* J. Torrey	-28.7	0.4
	Croton monanthogynus A. Michaux	-28.7	0.3
	Guterrezia texana (A. P. de Candolle) J. Torrey & A. Gray	-29.3	0.1
	Ratibida columnifera (T. Nuttall) E. Wooton & P. Standley	-28.5	0.4
	Wedelia texana (A. Gray) B. L. Turner	-28.6	0.1
C_3 graminoid	*Bromus* C. Linnaeus spp.	-30.5	0.3
	Carex planostachys G. Kunze	-28.0	0.3
	Elymus C. Linnaeus spp.	-29.4	0.1
	Nassella leucotricha (K. von Trinius & F. Reprecht) R. Pohl*	-28.4	0.6
	Panicum oligosanthes J. A. Schultes	-27.8	0.5
Mean C_3 herbaceous		**-28.8**	**0.2**
Mean C_3		**-28.3**	**0.2**
C_4 grass	*Andropogon gerardii* F. Vitman*†	-12.3	0.2
	Aristida C. Linnaeus spp.	-14.6	0.7
	Bothriochloa ischaemum (C. Linnaeus) Y. Keng	-14.9	1.0
	Bothriochloa laguroides (A. P. de Candolle) W. Herter	-12.8	0.1
	Bouteloua curtipendula (A. Michaux) J. Torrey	-14.4	0.1
	Bouteloua hirsuta M. Lagasca y Segura	-14.1	0.8
	Bouteloua rigidiseta (E. von Steudel) A. Hitchcock	-15.1	0.6
	Buchloë dactyloides (T. Nuttall) G. Engelmann	-15.7	1.0
	Eragrostis N. von Wolf spp.	-13.6	0.3
	Schizachyrium scoparium (A. Michaux) G. Nash†	-13.1	0.1
	Sorghastrum nutans (C. Linnaeus) G. Nash†	-13.6	0.4
Mean C_4		**-14.0**	**0.3**
CAM	*Opuntia engelmannii* J. Salm-Reifferscheid-Dyck	-13.5	0.2
	Tillandsia C. Linnaeus spp.**	-16.1	0.3
Mean CAM		**-14.8**	**1.3**
Mean C_4 + CAM		**-14.8**	**0.7**
Other	Lichen‡	-22.3	0.3

dead leaves and stem material collected below the plant. In an attempt to capture within-species variation in $\delta^{13}C$, tissue samples were collected from different habitats (upland vs. lowland) and/or different seasons (early summer vs. early fall).

When performing mass balance calculations on grassland samples, the mean $\delta^{13}C$ value of C_4 grass species was used for the 100%-C_4 endpoint (i.e., $\delta^{13}C_{C4}$ = -14.0‰; Table 2). For the 100%-C_3 endpoint in the grassland calculations, the mean $\delta^{13}C$ value of C_3 herbaceous (grass and forb) species was used (i.e., $\delta^{13}C_{C3}$ = -28.8‰). For the live oak and juniper habitats, $\delta^{13}C_{C3}$ was calculated using the pooled mean $\delta^{13}C$ of both herbaceous and woody C_3 species (-28.3 ± 0.2‰). The grassland $\delta^{13}C_{C4}$ value was also used for juniper woodland calculations. Because CAM species were more abundant at live oak cluster sites than in grasslands or the Ashe juniper woodland, and since the $\delta^{13}C$ values of CAM plants differed slightly from those of C_4 plants (Table 2), an integrated $\delta^{13}C$ value of C_4 and CAM species was used for $\delta^{13}C_{C4}$ for the oak clusters. This weighted value was calculated as follows: $\delta^{13}C_{C4}$ = [(3 × *Tillandsia* $\delta^{13}C$) + (1 × *Opuntia* $\delta^{13}C$) + (4 × mean $\delta^{13}C$ for C_4 plants)]/8 = (-14.8 ± 0.7‰). Although C_4 species do not appear to be present in these clusters currently, they may have been present in these sites at some point in the recent past. Therefore, this $\delta^{13}C_{C4}$ value was calculated such that C_4 and CAM species received equal weight. Within the CAM component, however, *Tillandsia* contributors were given more weight than that of *Opuntia*, since field observations indicated that *Tillandsia* spp. were more abundant than *Opuntia* spp. Also, although lichen samples were obtained for $\delta^{13}C$ analysis, they were not included in the mass balance calculations because they exhibited $\delta^{13}C$ values intermediate between C_3 and C_4 taxa (ca. -22‰; Table 2).

In addition to the actual $\delta^{13}C$ values of SOM, "adjusted $\delta^{13}C$" values were calculated to account for the +1-3‰ disparity commonly observed between litter and soil $\delta^{13}C$ values due to sources independent of vegetation change (Nadelhoffer and Fry 1988, Balesdent et al. 1993, Boutton 1996, Biedenbender 1999). For these adjusted $\delta^{13}C$ values, soil $\delta^{13}C$ values were decreased by a rate of 0.5‰ every 5 cm in depth, such that -0.5, -1.0, -2.0, and -3.0‰ were added to depth increments of 0–5, 5–10, 10–20, and 20–30 cm, respectively.

Some component of the observed 1–3‰ enrichment with depth is likely due to differences between the $\delta^{13}C$ values of above- and belowground plant biomass in the current vegetation community (Biedenbender 1999). Therefore, these adjusted $\delta^{13}C$ values should also remove any bias between the $\delta^{13}C$ values of the current and historic vegetation, caused by excluding root tissue from the $\delta^{13}C$ measurement of the current community. However, because this adjustment represents the maximum shift that such an enrichment in $\delta^{13}C$ has been typically observed to exhibit (i.e., a $\delta^{13}C$ shift of +3‰ within the upper 30 cm of the soil profile), this adjustment may overestimate the true disparity between litter and soil $\delta^{13}C$ values. Thus, the actual and adjusted $\delta^{13}C$ values provided here for soil at each depth increment should represent the endpoints, between which, the true $\delta^{13}C$ of the previous vegetation would be expected to lie.

Statistical analyses

Data from the study analyzing seasonal variation in $\delta^{13}C$ of grassland standing crop biomass were analyzed using a split plot factorial design (Kirk 1995) where transect was the whole plot and a random variable, and season was the split-plot and a fixed variable. Due to differences in sampling design, degree of replication, and site topography, the soil and litter data comparing grassland and live oak habitats were analyzed separately from the juniper woodland data. For the comparison of live oak and grassland habitats all variables except $\delta^{13}C$ were tested initially in a multivariate analysis of variance (MANOVA). The significant MANOVA test was followed by a separate univariate analysis of variance (ANOVA) test for each dependent variable. For these ANOVA analyses, data were analyzed in a split plot factorial design, with habitat type as the main plot and a fixed variable, and depth increment as the split-plot and a fixed variable. Since the $\delta^{13}C$ data were obtained from a different soil component than the other variables (i.e., older SOM vs. older SOM and the light fraction) and contained more replicates (6 rather than 5), these data were not included in the MANOVA analysis. The $\delta^{13}C$ data were analyzed separately in a univariate split plot factorial design, where habitat was the main plot and a fixed variable, and depth increment was the split-plot and a fixed variable. If the assumption of multisample sphericity was not tenable for the split-plot factorial designs, the *F* statistics were tested using adjusted (more conservative) critical values (Kirk 1995). The $\delta^{13}C$ data from the juniper woodland study were analyzed in a completely randomized factorial design (Kirk 1995) with site and depth as fixed factors.

Contrasts for pre-planned simple effects were performed using Student's t test (if contrasts were orthogonal), or Holm's sequentially rejective Bonferroni test (if contrasts were non-orthogonal) (Kirk 1995). In most cases, the assumption of homogeneity of variances was not met, and thus the t statistic was replaced with the t' statistic in either of the two previous tests and used with Welch's modified degrees of freedom (Kirk 1995). MANOVA and ANOVA tests were performed using SPSS and SAS software, while all simple effect contrasts were calculated by hand. Differences were considered statistically significant when $P \leq 0.05$.

RESULTS

Soil characterization

The texture of surface soils (0–20 cm) averaged about 40% clay, 36% silt, and 23% sand for grasslands, live oak clusters, and the Ashe juniper woodland, and did not differ significantly ($P \geq 0.50$) between habitats (Table 3). Soil pH was highest in live oak clusters (7.4) and least in grasslands (6.4). Levels of NO_3-N and P were also considerably greater in oak soils than in either the juniper woodland or grasslands (Appendix A).

Total organic carbon in soil was significantly ($P = 0.002$) greater beneath live oaks than in grasslands (Fig. 6a, Table 4). When averaged over all depths (0–30 cm), mean soil organic carbon beneath live oaks was ca. 55 g kg^{-1}, compared to 29 g kg^{-1} for grassland sites. Differences were most pronounced at the 0–5 cm depth, where SOC (mean ± SE) was more than two-fold greater beneath live oaks (108.2 ± 1.1 g kg^{-1}) than upland grasslands (40.2 ± 0.5 g kg^{-1}). Within soils of both habitats, organic carbon decreased significantly with depth ($P < 0.05$) to ca. 20 g kg^{-1} between 20–30 cm. Soil organic carbon in the Ashe juniper woodland was intermediate between that of live oak and grassland sites (Fig. 6a, Table 4). Specifically, organic carbon beneath junipers averaged ca. 48 g kg^{-1} across all soil depths, and was 81.7 ± 6.1 g kg^{-1} at 0–5 cm. Soil nitrogen content was also significantly greater beneath oak clusters than grasslands

Table 3. Texture of surface soils (0-20 cm) in grasslands, live oak clusters, and Transect 1 of the Ashe juniper woodland on the Freeman Ranch. Soils were collected in July 2000. Data are means ± 1 SE; n = 3.

