RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2002; **16**: 2184–2189
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.767

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Evolution of the $\delta^{13}C$ signature related to total carbon contents and carbon decomposition rate constants in a soil profile under grassland†

Frederik Accoe[1,2]*, Pascal Boeckx[1], Oswald Van Cleemput[1], Georges Hofman[2], Ying Zhang[3], Rong hua Li[3] and Chen Guanxiong[3]
[1]Department of Applied Analytical and Physical Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Belgium
[2]Department of Soil Management and Soil Care, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Belgium
[3]Institute of Applied Ecology, Chinese Academy of Science, Shenyang, China

Received 19 April 2002; Revised 26 June 2002; Accepted 26 June 2002

Evolution of the total carbon (C) content and the ^{13}C enrichment ($\delta^{13}C$ signature) of soil organic matter (SOM) with increasing depth in a soil profile under permanent grassland (C_3 vegetation) were investigated. The relationship between the total C content and the $\delta^{13}C$ signature at different depths in the upper 30 cm of the soil profile could be well fitted by the Rayleigh equation ($y = -29.8 - 2.3x$, $R^2 = 0.95$, $p < 0.001$), describing the enrichment in ^{13}C as resulting from isotopic fractionation associated with C mineralization (isotope enrichment factor $\varepsilon = -2.3‰$). Potential C dynamics of SOM in four depth intervals of the profile (0–10, 10–20, 20–30 and 30–40 cm depth) were investigated through an incubation study. The C decomposition rate constants decreased with increasing sampling depth from 0.0479 yr^{-1} (0–10 cm sampling depth) to 0.0256 yr^{-1} (30–40 cm sampling depth) and were highly correlated ($y = 0.02 + 0.13x$, $R^2 = 0.93$, $p < 0.05$) with the corresponding $\Delta\delta^{13}C$ values (average change of the $\delta^{13}C$ signature per depth increment). These results suggest that changes of the $\delta^{13}C$ signature of SOM in undisturbed soil profiles under continuous C_3 vegetation may serve as an indicator of the variation of SOM quality with increasing depth.

The $^{13}C/^{12}C$ ratio or $\delta^{13}C$ signature (expressed in ‰) of soil organic matter (SOM) is mainly determined by the $\delta^{13}C$ signature of the plant litter from which it is derived. Plants discriminate against $^{13}CO_2$ during photosynthesis and the extent of this discrimination is dependent on their photosynthetic pathway type (C_3, C_4 or CAM).[1] As C_3 plants discriminate more against $^{13}CO_2$ than C_4 and CAM plants, C_3 plants have $\delta^{13}C$ signatures ranging from approximately −32‰ to −22‰, while C_4 and obligate CAM plants have $\delta^{13}C$ values ranging from approximately −17‰ to −9‰.[2] During the decomposition process and incorporation into the SOM pool, the $\delta^{13}C$ signature of the plant litter may be gradually altered.[3–5] A possible source of alteration of the isotopic signature may be the different decomposition rates of isotopically distinct biochemical components of plant litter.[2,4,6] However, several studies have shown that this differential preservation does not appear to induce significant shifts in the $\delta^{13}C$ values of the residual litter or the associated SOM pool in well-drained mineral soils.[2,4,5,7] Shifts in the $\delta^{13}C$ signature may also be induced by discrimination against ^{13}C relative to ^{12}C associated with microbial decomposition of SOM, which may induce a gradual ^{13}C enrichment in the residual SOM.[8,9]

The first aim of this research was to investigate the evolution of the total C content and the $\delta^{13}C$ signature of SOM with increasing depth in a soil profile under permanent grassland (C_3 vegetation), and the relationship between total C contents and $\delta^{13}C$ signatures at different depths in this profile. The second aim was to study the relationship between changes in the $\delta^{13}C$ signature per depth increment ($\Delta\delta^{13}C$ values) and potential C mineralization dynamics of SOM at different depth intervals in this profile.

EXPERIMENTAL

Site and soil sampling

Soil samples were taken in April 2000 from a permanent grassland soil at the experimental agricultural station of the Ghent University located at Melle in Belgium (3°47′E, 50°59′N). The soil is a moderately drained Hapludalf with a degraded argillic horizon[10] and a sandy loam texture (9% clay, 36% silt). This is a very common soil in the sandy loam region of Belgium, with characteristics that are common for

*Correspondence to: F. Accoe, Department of Applied Analytical and Physical Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure Links 653, 9000 Gent, Belgium.
E-mail: Frederik.Accoe@rug.ac.be
†Presented at SIMSUG 2002, Belfast, UK.
Contract/grant sponsor: Belgian Ministry of Small Enterprises, Traders and Agriculture.

