



Reconstructing palaeoenvironment from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of soil organic matter: A calibration from arid and wetter elevation transects in Ethiopia

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ABSTRACT

One difficulty with reconstructing palaeoclimate from stable isotopic analyses of continental proxies is to determine whether changes occurred in temperature and/or precipitation. The resolution with which $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of soil organic matter (SOM) can be used to infer climate characteristics were examined from soils along two elevation transects in Ethiopia. Two transect characteristics permitted evaluation of temperature and precipitation effects separately and in tandem on δ values. First, transects differed from one another in precipitation but not in temperature. In addition, precipitation did not co-vary with elevation or temperature in the wetter transect. Vapour pressure deficits (physiologically meaningful measures of aridity affected by both temperature and precipitation) thus decreased more with elevation gain in the drier than in the wetter transect. In both transects, $\delta^{13}\text{C}$ values of surface (<10 cm depth) SOM were highest at middle elevations and lowest at both the highest and lowest elevations. This humped relationship was preserved in presumably older SOM samples up to 300 cm depth. These trends support hypotheses about climate influences on $\delta^{13}\text{C}$ values of SOM only from the middle to highest elevations. From the lower to middle elevations, the trends suggest the hypothesis that historical differences in land use pressures may have a greater and opposing influence than climate on $\delta^{13}\text{C}$ values of SOM. The $\delta^{15}\text{N}$ values were negatively related to elevation in the drier transect alone, supporting hypotheses that precipitation is the principal influence on $\delta^{15}\text{N}$ values of SOM. Elemental analyses provide some affirmation for the hypothesis that the influence of precipitation on openness of local nitrogen cycling can cause $\delta^{15}\text{N}$ values to increase with aridity. A problem in reconstruction of continental environments, per se, is to discern changes in climate from changes in land use. If differences in land use history have larger effects than climate on $\delta^{13}\text{C}$ values of SOM then $\delta^{15}\text{N}$ values of SOM may be valuable in conjunction with $\delta^{13}\text{C}$ analyses for reconstructing aspects of land use and climate.

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1. Introduction

At least four factors place Ethiopia among the most critically important locations for testing hypotheses about relationships between environmental changes and fundamental developments in human communities (e.g., Carneiro, 1970). First, it is a contender for the longest history of habitation by *Homo sapiens* (White et al., 2003). Second, a multi-millennial succession of highly organized kingdoms and empires has resided in the highlands of its northern half. These include a

probable centre of origin of agriculture and the once internationally powerful commercial empire, Aksum (Phillipson, 1998). These two factors alone; lengthy, and varied human history, make Ethiopia a compelling region for reconstructing past environments.

Third, Ethiopia's range of elevations (ca. –120 to 4620 m a.s.l.), and tropical latitudes (ca 3–15° N) offer climates ranging from those with regular frosts to among the hottest on terrestrial earth. This virtually unexploited factor makes Ethiopia a superior location in which to calibrate continental palaeoproxies for climate.

Past land use is an important aspect of palaeoenvironmental reconstruction for understanding developments in human societies and may provide keys to predict future societal developments as well (e.g., Diamond, 2005; for Ethiopia, Butzer, 1981). Land use may also confound efforts to reconstruct climate, however (Butzer, 2005). Studies of modern analogues could be as useful for identifying proxies

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for past land use as for calibrating palaeoclimate. Ethiopia's fourth attribute is that ancient land uses are more widely practiced in Ethiopia today than are modern technologies (Bard, 1997; Bard et al., 1997, 2000).

Factors three and four mean that Ethiopia has an unusually good array of modern analogues with which to develop means of separating climate from land use in environmental reconstruction. Herein, we make use of factors 3 and 4 to calibrate relationships of stable carbon and nitrogen isotopic compositions of soil organic matter to climate, and land use.

Soil organic matter (SOM) is among the most ubiquitous of terrestrial materials with potential to provide insights about past environments. An increasingly facile avenue for obtaining those insights is through carbon and nitrogen stable isotopic analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of SOM. Analyses of $\delta^{13}\text{C}$ values in SOM have become regular means of reconstructing past C_3/C_4 compositions of vegetation and their associated environmental conditions (Boutton et al., 1998) because $\delta^{13}\text{C}$ values of C_3 plants are, on average, 15‰ lower than those of C_4 plants (O'Leary, 1988), and plants are the primary sources of SOM. The proportions of C_4 and C_3 vegetation in a landscape are often related to climate (Ehleringer et al., 1997; Sage et al., 1999; Huang et al., 2001; Street-Perrott et al., 2004).

Biogeochemical factors can weaken the resolution of vegetation reconstructions from $\delta^{13}\text{C}$ values of bulk SOM. Most notably,

differences in decomposition rates among compounds that vary in $\delta^{13}\text{C}$ can cause carbon isotopic fractionations in SOM (Agren et al., 1996). In addition, <200 y old SOM is ^{13}C depleted by ca. 1.5‰ due to increases in organically derived CO_2 in the atmosphere that, in turn, is fixed by plants (Balesdent and Mariotti, 1996). Although the effects of decomposition on the $\delta^{13}\text{C}$ values of SOM can be eliminated by analyzing specific, highly refractory compounds (Huang et al., 1999), such effects are apparently small relative to those caused by shifts in vegetation cover, even after millions of years (Cerling et al., 1989). Consequently, $\delta^{13}\text{C}$ analyses of bulk material remain common for reconstructing past vegetation (Koch, 1998).

In contrast to $\delta^{13}\text{C}$, the principal source of an association of $\delta^{15}\text{N}$ values of bulk soil organic matter with environment is via biogeochemical factors. Specifically, ^{14}N is favoured in many outfluxes of inorganic nitrogen, leaving the remaining soil pool enriched in ^{15}N . SOM pools from more closed biogeochemical cycles may have less ^{15}N enrichment than SOM pools in more open cycles because there will be less ^{14}N loss from denitrification and more ^{14}N return of dead plant matter in the former than in the latter (Högberg, 1993). How closed or open an ecosystem's nitrogen cycle is may be influenced by climate and land use (Eshetu and Högberg, 2000a,b; Amundson et al., 2003; Miller et al., 2004).

Most of the change in $\delta^{15}\text{N}$ of SOM occurs within the first few tens of centimetres of the soil surface (Nadelhoffer and Fry, 1988; Martinelli

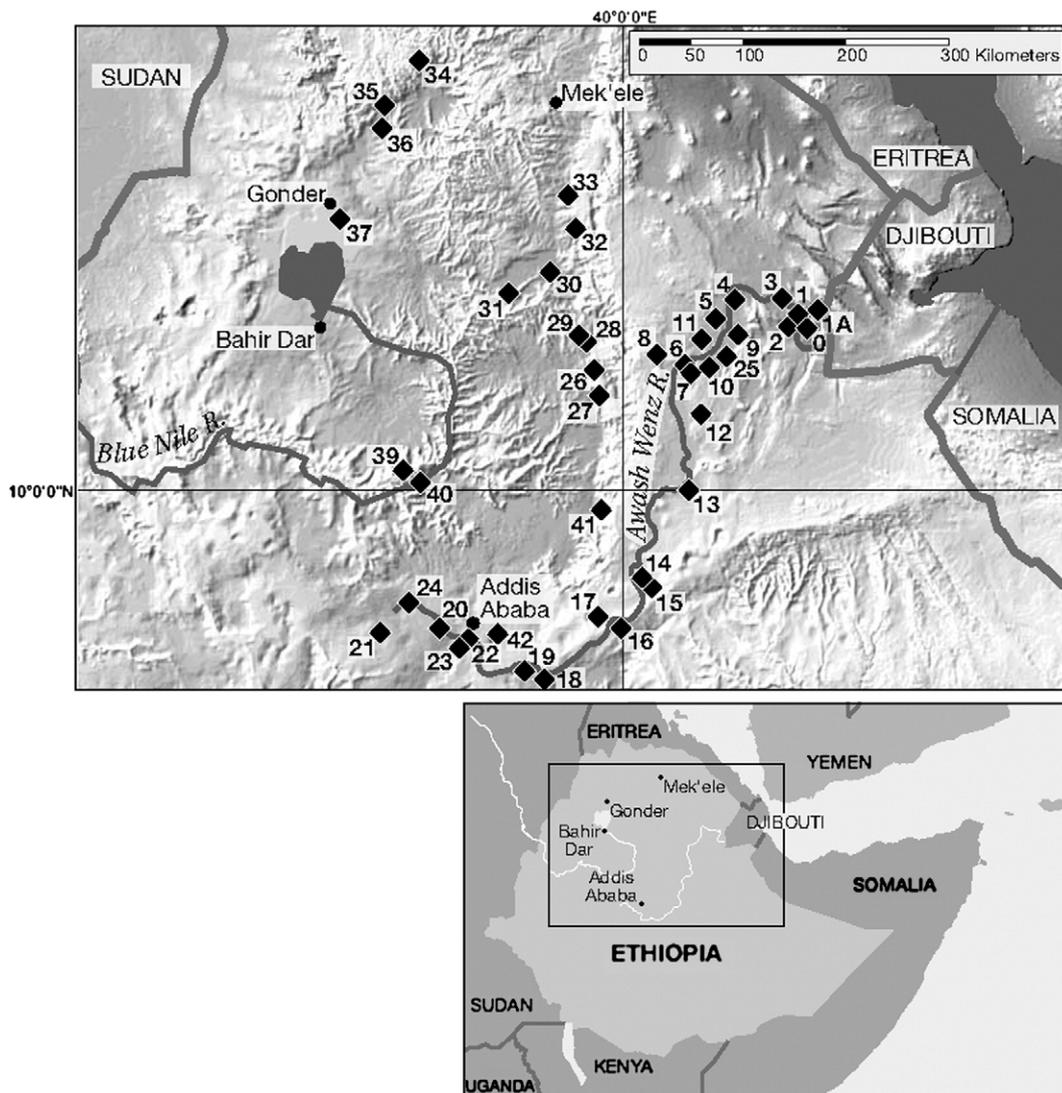


Fig. 1. Locations of sampling sites. See Table 1 for additional information.

et al., 1999). Consequently, $\delta^{15}\text{N}$ analysis of SOM in palaeosols is hypothesized to be a proxy for climatic reconstruction (Koch, 1998). Findings presently conflict, however, as to whether temperature, precipitation, or both influence $\delta^{15}\text{N}$ (e.g., Amundson et al., 2003; Aranibar et al., 2004). Part of the problem with discerning which climatic factor most influences $\delta^{15}\text{N}$ values of SOM is that temperature is often cross-correlated with rainfall (Terwilliger et al., 2002; Amundson et al., 2003).