Habitat	%sand	%silt	%clay
Grassland	23.0 (1.2)	35.3 (1.3)	41.7 (1.8)
Live oak cluster	22.3 (2.9)	37.3 (2.4)	40.3 (1.8)
Juniper woodland	22.3 (1.3)	35.3 (1.3)	39.0 (2.0)

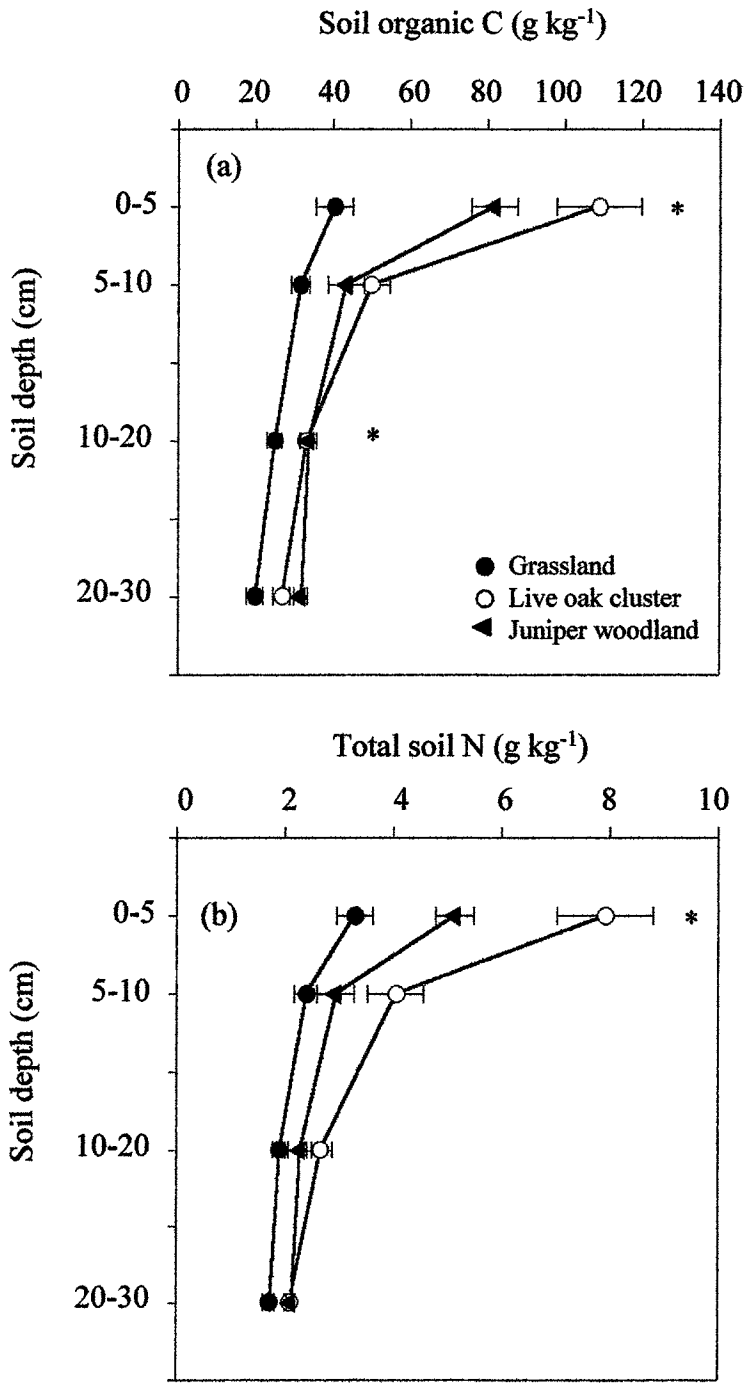


Figure 6. Depth profiles of soil organic carbon (a), and total soil nitrogen (b), for live oak clusters, grasslands, and Transect 1 of the Ashe juniper woodland on the Freeman Ranch. Data are means ± 1 SE; n = 5. Grassland and oak cluster habitats differed significantly (within a particular depth) at $P < 0.05$ when denoted by "*". In both graphs, all habitats changed significantly with depth at $P < 0.05$. Juniper woodland data were not compared statistically with the other habitats, due to site and sampling differences.

Table 4. Organic carbon, total nitrogen, stable nitrogen isotopes ($\delta^{15}N$), and C:N ratios of litter and soil organic matter from grasslands, live oak clusters, and Transect 1 of the Ashe juniper woodland on the Freeman Ranch. Data are means with 1 SE in parentheses; n = 5. Samples were collected in July 1999. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material.

Source	Depth (cm)	Habitat	Organic C g kg^{-1}	Total N g kg^{-1}	$\delta^{15}N$ (‰)	C:N
litter	surface	grassland	376.5 (7.8)	11.7 (0.7)	0.9 (0.2)	32.8 (2.2)
		live oak cluster	394.9 (12.7)	14.5 (0.8)	2.0 (0.3)	27.5 (0.9)
		juniper woodland	409.6 (15.0)	10.7 (1.2)	-1.8 (0.1)	39.5 (5.9)
soil	0-5	grassland	40.2 (4.8)	3.3 (0.3)	5.4 (0.3)	12.2 (0.4)
		live oak cluster	108.2 (10.9)	7.9 (0.9)	4.8 (0.4)	13.7 (0.4)
		juniper woodland	81.7 (6.1)	5.1 (0.4)	2.8 (0.2)	15.9 (0.5)
soil	5-10	grassland	31.3 (2.4)	2.4 (0.2)	7.0 (0.3)	13.2 (0.3)
		live oak cluster	49.7 (4.9)	4.0 (0.5)	7.1 (0.6)	12.5 (0.4)
		juniper woodland	43.3 (4.6)	2.9 (0.4)	6.0 (0.1)	14.8 (0.5)
soil	10-20	grassland	24.7 (1.9)	1.9 (0.1)	8.1 (0.2)	13.1 (0.4)
		live oak cluster	32.9 (1.4)	2.6 (0.2)	7.5 (0.6)	12.7 (0.8)
		juniper woodland	33.6 (2.2)	2.3 (0.2)	8.0 (0.3)	14.9 (0.4)
soil	20-30	grassland	19.7 (2.2)	1.7 (0.1)	8.7 (0.3)	11.5 (0.9)
		live oak cluster	26.6 (2.2)	2.1 (0.1)	8.1 (0.4)	12.8 (0.8)
		juniper woodland	31.5 (1.6)	2.1 (0.1)	8.4 (0.4)	14.9 (0.6)

(Fig. 6b, Table 4; $P = 0.002$). When averaged across all depths soil total N was ca. 4 g kg^{-1} beneath live oaks and 2 g kg^{-1} beneath grassland sites. The greatest difference between habitats occurred again in the 0–5 cm depth, where (mean ± SE) soil nitrogen (g kg^{-1}) was 7.9 ± 0.1 and 3.3 ± 0.1 for oak and grassland habitats, respectively. As with organic carbon, soil nitrogen of these two habitats decreased significantly with depth ($P < 0.05$), and converged at ca. 2 g kg^{-1} (at 20–30 cm). Juniper woodland soils were again intermediate between oak and grassland soils (Fig. 6b, Table 4) in soil total nitrogen. The juniper soils averaged ca. 3 g kg^{-1} of nitrogen across all depths, and contained 5.1 ± 0.4 g kg^{-1} between 0–5 cm.

Litter beneath oak clusters exhibited a lower mean C:N value (Table 4) than that of grasslands (27.5 ± 1.0 vs. 32.8 ± 2.2, respectively), but due to large site variability, these values were not statistically different ($P > 0.05$). Mean litter C:N in the Ashe juniper woodland was greater, but much more variable, than the other two habitats (39.5 ± 5.9). Soil C:N was similar at all depths between grassland and oak habitats, and slightly greater at all depths beneath the juniper woodland. In each habitat, although the soil C:N values were significantly different from the respective surface litter values ($P < 0.01$), they did not change significantly with depth ($P > 0.05$).

Stable carbon isotopes in upland grasslands and live oak clusters

In the seasonal analysis of grassland vegetation, the mean annual standing crop biomass within the grazing exclosure was 179.2 g m^{-2} (Table 5). Based on $\delta^{13}C$ data, C_4 species constituted ca. 77% of this annual standing crop, compared to 23% for C_3 species.