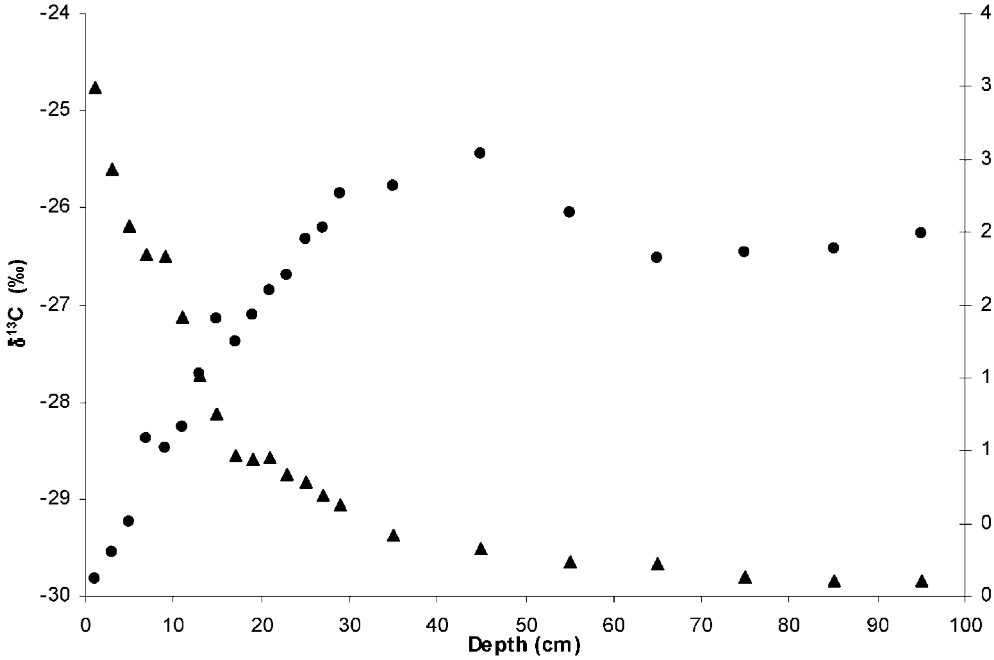

Figure 1. Evolution of the C content (triangles) and the δ^{13}C value (bullets) of the SOM down to a depth of 100 cm in the profile.

these soils in Europe. The site was arable land with only C_3 vegetation prior to the establishment of the permanent grassland (*Lolium perenne*) in 1966.

To study the evolution of the δ^{13}C signature and the total C content in the soil profile, eight replicate soil cores were taken from the 0–30, 30–60 and 60–100 cm depth intervals with a steel auger (respective auger diameters were 3.5, 2.5 and 2 cm). The 0–30 cm core was sectioned into 2-cm depth intervals, while the 30–60 and 60–100 cm cores were sectioned into 10-cm depth intervals. The replicate core sections were composited, mixed, air-dried, sieved through a 2-mm sieve and ground in a planetary ball mill (PM400, Retsch) for subsequent chemical and isotopic analysis.

For the incubation experiments, 16 replicate soil cores were taken from the 0–10, 10–20, 20–30 and 30–40 cm depth intervals with a steel auger (3.5-cm diameter). The replicate samples were composited, mixed, air-dried, sieved through a 2-mm sieve and stored until the start of the experiment.

Total C and δ^{13}C analysis

Measurements of total C content and ^{13}C natural abundance in the soil samples were performed using an ANCA-SL elemental analyzer coupled to an isotope ratio mass spectrometer (20-20, PDZ Europa, UK). The measured $^{13}C/^{12}C$ ratios are expressed as δ^{13}C values (‰) relative to the Pee Dee Belemnite (PDB) standard:

$$\delta^{13}C = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) * 1000$$

R_{sample} and $R_{standard}$ refer to the $^{13}C/^{12}C$ ratio in the sample and the standard, respectively. The working standard for the measurements was flour with a δ^{13}C value of -27.01 ± 0.04‰ (certified by Iso Analytical, UK). δ^{13}C analyses were always performed in triplicate.