We address 4 questions in this paper: 1) How well is $\delta^{13}\text{C}$ of SOM related to climate?, 2) Is there an association between $\delta^{15}\text{N}$ values of SOM and temperature as well as precipitation?, 3) Do $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ values reflect land use?, and 4) Can more information about environment be inferred from analyzing both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM than from only $\delta^{13}\text{C}$ values? We hypothesized that influences of land use as well as climate on biogeochemistry and/or vegetation might affect both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of SOM.

To address these questions, we collected soils from two elevation transects that differed in average annual rainfall. In addition, temperature and rainfall negatively co-varied with altitude along one transect but only temperature varied systematically with elevation along the other. Although studies are concerned with the extent to which temperature and/or precipitation affect $\delta^{13}\text{C}$ values of SOM, the proximal

physiological control on the $\delta^{13}\text{C}$ value of plants is mediated by the vapour pressure deficit (VPD), which is an interplay between these two environmental factors (Farquhar et al., 1989; Ehleringer, 1993). The two transects also differed in VPD (see Materials and methods).

2. Regional setting and sampling rationale

2.1. Transects

We selected 43 sites along two elevation transects in which to analyze soils (Fig. 1, Table 1). One transect (hereafter called “dry transect”) begins in the arid lowlands at Lake Gamari near the mouth of the Awash River (site 0, 350 m a. s. l.) and rises through the historically drought – affected highlands to near Mount Abune Yosef at Babasat (site 30, 3500 m a. s. l.). The other transect (hereafter called “wetter transect”) extends through a wetter region with no history of drought severe enough to lead to famine. The wetter transect begins near the Blue Nile south of Lake Tana (site 39, 1050 m a. s. l.) and rises to above Lake Wenchi (site 42, 3100 m a. s. l.). Topography and seasonal shifts of the Intertropical Convergence Zone (ITCZ) have large influences on the climates of the study sites.

Table 1
Information about sampling sites

Site	Vegetation	Elevation (m)	Latitude (N)	Longitude (E)	Geology ^a	Soil ^a
<i>Dry transect</i>						
1	Shrub	353	11° 30' 49"	41° 38' 50"	Quaternary sediments	Calcaric fluvisol
0	Shrub	354	11° 29' 18"	41° 39' 20"	Quaternary sediments	Calcaric fluvisol
2	Wetland	355	11° 31' 3"	41° 35' 56"	Quaternary sediments	Calcaric fluvisol
1A	Shrub	357	11° 30' 10"	41° 38' 56"	Quaternary sediments	Calcaric fluvisol
3	Shrub	367	11° 37' 39"	41° 24' 10"	Quaternary sediments	Calcic xerosol
4	Shrub	410	11° 41' 17"	40° 57' 21"	Quaternary sediments	Calcic xerosol
9	Shrub	414	11° 22' 20"	40° 56' 35"	Quaternary sediments	Eutric fluvisol
10	Shrub	462	11° 7' 44"	40° 45' 49"	Quaternary sediments	Eutric fluvisol
11	Shrub	470	11° 16' 56"	40° 45' 46"	Quaternary sediments	Eutric fluvisol
5	Shrub	483	11° 24' 46"	40° 45' 39"	Quaternary sediments	Calcaric fluvisol
7	Shrub	506	11° 6' 25"	40° 34' 52"	Quaternary sediments	Calcaric fluvisol
6	Shrub	561	11° 7' 17"	40° 33' 38"	Quaternary sediments	Calcic xerosol
8	Shrub	674	11° 13' 17"	40° 19' 8"	Quaternary sediments	Calcic xerosol
15	Grass	747	9° 14' 14"	40° 10' 19"	Quaternary sediments	Calcaric fluvisol
13	Wetland	758	10° 1' 49"	40° 35' 41"	Quaternary basaltic flows and related cones	Histosols
12	Grass	760	10° 42' 15"	40° 41' 40"	Quaternary basalts, intermediate and felsic volcanics	Haplic xerosol
16	Shrub	940	8° 50' 33"	40° 0' 43"	Tertiary volcanics, Magdala group	Haplic xerosol
17	Shrub	978	8° 55' 24"	39° 50' 37"	Quaternary basalts, intermediate and felsic volcanics	Calcaric fluvisol
25	Shrub	1143	11° 13' 29"	40° 55' 17"	Trap series, Tertiary volcanics	Eutric regosol
34	Shrub	1260	13° 45' 28"	38° 12' 48"	Upper Proterozoic, Precambrian metamorphics	Orthic Acrisol
27	Wetland	1474	10° 52' 22"	39° 48' 37"	Trap series, Tertiary volcanics	Cromic vertisol
32	Wetland	1480	12° 17' 59"	39° 35' 59"	Quaternary sediments	Eutric cambisol
18	Grass	1545	8° 23' 25"	39° 19' 48"	Quaternary sediments	Eutric regosol
29	Grass	1674	11° 22' 40"	39° 38' 31"	Trap series, Tertiary volcanics	Eutric fluvisol
35	Shrub	1747	13° 21' 34"	37° 55' 24"	Trap series, Tertiary volcanics	Eutric cambisol
28	Wetland	1933	11° 20' 21"	39° 41' 36"	Trap series, Tertiary volcanics	Eutric cambisol
26	Grass	1967	11° 5' 27"	39° 45' 30"	Trap series, Tertiary volcanics	Eutric cambisol
41	Grass	2015	9° 53' 4"	39° 49' 40"	Quaternary basalts, intermediate and felsic volcanics	Eutric cambisol
33	Grass	2468	12° 35' 47"	39° 31' 38"	Quaternary sediments	Eutric fluvisol
36	Grass	2870	13° 11' 18"	37° 53' 38"	Trap series, Tertiary volcanics	Eutric cambisol
31	Grass	3166	11° 45' 20"	39° 1' 4"	Trap series, Tertiary volcanics	Lithosol
30	Grass	3487	11° 55' 52"	39° 22' 31"	Trap series, Tertiary volcanics	Lithosol
<i>Wetter transect</i>						
39	Shrub	1049	10° 4' 32"	38° 11' 26"	Adigrat Sandstone of the Central Plateau, Mesozoic sediments	Arenosol
40	Shrub	1554	10° 3' 34"	38° 13' 30"	Adigrat Sandstone of the Central Plateau, Mesozoic sediments	Arenosol
19	Shrub	1604	8° 28' 47"	39° 9' 20"	Tertiary volcanics, Magdala group	Eutric fluvisol
37	Grass	1912	12° 24' 29"	37° 31' 52"	Quaternary sediments	Eutric cambisol
22	Grass	2010	8° 42' 21"	38° 36' 19"	Tertiary volcanics, Magdala group	Pellic vertisol
23	Grass	2017	8° 42' 19"	38° 36' 4"	Tertiary volcanics, Magdala group	Pellic vertisol
20	Grass	2071	8° 50' 59"	38° 24' 39"	Tertiary volcanics, Magdala group	Pellic vertisol
24	Grass	2239	9° 1' 36"	38° 7' 49"	Tertiary volcanics, Magdala group	Chromic vertisol
38	Grass	2425	–	–	Adigrat Sandstone of the Central Plateau, Mesozoic sediments	Nitisol
21	Grass	2899	8° 47' 56"	37° 53' 32"	Tertiary volcanics, Magdala group	Humic cambisol
42	Grass	3102	8° 45' 57"	37° 52' 33"	Trap series, Tertiary volcanics	Lithosol

A map of locations is in Fig. 1.

^a From Geography Division (1988) and most representative of the region at large.

The sites are at elevation intervals no greater than 150 m. All sites are dominated by perennial grasses and forbs and are grazed by livestock. However, some sites are entirely grass (grass), some have shrubs (shrub), and some had saturated soils all year (wet) (Table 1).

The landscapes of both transects are characterized by several high (>4000 m) mountain peaks and flat plateaus dissected by deep gorges (Fig. 1). The eastern portion of the study region slopes gradually towards the East African Rift Valley, and our dry sites follow this gradient downward to the Afar Depression. The wetter transect extends from the upper elevations of the Awash River Basin to points south of Lake Tana along the Blue Nile. We sampled from flat surfaces that were minimally subjected to erosive soil transport in a variety of soil types and lithologies (Table 1).

We compiled mean annual temperature, rainfall, and actual vapour pressure records (usually >30 y) for all weather stations along each transect (WHO, 1987) (Fig. 2). Mean annual temperatures do not differ significantly at given elevations between transects and decrease at 5.9 °C 1000 m⁻¹ elevation in both transects (Ancova; regression of temperature averages for all stations against elevation, $p < 0.0005$, $r^2 = 0.87$).