Table 5. Stable carbon isotopes ($\delta^{13}C$), standing crop biomass (standing live and dead), and percent C_3 and C_4 in biomass over an annual growth cycle in a 5-year grazing exclosure on the Freeman Ranch. Data are means ± 1 SE; n = 10. *To calculate the year average $\delta^{13}C$ value, standing crop biomass values from each sampling date were first summed, then the fraction that each season contributed to the sum was determined. The $\delta^{13}C$ values for each sampling date were then weighted by their respective fraction of biomass production, and added together.

		Mass balance calculations			**Dry weight measurements**		
Sampling date	$\delta^{13}C$ of sample (‰)	C_3 biomass (%)	C_4 biomass (%)	Standing crop biomass (g/m^2)	C_3 forb in biomass (%)	C_3 graminoid in biomass (%)	C_4 grass in biomass (%)
30 July 1999	-17.4 (0.6)	23	77	273.46 (73)	7.6	2.8	89.6
30 Oct 1999	-15.9 (0.8)	13	87	217.03 (60)	0.4	3.0	96.6
30 Jan 2000	-16.9 (0.9)	20	80	103.26 (13)	0.0	17.8	82.2
30 Apr 2000	-20.4 (0.8)	43	57	122.96 (26)	23.9	14.4	61.7
Year avg*	**-17.4**	**23**	**77**	**179.18**	**7.1**	**7.0**	**85.9**

The contribution of C_4 species to aboveground biomass was also greater than that of C_3 species at each sampling time (Fig. 7). The C_3 species did, however, show a significant increase ($P < 0.01$) in relative standing crop biomass between the October (ca. 13%) and April (ca. 43%) sampling times. Dry weight measurements revealed this was primarily due to an increase in C_3 forb biomass during the early spring (Table 5).

A $\delta^{13}C$ value calculated from the average contribution of C_3 and C_4 plants during the year in this seasonal study was -17.4‰. Because this value was similar to the mean value for the July sampling period in this study (-17.4 ± 0.6‰), it was concluded that the $\delta^{13}C$ values of grassland litter samples that were collected in July (to compare upland grasslands with live oak clusters) provided a reasonable estimate of integrated annual C_3/C_4 composition in these grasslands.

Within the upland oak and grassland study, the $\delta^{13}C$ values for current organic matter inputs were -26.7 ±0.2‰ for live oak clusters and -20.8 ± 1.3‰ for grasslands. These values equate to current C_3-plant contributions of ca. 88 ± 2% and 46 ± 9% to aboveground biomass in oak and grassland habitats, respectively (Fig. 8, Table 6). Because C_4 grasses are currently rare or absent in the live oak clusters, most or all of the non-C_3 contribution to this habitat (ca. 12%), is presently derived from the CAM species (i.e., *Tillandsia* and *Opuntia* spp.).

Soil $\delta^{13}C$ increased with depth in both the grassland and live oak habitats (Fig. 9). At the lowest sampled depth increment (20–30 cm) the $\delta^{13}C$ of SOC averaged -16.7 ± 0.3‰ beneath oak clusters, and -13.2 ± 0.1‰ beneath grasslands. Results of the ANOVA analysis for grassland and live oak $\delta^{13}C$ indicated a non-significant interaction term between habitat type and depth ($F_{4,40} = 1.44$, $P = 0.24$), but significant main effects for

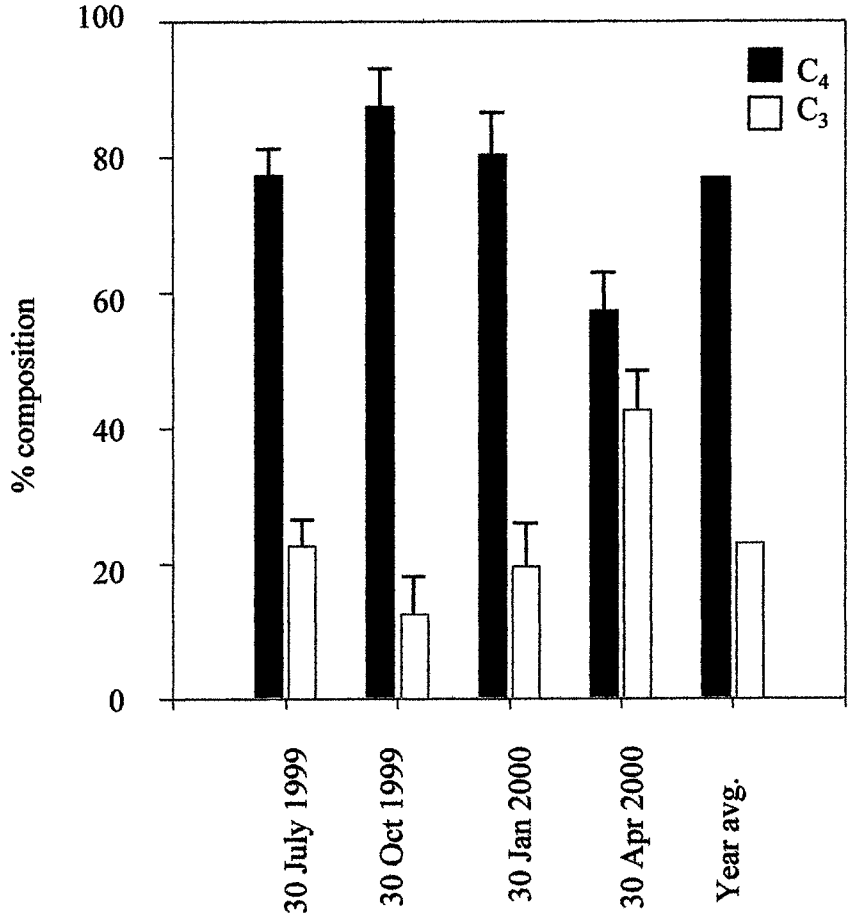


Figure 7. Mean (± 1 SE) percent contribution of C_4 and C_3 to total standing crop biomass for ungrazed grassland plots in a 5-year grazing exclosure on Freeman Ranch. Data are calculated by mass balance from mean $\delta^{13}C$ values; n = 10.

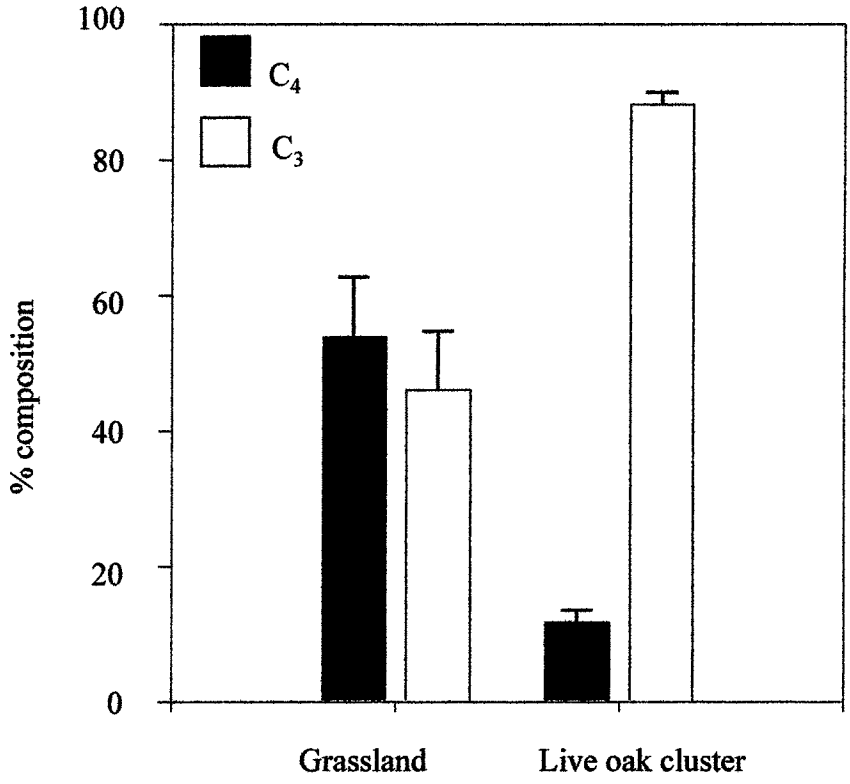
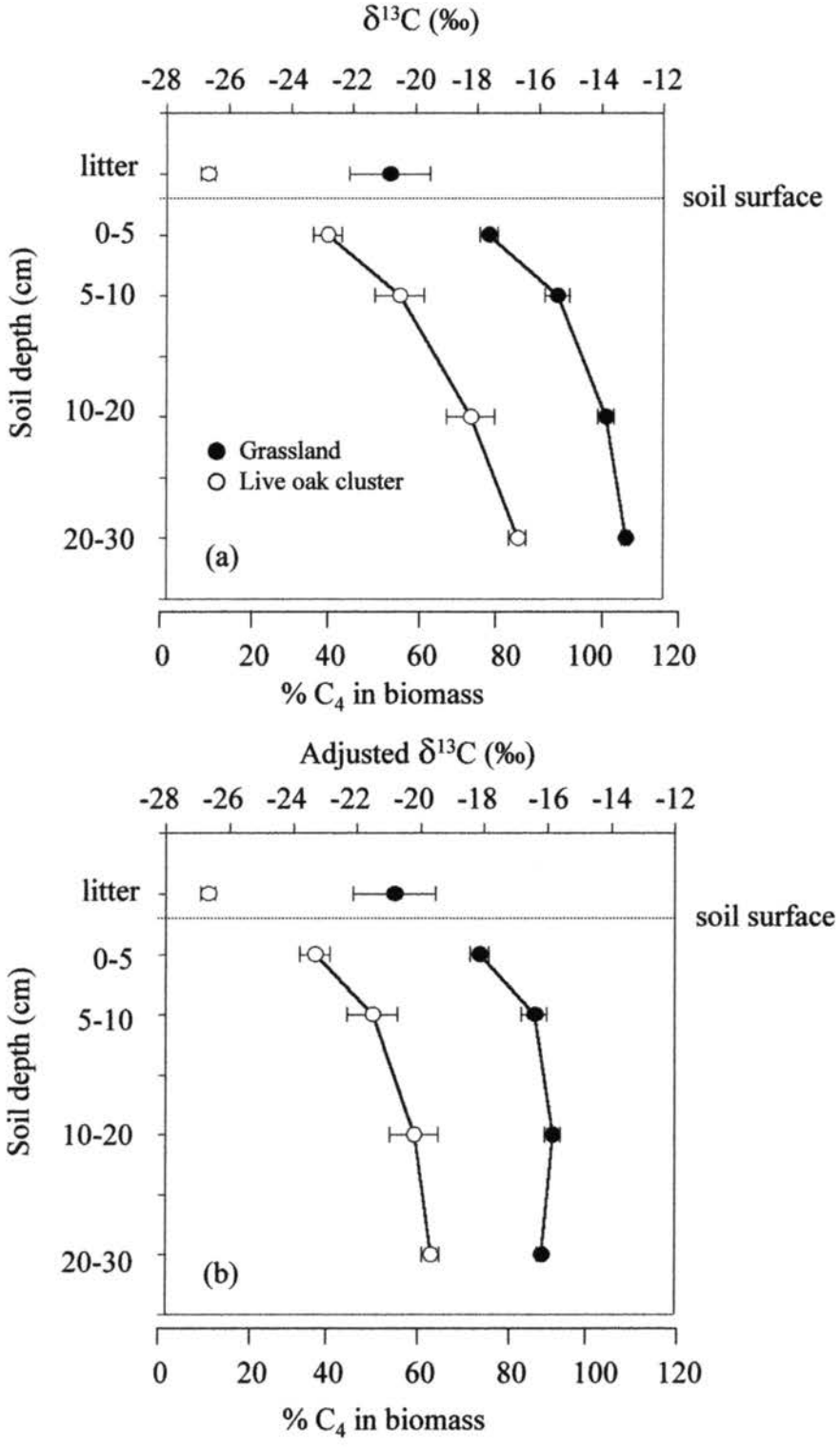


