



10-31-2000

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Recommended Citation

Sinton, Penelope; Seastedt, Tim; Bennett, Barry; Bock, Carl; and Bock, Jane (2000) "C₃ and C₄ Species

Changes Identified by $\delta^{13}\text{C}$ values of soil organic matter in a Colorado prairie," *Western North American Naturalist*. Vol. 60 : No. 4 , Article 12.

Available at: <https://scholarsarchive.byu.edu/wnan/vol60/iss4/12>

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C₃ AND C₄ SPECIES CHANGES IDENTIFIED BY $\delta^{13}\text{C}$ VALUES OF SOIL ORGANIC MATTER IN A COLORADO PRAIRIE

Penelope Sinton^{1,2,3}, Tim Seastedt^{1,2,4}, Barry Bennett¹, Carl Bock¹, and Jane Bock¹

ABSTRACT.—We measured carbon isotope signatures ($\delta^{13}\text{C}$) from 0–10 cm and 10–20 cm soil depth intervals for grassland soils near Boulder, Colorado. These grasslands included tall-, short-, and mixed-grass prairies that were grazed, ungrazed, or hayed. Soils exhibited $\delta^{13}\text{C}$ signatures consistent with observations that current sites are a mix of C₃ and C₄ species, with C₃ plants more abundant in mixed-grass than in native tall- or shortgrass prairies. The $\delta^{13}\text{C}$ signatures were not significantly different for grassland types; however, management treatments (grazing, no grazing, haying) significantly influenced changes in soil $\delta^{13}\text{C}$ signatures from the 0–10 cm to 10–20 cm soil depth intervals. We observed a correlation ($r = 0.63$) between isotopic values of surface soils and percent native species in total vegetation cover. Overall, the community type with the lowest percentage of nonindigenous species cover had the most enriched $\delta^{13}\text{C}$ signature.

Sites currently grazed by prairie dogs, cattle, or both herbivores had stronger C₃ signatures, indicating that grazing may have increased C₃ plant productivity in these communities at the expense of C₄ grasses. This finding differs from studies of native shortgrass steppe where grazing has the opposite effect on the relative abundance of these 2 functional groups of plants. This result, along with the correlation between C₃ isotopic values and nonnative vegetation abundance, provides evidence that management practices that maintain dominance of C₄ grasses should be encouraged.

Key words: $\delta^{13}\text{C}$, C₃, C₄, carbon, grassland, Colorado, prairie.

Human activities have caused wide-scale alteration of grassland communities. Cattle grazing, irrigation, and fire suppression have been practiced along the Front Range of Colorado for about 130 yr. In addition, many nonindigenous plant species have been introduced into the area (Bennett 1997). Increased atmospheric deposition of inorganic nitrogen (Williams et al. 1996), in conjunction with increases in atmospheric CO₂ and climatic changes, are also believed to be influencing ecosystem processes of grasslands that historically contained both C₃ and C₄ species (e.g., Wedin and Tilman 1996, Tieszen et al. 1997).

Bennett (1997) completed a survey of grassland plant communities around Boulder, Colorado, and found higher abundances of C₃ plants in these areas than previously reported (Vestal 1914). The significance of a shift in vegetative composition in temperate grassland from C₄ to C₃ species dominance implies changes in several ecosystem characteristics that affect soil characteristics and subsequent plant species composition and productivity (Seastedt 1995, Wedin and Tilman 1996).

Soil organic matter contains a record of the relative composition of C₃ and C₄ species (O'Leary 1981). Atmospheric CO₂ is composed of approximately 1.1‰ ¹³C and 98.9‰ ¹²C (O'Leary 1981, Farquhar et al. 1989). During photosynthetic CO₂ fixation, plants discriminate against the heavier isotope, ¹³C, while favoring the lighter isotope, ¹²C (O'Leary 1988). C₃ plants discriminate against ¹³CO₂ to a greater extent than do C₄ plants, resulting in distinctly different ranges of $\delta^{13}\text{C}$ values for C₃ and C₄ plants (O'Leary 1981, Dzurec et al. 1985, Farquhar et al. 1989, Ehleringer et al. 1991, Wang et al. 1993). $\delta^{13}\text{C}$ values for C₃ plants are between –23‰ and –34‰, with an average of –26‰; C₄ plant values are between –9‰ and –17‰ and have an average of –12‰ (O'Leary 1988). Average difference in $\delta^{13}\text{C}$ values between C₃ and C₄ plants is therefore about 14‰ (Dzurec et al. 1985, Wang et al. 1993).