One reason for examining two transects that differed in aridity was to permit some separate insights about relationships between δ values and temperature or rainfall. This objective was further enhanced because

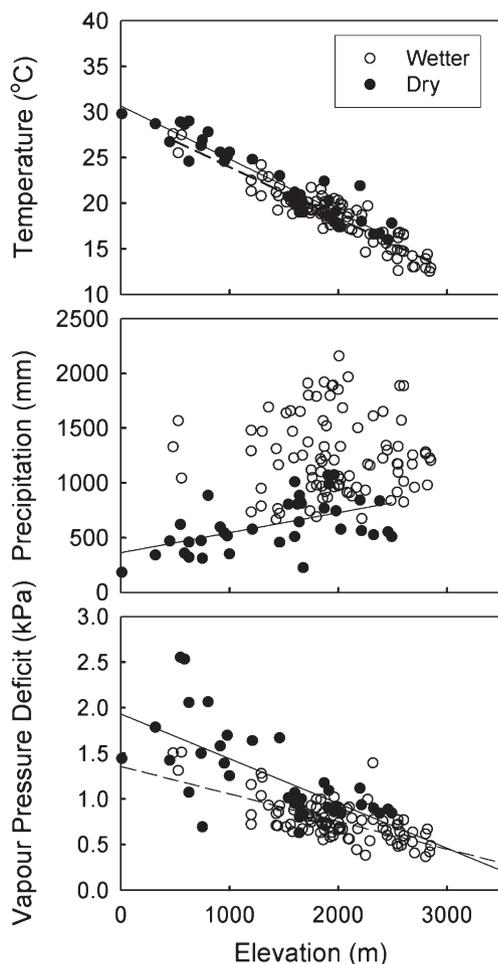


Fig. 2. Changes in average annual temperature, precipitation, and vapour pressure deficit with elevation at weather stations along the wetter (dashed line) and drier (solid line) transects (obtained or calculated from World Meteorological Organization, 1997). Temperature lines do not differ significantly from one another in intercept or slope. There is a significant relationship between precipitation and elevations in only the dry transect. Vapour pressure deficit lines differ significantly from one another in slope (see text).

temperature and rainfall are negatively correlated in the dry transect ($p = 0.002$, $R = -0.49$) but do not significantly co-vary in the wetter transect. Furthermore, average annual rainfall increases at 184 mm ppt per 1000 m gain in elevation ($p < 0.001$ in the dry transect, $r^2 = 0.27$) but is unrelated to altitude in the wetter transect (Fig. 2).

A physiologically meaningful measure of aridity; vapour pressure deficit (VPD), was calculated as the difference between vapour pressure at saturation (VP_s) and actual vapour pressure values from the compiled weather station records. VP_s (in kPa) was calculated from average annual air temperatures (t in °C) using the formula (WMO, 2006).

$$VP_s = 0.6112 \exp^{(17.62t)/(t+243.12)} \quad (1)$$

Vapour pressure deficit decreases with rise in elevation along both transects but VPD changes less per unit change in elevation along the wetter than along the dry transect (Ancova, $p \leq 0.0001$) (Fig. 2).

Over 90% of the average annual precipitation falls from February–October and July–August are the months with maximum precipitation in all sites. There is considerable inter-annual and inter-site variability in rainfall distribution, however. Some sites have a single rainy season and others have a weak spring rainy season followed by a main rainy season. Sites in the dry transect with spring rains usually have a longer, drier period before the summer rains than sites with spring rains in the wetter transect. Otherwise, we could find no clear relationship between characteristics of rainfall distribution and elevation or transect.

2.2. Sampling designs

Five approximately 0.25 kg soil samples were obtained in the first 10 cm of soil within a 10 ha area at all 42 sites. We assumed that by being closest to the surface, the SOM in these soils was the most modern. In addition, during a preliminary field trip one sample per site was obtained with a soil auger at successive depths to a maximum of 3 m in sites 1–5, 7–14, and 16–23 (Table 1). These sites were all along the Awash River basin but the highest elevations (sites 20–23) were in the wetter transect and the rest were in the dry transect. We assumed these samples to have older SOM and report the results of their analyses as a source of comparison with modern trends (*sensu* Eshetu and Högberg, 2000a,b). Some vegetation was collected near or in a few sites for data to estimate the relative isotopic contributions of C_3 and C_4 plants to SOM. Specifically, papyrus (*Cyperus papyrus* L.) leaves were collected near sites at the three elevations (758, 1480, and 2899 m a. s. l.), and leaves of dominant C_3 forbs and C_4 grasses were collected at 940 and 978 m in sites 16 and 17, respectively (Table 1). *C. papyrus* was selected because it was a single species found in the widest range of elevations.

3. Materials and methods

3.1. Elemental and stable isotopic analyses

Soil and plant samples were air dried upon collection. After completion of field work, samples were oven dried at 50 °C for four days. After removing particles > 2 mm diam and coarse organic debris, the remaining fractions of the soil samples were ground in a ball mill (Spex CertiPrep 6000; Metuchen, NJ, USA). Aliquots of each soil sample were tested for presence of carbonates with drops of 10% HCl. Carbonates were removed using a slight modification of the methods of Harris et al. (2001). Specifically, Harris et al. (2001) put ca. 30 mg samples of soil into silver capsules, wetted them with 50 μ L water, and fumigated them with 12 N HCl for 6–8 h. We weighed ca. 7 mg soil samples into dry silver 3 \times 5 mm capsules that had been washed in hexane followed by 10% HCl, added 10 μ L water to each sample, and found 4 h to be an ample fumigation time.

Bulk samples were analyzed for total percent organic carbon (TOC), total percent nitrogen (TN), $\delta^{13}C$, and $\delta^{15}N$ with an elemental analyzer

(Carlo Erba Instruments, NA 2500 series) coupled via continuous flow to a stable isotope ratio mass spectrometer (ConFloII to Delta Plus XL, ThermoFinnigan). Both nitrogen and carbon were analyzed from single ca. 2 mg samples of plants and from 7–35 mg of each soil sample depending upon TN. As was found in Harris et al. (2001), the procedures for removing carbonates could alter $\delta^{15}\text{N}$ values. Therefore, in soil samples containing carbonates, $\delta^{15}\text{N}$ values were obtained from samples prior to carbonate removal and $\delta^{13}\text{C}$ values were obtained after removing inorganic carbon. Acetanilide was used as a reference standard. The δ values of each element (X) are calculated according to the equation:

$$\delta X = [(R_{X\text{sample}}/R_{X\text{standard}})-1]1000 (\text{‰}) \quad (2)$$

where R is the ratio of heavier to lighter isotopes of the element and standards are Pee Dee Belemnite for carbon (Craig, 1953) and atmospheric N_2 for nitrogen (Mariotti, 1983). The precision of the analyses was $\pm 0.2\text{‰}$. All elemental and stable isotopic analyses were performed at the Geophysical Laboratory of the Carnegie Institution of Washington, D.C.

3.2. Data analyses

Parameters that might be cross-correlated, such as temperature and precipitation, were examined by Pearson's correlation analysis. Trends with elevation were established with linear regression or, where appropriate, curving fitting regression procedures. Data were tested for normality using Anderson Darling normality tests and for equality of variances using Bartlett's tests. Non-normally distributed data with unequal variances were transformed using Box-Cox procedures. Significance of slopes of trends with elevation and differences in slopes and intercepts of trends between transects were examined using analyses of covariance via general linear models. Differences in relationships between dependent variables and elevation among vegetation types were established by Ancova models and Tukey comparisons. Statistical packages used include Minitab, DataDesk, and SigmaStat. Significant results had $p \leq 0.05$.

Proportional contributions of C_4 carbon to TOC in soil (ρ) were calculated using the mass balance approximation (Trouve et al., 1994):

$$\delta^{13}\text{C}_{\text{SOM}} = \rho(\delta^{13}\text{C}_{\text{C}_4}) + (1-\rho)\delta^{13}\text{C}_{\text{C}_3} \quad (3)$$

and were then converted to percentages.

4. Results

4.1. $\delta^{13}\text{C}$ values of C_3 and C_4 plants

The average $\delta^{13}\text{C}$ of all C_3 plants was $-28.3 (\pm 0.35 \text{ SE}) \text{‰}$ ($n=8$). The $\delta^{13}\text{C}$ values of C_4 grasses averaged -14.5‰ and ranged from -15.5 to -13.5‰ ($n=3$). We used these averages as constants for $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ in the estimates of percent inputs of C_4 carbon into the organic carbon of soil (see Eq. (3)). The $\delta^{13}\text{C}$ values of *C. papyrus*, the species sampled over a wide range of elevations, suggested no relationship to elevation that could be used to compute $\delta^{13}\text{C}_{\text{C}_3}$. They were -26.8‰ at 758 m, -28.8‰ at 1480 m, and -27.6 at 2899 m; a range of values that encompassed the remaining C_3 plants analyzed. We recognize that environmental factors may have led to differences in $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ values of plants along and between transects that were beyond the scope of vegetation sampling possible in this study. The most likely of these factors and their consequences for our estimates of percent inputs of C_4 carbon into the organic carbon of soil are considered in the discussion.

4.2. Relationships of $\delta^{13}\text{C}$ to elevation and vegetation type (SOM from <10 cm)

The $\delta^{13}\text{C}$ values of modern SOM (from <10 cm depth) and estimated percent contribution of C_4 carbon to SOM did not change linearly with elevation in either transect. These variables did, however, vary quadratically (convex curve) such that the highest $\delta^{13}\text{C}$ values and greatest relative contributions of C_4 carbon were at the middle elevation sites and the lowest $\delta^{13}\text{C}$ values with lowest relative contributions of C_4 carbon were at both the highest and lowest elevations (model $Y=aX+bX^2+c$, $p<0.0005$, r^2 dry transect=0.24, r^2 wetter transect=0.77) (Fig. 3). The curve for the dry transect differed from that of the wetter transect (Ancova, $p=0.013$). The difference in curves between transects was not an artifact of the dry transect extending into lower elevations than the wetter as it was significant even when the values from the dry transect at lower elevations with no wetter transect counterparts were omitted from analysis (Ancova, $p=0.043$).

Although changes in vegetation type could have contributed to the quadratic trend in percent contributions of C_4 carbon and $\delta^{13}\text{C}_{\text{SOM}}$ with elevation, this trend also occurred within specific vegetation types. Relationships of $\delta^{13}\text{C}_{\text{SOM}}$ to elevation in grass sites alone were quadratic (regressions: $p<0.0005$ for each transect) and differed between the dry and wetter transects (Ancova, $p=0.007$). Wet vegetation sites were only present in the dry transect and from lower to middle elevations but their $\delta^{13}\text{C}_{\text{SOM}}$ values also exhibited a significant quadratic relationship to elevation ($r^2=0.28$, $p=0.032$). Shrub (grassy, some woody plants) sites were only present in the lower to middle elevations of both transects (Table 1). In the wetter transect, $\delta^{13}\text{C}$ values of shrub sites increased with gain in elevation (insufficient sites for statistical analysis) whereas $\delta^{13}\text{C}$ values of shrub sites in the dry transect were unrelated to elevation. Some differences occurred in relationships between $\delta^{13}\text{C}_{\text{SOM}}$ and elevation between vegetation types (Ancova on quadratic model, dry transect only: $p<0.0005$; Tukey comparisons: grass vs shrub, $p<0.00005$; grass or shrub vs wet, $p=0.0003$). In the cases where comparisons could be made over the same elevation ranges, however, no differences in $\delta^{13}\text{C}_{\text{SOM}}$ emerged between vegetation types.