Figure 8. Mean (± 1 SE) relative biomass, calculated from $\delta^{13}C$ for litter within live oak cluster and grassland habitats on the Freeman Ranch in July 1999; n = 6. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material. $\delta^{13}C$ values differed significantly ($P < 0.001$) between habitats.

Table 6. Stable carbon isotopes ($\delta^{13}C$ and adjusted $\delta^{13}C$) and percent C_3 and C_4 in biomass, for litter and soil organic matter, from grasslands and live oak clusters on the Freeman Ranch. Data are means ± 1 SE; n = 6. Samples were collected in July 1999. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material. Adjusted $\delta^{13}C$ values were calculated to account for the 1–3‰ enrichment with depth independent of vegetation change (Nadelhoffer and Fry 1988, Balesdent et al. 1993, Boutton 1996), by adding to soil $\delta^{13}C$ values, a constant rate of -0.5‰ every 5 cm in depth (i.e., -0.5, -1.0, -2.0, and -3.0‰ have been added to respective depths 0–5, 5–10, 10–20, and 20–30 cm). Percent contributions were calculated from both actual and adjusted $\delta^{13}C$ values.

		Grassland				**Live oak cluster**			
Source	Depth (cm)	$\delta^{13}C$ (‰)	Adj. $\delta^{13}C$ (‰)	$\%C_3$	$\%C_4$	$\delta^{13}C$ (‰)	Adj. $\delta^{13}C$ (‰)	$\%C_3$	$\%C_4$
litter	surface	-20.8 (1.3)	-20.8	46	54	-26.7 (0.2)	-26.7	88	12
soil	0-5	-17.6 (0.3)	-18.1	24-28	72-76	-22.8 (0.5)	-23.3	60-64	37-40
soil	5-10	-15.4 (0.4)	-16.4	9-16	84-91	-20.5 (0.8)	-21.5	43-50	50-57
soil	10-20	-13.9 (0.3)	-15.9	0-12	88-100	-18.2 (0.8)	-20.2	26-40	60-74
soil	20-30	-13.2 (0.1)	-16.2	0-14	85-100	-16.7 (0.3)	-19.7	14-37	64-86

Figure 9. Mean (± 1 SE; n =6) $\delta^{13}C$ (a), and adjusted $\delta^{13}C$ (b) for live oak clusters and grasslands on the Freeman Ranch. Samples were collected in July 1999. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material. "Adjusted $\delta^{13}C$ values" were calculated to account for the 1–3‰ enrichment with depth independent of vegetation change (Nadelhoffer and Fry 1988, Balesdent et al. 1993, Boutton 1996), by adding to soil $\delta^{13}C$ values a constant rate of -0.5‰ for every 5 cm in depth (i.e., -0.5, -1.0, -2.0, -3.0‰ were added to respective depths 0–5, 5–10, 10–20, and 20–30 cm). Habitats differed significantly ($P < 0.001$) at all depths. In (b), both habitats changed significantly with depth ($P < 0.01$).


δ13C (‰)
-28 -26 -24 -22 -20 -18 -16 -14 -12
litter
soil surface
0-5
5-10
10-20
20-30
Soil depth (cm)
Grassland
Live oak cluster
(a)
0 20 40 60 80 100 120
% C4 in biomass
Adjusted δ13C (‰)
-28 -26 -24 -22 -20 -18 -16 -14 -12
litter
soil surface
0-5
5-10
10-20
20-30
Soil depth (cm)
(b)
0 20 40 60 80 100 120
% C4 in biomass

habitat type ($F_{1, 10} = 91.97$, $P < 0.001$) and depth ($F_{4, 40} = 86.79$, $P < 0.001$). These results indicate that: (1) $\delta^{13}C$ values differed significantly ($P < 0.001$) between the two habitats at all depths, and (2) $\delta^{13}C$ profiles in both habitats increased significantly ($P < 0.001$) with depth.

When mass balance calculations are performed on the actual soil $\delta^{13}C$ values from 20–30 cm depth in grasslands, the calculated relative contribution from C_4 species is >100% (i.e., 105.5%). The reason for this is that the actual mean soil $\delta^{13}C$ value at this depth in grasslands (-13.2 ± 0.1‰) is more enriched in $\delta^{13}C$ than the value used to represent a community composed entirely of C_4 species (i.e., $\delta^{13}C_{C4}$ = -14.0‰). The occurrence of $\delta^{13}C$ values for SOC that are higher than the 100%-C_4 endpoint could be due to two reasons: (1) the +1–3‰ enrichment of soil $\delta^{13}C$ with depth that occurs independent of vegetation change (Nadelhoffer and Fry 1988, Balesdent et al. 1993, Boutton 1996, Biedenbender 1999), and/or (2) actual differences between the true $\delta^{13}C_{C4}$ value for the current grassland vegetation and the true $\delta^{13}C_{C4}$ value for the historic grassland vegetation. For example, such a disparity between the current and historic $\delta^{13}C_{C4}$ values could be caused by a greater historical contribution from the C_4 species that exhibit higher $\delta^{13}C$ values than C_4 species currently exhibit on average. Indeed, the three primary grasses that are thought to have been the historical dominants of upland grasslands in this area (i.e., *Andropogon gerardii*, *Schizachyrium scoparium*, and *Sorghastrum nutans*) all happen to exhibit $\delta^{13}C$ values that are higher than the mean C_4 value ($\delta^{13}C_{C4}$) calculated here for the current community (Table 2). Regardless of the cause of this disparity in $\delta^{13}C$ between current litter and SOC, however, the adjusted $\delta^{13}C$

values reported here appear to account for this disparity and consequently yield relative contributions from C_4 species that are substantially less than 100% at all depths.

Figures 9a and 9b illustrate the actual and adjusted soil $\delta^{13}C$ profiles for the upland oak clusters and grasslands. A comparison of these figures reveals that the adjusted $\delta^{13}C$ values more conservatively estimate the magnitude of change in $\delta^{13}C$ with depth due to vegetation change than do the actual $\delta^{13}C$ values. However, both sets of values exhibit a significant decrease ($P < 0.01$) in $\delta^{13}C$ from deeper in the profile to the surface. This indicates that C_4 productivity has declined significantly in both habitats during the recent past. From the actual and adjusted $\delta^{13}C$ values of SOC at the 20–30 cm depth, it is calculated that C_4 and/or CAM plants historically represented between 64–86% of the total biomass within oak cluster sites, compared to a current contribution of only about 12%. In grasslands, C_4 species historically represented ca. 85–100% of the total biomass, compared to only 54% on average, currently.