Potential C mineralization dynamics

An incubation experiment was conducted to examine the potential C mineralization dynamics of soil samples from the 0–10, 10–20, 20–30 and 30–40 cm depth intervals of the profile. Air-dried bulk soil samples from the four depth intervals were brought to a gravimetric water content of, respectively, 22.8, 19.3, 17.1 and 16.6%, corresponding to a water-filled pore space of 60% at the bulk density in the field (assuming a soil particle density of 2.65 Mg/m^3).

Three replicate subsamples of 150 g soil (air dried basis) were placed into 4.6-cm diameter PVC tubes, compressed manually to the corresponding bulk density (respectively, 1.32, 1.43, 1.51 and 1.53 g cm^{-3}), covered with Parafilm and pre-incubated at 15 °C for 10 days. After pre-incubation, the PVC tubes were placed in sealed 1000-cm^3 glass jars fitted with a rubber septum for gas sampling, and incubated at 15 °C for 76 days. The production of CO_2 in each jar was measured by analyzing a 1-cm^3 headspace sample for CO_2 using a gas chromatograph (CP 9000, Chrompack) after 2, 4, 7, 12, 20, 25, 30, 35, 40, 47, 61 and 76 days of incubation. Following each gas sampling, glass jars were opened and Parafilm was removed from the PVC tubes during 10–15 min to re-establish ambient conditions.

Statistical analysis

Linear regression analysis and statistical analysis of the data were performed using Excel 97 for Windows. The statistical significance of the differences between estimated regression coefficients and mean total C contents in the four sampling depths in the profile was analyzed using a *t*-test at the 95% confidence level.

RESULTS AND DISCUSSION

Evolution of C content and δ^{13}C signature of SOM in the profile

The evolution of the total C content and the δ^{13}C signature of the SOM down to a depth of 100 cm in the profile are shown in Fig. 1.

The total C content showed a strong decrease in the upper 40 cm of the soil profile, followed by a slight decrease down to a depth of 100 cm. The decreasing total C content in the upper 40 cm of the profile was accompanied by a gradual increase of the δ^{13}C signature of about 4‰ in relation to the δ^{13}C signature (−29.8‰) of SOM in the surface layer (0–2 cm depth), indicating an enrichment in ^{13}C relative to ^{12}C with increasing depth in the soil profile. Fresh plant material from the investigated grassland at the time of soil sampling had a δ^{13}C signature of −30.2‰, which means that plant litter serves as a continuous input of ^{13}C-depleted material into the SOM pool.

The observed trend of increasing δ^{13}C values with increasing depth has been reported in several other studies, investigating the evolution of the δ^{13}C signature of SOM in both well-drained[7,11–14] and poorly drained[13] undisturbed soil profiles with a permanent C_3 vegetation.

A possible mechanism explaining the observed trend is isotopic discrimination against ^{13}C during organic matter decomposition, combined with the increasing degree of transformation of SOM with depth in the profile.[7,11,14] In their metabolism, decomposing organisms would prefer ^{13}C-depleted molecules for respiration, while ^{13}C-enriched molecules tend to be utilized in the production of biomass and the end-products of the metabolism.[8,9] As a result, SOM decay may lead to a progressive ^{13}C enrichment in the mixture of residual substrate, microbial products and metabolites.[11] This is consistent with our findings that the microbial biomass (δ^{13}C value of −27.9‰) was enriched in ^{13}C relative to whole soil C (δ^{13}C value of −28.8‰) in the upper 20 cm of the investigated profile (data not shown). A gradual ^{13}C enrichment of the residual substrate, combined with an enhanced contribution of ^{13}C-enriched microbially (re-) synthesized compounds to the SOM pool, with increasing depth in the soil profile[15–17] might thus explain the observed trend of the δ^{13}C signature.

To further verify this hypothesis, we fitted the Rayleigh equation[18] to the observed C contents and corresponding δ^{13}C signatures at the different depths in the profile. The Rayleigh equation describes the gradual enrichment in ^{13}C of SOM, resulting from isotopic fractionation associated with C mineralization, where δ_o and C_o represent the initial δ^{13}C signature and the initial C content:

$$\delta = \delta_o + \varepsilon \ln[C/C_o]$$

δ_o and C_o were approximated by the δ^{13}C value and the total C content in the surface layer of the profile (0–2 cm depth). The relation between the total C contents and the corresponding δ^{13}C signatures of the SOM could be best fitted ($R^2 = 0.95$, $p < 0.001$) by the Rayleigh equation in the