4.3. Relationships of $\delta^{15}\text{N}$ to elevation and vegetation (SOM from <10 cm)

$\delta^{15}\text{N}$ values of SOM were negatively related to elevation in the dry transect ($p<0.0005$, $r^2=0.16$) but were unrelated to elevation in the wetter transect (Fig. 4). $\delta^{15}\text{N}$ values of SOM did not differ significantly

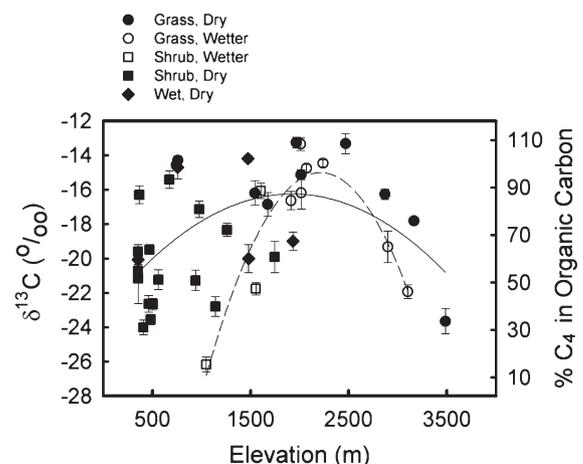


Fig. 3. Trends in $\delta^{13}\text{C}$ and estimated % of carbon from C_4 plants of SOM with elevation in the dry (solid line) and wetter (dashed line) transects.

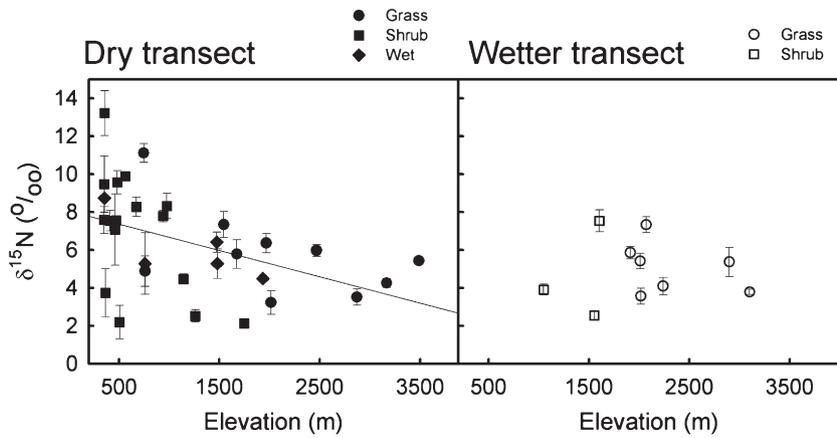


Fig. 4. Trends in $\delta^{15}\text{N}$ values of SOM with elevation in the dry (left frame) and wetter (right frame) transects. Significant trends were only found in the dry transect.

between transects (Ancova: p for elevation <0.0001 , p for transect n.s.), however. A significant decrease in $\delta^{15}\text{N}_{\text{SOM}}$ with elevation was observed for all three vegetation types in the dry transect ($p < 0.0005$ for grass and shrub; $p = 0.048$ for wet; r^2 grass = 0.24, r^2 shrub = 0.25, r^2 wet = 0.16). The slopes of relationships between $\delta^{15}\text{N}$ and elevation did not differ significantly among vegetation types if the higher elevations, which consisted solely of grass sites, were omitted. If all values were included, the slope of the relationship between $\delta^{15}\text{N}$ and

elevation was significantly steeper for the shrub and wet sites than for the grass sites (Ancova, parallel line test, $p = 0.042$).

4.4. Elemental analyses (SOM from <10 cm)

Carbon and nitrogen percents and C/N molar ratios provide some inferences about climate and the biogeochemical status of nitrogen in the soil that are related to the $\delta^{15}\text{N}$ of SOM. Like $\delta^{15}\text{N}$ values,

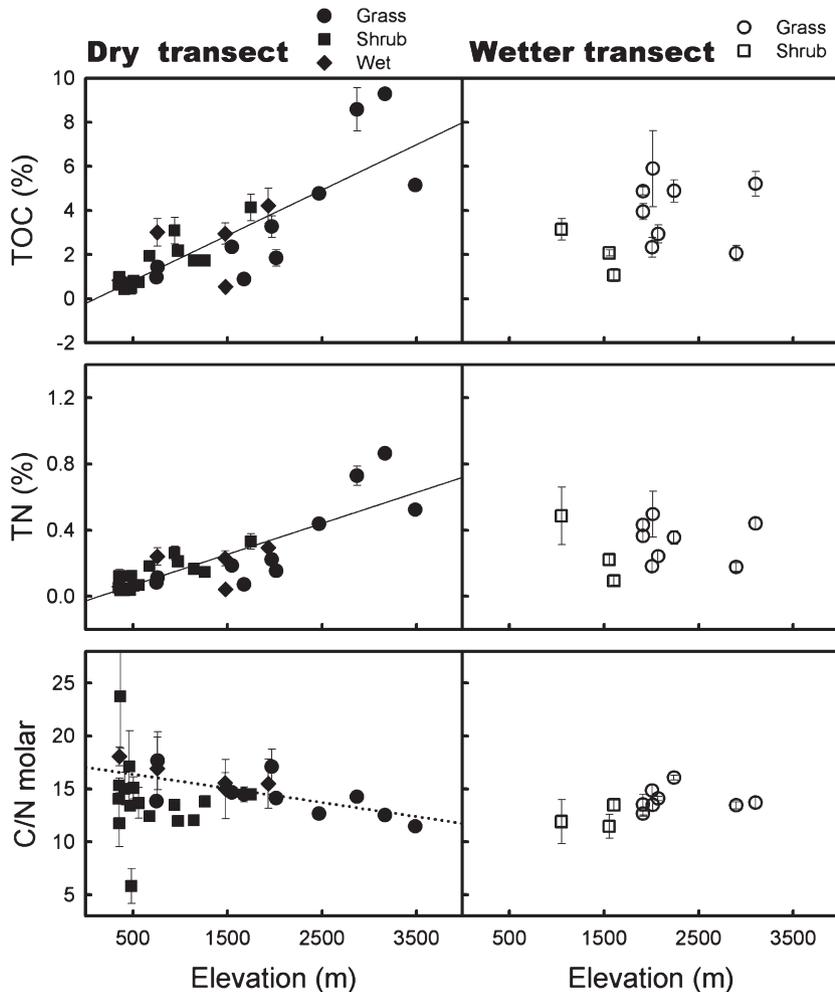


Fig. 5. Trends in total percent organic carbon (TOC) and nitrogen (TN) and C/N molar ratios in soil with elevation. Lines shown are for raw values to show the direction of the trends. Statistics quoted in text are for Box-Cox transformations of TOC and TN (because they had non-normal distributions and/or unequal variances) and for untransformed C/N ratios. Solid lines are for significant trends for all values in the dry transect. The dotted line means a significant trend was found for only the grass sites in the dry transect.

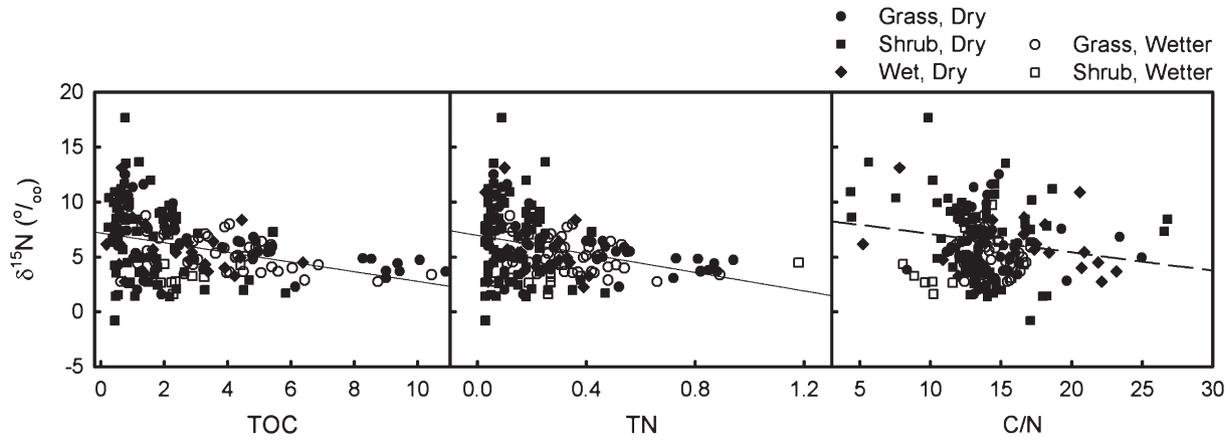


Fig. 6. Relationships between $\delta^{15}\text{N}$ values of SOM and total percent organic carbon (TOC), nitrogen (TN), and C/N molar ratios. Lines shown are for untransformed values to show the direction of the trends. Statistics quoted in text are for Box-Cox transformations of TOC and TN and for untransformed C/N ratios. Solid lines denote a significant relationship for all values in all sites. The dashed line indicates that only the values in the dry transect were significantly related.

relationships of total organic carbon (TOC), total nitrogen (TN), and C/N to elevation (and thus climate) were found only in the dry transect (Fig. 5).

TOC and TN were positively related to elevation in the dry transect, although they did not significantly differ between transects or vegetation types (Ancova: for elevation, $p < 0.0005$; transect, vegetation n.s. Dry transect: Ancova, elevation, $p < 0.0005$, vegetation type n.s.; regressions of TOC and TN to elevation, $p < 0.0005$, $r^2 = 0.57$ for TOC, $r^2 = 0.59$ for TN; Wetter transect: regressions of TOC and TN to elevation n.s.). C/N molar ratios decreased significantly with increase in elevation but only in the grass sites of the dry transect (regression $p = 0.001$, $r^2 = 0.20$).