Decomposition further modifies isotopic values. Respiratory CO₂ is lower in ¹³C than is soil organic matter, and there is usually a detectable increase in ¹³C abundance in older organic matter originating from C₃ sources

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(Nadelhoffer and Fry 1988). Long-term decomposition rates of C₃ and C₄ plant substrates are unknown, which adds some uncertainty to the analyses. However, the relatively large difference in carbon isotope signatures of the 2 plant groups should dominate soil organic matter signatures (Dzurec et al. 1985, Kelly et al. 1993, Boutton 1996). Assuming that soil organic matter is derived from past plant associations and not deposited as loess, we can therefore estimate relative historical C₃ and C₄ species abundances by assuming that the isotopic signature is correlated to relative productivity of each plant group (Dzurec et al. 1985, Wang et al. 1993).

Average age of soil carbon in temperate grassland soils is variable. As a general rule, less than half of soil organic matter is composed of recalcitrant materials, humus, and precursors of humus that may average over 1000 yr in age. The remainder is composed of relatively young material produced in the last decades (Parton et al. 1996). Older carbon is also found deeper in the soil (e.g., Tiezen et al. 1997). Identifying the photosynthetic pathway source of this carbon therefore provides information on the historical composition of the area as well as an indication of how much the current community may differ from the past.

C₄ species comprise most native grass species in the Boulder area (Bennett 1997). They have historically dominated both tallgrass and shortgrass prairies and are a significant component of mixed-grass prairie (Brown 1989). We hypothesize that (1) the tallgrass prairie will exhibit the strongest C₄ carbon isotopic signature because this community type is believed to have the strongest dominance of warm-season (C₄) grasses; and (2) given that all nonnative plants currently abundant in the Boulder area are C₃ species, we expect the strongest C₃ carbon isotopic signature will be found in nonnative vegetation areas. To test these hypotheses, we used carbon isotope signatures of soil organic matter as an index of change in the community composition of C₃ and C₄ plant species (e.g., Boutton 1991). We also evaluated whether isotopic signatures in the soil would identify functional shifts in vegetation due to grazing and haying activities.

STUDY SITE AND METHODS

Plots were sampled in Boulder Open Space grasslands (ca 40°N, 105°W, 1645 m elevation)

used in Bennett's (1997) study of grassland diversity. Precipitation averages about 46 cm per year, and soils are mostly cobbly clay loams or sandy clay loams classified as Aridic Argiustolls (Moreland and Moreland 1975). These grasslands were formed on alluvial and colluvial deposits generated from Pleistocene outwash from the Front Range. Parent materials are of igneous or metamorphic composition and are noncarbonaceous. Shale sites or sites with suspected carbonate materials were excluded from the study. Native vegetation in the area ranges from tallgrass sites dominated by *Andropogon gerardii* (C₄) and *Panicum virgatum* (C₄), to mixed-grass sites dominated by *Agropyron smithii* (C₄), *Stipa comata* (C₃), and *Koeleria pyramidata* (C₄), and grazed mixed-grass areas with extensive *Bouteloua gracilis* (C₄) and *Buchloe dactyloides* (C₄). These native grasses are often accompanied or dominated by nonnative C₃ species such as *Bromus* spp., *Poa pratensis*, and *Alyssum minus* (Bennett 1997). Bennett's data were summarized by Craig et al. (in press), who found that tall-, short-, and mixed-grass prairies consisted of 45%, 44%, and 29%, respectively, nonindigenous plant species. Hay meadows on former tall- and mixed-grass prairies averaged 85% nonnative species in terms of percent vegetation cover.

