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STABLE CARBON ISOTOPE STUDY OF THE PRAIRIE-FOREST TRANSITION SOIL IN LOUISIANA[1]

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The calcareous prairies of Louisiana are among the most endangered and the least understood ecosystems in North America. The major threat to this ecosystem is the invasion of woody plants, primarily eastern red cedar (*Juniperus virginiana* L.). We studied the vertical profile of soil organic C (SOC) and the stable carbon isotope ($\delta^{13}C$) from soil organic matter (SOM) to infer the vegetation history and to understand the C dynamics of these prairies. SOC decreased with depth (0–100 cm), but variation due to vegetation type (prairie, transition, forest) and landscape position were not statistically significant. The mean total SOC stock within the 0–30 cm depth from three sites, three vegetation types, and two landscape positions was 7.6 ± 0.4 kg/m^2 (mean ± SE, n = 18). The SOM $\delta^{13}C$ values within the 0–10 cm depth showed that although the forest vegetation was exclusively C3, the transition and the prairie vegetation were composed of a mixture of C3 and C4 plant functional forms. Statistical comparison of SOM $\delta^{13}C$ values from the forest, transition, and prairie with depth showed that the SOM $\delta^{13}C$ from the prairie and transition are significantly different from that of the forest up to the 30–40 cm depth. SOM $\delta^{13}C$ values of the prairie and transition were not significantly different at all depths, suggesting that the invasion of C3 trees and shrubs observed within the transition may be a recent phenomenon coinciding with fire suppression. SOM $\delta^{13}C$ below 30–40 cm indicated that in the past, a C4 community might have dominated the entire site. (Soil Science 2003;Volume 168:783–792)

Key words: ^{13}C natural abundance, soil organic matter, Louisiana, calcareous prairie soil, soil organic carbon, eastern red cedar, woody invasion.

THE calcareous prairies of Louisiana are among the most endangered ecosystems in North America (Noss, 1997). The major threat to this ecosystem is invasion by woody plants, primarily eastern red cedar (*Juniperus virginiana* L.). Recent estimates show that <1% of the original Louisiana prairies remain (MacRoberts and MacRoberts, 1997a). Floristically, these prairies are classified as tallgrass prairies (MacRoberts and MacRoberts, 1997b) and resemble the Jackson Prairies of Mississippi (Moran, 1995). Unlike the well known Midwestern prairies, the ecology of the calcareous Louisiana prairies is poorly understood. The Louisiana Natural Heritage Program has designated these prairies as a 'research natural area' so they can be protected and serve as a baseline area for measuring long-term ecological changes, as well as to monitor effects of resource management techniques and practices (Hyatt, 1999). These isolated calcareous prairies exist as mosaics within short leaf pine-oak-hickory forest (Smith et al., 1989) and are unique and anomalous considering the region's humid climatic features and the predominant forest vegetation that covers the surrounding landscape. They are important because all contain either rare or endangered species (MacRoberts and MacRoberts, 1997b).

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The changes in vegetation of the calcareous prairies of Louisiana are not unique. Invasion of grasslands by woody plants is one of the dominant life form changes in the earth's vegetation occurring during the last two centuries (Brown and Archer, 1999). Implicated causes for woody invasion include overgrazing, climate change, and fire suppression (Archer et al., 1995). For Louisiana prairies, fire suppression is believed to be the main culprit (Smith et al., 1989; Hyatt, 1999). Woody invasion elsewhere has been associated with changes in ecosystem function because vegetation change is often accompanied by changes in site hydrology, soil organic matter (SOM), and other soil chemical characteristics (Vitousek et al., 1987; Boutton and Archer, 1998; Gill and Burke, 1999; Jackson et al., 2002). Similar to other invading woody species, eastern red cedar affects the soil and ground cover beneath its canopy (Gehring and Bragg, 1992; Barton and Wallenstein, 1997). It alters soil pH (Kourtev et al., 1998) and species richness (Gehring and Bragg, 1992), which, in turn, affects ecosystem function (Chapin et al., 2000). Therefore, knowledge of the extent and impact of woody invasion of relict ecosystems is important in understanding ecosystem processes that may be useful in designing appropriate management plans and future controlled studies (Lubchenco et al., 1991; Jackson, 1997).