Stable carbon isotopes in the Ashe juniper woodland

Litter and soil $\delta^{13}C$ values for the Ashe juniper woodland are shown in Figure 10 and Table 7. The $\delta^{13}C$ values for litter (ca. -27‰) were similar among the three sampling sites that are currently occupied by juniper woodland (i.e., sites T1–T3). The present-day savanna sites (i.e., T4 and T5) however, had litter $\delta^{13}C$ values (-20.3 and -22.8‰, respectively) that were significantly different ($P < 0.01$) from the woodland sites. These litter $\delta^{13}C$ values translate to current contributions from C_3 species of ca. 90% to total aboveground biomass within the woodland and ca. 40–60% in the savanna.

Figure 10. Mean (± 1 SE; n = 3) $\delta^{13}C$ (a), and adjusted $\delta^{13}C$ (b) with depth for the Ashe juniper woodland on the Freeman Ranch. Samples were collected in July 1999. Legend: T1 = woodland near cliff, T2 = woodland interior, T3 = woodland/savanna boundary, T5 = savanna, 80 m from woodland boundary. The profile for T4 (savanna, 20 m from woodland boundary) is not shown for simplicity, though it resembles T5. Values for all sites can be seen in Table 7. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material. "Adjusted $\delta^{13}C$ values" were calculated to account for the 1–3‰ enrichment with depth independent of vegetation change (Nadelhoffer and Fry 1988, Balesdent et al. 1993, Boutton 1996), by adding to soil $\delta^{13}C$ values a constant rate of -0.5‰ for every 5 cm in depth (i.e., -0.5, -1.0, -2.0, and -3.0 were added to respective depths 0–5, 5–10, 10–20, and 20–30‰). Within a given depth, a significant difference ($P < 0.05$) between T1 and T5 is denoted by "*". A significant difference between T1 and all sites is denoted by "**". In (b) all sites changed significantly with depth ($P < 0.001$).

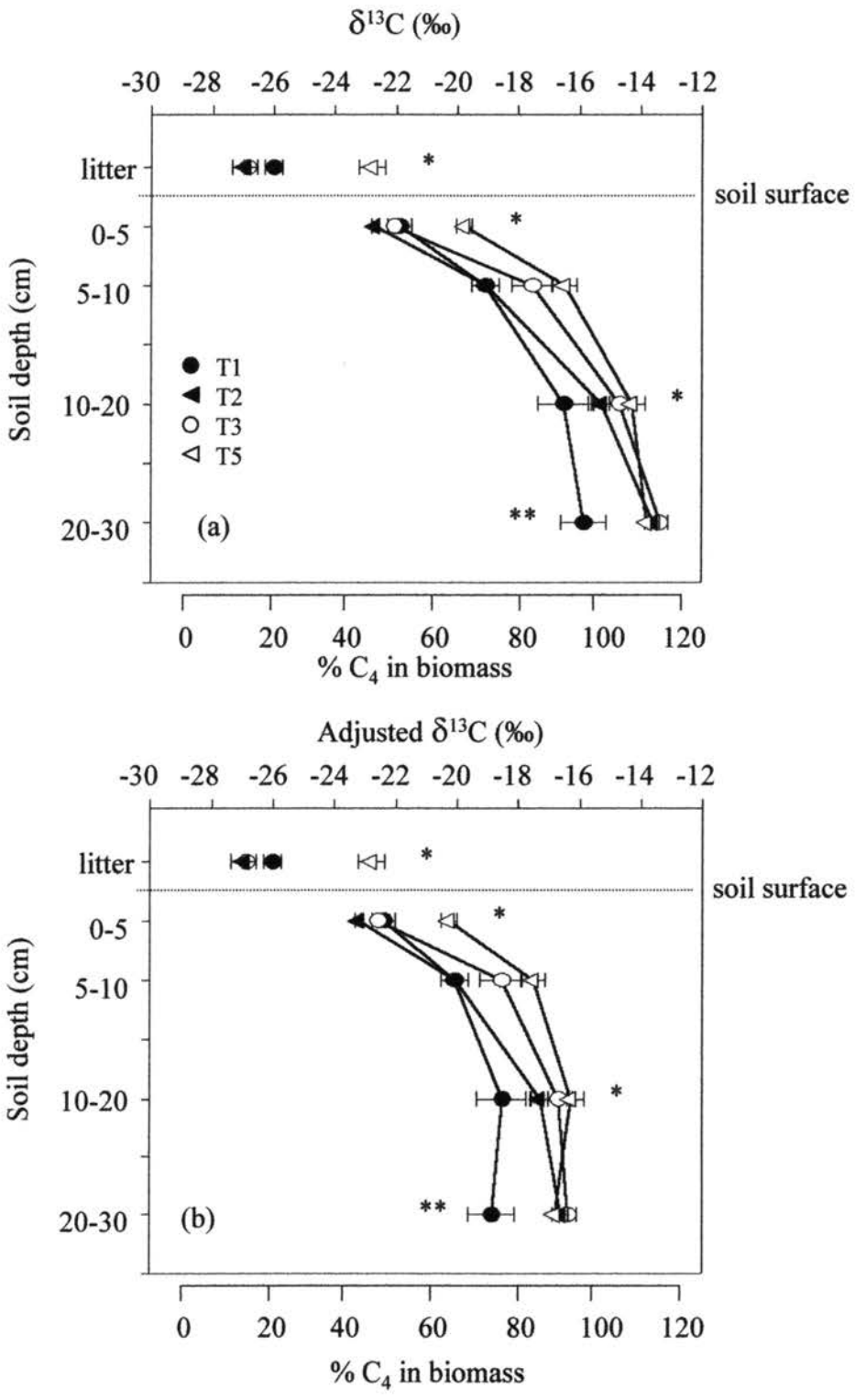

δ13C (‰)
-30 -28 -26 -24 -22 -20 -18 -16 -14 -12
litter
soil surface
0-5
5-10
10-20
20-30
Soil depth (cm)
T1
T2
T3
T5
(a)
*
*
*
**
0 20 40 60 80 100 120
% C4 in biomass
Adjusted δ13C (‰)
-30 -28 -26 -24 -22 -20 -18 -16 -14 -12
litter
soil surface
0-5
5-10
10-20
20-30
Soil depth (cm)
(b)
*
*
*
**
0 20 40 60 80 100 120
% C4 in biomass

Table 7. Stable carbon isotopes ($\delta^{13}C$ and adjusted $\delta^{13}C$), and percent C_3 and C_4 in biomass for litter and soil organic matter from the Ashe juniper woodland site on Freeman Ranch. Data are means ± 1 SE; n = 3. Samples were collected in July 1999. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material. *Transect locations are illustrated in Figure 5. **Adjusted $\delta^{13}C$ values were calculated to account for the 1–3‰ enrichment with depth independent of vegetation change (Nadelhoffer and Fry 1988, Balesdent et al. 1993, Boutton 1996), by adding to soil $\delta^{13}C$ values, a constant rate of -0.5‰ every 5 cm in depth (i.e., -0.5, -1.0, -2.0, and -3.0‰ have been added to respective depths 0–5, 5–10, 10–20, and 20–30 cm). Percent contributions were calculated from both actual and adjusted $\delta^{13}C$ values.

Transect* (location)	Source	Depth (cm)	$\delta^{13}C$ (‰)	**Adj. $\delta^{13}C$ (‰)	$\%C_3$	$\%C_4$
T1	litter	surface	-26.0 (0.3)	-26.0	84	16
(woodland	soil	0–5	-21.9 (0.4)	-22.4	55-58	41-45
near cliff)	soil	5–10	-19.1 (0.1)	-20.1	36-43	58-65
	soil	10–20	-16.5 (0.8)	-18.5	18-32	68-82
	soil	20–30	-15.9 (0.7)	-18.9	13-34	66-87
T2	litter	surface	-26.9 (0.1)	-26.9	90	10
(woodland	soil	0–5	-22.1 (0.2)	-22.6	57-60	40-43
interior)	soil	5–10	-17.6 (0.7)	-18.6	25-32	68-75
	soil	10–20	-14.7 (0.9)	-16.7	5-19	81-95
	soil	20–30	-13.4 (0.3)	-16.4	0-17	83-100
T3	litter	surface	-27.0 (0.4)	-27.0	91	9
(woodland	soil	0–5	-22.7 (0.1)	-23.2	61-65	36-39
boundary)	soil	5–10	-19.1 (0.5)	-20.1	36-43	58-65
	soil	10–20	-15.3 (0.3)	-17.3	9-23	77-91
	soil	20–30	-13.6 (0.3)	-16.6	0-18	82-100
T4	litter	surface	-20.3 (0.3)	-20.3	42	58
(savanna	soil	0–5	-20.4 (0.3)	-20.9	43-47	53-57
20 m)	soil	5–10	-17.4 (0.1)	-18.4	23-30	70-77
	soil	10–20	-14.7 (0.1)	-16.7	5-18	82-95
	soil	20–30	-13.7 (0.3)	-16.7	0-18	82-100
T5	litter	surface	-22.8 (0.4)	-22.8	59	41
(savanna	soil	0–5	-19.8 (0.3)	-20.3	39-42	58-61
80 m)	soil	5–10	-16.5 (0.4)	-17.5	17-24	77-83
	soil	10–20	-14.3 (0.1)	-16.3	2-15	85-98
	soil	20–30	-13.8 (0.1)	-16.8	0-19	81-100

As was the case for the grassland and live oak habitats, all soil $\delta^{13}C$ values within the juniper woodland increased significantly with depth ($P < 0.01$), indicating that relative C_4-plant productivity has similarly declined in this habitat in the recent past. In addition, soil $\delta^{13}C$ values for the outermost woodland transects (T2 and T3) converged with those of the savanna sites (T4 and T5) at depth, such that at 20–30 cm, soil $\delta^{13}C$ values from transects T2–T5 were similar, and significantly different ($P < 0.05$) from those of the most interior woodland transect (T1). Relative C_3 productivities at 20–30 cm depth were between 0–19% for transects T2–T5, and 13–34% for T1.