Because of the similarities in the direction of trends with elevation between $\delta^{15}\text{N}$ of SOM and the elemental analyses, we examined direct relationships between $\delta^{15}\text{N}$ values and TOC, TN, or C/N (Fig. 6). $\delta^{15}\text{N}$ values significantly decreased with decrease in TOC and also in TN, regardless of transect or vegetation type (Ancovas: TOC or TN, $p < 0.0005$; transect, vegetation type, n.s.; regression of $\delta^{15}\text{N}$ to TOC or TN: $p < 0.0005$, $r^2 = 0.14$ for TOC, $r^2 = 0.10$ for TN). The relationships between $\delta^{15}\text{N}$ and TOC were significant in both transects (regressions on TOC: $p < 0.0005$, $r^2 = 0.12$ for dry transect, $p = 0.032$, $r^2 = 0.10$ for wetter transect; regressions on TN: $p = 0.001$, $r^2 = 0.07$ for dry transect, $p = 0.012$, $r^2 = 0.11$ for wetter transect) and did not differ significantly from one another.

Relationships between $\delta^{15}\text{N}$ and C/N did not vary between transects or vegetation types. Furthermore, there was only a significant relationship of $\delta^{15}\text{N}$ to C/N in the dry transect and then with even more unexplained variation than for relationships of $\delta^{15}\text{N}$ to TOC or TN (regression: $p = 0.006$, $r^2 = 0.06$).

4.5. Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with soil depth

As occurred in surface soils, there was a quadratic relationship between elevation and the combined $\delta^{13}\text{C}$ values of all samples taken from the up to 3 m cores (regression on quadratic model: $p < 0.0001$, $r^2 = 0.41$) (Fig. 7). This finding suggests that the highest proportional contributions of C_4 plants to the soil organic carbon pool had long been in the middle elevations.

Changes in $\delta^{13}\text{C}$ values of SOM and estimated % carbon from C_4 plants with depth were rarely systematic. We considered values of $\delta^{13}\text{C}$ that differed by $\leq 1\text{‰}$ to be equivalent to one another (O’Leary, 1988). No single pattern of change in $\delta^{13}\text{C}$ with depth clearly predominated among the 15 sites at lower (<1000 m) elevation (Fig. 8). Of the 5 sites corresponding to the middle elevation range (>1500 to <2100 m elev), $\delta^{13}\text{C}$ values of SOM and estimated % carbon

from C_4 plants in the modern soil were only out of the range of values for other depths in one site (1545 m). The $\delta^{13}\text{C}$ values were equivalent at all depths sampled in one of the middle elevation sites (2017 m). The $\delta^{13}\text{C}$ values of SOM and estimated % carbon from C_4 plants clearly decreased with depth from the surface at the highest elevation site (2899 m). The 7‰ difference in $\delta^{13}\text{C}$ of SOM between the highest and lowest depth sampled was at least 2‰ larger than in any other site where $\delta^{13}\text{C}$ values changed systematically with depth and there was only one site where a greater difference in $\delta^{13}\text{C}$ existed between any two sample depths (410 m elev).

Some or all of the samples taken below 10 cm depth in 6 of the 14 sites under 1000 m elevation yielded no detectable nitrogen even after four times the usual sample weight was analyzed (Fig. 9). Although total nitrogen contents were always higher in soils at <10 cm depth than below ($p < 0.0005$), TN followed no systematic relationship with depth below 10 cm. As a result, depths that yielded reliable $\delta^{15}\text{N}$ values sometimes underlay depths that had no detectable nitrogen (e.g., elevation 940 m, Fig. 9).

There were no significant relationships of the combined $\delta^{15}\text{N}$ values for all depths to elevation. Furthermore, and in contrast to results on just the surface (<10 cm) soils, $\delta^{15}\text{N}$ values were not significantly related to (Box-Cox transformed, see Materials and methods) TOC, TN or C/N ratios. Finally, no systematic relationships were found between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, i.e., the direction of change with depth in these values was as likely to be in the same direction as in the opposite direction. In 12 of the 19 sites for which such a comparison

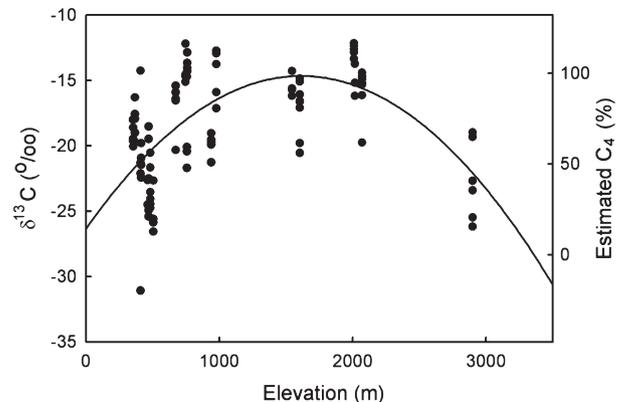


Fig. 7. Trends in $\delta^{13}\text{C}$ values and estimated % of carbon from C_4 plants in SOM ($\pm\text{SE}$) with elevation for sites sampled to depth. Values from all depths are plotted.

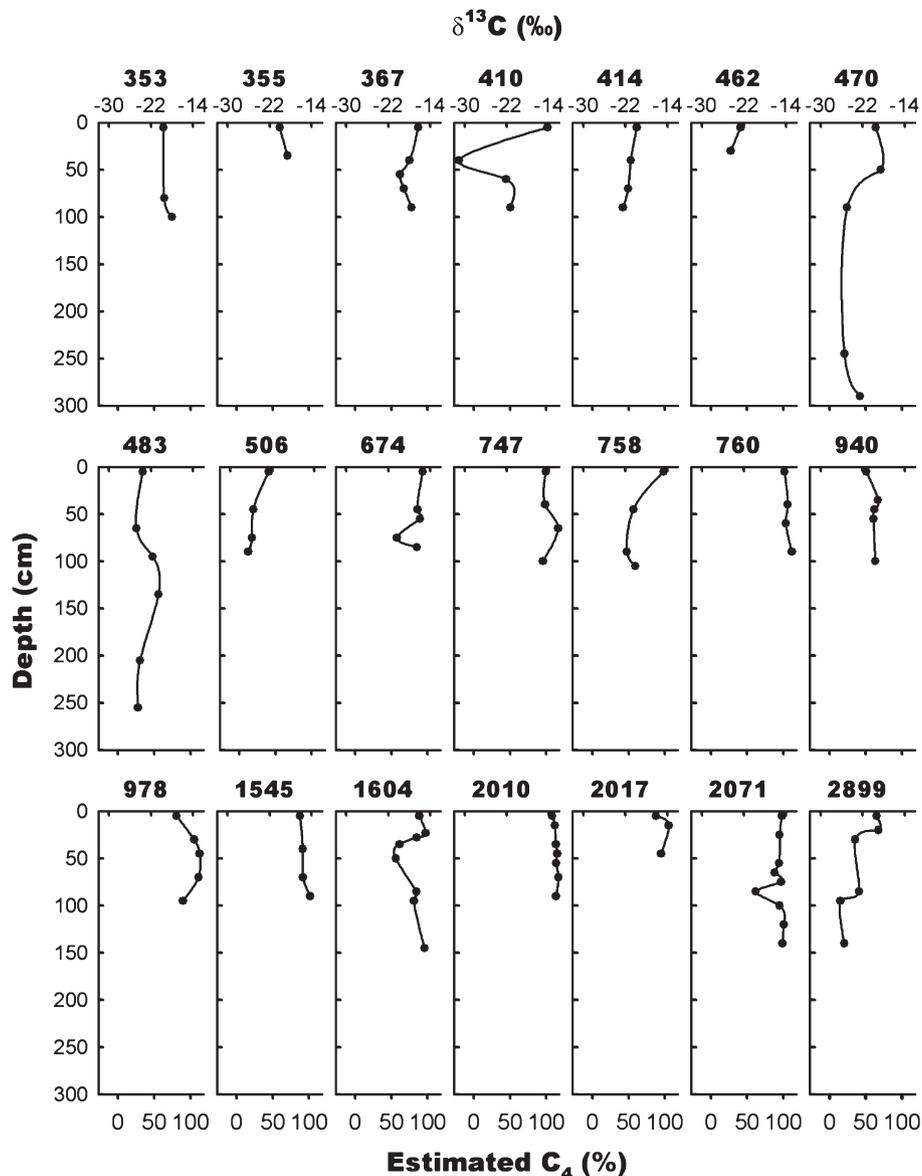


Fig. 8. Changes in $\delta^{13}\text{C}$ values of SOM for with depth at all elevations sampled. Numbers on the upper x axes of each plot refer to the elevation of the site sampled. Additional information about site coordinates and transect are in Fig. 1 and Table 1.

could be made, $\delta^{15}\text{N}$ values did not decrease and increased by more than 1‰ from the soil surface to at least one lower depth within 80 cm (in 353, 355, 367, 410, 483, 674, 758, 760, 940, 1545, 2010, 2899 m). Otherwise, changes in $\delta^{15}\text{N}$ values from the second to final depth sampled showed no consistent pattern within or between sites.

5. Discussion

An unanticipated finding from this study is that $\delta^{13}\text{C}$ values of bulk SOM are least negative at the middle elevations of our transects (Fig. 3). This result implies that the highest contributions of carbon to the soil from C_4 plants relative to the carbon from C_3 plants are at the middle rather than at the hottest, driest bottom elevations (Fig. 3 and Eq. (3)). Furthermore, although this result in no way precludes a relationship of $\delta^{13}\text{C}$ to climate, it clearly complicates a description of that relationship.

Our choice of $\delta^{13}\text{C}$ values from C_3 and C_4 plants did not account for the possibility that the $\delta^{13}\text{C}$ values of source plants varied along the transects due to climatically influenced factors. The humped relationship of % C_4 carbon in SOM to elevation may just be an artefact of error

in the estimates of % C_4 plant derived carbon in the SOM (Eq. (3) and Section 4.1). Alternatively, our estimates of % C_4 plant derived carbon in SOM may be reasonably correct. In this case, the humped relationship may be because factors other than climate sometimes have the strongest influences on the distributions of C_3 and C_4 vegetation.