Evidence from the stable carbon isotope $\delta^{13}C$ of SOM was examined in this study to infer the vegetation history and dynamics of the remnant calcareous prairies of Louisiana. The utility of $\delta^{13}C$ of SOM is based on the systematic isotopic variation between C3 (trees, shrubs and cool season grasses) and C4 plants (warm season grasses). The $\delta^{13}C$ value of SOM in the upper soil profile (0–10 cm) for a given site is similar to that of the vegetation at the site. Isotopic signals from antecedent vegetation persist deeper in the soil profile (Boutton, 1996). Thus, changes in the relative proportions of C3 and C4 plants can be detected by a measured difference between the isotopic composition of the current plant community and that of the SOM at various depths. In interpreting $\delta^{13}C$ of SOM at various depths, other known sources of $\delta^{13}C$ variation with depth must be considered. Older organic matter found deeper in the soil profile has $\delta^{13}C$ values that are generally 1–3% greater than those for organic carbon in the upper profile (Balesdent et al.,1987; Mariotti and Peterschmitt, 1994; Boutton, 1996; Ehleringer et al., 2000). This enrichment with depth has been attributed to one or more of the following processes: (i) the Suess effect (the $\delta^{13}C$ decrease in atmospheric CO_2 over the last 200 years), (ii) organic matter decomposition process which favors the loss of ^{12}C, and (iii) translocation of relatively undecomposed (^{13}C enriched) soluble carbon fractions downprofile. However, the 1–3% difference rarely affects interpretation of SOM $\delta^{13}C$ data because of the wide range between the mean $\delta^{13}C$ of C3 plants (−27%) and C4 plants (−13%). This approach has been applied successfully at various locations around the world (Schwartz et al., 1986; Ambrose and Sikes, 1991; McPherson et al., 1993; Mariotti and Peterschmitt, 1994; Victoria et al., 1995, Boutton et al., 1998; Eshetu and Hogberg, 2000).

Evidence of woody invasion of Louisiana calcareous prairies is based on aerial photography and personal accounts (MacRoberts and MacRoberts, 1997a). To date, there has been no isotopic study of the vegetation and SOM of these ecosystems. We hypothesize that the prairie soil has a distinct SOM $\delta^{13}C$ that distinguishes it from the adjoining transition soil and forest soil. If the transition soil was once under prairie vegetation, the SOM $\delta^{13}C$ of the transition soil with depth would resemble the prairie soil. A transition soil is defined here as a soil found between the prairie and the forest that is heavily encroached by eastern red cedar. The objectives of this research were (i) to reconstruct the vegetation history and dynamics of the calcareous prairies of Louisiana using stable C isotope data from SOM; and (ii) to assess the impact of recent vegetation on soil organic carbon (SOC) of the calcareous prairies of Louisiana.

MATERIALS AND METHODS

Study Area

The study was conducted within the Winn Ranger District of the Kitsatchie National Forest, Winn Parish, Louisiana (Fig. 1). The dominant remnant prairies of Louisiana, also known as the Keiffer calcareous prairie/forest complex, consist of a series of natural grassland openings occurring in a mosaic with calcareous hardwood-pine forest (Smith et al., 1989).

The Keiffer calcareous prairie/forest complex has developed on Eocene-age marly clays of the Tertiary period (Smith et al., 1989). The sediments belong to the Jackson group (Spearing, 1995). The Keiffer prairies occur on Keiffer loam soil that has $CaCO_3$ throughout the profile. The $CaCO_3$ was formed from the remains of marine life forms (USDA, 1998). Taxonomically, the soil

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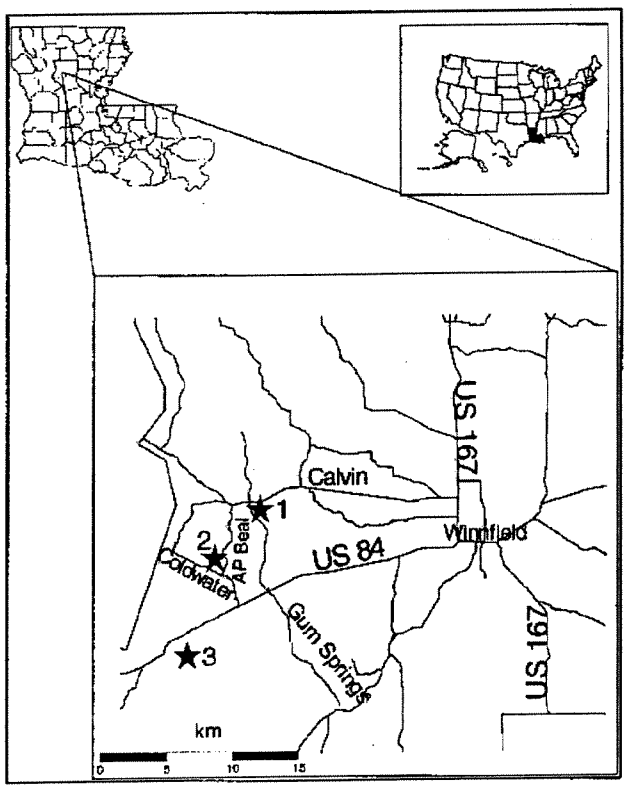