Stable nitrogen isotopes

$\delta^{15}N$ of litter (Fig. 11, Table 4) differed significantly between upland oak and grassland habitats (2.0 ± 0.3 and 0.9 ± 0.1‰, respectively; $P < 0.05$). Values of soil $\delta^{15}N$ however, were similar between oak clusters and grasslands at all depths, and increased significantly ($P < 0.01$) with depth, from ca. 5‰ at 0–5 cm to ca. 8‰ at 20–30 cm for both habitats. $\delta^{15}N$ values in the juniper woodland (T1) changed more dramatically with depth than did those of oak or grassland sites (Fig. 11), from -1.8 ± 0.1‰ in litter, to 8.4 ± 0.4‰ at 20–30 cm depth.

Soil $\delta^{15}N$ averaged across all depths was slightly greater in grasslands (7.4 ± 0.3‰), than for oaks (7.0 ± 0.3‰). The average soil value for the juniper woodland, however, was much less than in the other habitats (4.7 ± 1.9‰). Soil $\Delta^{15}N$ values (defined here as $\delta^{15}N_{\text{20–30cm depth}} - \delta^{15}N_{\text{surface soil}}$) were similar for grassland and oak sites

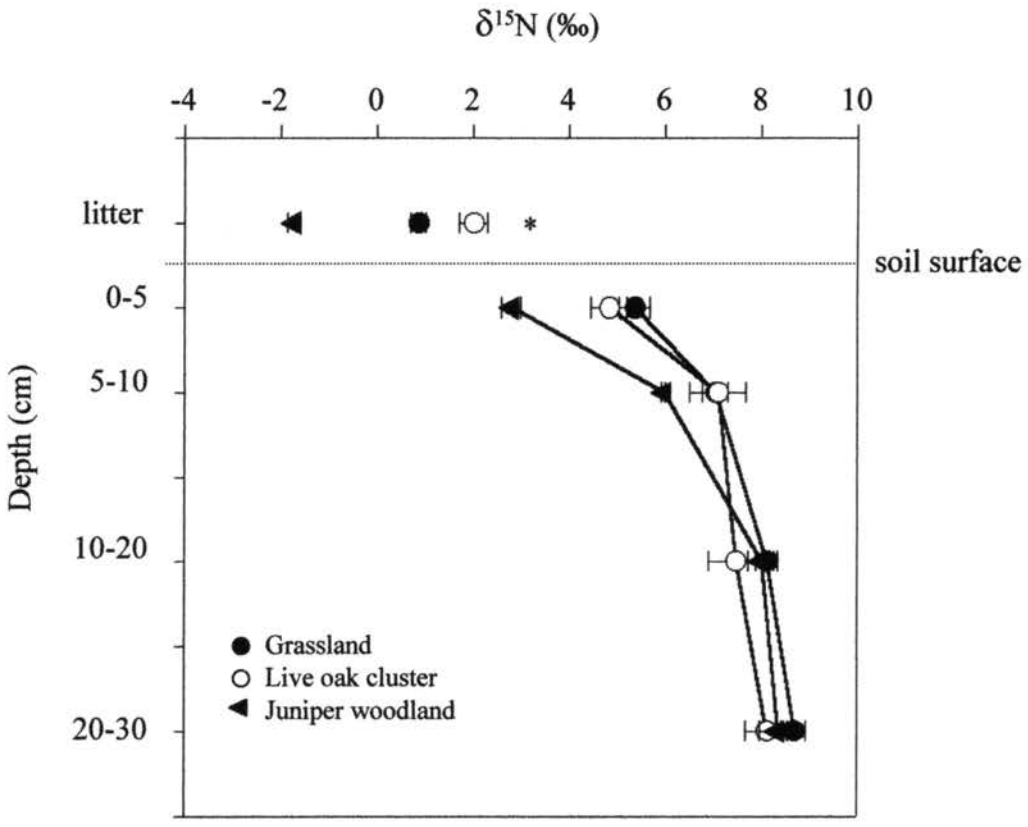


Figure 11. Mean (± 1 SE) $\delta^{15}N$ with depth for live oak clusters, grasslands, and Transect 1 of the Ashe juniper woodland on the Freeman Ranch. N = 5 for live oak and grassland habitats, and n = 3 for the juniper woodland. Samples were collected in July 1999. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material. A significant difference ($P < 0.05$) between live oak and grassland habitats (within a particular depth) is denoted by "*". Data from the juniper woodland were not compared statistically with the other two habitats due to site and sampling differences. Soil values differed significantly from respective litter values ($P < 0.01$), and with depth in the soil profile ($P < 0.01$), for all habitats.

(3.3 ± 0.5 and 3.3 ± 0.3, respectively), and greatest for the juniper woodland (5.6 ± 0.6). Soil $\delta^{15}N$ correlated best with soil total nitrogen ($R^2 = 0.575$, $P < 0.0001$) and soil organic carbon ($R^2 = 0.532$, $P < 0.0001$).

DISCUSSION

Stability of upland grasslands

The $\delta^{13}C$ data from grassland litter and soils in this study showed a significant increase with depth, supporting the initial hypothesis that relative C_4-biomass production has decreased in upland grasslands at this site on the Edwards Plateau. It appears that the C_4 species represented about 85–100% of the grassland biomass at some point in the past. Currently however, although there are still more species of C_4 grasses (ca. 75%) than C_3 grasses (ca. 25%) as might be expected at this latitude (Terri and Stowe 1976), the C_4 species only constitute about 54% of the grassland biomass on average.

In an attempt to establish a general time frame for which these changes in the relative C_4 contribution may have occurred, an indirect approach was used to estimate the age of SOC at this site. Boutton et al. (1998) used radiocarbon (^{14}C) dating to find mean residence time of SOC at a mesquite/shrub savanna in southern Texas, which exhibits a similar mean annual temperature to that of Freeman Ranch (ca. 20°C), but a lower %clay content (30% vs. 40%, respectively). Schimel et al. (1994) showed that given the same mean annual temperature, lower % clay content should lead to shorter SOC turnover times. Since Boutton et al. found the mean residence time of SOC to be ca. 40–100 years between 0–15 cm and ca. 300–500 years between 15–30 cm at the mesquite/shrub savanna, SOC of similar depths at the Freeman Ranch is likely at least of the same age.

This estimation of SOC age suggests that though soil samples could only be obtained to 30 cm depth in this study, the $\delta^{13}C$ of SOC reported here should provide a historical record of vegetation that extends >300 years prior to the present and thus extends beyond the time of European settlement of the area (150–200 years ago). Also, since most of the $\delta^{13}C$ shift observed in the grassland profiles at Freeman Ranch (or virtually all of the shift according to the adjusted $\delta^{13}C$ values, Fig. 9b) occurred within the top 10 cm of soil, the decline of C_4 grasses probably occurred recently, perhaps corresponding to the introduction of livestock grazing on the Freeman Ranch.

Floristic analyses of the contemporary grassland vegetation of Freeman Ranch seem to implicate grazing as the principal cause of C_4-decline at this site. First, the current co-dominant herbaceous species in these grasslands (Texas grama and Texas wintergrass) are shortgrass species that are known to increase with grazing (Launchbaugh 1955, USDA 1984, Smeins and Merrill 1988, Boutton 1996). While conversely, the taller grass species that are thought to dominate the potential climax community at this site (e.g., little bluestem, big bluestem, and Indiangrass) tend to decrease in grazed pastures on the Edwards Plateau (Launchbaugh 1955, USDA 1984, Smeins and Merrill 1988), and are presently rare or absent throughout the Freeman Ranch. Also, the 5-year absence of grazing from the Pasture 1 exclosure (used for the seasonal analysis of C_3/C_4 composition in grasslands) appeared to have already resulted in differences between the vegetation of the exclosure and the typical upland grassland community of Freeman Ranch. The exclosure not only contained patches of the rare taller grasses (i.e., little bluestem and Indiangrass), but it also exhibited a greater average C_4 contribution (ca. 77% for the year in the seasonal analysis) than on average throughout Freeman Ranch (ca. 55%).