Plots were located in areas that were (1) grazed by cattle, prairie dogs, or both herbivores; (2) hayed; or (3) in enclosures where there was no grazing or haying for about the last decade. We classified plots as either mixed-grass or tallgrass prairie. A subset of the mixed-grass sites had vegetation characteristics consistent with shortgrass steppe, but we lacked sufficient replicates of these sites for a separate analysis.

Soil cores of 1.7-cm diameter to a depth of 20 cm were obtained at each site. Of the 66 plots surveyed by Bennett (1997), only 42 could be sampled in this manner. Sites with shale substrate were excluded from analysis because of high CaCO₃ content. We assumed there was very little or no CaCO₃ at sites with soils derived from noncarbonaceous parent material. Each soil core was divided into samples 0–10 cm deep and 10–20 cm deep. Soil samples were air-dried, and the dry soil was shaken onto a series of increasingly smaller-diameter sieves. Visible particulate debris such as

roots and rocks was manually removed. Samples were ground to a fine powder and combusted in a VG Isochron mass spectrophotometer to measure the amount of ^{13}C present.

Results here are expressed in standard $\delta^{13}\text{C}$ notation where

$$\delta^{13}\text{C}\text{‰} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

and $R = ^{13}\text{C}/^{12}\text{C}$ (Craig 1957). All samples are reported relative to the international PDB standard (O'Leary 1988). Repeated measurements of a soil standard yielded a precision of 0.1‰. We performed two 2-way analysis of variance (ANOVA) procedures to test for isotopic differences attributable to grassland type and land use treatment. We compared average isotopic value for the entire 20-cm profiles (weighted equally for each horizon) in 1 analysis, and in the 2nd we tested for changes in $\delta^{13}\text{C}$ values between the 0–10 cm and 10–20 cm cores related to grassland type and land use effect. This 2nd test assumed that larger differences in the top-to-bottom $\delta^{13}\text{C}$ values would potentially identify more recent changes in the functional composition of grasslands. Younger carbon is assumed to dominate surface horizons, while older, more recalcitrant forms of carbon occupy lower horizons (Nadelhoffer and Fry 1988). We used the Student-Newman-Keuls multiple range test when significant differences were detected to identify cell differences in land use type (SAS 1988).

We also used a Pearson's correlation analysis to relate our soil isotope values with summary findings from Bennett's (1997) vegetation analysis. Isotopic values from each plot were correlated with the value of native plants as percent of total vegetation cover.

RESULTS

The 0–10 cm depth interval averaged $\delta^{13}\text{C}$ values of about -21‰ , with a range from -16.3‰ to -23.8‰ , while soils at lower depths exhibited a less negative $\delta^{13}\text{C}$ signature and ranged from -13.8‰ to -22.7‰ (Table 1). Surprisingly, soils with the highest ^{13}C content were from ungrazed mixed-grass sites. Grazed mixed-grass sites with or without prairie dogs, or sites identified as potential shortgrass sites, all showed similar values (Table 1).

Hay meadows are dominated by nonnative C_3 grasses (Bennett 1997) and therefore were expected to have a stronger C_3 influence than soils of native grasslands; this trend was evident (Table 1). Grazing also produced significantly different $\delta^{13}\text{C}$ values than ungrazed sites (SNK test; Fig. 1). Isotope values of grazed mixed-grass sites indicated a larger C_3 increase than did grazed tallgrass sites (Fig. 1), which produced a marginally significant ($P = 0.06$) interaction effect between treatment and prairie type.

Differences in isotopic abundance between upper (0–10 cm) and lower (10–20 cm) soil depths showed no effect due to plant community type, but did show a significant effect of treatment type ($F_{2,36} = 9.94$, $P < 0.001$) and a strong interaction effect ($F_{2,36} = 5.53$, $P = 0.008$) between community and treatment. Of the plant communities, tallgrass prairie showed the largest changes in isotopic abundance between upper and lower soil depths (Table 1). While this difference for tallgrass communities was maximized for hay meadows, the difference was minimized in hay meadows of mixed-grass sites, thereby producing the interaction (Table 1).

TABLE 1. Carbon isotope signatures from 0–10 and 10–20 cm deep soil samples from grasslands near Boulder, Colorado. Values are means with standard errors in parentheses.