Fig. 1. Map showing study locations. Site 1: Milam Branch Prairie North; Site 2: White Oak Creek South Prairie; Site 3: Couley Church Prairie.

is classified as fine-silty, carbonatic, thermic Rendollic Eutrochrepts. Immediately adjacent to the prairies are forests on calcareous soils dominated by Hollywood (fine, smectitic, thermic Typic Pelluderts) and Oktibbeha (very-fine, smectitic, thermic Vertic Hapludalfs) soils. Vaiden (very-fine, smectitic, thermic Vertic Hapludalfs) and Bellwood (very-fine, smectitic, thermic Aquentic Chromuderts) soils also occur adjacent to the prairies. All of the prairies have been heavily grazed, and a few were farmed for short periods of time (Smith et al., 1989). Sites chosen for this study have not been farmed, and they have not been burned for the last 30 years. They are invaded by eastern red cedar and other woody plants. The sites receive mean annual precipitation (MAP) of 1250 mm and have a mean annual air temperature of 20 °C.

Field Methods

Three relatively undisturbed prairie/forest associations were selected for this study. According to MacRoberts and MacRoberts (1997a), the largest, relatively best preserved, and protectable prairie remnants are found within the Winn Ranger District of the Kisatchie National Forest. The criteria for site selection were established so that all three prairies would have: (i) similar adjacent soil units derived from the same parent material: Oktibbeha and Hollywood soils were soils adjacent to Keiffer soil derived from the same parent material; and (ii) minimum disturbance (e.g., roads, horse trails, and history of other land use). The selected sites are shown in Fig. 1. The sizes of the prairie portion of the sites are approximately 0.3 ha (Site 1), 0.9 ha (Site 2), and 1.4 ha (Site 3). The sites are separated by approximately 5 km. Typical tallgrass species in the prairies include little bluestem (*Schizachyrium scoparium*), big bluestem (*Andropogon gerardii*), tall dropseed (*Sporobolus asper*), and Indian grass (*Sorghastrum nutans*). The dominant vegetation within the forest is oak (*Quercus* spp). Woody plants other than eastern red cedar present at the

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transition include hawthorns (*Crataegus* spp.), persimmon (*Diospyros virginiana*), and rattan vine (*Berchemia scandens*). Most of the eastern red cedar trees at the transition had a height exceeding 2 m. Increment borer age estimate taken at breast height for the tallest trees was approximately 50 years. Smaller seedlings of eastern red cedar were also present within the prairie.

Soil samples were collected from each site using a push probe at 10-cm increments to a depth of 1 m. Two sets of samples (referred to as 0–100 cm sets hereafter to distinguish from the 0–30 cm sets) were collected from each of prairie, transition, and forest sites, each representing contrasting slope positions (upslope and downslope). Soil samples were collected from two contrasting slope positions to account for topographic variability in organic C and $\delta^{13}C$ of SOM (Barnes et al., 1983; Garten et al., 2000). All sampling points were similar in aspect. To obtain bulk density and estimate the total organic C stock within the 0–30 cm depth, separate sets of soil samples (0–30 cm sets) were collected from locations adjacent to the 0–1-m set of samples using a core sampler of known volume (length = 30 cm, internal diameter = 4.7 cm). For stable C isotope analysis, live plant specimens (leaves) were collected from the prairie, transition, and forest using a 1 × 1-m quadrant in the prairie and a 5 × 5-m quadrant in the transition and forest. To quantify seasonal variations in the organic matter input from C3 and C4 functional forms, the plants were collected at two different times of the year, in late March 2000 and late August 2000.