Boutton et al. (1993) reported that grazing had similarly decreased C_4 contribution to grasslands at a tallgrass prairie in east-central Texas. In that study, Boutton et al. compared a site that had been grazed by livestock for 20 years with an adjacent site having no record of grazing since 1832. In this study they found that though C_4 contribution was significantly lower in the current vegetation of the grazed site, with depth in the soil profile, soil $\delta^{13}C$ values for the grazed site became more similar to the soil values of the ungrazed site. This indicated that C_4 grasses had dominated both sites historically, but had declined significantly in the grazed site. Additionally, some of the same C_3 plants that dominate the contemporary Freeman Ranch grasslands (e.g., Texas wintergrass), were also found by Boutton et al. to have increased on the grazed site relative to the ungrazed site in that study. Overall, together with the timing of change in the soil $\delta^{13}C$ profiles, these findings support the hypothesis that grazing was the primary force leading to the decline of C_4 productivity in the grasslands of Freeman Ranch.

Stability of upland live oak clusters

In agreement with the initial hypothesis, the soil $\delta^{13}C$ profiles of live oak clusters were significantly different, at all depths, from adjacent grassland patches. Based on the general estimate of turnover time made previously, this indicates that the vegetation of discrete live oaks and grasslands has been distinct for likely >300 years. The exact age of the live oaks in the sampled clusters is not known. Because these oaks (like many other oak species (White 1966)) possess very hard wood, complete intact cores from this species are difficult to obtain (Norma Fowler, *personal communication*). Some studies

have suggested that live oaks can attain ages of at least 300 years of age (Harlow et al. 1996). Additionally, annual ring studies of the deciduous blue oak (*Q. douglasii*) in the oak-annual grassland savannas of California have demonstrated that some oak species of similar landscapes can live 400+ years (White 1966, Burns and Honkala 1990).

While the soil $\delta^{13}C$ values of the live oak clusters are distinct from those of the adjacent grasslands, the profiles beneath oak clusters also indicate that relative C_3 productivity has increased in the live oak cluster systems at a rate that is similar to that which occurred within grasslands. In the absence of data regarding the age of these oaks, it is impossible to definitively conclude that the C_3-signal from the lowest depth increment (20–30 cm) beneath these oak clusters is from the contemporary live oaks, rather than other C_3 plants that may have previously occupied the site. However, anecdotal accounts report that plateau live oaks may have been the only major woody species living in these upland landscapes at the time of European settlement (Olmstead 1857, Buechner 1944). Also, the steady increase in C_3 contribution exhibited by the soil $\delta^{13}C$ profiles beneath these live oak clusters is compatible with the expected increase in C_3 productivity following colonization and expansion of a live oak cluster, as the central oak increased in biomass and canopy cover. As it grew and developed, the increasing canopy could have progressively shaded out understory C_4 grasses. Furthermore, in accordance with the typical pattern of woody cluster formation beneath a nurse plant (Archer et al. 1988, Fowler 1988) understory C_3 shrubs may have established beneath the existing live oak and added more C_3 biomass, while concurrently contributing to the loss of C_4 grasses. Additionally, since livestock are often seen congregating beneath large live oaks, it is possible that some small part of the increase in C_3 contribution beneath oaks is

due to feces deposition (from livestock or other animals), reflecting the simultaneous increase of C_3 contribution to adjacent grassland communities. Overall, if the $\delta^{13}C$ signature revealing an increase in C_3 contribution at oak cluster sites does represent a record of the colonization and development of the oak cluster, one might expect to find the soil $\delta^{13}C$ values beneath these live oaks to finally converge with those of the adjacent grassland patches at depths slightly below 30 cm.

The development of the present-day shrub understory of these oaks was likely facilitated by some interaction between fire and grazing. Even before European settlement of the region, when fires are thought to have been more frequent (Smeins et al. 1997), these live oaks may have periodically served as "safe sites" for junipers and other fire-sensitive woody species, since fires beneath live oaks are not typically as hot or as uniform as beneath other nurse species of central Texas, such as the deciduous post oak (*Q. stellata*) (Fonteyn et al. 1988). Alternatively, if Freeman Ranch experienced episodes of heavy grazing following European settlement as is suggested by the grassland $\delta^{13}C$ data in this study, grazing may have reduced fire frequency and/or intensity through a reduction of fine fuel loads, also contributing to the success of woody shrubs in these clusters (Fuhlendorf and Smeins 1997).

Stability of the Ashe juniper woodland

Previous studies on Ashe juniper in the Edwards Plateau have indicated that this species has existed in the region for thousands of years (Bryant and Shafer 1977). Despite being an aggressively-invasive species, it may have been restricted in its range prior to

European settlement by more frequent and more intense fires set by lightning strikes and/or Native Americans (Foster 1917, Smeins 1980, Fuhlendorf et al. 1996). This study tested for evidence of pre-settlement existence of an Ashe juniper woodland at a site on the Freeman Ranch. This site, because of its topography (i.e., located near a north-facing steep cliff and bordering a large intermittent drainage) would be expected to provide a cooler and moister habitat that may have been able to sustain Ashe juniper even during times of fire. Additionally, this study tested the stability of the boundary between the present juniper woodland at this site and an adjacent savanna.

The $\delta^{13}C$ data for the transects located in the adjacent savanna (i.e., transects T4 and T5) showed the same significant increase with depth in the soil profile that was observed in the upland grasslands throughout the Freeman Ranch, indicating that these sites were likely grassland >300 years ago, and contained a greater C_4 component than they do today. The $\delta^{13}C$ data for two of the Ashe juniper woodland transects (i.e., T2 and T3) converged with those of the adjacent savanna with depth such that at the 20–30 cm depth, the soil $\delta^{13}C$ values of these woodland sites were virtually identical to those of the savanna sites. This indicates that sites T2 and T3, though currently within the woodland, were vegetated by C_4-dominated grasslands several hundred years ago and have since been converted to woodland. These findings agree with the initial hypothesis that the woodland/savanna boundary has not been static at this site, and they correspond with the well-documented instability of such boundaries throughout the world (e.g., Steuter et al. 1990, McPherson et al. 1993, Schwartz et al. 1996, Boutton et al. 1998).

In contrast to transects T2 and T3, the other woodland transect (T1, located adjacent to the cliff) showed $\delta^{13}C$ values that differed distinctly from those of the savanna

sites at all depths in the soil profile, indicating that there has been a greater C_3 component at this woodland site for at least 300 years. A comparison of the juniper woodland $\delta^{13}C$ profiles with the profiles from the upland live oak clusters and grasslands on Freeman Ranch can be seen in Figure 12. This comparison indicates that though the C_3 component was greater at the juniper woodland cliff site than the adjacent savanna sites at the time corresponding to 20–30 cm depth, the C_3 signature for this woodland was not as strong as that found beneath oak clusters at the same depth. Though junipers may have been established near the cliff > 300 years ago, this comparison implies that they were likely not very large and/or very dense.

Finally, although at some point in the past the $\delta^{13}C$ profiles from woodland sites T2 and T3 both separated from the $\delta^{13}C$ signature of the savanna and converged with that of the woodland, the profile for T2 did this at a point deeper in the soil profile (i.e., further back in time) than did that of T3 (Fig. 10). This indicates that the woodland growth was initiated from the cliff (T1) and expanded away from it into the adjacent savanna (i.e., it expanded first into site T2 and then site T3). Basal diameter measurements also support these $\delta^{13}C$ results, since the largest Ashe juniper individuals (exhibiting basal diameters >1.0 m) were located near the cliff (on or near transect T1). The expansion of the woodland at this Freeman Ranch site agrees with previous findings of increasing woody abundance (especially of Ashe juniper) throughout the Edwards Plateau (Smeins 1980, Smeins and Merrill 1988, Van Auken 1993).

Some anecdotal accounts suggest that frequent clearing of Ashe juniper woodlands (e.g., for removing pests or for obtaining building materials) following settlement may have dramatically reduced the number and extent of these woodlands