Grassland	Treatment	Number of samples	Soil depth (cm)		Difference (top–bottom)
			0–10	10–20	
Tallgrass	Ungrazed	4	-20.6 (0.5)	-17.4 (0.2)	3.1 (0.4)
	Grazed	3	-22.6 (1.3)	-20.2 (1.7)	2.4 (0.5)
	Hay meadow	5	-23.7 (0.6)	-19.0 (0.6)	4.7 (0.5)
Mixed-grass	Ungrazed	10	-16.3 (1.4)	-13.8 (1.6)	2.5 (0.5)
	Grazed (total)	13	-20.9 (0.6)	-18.3 (0.9)	2.6 (0.5)
	(with prairie dogs)	6	-20.5 (1.2)	-17.4 (1.7)	3.0 (1.0)
	(without prairie dogs)	7	-22.1 (0.6)	-19.2 (1.4)	2.9 (0.8)
	(shortgrass)	3	-20.3 (0.9)	-18.8 (0.7)	1.5 (0.2)
	Hay meadow	7	-23.8 (0.3)	-22.7 (0.3)	1.0 (0.3)

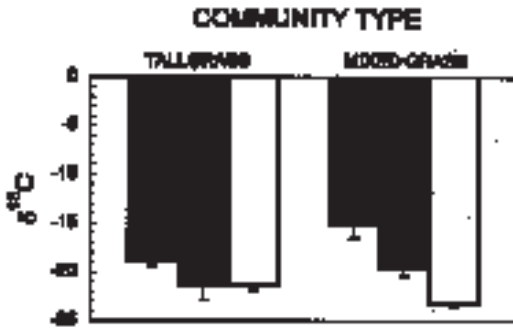


Fig. 1. Carbon isotopic values for soils from tallgrass and mixed-grass sites near Boulder, Colorado. Treatments include ungrazed areas (shaded bars), grazed areas (hatched bars), and hayfields (clear bars). Vertical hatched bars designate 1 standard error.

We observed a positive correlation between isotopic values and percent exotic vegetation at each site (Fig. 2). This relationship exists for the entire data set ($r = 0.62$), as well as when hayfields were excluded from the analysis ($r = 0.63$).

DISCUSSION

Changes in plant species composition can produce feedbacks that further alter ecosystem components (D'Antonio and Vistousek 1992, Bowman and Steltzer 1998). In particular, the shift from C_4 to C_3 species dominance in temperate grasslands is thought to be associated with higher levels of nitrogen availability (Wedin and Tilman 1996), which would benefit nitrogen-loving species, including many weeds. Understanding how changes in land use practices have affected particular grassland communities is difficult because the effects of one practice are often compounded with those of others. Collectively, these changes have undoubtedly created a new environment for remaining plant communities. At a minimum, these communities are clearly outside the historical range of variability in natural disturbance agents (such as fire) responsible for the vegetation composition occurring prior to European settlement (e.g., Hobbs and Huenneke 1992, Swanson et al. 1993).

We reject our 1st hypothesis that tallgrass communities in the Boulder area would exhibit the strongest C_4 carbon isotope signature. Average isotopic signatures observed in soils from

the Boulder area indicate that C_3 grasses are present in all community types. Indeed, $\delta^{13}C$ values were very different from the average of -13‰ reported for tallgrass soils in eastern Kansas (Smith and Johnson 1997). We conclude instead that Front Range tallgrass areas likely had a higher C_3 species component than their eastern counterparts.

The more depleted $\delta^{13}C$ values in surface soils are consistent with increases in C_3 species contribution to soil organic matter. These results correlated with Bennett's (1997) findings, lending support to his suggestion of an overall shift in community dominance from C_4 to C_3 species in the grasslands around Boulder. We therefore accept our 2nd hypothesis that the C_3 carbon isotopic signature is associated with nonnative (C_3) vegetation. These observations are also consistent with other studies that suggest a trend toward more C_3 -dominated grasslands in North America (Tieszen et al. 1997). Causal mechanisms for these changes remain unidentified, but atmospheric nitrogen enrichment, fire suppression, and the introduction of a large number of nonindigenous C_3 plants into the region could all contribute to the trend.