Laboratory Methods

The 0–1-m soil samples were air dried for several weeks and ground to pass a 2-mm sieve. Soil samples from each depth were treated with 1 *N* HCl at 25 °C for 3 days to remove carbonate C (Boutton, 1991). Each sample was washed to neutrality with distilled water to remove excess chloride, dried, and pulverized with a ball mill. Acid treatment did not affect $\delta^{13}C$ of SOM (Midwood and Boutton, 1998). SOM $\delta^{13}C$ was measured using a Finnigan MAT 262 Mass Spectrometer. Duplicate measurement of the same sample was within the error range of the instrument (± 0.2). All the $\delta^{13}C$ values are reported relative to the international standard Vienna Pee Dee Belemnite (V-PDB) and calculated as:

$$\delta^{13}C(‰) = \left(\frac{R_{sample} - R_{s\,tan\,dard}}{R_{s\,tan\,dard}}\right) \times 1000 \quad (1)$$

where $\delta^{13}C$ is the difference between the $^{13}C/^{12}C$ ratio (R) of the sample and that of the standard. Increases in the δ value denote increases in the relative amount of the heavy isotope component (^{13}C) or a reciprocal decrease in the light isotope component (^{12}C).

Organic C was analyzed with a CHN analyzer (EA 1108 Elemental Analysis, Fison Instruments). The plant specimens were oven dried (65 °C) and ground to a fine powder with a ball mill prior to isotopic analysis.

The 0–30-cm soil samples were used for obtaining bulk density after oven drying (105 °C). Organic C was also analyzed on these samples after all pretreatments were performed as described above. A paired *t* test on SOC concentration obtained from the 0–30 cm soil samples and those obtained by averaging the 0–10, 10–20, and 20–30 cm depths obtained from the 0–1 m soil samples showed nonsignificant differences at 5% probability level. Therefore, computation of total soil organic C stock in the 0–30 cm depth was based on the SOC measurements from the 0–30 cm soil samples. The total SOC content (TSOCC) within the 0–30 cm depth in kg/m^2 was calculated as (Amundson, 2001):

$$TSOCC = (SOC) \times (BD) \times \left(\frac{\%NG}{100}\right)xz \quad (2)$$

where SOC is soil organic carbon concentration (g/kg), BD is the bulk density (g/cm^3), %NG is the percent of soil that is nongravel (vol/vol), and z is the depth (m). In our case z = 0.3 m. All reported SOC concentration was calculated relative to the bulk soil sample.

The proportion of organic carbon derived from C4 sources, x, in the soil samples was estimated by a mass balance equation (Boutton, 1996) as follows:

$$x = \frac{\delta^{13}C_{soil} - \delta^{13}C_{C3}}{\delta^{13}C_{C4} - \delta^{13}C_{C3}} \quad (3)$$

Where $\delta^{13}C_{soil}$ is the $\delta^{13}C$ of SOM samples, $\delta^{13}C_{C4}$ is the average $\delta^{13}C$ value of C4 plants at the site (−13.2%), and $\delta^{13}C_{C3}$ is the average $\delta^{13}C$ value of C3 plants at the site (−27.4%). The proportion of carbon derived from C3 plant sources is 1-x.

Data Analysis

The 0–100 cm data were analyzed as a split-split plot design using the PROC MIXED procedure of SAS (SAS Institute, 1999). The split-split plot design was used because of the randomization restriction associated with the subplot and

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sub-subplot treatments (Kuehl, 1994). The statistical model used was:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + e_{ij}^{A} + \gamma_k + (\beta\gamma)_{jk} + e_{ijk}^{B} + \delta_l + (\beta\delta)_{jl} + (\gamma\delta)_{kl} + (\beta\gamma\delta)_{jkl} + e_{ijkl}^{C}$$

where μ is the overall mean, α_i is the random block (site) effect, β_j is the vegetation (main plot) effect, γ_k is the landscape position (subplot) effect, and δ_l is the depth (sub-subplot) effect. Thus, site*vegetation (e_{ij}^{A}) was the error term used for testing the main effect of vegetation, and site*vegetation*landscape position (e_{ijk}^{B}) was the error term used for testing the effects of landscape position and vegetation x landscape position interaction. The 0–30 cm data were analyzed as a split-plot design using a procedure similar to the 0–100 cm data. For the 0–30 cm data, vegetation was the main plot effect and landscape position was the subplot effect. Depth effect was not tested for the 0–30 cm data sets because we had only one measurement of bulk density for the entire 0–30 cm depth upon which the computation of total organic C content was based. Site was considered random in both cases because the selected sites would represent other Keiffer prairies in Louisiana. Smith et al. (1989) reported the presence of more than 45 Keiffer prairies.