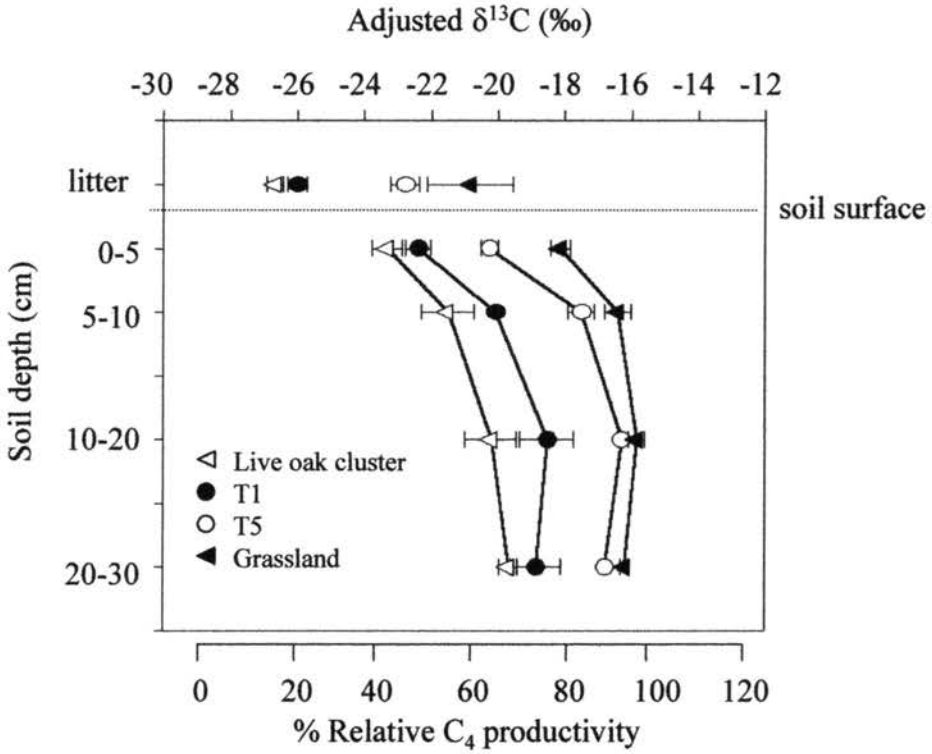


Figure 12. Mean (± 1 SE) adjusted $\delta^{13}C$ comparing profiles from the most extreme sites in the Ashe juniper woodland study [i.e., T1 (woodland near cliff), and T5 (savanna, 80 m from woodland boundary)] with profiles from the live oak cluster and grassland habitats on the Freeman Ranch. For juniper woodland sites, n = 3; for live oak clusters and grasslands, n = 6. "Litter" = surface litter (from woody and herbaceous plants) as well as live and standing dead herbaceous plant material.

for some time (Foster 1917, Krueger 1976, Smeins et al. 1997). Signs of such clearing are evident at another cliff site on the Freeman Ranch (in Pasture 8) that shares many of the same topographic features as the site used in this Ashe juniper woodland study (found in Pasture 9). At the Pasture 8 cliff site (which currently contains a mixed woodland of many species including some small juniper) numerous Ashe juniper stumps are present, some of which are relatively large. In contrast, such stumps are rare at the Pasture 9 cliff site, suggesting that the Ashe juniper woodland used in this study largely escaped the post-settlement clearing. Assuming that the selection of junipers for harvest was partly determined by the proximity of junipers to the homestead in those times, this woodland may have escaped since it is further away from the homestead location, than is the Pasture 8 cliff site.

Old-growth juniper woodlands are known to provide not only food and shelter for deer, small mammals, and birds of the Edwards Plateau (Rollins and Armstrong 1997), but they are also the habitat for the endangered golden-cheeked warbler (*Dendroica chrysoparia*), which has been sighted at this woodland (Beth Banks *personal communication*). Because old-growth juniper stands are likely currently rare on the Edwards Plateau (Smeins et al. 1997), this Ashe juniper woodland on Freeman Ranch likely serves a critical role as a benchmark community in the contemporary ecosystem.

Effects of woody patches on biogeochemistry

Initially, it was hypothesized that woody patches (i.e., live oak clusters and the Ashe juniper woodland) at this site on the eastern edge of the Edwards Plateau would exhibit greater soil nutrient storage than upland grasslands. In agreement with this hypothesis the results showed that SOC and total N were greatest in soils beneath live oaks, followed by those of Ashe juniper, and lowest in soils of nearby grassland patches. These findings also agree with those of Marshall (1995), who found similar results for a juniper-oak savanna in the western Edwards Plateau, and are consistent with the more general finding of nutrient enriched soils associated with woody plants in other savanna ecosystems (Klopatek 1987, Schlesinger et al. 1990, Jackson et al. 1990, McPherson et al. 1993). It is possible however, that soil C and N levels in these upland grasslands of Freeman Ranch have declined with the changing grassland vegetation over the recent past. Archer et al. (2000) modeled the effects of vegetation change on biogeochemistry at a savanna site in southern Texas and reported that SOC had likely decreased within the grasslands following heavy grazing. However, they also found that, with time, SOC and total N of soils beneath invading woody plants surpassed the levels in the pristine grassland. Thus, though Freeman Ranch grasslands may have experienced a loss of soil C and N following the introduction of livestock, the increase in productivity from C_3 plants (in oak cluster development and in expansion of juniper woodlands) has likely led to greater levels of soil C and N at the Freeman Ranch. Overall, together with the observation of woody plant increase on the Edwards Plateau (this study, Smeins and Merrill 1988, Van Auken 1993), the increased levels of nutrient storage found beneath

woody plants in this region support the notion that woody plant encroachment of grasslands and savannas can be an important avenue of carbon and nitrogen sequestration (Ciais et al. 1995, Scholes and Archer 1997).

Along with increased nutrient storage beneath woody plants, it was hypothesized that woody patches would exhibit altered patterns of nitrogen cycling, such that differences in $\delta^{15}N$ profiles might be observed between live oak cluster and grassland habitats on the Freeman Ranch. However, the $\delta^{15}N$ results proved to be largely inconclusive. For example, although litter $\delta^{15}N$ values were found to differ slightly between grassland and live oak habitats in this study, the $\delta^{15}N$ values for soil were similar between these two habitats. The similarity of soil $\delta^{15}N$ profiles between these two habitats at the Freeman Ranch site do not necessarily reflect N-cycles that are similar, as they may simply represent a similar net signature from very different N-cycling systems (Handley and Scrimgeour 1997).

Litter and shallow soils of the Ashe juniper woodland however, did show $\delta^{15}N$ values that differed from those in grasslands or live oak clusters. This difference can likely be attributed to differences in plant fractionation during uptake and resulting dilution of shallow soils due to litterfall (Nadelhoffer and Fry 1988). Thus, juniper tissue appears to be more enriched in ^{14}N-N than grassland or oak cluster vegetation, perhaps due to greater utilization of NO_3^--N relative to NH_4^+-N (Högberg 1997) by junipers than the other vegetation types.

Between the three habitats in this study (live oak clusters, grasslands, and juniper woodland) the range of $\delta^{15}N$ values for litter (-1.75 to 1.99‰) and soil at 20–30 cm depth (8.12 to 8.35‰) fell within the ranges suggested by Nadelhoffer and Fry (1994) for

terrestrial ecosystems (-5 to +2‰ for vegetation and +8 ± 2‰ for the corresponding soil). However, the soil $\delta^{15}N$ profiles beneath the juniper woodland increased more overall than did the profiles of the other two habitats. One potential explanation for this greater magnitude of ^{15}N-enrichment with soil depth ($\Delta^{15}N$) in the Ashe juniper woodland, and the convergence of $\delta^{15}N$ between habitats with depth, is that there are more pathways of loss for ^{14}N at this site than at grassland or live oak sites. Due to the topography of this specific site in the juniper woodland (i.e., all soil for $\delta^{15}N$ measurements within the juniper woodland were extracted from transect 1, which contained extensive areas of exposed limestone punctuated by deep soils in the interspaces), excavation sites may have coincided with local areas where water from rainfall events had typically concentrated. As a result, these sites might exhibit greater leaching of ^{14}N-enriched N (Högberg 1997) and perhaps greater rates of nitrogen cycling than typical upland grassland or live oak sites, resulting in these differences in $\Delta^{15}N$.

CONCLUSIONS

This study supported the hypothesis that upland grasslands on the Freeman Ranch were more strongly dominated by C_4 grasses prior to European settlement and suggests that the decline of the C_4 component was due to grazing. Discrete live oaks appear to have been present at least 300 years ago within these upland grasslands. Also, the $\delta^{13}C$ signature beneath these clusters appears to trace the growth and development of the live oak and shrubby understory, while simultaneously reflecting the displacement of C_4 and/or CAM species at these sites. Finally, the study within the Ashe juniper woodland suggested that some junipers were established adjacent to the steep north-facing cliff of Pasture 9 in Freeman Ranch prior to European settlement and have since increased in density and range as they expanded into the adjacent grassland.

Overall, the $\delta^{13}C$ data reveal considerable shifts in the C_3/C_4 composition of the vegetation at this site on the eastern Edwards Plateau. The data suggest that this site has experienced an increase in the abundance of woody plants in recent times, and because these woody plants exhibit increased levels of nutrient storage relative to adjacent grasslands, this site has likely served as a net sink for atmospheric CO_2 in recent times.

APPENDIX

Soil nutrient content (ppm) of surface soils (0-20 cm) from grasslands, live oak clusters, and transect 1 of the Ashe juniper woodland on the Freeman Ranch. Soils were collected in July 2000. Data are means ± 1 SE; (n = 3).

Habitat	pH	NO_3-N	P	K	Ca	Mg	Na	S
Grass	6.4 (0.1)	4.7 (0.3)	2.3 (0.3)	220 (46)	5359 (356)	233 (13)	223 (6.6)	32.7 (2.2)
Oak	7.4 (0.2)	7.0 (0.0)	22.7 (8.4)	779 (287)	7733 (515)	419 (86)	223 (20)	29.3 (2.9)
Juniper	7.0 (0.1)	5.0 (0.0)	3.0 (0.6)	163 (11)	8921 (129)	206 (23)	239 (19)	25.3 (1.5)

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