In Section 5.1, we consider known mechanisms for an influence of climate on $\delta^{13}\text{C}$ values of bulk SOM and conclude that the first “varied climatic influences” hypothesis is a less credible explanation for our results than the second “factors other than climate” hypothesis. Sections 5.2 and 5.3 consider present knowledge of what “other factors” may be and their relative importance as influences on both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bulk SOM. In Section 5.4, we outline a model for future research that uses $\delta^{15}\text{N}$ trends to differentiate the effects of climate from “other factors” on $\delta^{13}\text{C}$ values of bulk SOM.

5.1. How well are $\delta^{13}\text{C}$ values of SOM related to temperature and rainfall?

After quantitatively considering the following causes of variation in $\delta^{13}\text{C}$ values of SOM, we found that the humped relationship of % C_4

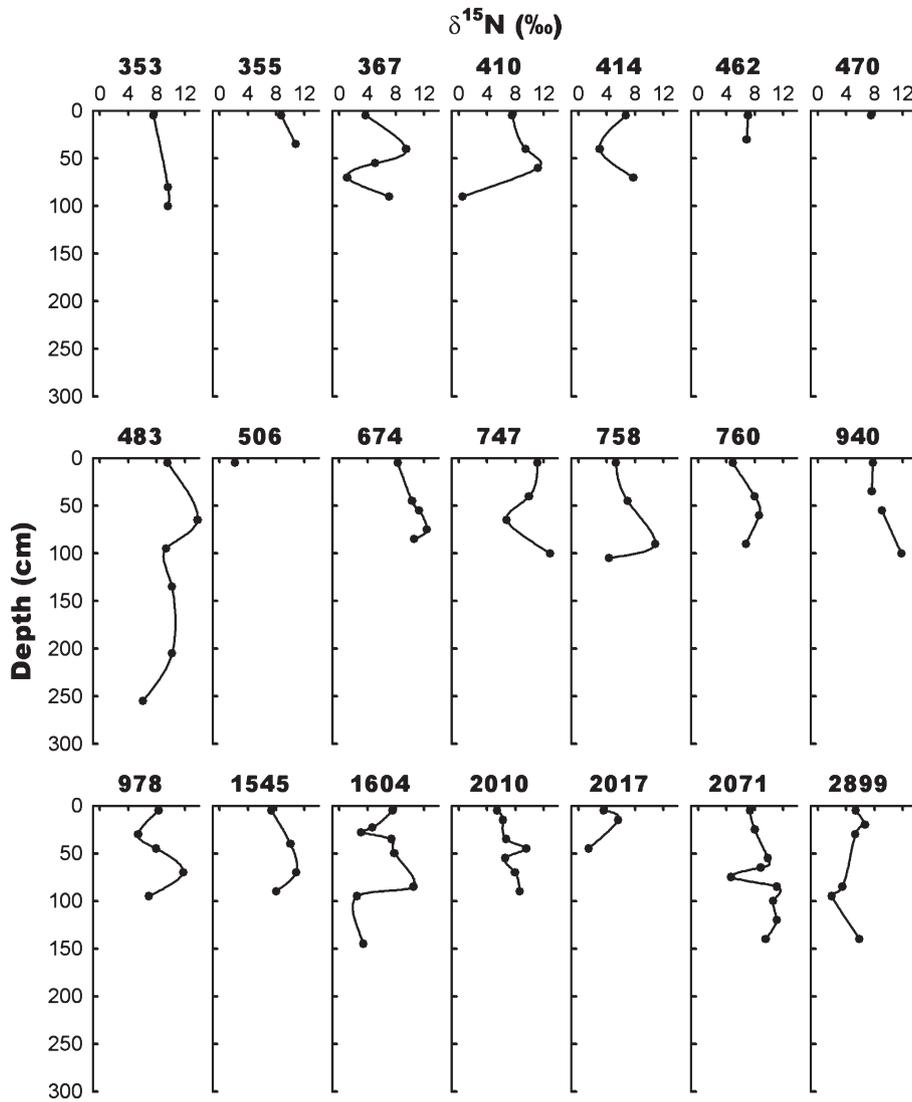


Fig. 9. Changes in $\delta^{15}\text{N}$ values of SOM for with depth at all elevations sampled. Numbers on the upper x axes of each plot refer to the elevation of the site sampled. Additional information about site coordinates and transects are in Fig. 1 and Table 1.

derived carbon to elevation was robust and not realistically explained by known climatic influences alone. Four categories of factors affect the $\delta^{13}\text{C}$ values of bulk SOM and they may all be linearly influenced by climate. The photosynthetic pathways of the vegetation sources are the most significant of these. A second factor is the ratio of CO_2 partial pressure at the site of carbon fixation by ribulose biphosphate carboxylase oxygenase (Rubisco) within leaves to the partial pressure of CO_2 in the atmosphere (p_i/p_a) (Farquhar et al., 1982, 1989). The third factor is the $\delta^{13}\text{C}$ value of atmospheric CO_2 that will enter the leaves and the fourth is decomposition of SOM (Nadelhoffer and Fry, 1988; Boutton et al., 1999; Ehleringer and Buchmann, 2000).

We recognized two photosynthetic pathways, C_3 and C_4 , as the likely sources of carbon in most of the SOM. Nonetheless, there are several C_4 subpathways (described in Sage et al., 1999) that differ somewhat in their $\delta^{13}\text{C}$ values and climatic distributions. Three principal C_4 subpathways differ in their use of nicotinamide adenine dinucleotide phosphate (NADP), nicotinamide adenine dinucleotide (NAD), and phosphoenolpyruvate carboxykinase (PCK). NADP plants tend to have the least negative $\delta^{13}\text{C}$ values and to be most prevalent in more mesic grasslands than where NAD and PCK subpathway plants are most common (Hattersley, 1982). In East Africa, Cerling et al. (2003) found mesic NADP subpathway dominated grasslands to have $\delta^{13}\text{C}$ values as high as

–11.5‰ while xeric grasslands dominated by plants with the other two subpathways had $\delta^{13}\text{C}$ values as low as –14.4‰.

The following modification of Farquhar et al.'s (1982, 1989) model with Eq. (3) summarizes other influences the four factors may have on $\delta^{13}\text{C}$ values of SOM:

$$\delta^{13}\text{C}_{\text{SOM}} = \rho \left[\delta^{13}\text{C}_{\text{CO}_2\text{A}} - a - (b-a)p_i/p_a \right]_{\text{C}_4} + \left[(1-\rho)\delta^{13}\text{C}_{\text{CO}_2\text{A}} - a - (b-a)p_i/p_a \right]_{\text{C}_3} + d \quad (4)$$

where $\text{CO}_{2\text{A}} = \text{CO}_2$ in air ($\delta^{13}\text{C}_{\text{CO}_2\text{A}} \approx -8\text{‰}$; Keeling, 1958) (factor 3) a = isotopic changes during diffusion into leaves ($\approx 4.4\text{‰}$; Craig, 1953) b = isotopic changes during carboxylation in leaves (factor 1, see below) and d = isotopic changes in the soil (factor 4; $< 1\text{‰}$ in modern soils, 1–3‰ in most older soils (Balesdent and Mariotti, 1996; Huang et al., 1996).

The proportions of CO_2 that are fixed by phosphoenol pyruvate carboxylase (PEPc) (β) and Rubisco ($1-\beta$) assure that “ b ” and $\delta^{13}\text{C}$ differ markedly between C_3 and C_4 plants as follows (sensu Farquhar et al., 1989):

$$b = (1-\beta)(b_r) + \beta(b_p) \quad (4a)$$

where b_r = fractionation of CO₂ by Rubisco = 29‰ (Roeske and O'Leary, 1984) and b_p = fractionation of CO₂ by PEPc = -5.7‰ (O'Leary, 1988).

In C₃ plants, β is assumed to be about 0.057 so that $b \approx 27\%$ (Holbrook et al., 1984). Evidence are accumulating that β is not constant among C₃ plants (Terwilliger et al., 2001; Bathelier et al., 2007). Effects of climate on β have not been examined but cannot be eliminated as a source of variation in $\delta^{13}\text{C}$ values of C₃ plants. In C₄ plants, $\beta \approx 1$ so that $b \approx -5.7\%$.

Other than C₄ subpathway, most of the local deviation from the 15‰ average global difference in $\delta^{13}\text{C}$ between C₃ and C₄ plants may be caused by variation in p_i/p_a among C₃ plants (factor 2) (von Caemmerer and Farquhar, 1981; O'Leary, 1988). Vapour pressure deficit is a climatic factor that could influence p_i/p_a either as a direct source of water stress to plants or by affecting soil water potential. Where VPD is high, water vapour loss from photosynthesizing leaves may be curtailed by reducing stomatal aperture and consequently p_i/p_a . When this response occurs, the $\delta^{13}\text{C}$ values of C₃ plants will be positively related to VPD. For example, Madhavan et al. (1991) grew plants in several humidity treatments and the resulting $\delta^{13}\text{C}$ values of C₃ species were positively related to VPD.

Plant species may exhibit other adjustments to VPD gradients under natural conditions, however. Variations in morphology and function of plants of the same species may prevent p_i/p_a and $\delta^{13}\text{C}$ from changing along VPD gradients (Williams and Ehleringer, 1996) or even cause p_i/p_a to be positively related and $\delta^{13}\text{C}$ to be negatively related to VPD (e.g., Körner et al., 1988; Vitousek et al., 1990; Cordell et al., 1999). In the most comprehensive study of $\delta^{13}\text{C}$ values along elevation gradients in the southwestern United States, van de Water et al. (2002) concluded that $\delta^{13}\text{C}$ values were negatively related to elevation in arid climates and only positively related to elevation in humid climates. The changes in $\delta^{13}\text{C}$ with elevation that they found were so small, however, that they did not recommend correcting for elevation effects when making inferences from $\delta^{13}\text{C}$ values.