The LSMEANS statement was used to separate main effects of vegetation. Pairwise tests of significant main effects were evaluated with the PDIFF option (SAS Institute, 1999). Where soil depth*vegetation interaction was significant, for the 0–1 m soil data, the SLICE option was used to compare soil depth for each vegetation type and vice versa. The SLICE option evaluates the simple effects of vegetation at each depth, and the simple effects of depth for each vegetation type (Littell et al., 1996). This is useful inasmuch as one of our objectives was to investigate the impact of recent vegetation on SOC and $\delta^{13}C$ of SOM at each depth. All statistical significance was judged at the 10% probability level. The proportion of organic carbon derived from C4 sources in the soil samples from each depth was estimated using Eq. 3.

RESULTS AND DISCUSSION

Soil Organic C

There was no significant difference in the Soil Organic C concentration caused by vegetation and landscape position (Table 1). As a result, the SOC concentration data from the three vegetation types and two landscape positions were pooled together. SOC concentration declined with depth in all three vegetation types (Fig. 2). Mean SOC concentration ranged between 33.5 ± 2.2 g/kg (mean ± standard error, n = 18) at the 0–10 cm depth and 4.0 ± 0.7 g/kg at the 90–100 cm depth. There was no vegetation by depth interaction effect on SOC concentration, indicating that the vertical distribution of SOC is similar among the three vegetation types with depth (Table 1).

TABLE 1

Analysis of variance table showing the effect of vegetation (VEGE), landscape position (LSPN), soil depth (DEPTH), and interaction terms on $\delta^{13}C$ of SOM, and soil organic C (SOC) content from three Keiffer prairie-forest associations, Louisiana. (F = F statistic)

	DF	SOC F	$\delta^{13}C$ F
VEGE	2	0.3NS	4.9*
LSPN	1	0.0NS	0.1NS
DEPTH	9	86.2****	9.4****
VEGE*LSPN	2	0.1NS	0.5NS
VEGE*DEPTH	18	1.0NS	1.5*
VEGE*LSPN*DEPTH	27	0.7NS	0.4NS

*Significant at $P < 0.1$
****Significant at $P < 0.001$
NS = Not significant

Total SOC stock within the 0–30 cm depth did not differ among the vegetation types (F = 0.09, P = 0.915) and landscape position (F = 0.62, P = 0.462). The mean total SOC stock within the 0–30 cm depth from the three sites, three vegetation types, and two landscape positions was 7.6 ± 0.4 kg/m^2(n = 18). A similar result would be expected for the 0–100 cm depth for two reasons: (i) woody invasion is expected to influence carbon cycling in surface soils because soil C kinetics are typically more rapid near surface than at lower depths (Trumbore et al., 1995; Gill and Burke, 1999), and (ii) variability in bulk density among vegetation types is generally small at greater depths (>30 cm). Other studies (Vitousek et al., 1987; Boutton and Archer, 1998; San Jose et al., 1998; Gill and Burke, 1999) showed increased SOC following woody invasion. As a result, woody invasion into grasslands is believed to be an important component of the terrestrial carbon sink (Pacala et al., 2001). However, a recent comprehensive study (Jackson et al., 2002) involving a global analysis of more than 2700 SOC profiles suggests that the relationship between woody invasion and a change in SOC is not con-

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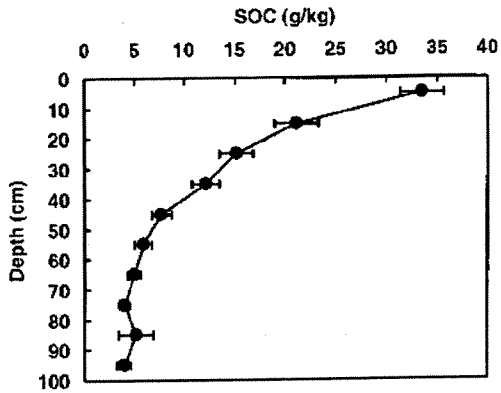

Fig. 2. Vertical distribution of Soil Organic C (SOC) within Keiffer prairies, Louisiana. Error bars represent standard error from three sites, three vegetation types, and two topographic positions (n = 18) that did not differ statistically.