Results from this study suggest that we need to reconsider our preconceptions regarding the historic composition of tallgrass, mixed-grass, and shortgrass communities. Strongest C_4 signatures in soils were not found in tallgrass sites, but in areas of currently ungrazed, mixed-grass prairie. These sites appear to have a higher contribution of warm-season C_4 grasses than the tallgrass areas. Grazing by cattle, prairie dogs, or both species has increased the relative abundance of C_3 species in all sites, but particularly in mixed-grass sites (Fig. 1). In the Boulder area, grazing in tallgrass prairie is often restricted to winter or early spring, which would favor the persistence of warm-season C_4 grasses. Nevertheless, our data suggest that this grazing regime has resulted in an increase in relative C_3 productivity.

Prairie dog colonies are known to favor forb growth over grasses (e.g., Whicker and Detling 1988) and should therefore enhance the C_3 carbon isotopic signature. However, grazing by ungulates has been reported to enhance C_4 species abundance in more xeric grasslands (Mulchunas et al. 1989, Frank et al. 1995). Our results indicate a consistent effect of grazing toward C_3 species dominance. While interpre-

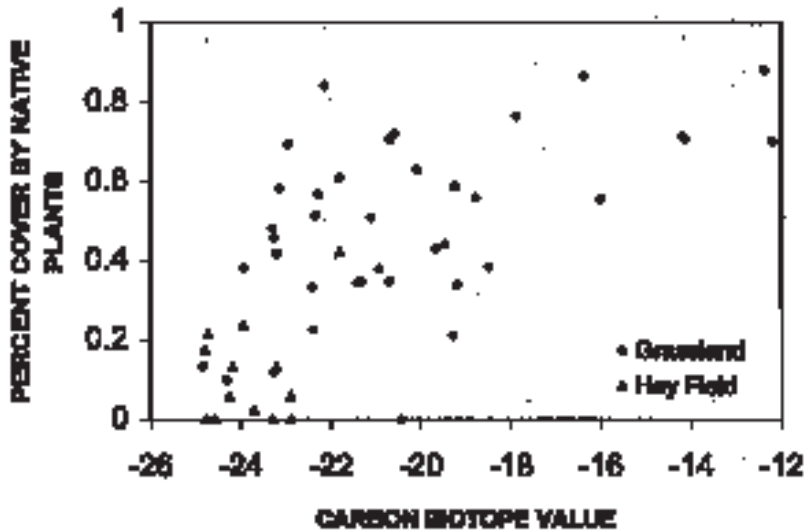


Fig. 2. Correlation between surface (0–10 cm) soil $\delta^{13}\text{C}$ signatures and native plant abundance as percentage of total vegetation cover. Square symbols are from hayfields; diamonds are from tall-, mixed-, and shortgrass prairie.

tations remain speculative, the increase in non-indigenous, largely C_3 species such as cheatgrass (*Bromus tectorum*) and a variety of forbs on these lands (e.g., Bennett 1997, Reeve-Morgahan and Seastedt 1999) is hypothesized as the causal mechanism for the soil carbon isotope grazing response observed in the Boulder area. Indeed, the community type with the highest contribution of C_4 carbon to the soil, the mixed-grass prairie, correlated with the lowest percentage composition of non-indigenous species (29% vs. over 44% for all other sites) reported by Craig et al. (in press).

Changes in plant community composition will likely affect community productivity, soil carbon storage, and other ecosystem properties that can impact surviving prairie grasslands around the Boulder area. If the dominance of native, warm-season grasses in these systems is a priority management goal, more proactive measures to enhance C_4 grasses in this area are warranted.

ACKNOWLEDGMENTS

We thank Lynn Reidel and the Boulder Open Space Department for access to sites and for supplementary information. Isotopic data were obtained by Dan Ruess at the Colorado Laboratory for Environmental Mass

Spectrometry at Colorado State University. Ideas expressed herein benefited from discussions with Alan Townsend and from comments and suggestions from several anonymous reviewers. This research was supported by an NSF-REU grant and the Undergraduate Research Opportunity Program at University of Colorado.

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Received 31 August 1998
Accepted 3 December 1999