Although their anatomy keeps p_i/p_a effectively close to zero, some variation in effective p_i/p_a may occur among C₄ plants. According to Eq. (4), $\delta^{13}\text{C}$ values of C₄ plants have the opposite relationship to p_i/p_a than that of the $\delta^{13}\text{C}$ values of C₃ plants. Madhavan et al.'s (1991) experiments supported the hypothesis that the p_i/p_a of both C₃ and C₄ plants can decrease as conditions become drier but with opposing effects on $\delta^{13}\text{C}$ values. In contrast, however, van de Water et al. (2002) found the $\delta^{13}\text{C}$ values of C₄ species to either not change with elevation or, as in C₃ plants, to be negatively related to elevation.

The aforementioned factors should each produce linear changes in $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ values with elevation or at least have their minimal and maximal effects at the elevation extremes. There is less reason to assume that $\delta^{13}\text{C}_{\text{CO}_2\text{A}}$ (factor 3) or decomposition rates (factor 4) changed in a single direction with elevation (climate) along our transects. Atmospheric CO₂ (factor 3) is usually very well-mixed isotopically. Nonetheless, localized variations in $\delta^{13}\text{C}_{\text{CO}_2\text{A}}$ can occur that affect the $\delta^{13}\text{C}$ values of plants in environments where obstacles such as closed tropical rainforest slow the mixing of high inputs of respired CO₂ into the global atmosphere (Sternberg et al., 1989). Variations in inputs of respired CO₂ may have occurred along our transects but none of the sites were in sufficiently closed ecosystems to impede their dispersion into a well-mixed airstream. It is unlikely that the organic matter in our surface soils had been decomposing long enough for their $\delta^{13}\text{C}$ values to have significantly changed. Nonetheless, the mix of temperature and humidity that would have permitted the fastest decomposition rate need not have been at any of the elevation extremes.

We made several series of adjustments to $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ values in Eq. (3) to determine whether failure to account for a combination of the factors described above could realistically have masked a linear relationship between % contribution of C₄ to SOM and elevation. We made adjustments to account for all possible linear changes in $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ with elevation even though some of those changes were

highly improbable. We sought to make the changes in $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ with elevation as large as realistically possible. Wooller et al.'s (2001) survey of $\delta^{13}\text{C}$ values of graminoids and sedges from 1960 – ca. 4500 m a.s.l. on Mount Kenya produced the same average $\delta^{13}\text{C}$ value as ours for C₃ plants but the widest range of values for East Africa that we could find. We used their lowest (-34.3‰) and highest (-24.9‰) values as $\delta^{13}\text{C}_{\text{C}_3}$ end-members. They did not relate their $\delta^{13}\text{C}$ values to elevation and the difference between these end-members is larger than any presently known along elevation gradients. Their $\delta^{13}\text{C}$ values of C₄ plants ranged from -13.9 to -10‰. We used their -10‰ and our average of -14.5‰ as end-members for $\delta^{13}\text{C}_{\text{C}_4}$, again using values for East Africa with greater than known ranges in $\delta^{13}\text{C}$ with elevation. We estimated eight sets of % contributions of C₄ carbon to SOM Eq. (3) per transect by adjusting $\delta^{13}\text{C}_{\text{C}_3}$ and/or $\delta^{13}\text{C}_{\text{C}_4}$ values to change as follows with increase in elevation: 1) linear decrease in $\delta^{13}\text{C}_{\text{C}_3}$, no change to original $\delta^{13}\text{C}_{\text{C}_4}$; 2) linear increase in $\delta^{13}\text{C}_{\text{C}_3}$, no change to original $\delta^{13}\text{C}_{\text{C}_4}$; 3) no change to original $\delta^{13}\text{C}_{\text{C}_3}$, linear increase in $\delta^{13}\text{C}_{\text{C}_4}$; 4) no change to original $\delta^{13}\text{C}_{\text{C}_3}$, linear decrease in $\delta^{13}\text{C}_{\text{C}_4}$; 5) both $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ increase; 6) both $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ decrease; 7) $\delta^{13}\text{C}_{\text{C}_3}$ increases, $\delta^{13}\text{C}_{\text{C}_4}$ decreases; 8) $\delta^{13}\text{C}_{\text{C}_3}$ decreases, $\delta^{13}\text{C}_{\text{C}_4}$ increases.

The best model for the relationship between all 8 sets of estimates for % C₄ derived carbon in SOM (Y) and elevation (X) for both transects was quadratic and humped so that the highest % C₄ values tended to be at the middle elevations (model: $Y = aX + bX^2 + c$; $p < 0.0005$ for all sets of estimates). Deviation from a humped relationship was most likely to occur in the dry transect since that was where $\delta^{13}\text{C}$ values of SOM changed the least with elevation. That these results occurred for the dry transect thus particularly strongly suggests that % C₄ plant derived carbon is, in reality, highest in SOM at the middle elevations.

We then examined whether non-linear changes in the largest physiologically possible range of $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ values could produce the expected negative relationship of % C₄ derived carbon in SOM to elevation. The criteria for set 5 are most mathematically likely to produce such a result. We used set 5 criteria for as broad a range of $\delta^{13}\text{C}_{\text{C}_3}$ (-33 to -22‰) and $\delta^{13}\text{C}_{\text{C}_4}$ values (-16 to -9‰) as seemed physiologically possible (Deines, 1980). We also made the $\delta^{13}\text{C}_{\text{C}_3}$ and $\delta^{13}\text{C}_{\text{C}_4}$ values change more with elevation for sites at lower and upper elevations than for the middle elevations. While this series produced less difference in % C₄ derived carbon between the lower and middle elevations than between the middle and upper elevations, the changes in % C₄ contribution with elevation still best fit a humped, quadratic curve (dry transect: $r^2 = 0.29$, $p < 0.0005$). We thus conclude that the tendency of highest $\delta^{13}\text{C}$ values of SOM to be at the elevations of intermediate VPD was because % contributions of C₄ carbon to SOM are highest at those intermediate elevations.

Empirical evidence and logic suggest that climate strongly influences the distributions of C₃ and C₄ vegetation (e.g., Teeri and Stowe, 1976; Sage et al., 1999). The energy costs of C₄ photosynthesis are higher than those of C₃ photosynthesis but energy consumptive photorespiration is eliminated in C₄ plants (Nobel, 1999). Therefore, C₄ plants should be favoured under environmental conditions evoking high energy losses to photorespiration by the C₃ pathway. Two such conditions are a climate that favours water conservation in plants and low p_a . Studies along elevation and latitudinal gradients have repeatedly shown robust positive relationships between abundance of monocotyledonous C₄ species and temperature and between dicotyledonous C₄ species and high temperature with high VPD (reviewed in Ehleringer et al., 1997). C₄ plants may have had a more expansive global distribution during the last glacial maximum than at present due to the low partial pressure of CO₂ compared to now (Sage, 1995). The high abundance of C₄ monocots above tree line on Mount Kenya may also be influenced by low p_a (Wooller et al., 2001).

The $\delta^{13}\text{C}$ trends from middle to upper elevations are consistent with studies showing relationships of relative abundance of C₄ carbon to climate. The $\delta^{13}\text{C}$ trends from low to middle elevations oppose a climatic explanation. Changes in p_a would only explain our results if

there was logical reason for p_a to be much lower at the middle elevations than at the upper and lower elevations. Factors other than climate or p_a may have most significantly affected the relative contributions of C_4 from at least the lower to middle elevations.

5.2. Is $\delta^{15}N_{SOM}$ associated with temperature as well as rainfall?

Our results augment a growing number of findings linking climate to $\delta^{15}N$ values of bulk SOM. Handley et al.'s (1999) global analyses most strongly suggest that $\delta^{15}N$ is negatively related to precipitation. Additionally, Amundson et al.'s (2003) global latitudinal analyses found a positive relationship between $\delta^{15}N$ and temperature. Cross-correlation of temperature and rainfall may have been a factor in both studies, however. Aranibar et al. (2004) found $\delta^{15}N$ values of SOM to increase as precipitation decreased along a gradient in the Kalahari Sands that did not vary in temperature or soil texture.

Our dry transect is another gradient with a negative relationship between mean annual precipitation and temperature (Fig. 2). The wetter transect is unique because only temperature changes systematically with elevation. $\delta^{15}N$ values of SOM increased as elevation decreased but only along the dry transect (Fig. 4). This result supports the hypothesis that $\delta^{15}N$ values of SOM are affected by precipitation amounts. It also suggests that, at least for the range of temperatures examined, temperature changes alone do not discernibly affect $\delta^{15}N$.

Nonetheless, Martinelli et al. (1999) found $\delta^{15}N$ values of tropical forest SOM to be close to 8‰ higher than the $\delta^{15}N$ values of temperate forest SOM. Mean annual precipitation was higher in the tropical than in the temperate sites suggesting the opposite relationship between $\delta^{15}N$ and precipitation to that found in other studies. Presumably, vapour pressure deficits were much higher in the tropical than in the temperate sites. VPD varied in all of the aforementioned studies, regardless of whether temperature and/or precipitation varied. In our study, VPD changed more with temperature in the dry than in the wetter transect. An emerging hypothesis to test is that VPD may be the most strongly related of climatic characteristics to $\delta^{15}N_{SOM}$.

$\delta^{15}N_{SOM}$ may be related to climate through the effect of climate on biogeochemistry. Specifically, climate may have large effects on inputs, microbial transformations and outputs of nitrogen that influence the openness (losses relative to turnover) of the nitrogen cycle in a given ecosystem. The $\delta^{15}N$ of a given pool of SOM is affected by the $\delta^{15}N$ of fluxes into it (e.g., plants, deposition, and fixed N_2) and of outfluxes (e.g., denitrification, ammonia volatilization, and uptake by plants). Processes that cause gaseous outfluxes of nitrogen from ecosystems, such as denitrification and ammonia volatilization, usually favour ^{14}N and lead to ^{15}N enrichment of the remaining soil nitrogen pool (Högberg, 1997).

The connections between climate, ecosystem openness, and soil $\delta^{15}N$ values can be indirectly examined to a limited extent from trends in carbon and nitrogen elemental analyses. Open environments are expected to have lower TOC, TN, and C/N both due to lower biomass and to the greater ecosystem losses that could lead to ^{15}N enrichment of the remaining SOM. Furthermore, low C/N may specifically contribute to ^{15}N enrichment by providing a favourable environment for mineralization (Brady and Weil, 2002) and subsequent gaseous nitrogen losses (Aranibar et al., 2004).