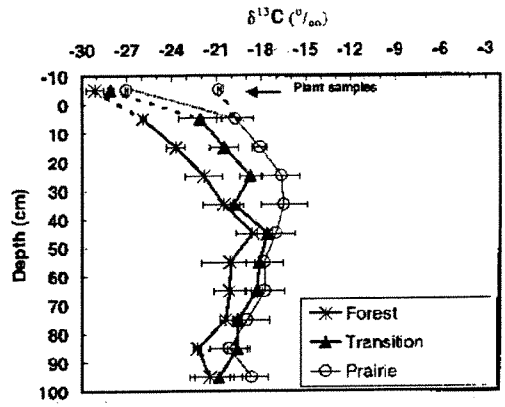

Fig. 3. $\delta^{13}C$ values of plants, and soil organic matter of prairie, transition and forest vegetation in Keiffer prairies, Louisiana. Error bars represent standard error from three sites and two topographic positions (n = 6) that did not differ statistically. Plant $\delta^{13}C$ values from prairie represent two seasonal values.

sistent along a precipitation gradient. Jackson et al. (2002) found that woody plant invasion increased content of SOC at drier sites (MAP < 660 mm) and decreased at wetter sites (MAP > 660 mm). Our study sites have a MAP of 1250 mm and are considered wet. The lack of significant difference in SOC among the vegetation types at this site (MAP = 1250) prompts the need for assessing the C contents of the litter and standing biomass of each vegetation type to allow evaluation of the net effects of woody invasion in this ecosystem.

$\delta^{13}C$ of SOM and Plants

There were significant vegetation, depth, and vegetation × depth effects on $\delta^{13}C$ of SOM (Table 1). Since there was no significant difference in $\delta^{13}C$ of SOM from the two topographic positions, the values were pooled together for plotting and discussion purposes. The mean $\delta^{13}C$ of SOM within the 0–10 cm depth of the forest soil was −25.9 ± 0.5% (n = 6) (Fig. 3). The mean $\delta^{13}C$ of SOM within the 0–10 cm depth of the prairie soil was −19.7 ± 0.9%. The value increased to −16.5 ± 1.0% at a depth of 30–40 cm and decreased to −20.0 ± 1.8% at 80–90 cm depth. The vertical distribution of $\delta^{13}C$ of SOM of the transition lies between that of the forest and the prairie (Fig. 3). The mean values were −22.1 ± 1.0% at 0–10 cm depth. The increase with depth of SOM $\delta^{13}C$ is a common trend (Balesdent et al., 1990; Kindscher and Tieszen, 1998; Boutton et al., 1998). The increasing age and degree of decay of organic carbon explain, in part, this enrichment in the heavier isotope with depth. Because organic matter migrates downwards as it decays, the relative proportion of old carbon increases with depth and is thus larger in the subsoil compared with the surface (Balesdent et al., 1990).

The $\delta^{13}C$ of above ground plant samples was −28.9 ± 0.1% (n = 6) for the forest, −28.0 ± 0.3% for the transition, and −27.0 ± 0.4% for the prairie for samples collected in late March 2000. Plant samples resampled in late August 2000 had $\delta^{13}C$ values of −22.6 ± 0.8% for the prairie, whereas there were no changes for the forest and transition zones. Similar to this observation, Ode et al. (1980) and Tieszen and Steuter (1991) documented a variation in $\delta^{13}C$ values from about −26% in early spring to about −20% in late summer for the Ordway Prairie, north-central South Dakota. The seasonal change in $\delta^{13}C$ values of forests is generally insignificant (Garten and Taylor, 1992). These results indicate that prairie SOM $\delta^{13}C$ is a time-integrated signal and provides evidence on the temporal C3 and C4 community dynamics where these plant communities co-exist.

The 0–10 cm SOM was enriched with the heavier isotope (^{13}C) relative to the plant samples by approximately 3% for the forest, 6% for the transition, and a seasonal average of 5.1% for the prairie. These differences are a consequence of several factors, including differences in $\delta^{13}C$ of plant parts, litter chemistry, and decomposition

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rates among plant species. Generally, leaves are depleted in the heavier isotope compared with roots (von Fischer and Tieszen, 1995). Litter derived from trees and shrubs contains high concentrations of lignin and secondary compounds that retard decomposition rates (Wedin et al., 1995). von Fischer and Tieszen (1995) reported that cellulose was enriched an average of 1.3%, whereas lignin was depleted by an approximately similar amount for four forest types in Luquillo, Puerto Rico. Wedin et al. (1995) analyzed changes in the isotopic composition of aboveground and belowground plant tissues of C3 and C4 grass species and associated changes in lignin concentration and isotopic composition during decomposition. Lignin fractions were consistently depleted in ^{13}C compared with bulk tissues (3.6 parts % more negative on average). The large enrichment of the 0–10 cm depth relative to leaves for this study may be a result of the combination of selective preservation of cellulose and other enriched components within the soil (Balesdent et al., 1990; von Fischer and Tieszen, 1995; Boutton, 1996). The relative mean $\delta^{13}C$ enrichment ($\Delta\delta$) with reference to $\delta^{13}C$ at 0–10 cm depth for SOM increased with depth to 30–50 cm for all vegetation types (Fig. 4). The maximum enrichment that can be obtained as a result of a combination of recent atmospheric ^{13}C depletion (1.5%) and decomposition process (1.5%) (Balesdent et al., 1987; Ehleringer et al., 2000) was estimated to be approximately 3.0%. Further enrichment was interpreted as the occurrence of a major vegetation shift, in this case the predominance in the past of C4 vegetation.