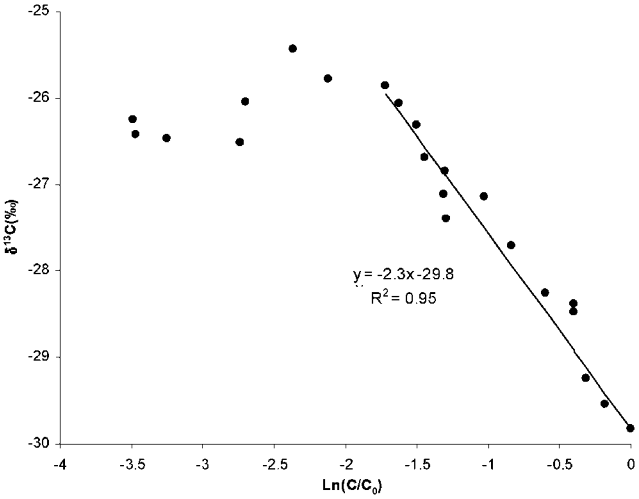

Figure 2. Relationship between ln(C/C_0) and the δ^{13}C values down to a depth of 100 cm in the soil profile (bullets), and fit by the Rayleigh equation (full line). 7 points, corresponding to the sampling depths below 30 cm in the profile, were excluded from the fit. C and C_0 stand for the C content in the different depth intervals and in the surface layer (0–2 cm depth), respectively.

upper 30 cm of the soil profile (Fig. 2). The enrichment factor ε associated with the observed data in the upper 30 cm of the soil profile was −2.3‰. This enrichment factor is larger (in absolute value) than the enrichment factors reported by Natelhoffer and Fry[7] (−0.8‰) and Balesdent and Mariotti[11] (−1.71‰), which were determined from short-term and long-term soil incubation experiments, respectively. Theoretically, the Rayleigh equation is applicable in the case of a simple monodirectional system: substrate → product.[18] However, the system studied here is not a simple reaction model, as, during the SOM decomposition process, both CO_2 and microbial biomass are formed as products. As microbial biomass may be re-incorporated into the residual SOM and may be recycled in the decomposition process, the enrichment factor ε calculated here should be interpreted rather as an apparent enrichment factor for total C transformation.

The current trend of the δ^{13}C signature in the profile may be partially influenced by the δ^{13}C signature and the amount of SOM, which was initially present in the profile, at the time of establishment of the grassland. As the soil was used for arable cropping until 1966, we may assume that the C content and the δ^{13}C signature were initially uniformly distributed in the upper layer of the profile (0 to ± 25 cm depth) due to ploughing. Consequently, the current evolution of the C content and the δ^{13}C signature in the profile must be mainly the result of a gradual accumulation of SOM derived from decomposing grass litter. As the δ^{13}C signature of this litter input (relatively 'young' C) was most probably more negative than the δ^{13}C signature of the originally present SOM (relatively 'old' C), this initial difference in δ^{13}C signatures between relatively 'young' and 'old' C might have enhanced to a certain extent the trend of increasing δ^{13}C signatures with depth, resulting in a larger enrichment factor ε. The ^{13}C enrichment in the upper soil layer could also be

Table 1. Total C contents, potential C mineralization rates, decomposition rate constants and $\Delta\delta^{13}C$ values in the 0–10, 10–20, 20–30 and 30–40 cm depth intervals of the profile (standard errors in brackets). Different letters in the same column indicate significant differences ($p < 0.05$)

Depth (cm)	C content (g C kg^{-1} soil)	C mineralization rate (mg CO_2-C kg^{-1} soil d^{-1})	Decomposition rate constant (yr^{-1})	$\Delta\delta^{13}C$ (‰ cm^{-1})
0–10	28.4 (0.7)^a	3.73 (0.07)^a	0.0479 (0.0009)^a	0.19 (0.04)^a
10–20	11.0 (0.1)^b	1.10 (0.02)^b	0.0366 (0.0007)^b	0.13 (0.04)^a
20–30	7.5 (0.1)^c	0.70 (0.02)^c	0.0339 (0.0009)^b	0.12 (0.01)^a
30–40	5.7 (0.1)^d	0.40 (0.01)^d	0.0256 (0.0006)^c	0.03 (0.01)^a

partially explained by the fact that the natural abundance of atmospheric CO_2 has decreased by about 1‰ since pre-industrial times, due to the input of depleted CO_2 into the atmosphere from fossil carbon burning and deforestation.[19] However, the good fit by the Rayleigh equation of the observed $\delta^{13}C$ profile suggests that the observed pattern of ^{13}C enrichment with increasing depth can be largely explained by isotopic discrimination associated with SOM decomposition.