TOC and TN were positively related to elevation but only in the dry transect (Fig. 5). $\delta^{15}N_{SOM}$ was negatively related to TOC and TN in both transects. Thus TOC and TN varied along the known precipitation and steepest VPD gradient in a manner consistent with openness of environment. These results are consistent with the hypothesis that changes in openness of environments with precipitation affect the $\delta^{15}N$ of SOM. Nonetheless, C/N ratios supported no hypothesized link that we know of between openness of the nitrogen cycle and climate. C/N ratios were negatively related to elevation in the grass sites of the dry transect (Fig. 5). Nonetheless, C/N ratios were positively related to $\delta^{15}N$ in the dry transect and were unrelated to $\delta^{15}N$ in the wetter transect.

Our results from the most modern SOM suggest that a relationship of climate to $\delta^{15}N$ values of soil emerged despite the fact that factors other than climate affect soil biogeochemistry and the openness of nitrogen cycles in ecosystems. For example, nitrogen fixation favours ^{14}N and, where it contributes significant nitrogen, it can result in ^{15}N depletion of plant and soil pools (Högberg, 1997). Fire, land clearing, and soil texture, can also affect the openness of N cycles. Although many soils in Ethiopia originated from volcanic substrata (Abebe, 1998), our study sites encompassed additional substrata and at least 7 soil orders (Table 1). Disturbances from land use such as vegetation clearance and fire have been practiced for millennia (Hurni, 1987). We therefore likely maximized the effects of factors other than climate on $\delta^{15}N_{SOM}$ values, yet still observed an association between the two.

5.3. Do $\delta^{13}C$ and/or $\delta^{15}N$ values reflect land use?

The trends in $\delta^{13}C_{SOM}$ values from this study probably reflect relative carbon inputs of C_4 and C_3 vegetation but only run in the expected direction for climate from the middle to upper elevations (Section 5.1, Fig. 3). The tendency for the % carbon derived from C_4 plants to be highest at the middle elevations had existed for a long time since it was retained even when a variety of deeper soils were included in the analyses (Fig. 7). In contrast, the negative relationship between $\delta^{15}N_{SOM}$ values and elevation in the dry transect (Fig. 4) is consistent with other findings that $\delta^{15}N_{SOM}$ is affected by precipitation or VPD but not by relative carbon inputs from C_3 and C_4 vegetation (Heaton, 1987). We conclude that a factor other than climate needs to be explored to best explain why $\delta^{13}C_{SOM}$ values are more negative in the hotter, lower elevations than in the middle. In contrast, trends in $\delta^{15}N_{SOM}$ are best associated with climate but factors other than climate may cause the noise in this association.

We hypothesize that history of intense disturbance from human activities may explain the deviation from a clear relationship between % C_4 carbon and climate. Our results specifically imply that a history of high grazing pressures on landscapes may have had more influence than climate on the relative contribution of C_4 derived carbon to SOM in the middle elevations of Ethiopia. Although hereafter we often use the term “land use” instead of “other factors”, this hypothesis is a direction for future research. Some of that research would be empirical study of relationships between $\delta^{13}C_{SOM}$ and C_3/C_4 vegetation composition from intensive analyses of overlying vegetation, pollen, and charcoal. At present, the results of this study do not support an explanation evoking atmospheric CO_2 partial pressure; the best studied alternative to climate that influences the distributions of C_4 plants (Sage, 1995).

Evidence suggest that C_4 photosynthesis and anatomy can give many species a competitive edge for growing in disturbed sites. Stowe and Teeri (1978) had difficulty associating the distributions of many dicotyledonous C_4 species with climate. They concluded that C_4 dicots had superior growth characteristics for invading disturbed sites even where the sites were in sub-optimal climates. Elmore and Paul (1983) found the percentage of weedy invasive species with C_4 photosynthetic pathways to be seventeen times greater than the percentage of known C_4 plant species of all known species worldwide. Their list of C_4 plant species that are weedy invasives include monocots and dicots of all photosynthetic subpathways. Areas that have the longest history of disturbance might be expected to have particularly large components of invasive C_4 plants.

Humans were using all of the study sites to graze livestock. Oral legend, archaeological, and environmental history research suggest that the middle elevations of our study sites have been under the most intensive use for crop and livestock production for the longest period of time (e.g., Pankhurst, 1989; McCann, 1995; Pankhurst, 1998; Phillipson, 1998; Bard et al., 2000; Fattovich et al., 2000). This land use history includes a probable centre of origin of agriculture complete with domesticated livestock that occurred perhaps more

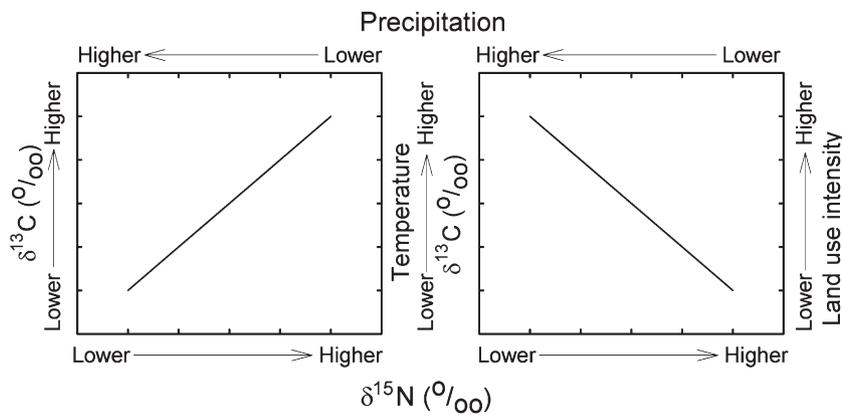


Fig. 10. Schematic showing changes in land use and climate that are associated with trends in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}_{\text{SOM}}$ suggested by this study. A positive relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is associated with changes in precipitation and temperature. A negative relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is best associated with changes in precipitation and intensity of land use (grazing). If diagenetic changes in $\delta^{15}\text{N}$ are very small in old, deep soils relative those influenced by climate in surficial organic matter, then use of both isotopes could be a powerful means of inferring past climate and history.

than 7000 years BP (Ehret, 1979). The lower elevations have a shorter history of lighter use by low populations of nomadic pastoralists that is preceded by a history of hunters and gatherers that may date back to the first *Homo sapiens* (White et al., 2003). Indications are that use of the highest elevations has been shorter, more intermittent, and less intensive than use of the middle elevations. Furthermore, benefits of having protected forests in the high elevation ranges of our sites were recognized more than 500 years ago (Eshetu and Högborg, 2000a,b). Population growth at the middle elevations has been leading to migration to and more intensive grazing of the highest elevations for three decades (Africa Watch, 1991). The increase in $\delta^{13}\text{C}_{\text{SOM}}$ values from deepest to shallowest soil at the highest elevation sampled below the surface (Fig. 8) opposes trends associated with decomposition or increasing CO_2 partial pressures and may be a reflection of the increasing grazing pressures.

5.4. Can $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM yield more information than $\delta^{13}\text{C}$ alone?

Some changes in $\delta^{13}\text{C}$ values of SOM in this study may be related to climate or other factors such as disturbances associated with history of land use pressure. Trends in $\delta^{15}\text{N}$ values may help discern whether it was climate or land use that most affected $\delta^{13}\text{C}_{\text{SOM}}$. Fig. 10 illustrates two examples using the relationships of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of SOM to climate and land use suggested by our results. A positive relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ corresponds to increasing mean annual temperature and decreasing precipitation. Land use may also influence the δ values but does not alter the qualitative inferences about climate and cannot be readily deciphered. A negative relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ corresponds to a decrease in precipitation as $\delta^{15}\text{N}$ values increase and a decrease in land use intensity as $\delta^{13}\text{C}$ values concomitantly decline. Prolonged land use may obscure any influence of temperature on $\delta^{13}\text{C}$. By extension, changes in $\delta^{13}\text{C}$ that are not accompanied by changes in $\delta^{15}\text{N}$ suggest shifts in land use intensity but not climate.

What now needs to be explored is whether plots of trends in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can be used to interpret similar environmental changes of the past. Processes affecting organic matter chemistry as it is incorporated into soil most link $\delta^{15}\text{N}$ values of SOM near the surface to climate. If changes in $\delta^{15}\text{N}$ values as it is buried are small relative to changes that occurred during the biogeochemical processes near the surface then the links of $\delta^{15}\text{N}$ to the earlier environment may be preserved. The lack of a relationship between $\delta^{15}\text{N}$ in soils up to 300 cm depth and elevation may mean that climate signals are not preserved in $\delta^{15}\text{N}$ values of older soils or that other factors including age differences masked a climate signal.

In sum, our data on surface soils suggest that analyses of $\delta^{13}\text{C}$ values do not stand alone as a means of interpreting environments but become a far more powerful tool of inference if coupled with analyses of $\delta^{15}\text{N}$. It is thus worthwhile to explore how well the initial effects of environment on $\delta^{15}\text{N}$ are preserved over time.

6. Conclusions

Numerous studies indicate that climate and atmospheric CO_2 partial pressures strongly influence the C_3/C_4 composition of plants and that this influence will ultimately be reflected in the $\delta^{13}\text{C}$ values of soil organic matter (Cole and Monger, 1994; Boutton et al., 1998; Lee et al., 2005). Our findings add a third possible factor, history of land use pressure. We hypothesize that a long history of land use for intensive grazing by livestock caused trends in $\delta^{13}\text{C}_{\text{SOM}}$ that ran counter to what would be expected had climate been the dominant influence. This is a potential problem for using $\delta^{13}\text{C}$ values of SOM alone to address questions about environmental change where human impact has been high. Nonetheless, in conjunction with a proxy that is most affected by climate, $\delta^{13}\text{C}$ analyses of SOM may become a powerful tool for reconstructing land use and/or climatic changes. Climate – and precipitation in particular – had the most dominant association with $\delta^{15}\text{N}$ values of modern SOM. If this association undergoes little alteration with time, $\delta^{15}\text{N}$ analyses have the potential to clarify factors most influencing trends in $\delta^{13}\text{C}$ values of SOM.

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