The approximate proportion of C3 and C4 contributions to SOC varied with depth within prairie, transition, and forest soils (Fig. 5). The isotope signal observed for the forest soil in the upper 10-cm depth was typical of C3 dominated communities. For the prairie, approximately 52% of the SOM within the 0–10 cm depth was derived from C4 vegetation, whereas for the transition, the C4 contribution was 35%. The proportion of C4 contribution increased with depth, reaching approximately 75% at the 30–40 cm depth for the prairie, 65% for the transition, and 60% for the forest at the 40–50 cm depth. The prairie, transition, and forest were similar in vegetation composition below 40–50 cm depth, with a slight decrease in C4 contribution to a depth of 80–90 cm. The statistical test from the SLICES option for the vegetation × depth effect (P = 0.09) on SOM $\delta^{13}C$ showed significant differences among vegetation types only for the 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm depths (Table 2). The pair wise test for significant vegetation effect indicated that compared with the prairie SOM, the transition SOM was consistently depleted in ^{13}C up to the 30–40 cm depth, but this depletion was not statistically significant (Fig. 3, Table 2). Forest SOM is significantly depleted in ^{13}C compared with both the transition SOM and prairie SOM up to the 30–40 cm depth (Fig. 3, Table 2). This indicates that the invasion of C3 trees and shrubs at the transition is a recent phenomenon.

The SOM in the upper 10-cm layer is indicative of recent vegetation, approximately 200 years (Boutton, 1996). Kindscher and Tieszen

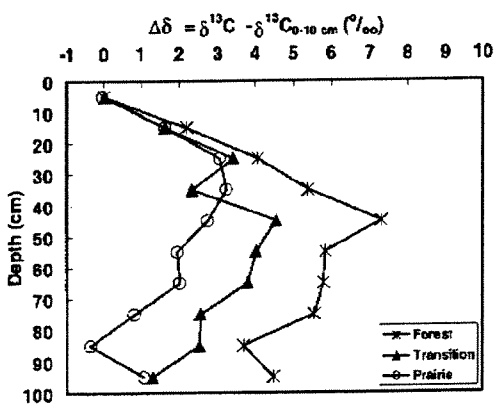

Fig. 4. Mean SOM $\delta^{13}C$ enrichment relative to the 0–10 cm depth from prairie, transition and forest vegetation in Keiffer prairies, Louisiana.

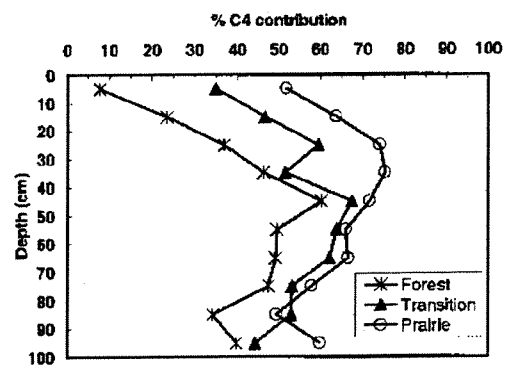

Fig. 5. Approximate proportion of soil organic carbon derived from C4 vegetation within prairie, transition, and forest in Keiffer prairies, Louisiana.

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TABLE 2

Results of analysis of variance for the simple effects of vegetation on the $\delta^{13}C$ of SOM at each depth and corresponding pair wise tests for three Keiffer prairie-forest associations, Louisiana, as evaluated by the SLICE and PDIFF options of SAS. (F = F statistic)

Depth (cm)	Vegetation effect	Pair wise comparison		
	F	ΔPF(‰)	ΔTF(‰)	ΔPT(‰)
0–10	8.7***	6.2****	3.8**	2.3 NS
10–20	7.2***	5.6***	3.2**	2.4 NS
20–30	5.4**	5.0***	3.1*	1.9 NS
30–40	4.2*	4.0**	0.7 NS	3.1*
40–50	0.6NS	1.6 NS	1.0 NS	0.6 NS
50–60	1.4NS	2.3 NS	2.0 NS	0.3 NS
60–70	1.4NS	2.4 NS	1.8 NS	0.6 NS
70–80	0.5NS	1.5 NS	0.8 NS	0.7 NS
80–90	1.6NS	2.2 NS	2.5 NS	-0.3 NS
90–100	1.5NS	2.5 NS	0.2 NS	2.3 NS

*Significant at $P < 0.1$; **Significant at $P < 0.05$; ***Significant at $P < 0.01$; ****Significant at $P < 0.001$; NS = Not significant.