Relationship between $\Delta\delta^{13}C$ values and potential C mineralization dynamics

As the largest variation in total C contents and $\delta^{13}C$ signatures was observed in the upper 40 cm of the profile, potential C dynamics of soil samples from the 0–10, 10–20, 20–30 and 30–40 cm depth intervals of the profile were investigated.

Total C contents in the bulk samples decreased from 28.4 g C kg^{-1} soil in the 0–10 cm depth interval to 5.7 g C kg^{-1} soil in the 30–40 cm depth interval (Table 1). The cumulative C mineralization curves from the four depth intervals are shown in Fig. 3. The nearly linear part of each curve (from day 30 to 76) was used to estimate the potential C mineralization rate by linear regression for each depth interval (Fig. 3). With increasing sampling depth in the profile, the potential C mineralization rates decreased with decreasing total C contents from 3.73 (0–10 cm sampling depth) to 0.40 mg C kg^{-1} soil day^{-1} (30–40 cm sampling depth) (Table 1).

Carbon decomposition rate constants, which are an indicator of the degradability of the SOM, were calculated by dividing the observed potential C mineralization rate by the corresponding total C content in each depth interval (Table 1). The C decomposition rate constants decreased from 0.0479 yr^{-1} (0–10 cm sampling depth) to 0.0256 yr^{-1} (30–40 cm sampling depth). In other words, the potential degradability of SOM in the 10–20, 20–30 and 30–40 cm depth intervals was, respectively, 25, 29 and 46% lower in relation to the potential degradability of SOM in the surface layer. This shows that the stability of SOM against microbial

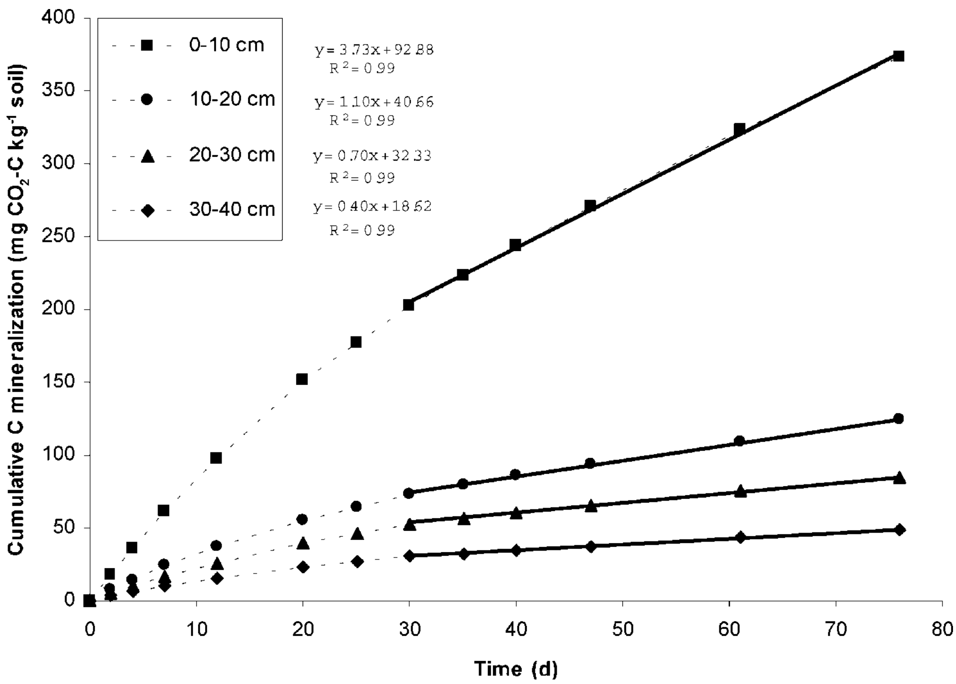

Figure 3. Evolution of the cumulative C mineralization of soil samples from the 0–10, 10–20, 20–30 and 30–40 cm depth intervals of the profile (day 0 to day 76 after pre-incubation) and linear regression of the curves (from day 30 to day 76 after pre-incubation).