$\Delta PF = SOM\ (\delta^{13}C_{Prairie} - \delta^{13}C_{Forest})$

$\Delta TF = SOM\ (\delta^{13}C_{Transition} - \delta^{13}C_{Forest})$

$\Delta PT = SOM\ (\delta^{13}C_{Prairie} - \delta^{13}C_{Transition})$

(1998) suggested that, based on their study of native prairies of northeast Kansas, the carbon isotopic signal at a depth around 25 cm is representative of an undisturbed prairie, with the assumption that turnover at this depth is slower than that near the surface. Given the warmer and more humid climate in Louisiana compared with Kansas, we expect the turnover rate to be higher within Louisiana prairies. This assumption is supported by the findings of Garten et al. (2000) that demonstrated increased turnover rate with increasing temperature. If the suggestion of Kindscher and Tieszen (1998) is correct, the carbon isotope signal at a depth of 40–50 cm may represent an undisturbed prairie signal for the Keiffer calcareous prairies. This depth is also where $\delta^{13}C$ values of the prairie, transition, and forest converge (mean SOM $\delta^{13}C = -7.7 \pm 0.4, n = 18$), suggesting that in the past the forest site may have been dominated by C4 vegetation. This depth may also be indicative of middle Holocene (6000 – 4500 yr BP) climatic conditions. Radiocarbon data for SOM from central Texas (Nordt et al., 1994) revealed the prevalence of maximum temperatures and minimum precipitation and, therefore, greater productivity of C4 plants during the middle Holocene. Humphrey and Ferring (1994) used similar methods to obtain evidence for more humid *early* Holocene (greater C3 productivity), drier *middle* Holocene, and more humid *early* Holocene for north-central Texas. These historic climatic fluctuations determine the C3/C4 species composition and productivity (Ehleringer et al., 1997) and, therefore, explain in part the fluctuation with depth of $\delta^{13}C$ of SOM.

The similarities and differences in the morphological characteristics of the Keiffer prairie soil compared with the adjacent Oktibbeha and Hollywood forest soils also support the conclusion drawn from the $\delta^{13}C$ data. The three soils are similar in morphology below approximately 40 cm in depth; they all have accumulations of $CaCO_3$ and exhibit intersecting slickensides. The Keiffer soil is at a slightly higher position and is calcareous to the surface. Oktibbeha and Hollywood forest soils do not contain $CaCO_3$ at the surface and are slightly acidic (USDA, 1998).

SUMMARY AND CONCLUSION

SOC data from three remnant calcareous prairies in north-central Louisiana varied with depth, but variation due to vegetation and landscape position were not statistically significant. The $\delta^{13}C$ values of SOM with soil depth served as a signal of the temporal vegetation dynamics. The $\delta^{13}C$ signatures from SOM show that while the forest is exclusively C3, the transition and the prairie are composed of a mixture of C3 and C4 plant functional forms. The C4 contribution to the 0–10 cm SOM pool within the prairie was 52%, whereas the C4 contribution to the SOM at a similar depth was lower (35%) in the transition soil. The $\delta^{13}C$ of SOM increased with depth with proportional increases in C4 productivity for each vegetation type to a depth of 40–50 cm. The observation that the isotope signature of transition soil is not significantly different from the prairie soil at all depths suggests that woody invasion at the transition is a recent event. The $\delta^{13}C$ of SOM at the 40–50 cm depth suggests that the entire site might have been occupied by a predominantly C4 vegetation (prairie). This result has implications for carbon cycling, C sequestration, and management of this ecosystem. To assess the net effects of woody plant invasion on the C cycling and C sequestration potential of this ecosystem accurately, future research must consider C contents of litter and the above- and below-ground biomass. Further, these relict prairies represent one end of the spectrum of climate (high MAP)-woody invasion continuum, and future research must take these prairies into consideration as they offer additional clues to the past environment of the southeastern

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United States as well as information on the relationship among SOC dynamics, woody invasion, and climate.

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