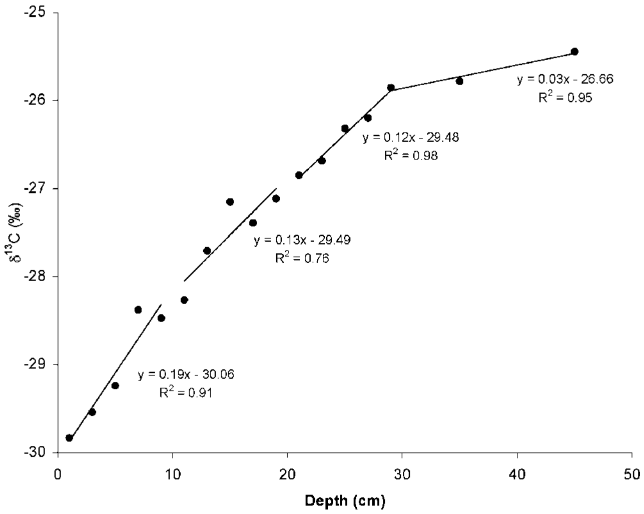

Figure 4. Linear regressions of the evolution of the δ^{13}C values in the 0–10, 10–20, 20–30 and 30–40 cm depth intervals of the profile.

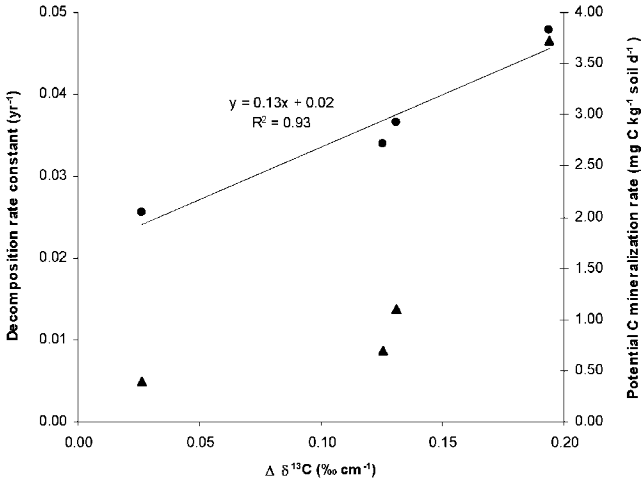

Figure 5. Decomposition rate constants (bullets) and potential C mineralization rates (triangles) plotted versus the corresponding $\Delta\delta^{13}$C values in the 0–10, 10–20, 20–30 and 30–40 cm depth intervals of the profile.

degradation increases with depth in the profile and with an advancing stage of decomposition.

As we observed a trend of increasing ^{13}C enrichment in the profile, we tried to quantify the relationship between the rate of change of the δ^{13}C values and the potential C dynamics. Therefore, we estimated the average change of the δ^{13}C signature per depth increment ($\Delta\delta^{13}$C value, expressed in ‰ cm^{-1}) for each depth interval, by means of the slope of a linear regression of the observed δ^{13}C values (Fig. 4). The $\Delta\delta^{13}$C value for the 30–40 cm depth interval was estimated using the δ^{13}C values from the 28–30, 30–40 and 40–50 cm intervals. These $\Delta\delta^{13}$C values decreased with increasing depth in the profile from 0.19‰ cm^{-1} in the 0–10 cm depth interval down to 0.03‰ cm^{-1} in the 30–40 cm depth interval. The potential C mineralization rates and decomposition rate constants were plotted versus the corresponding $\Delta\delta^{13}$C values in each depth interval (Fig. 5). The decomposition rate constants from the different sampling depths were highly correlated ($R^2 = 0.93$, $p < 0.05$) with the corresponding $\Delta\delta^{13}$C values. This suggests that the $\Delta\delta^{13}$C values may be interpreted as an indicator of varying soil C stability or quality with increasing depth in the soil profile. The relationship between the $\Delta\delta^{13}$C values and the potential C mineralization rates, however, was not so clear, based on the data observed in this profile. The relationship between the evolution of the ^{13}C enrichment and the decomposition rate constants of the SOM in the profile may be explained by the fact that its degradability is proportional to the labile fraction of the SOM. Fresh litter input, which has a more negative δ^{13}C signature than the SOM in the profile, serves as a continuous source of labile C, which is gradually incorporated into the soil profile. In this way, the evolution of the δ^{13}C signature might also be interpreted as a decreasing fraction of relatively 'young', labile C in the SOM with increasing depth in the profile. The soil type may have an influence on the evolution of the δ^{13}C signature with increasing depth, in terms of the rate at which solid and soluble compounds, originating from the surface layer, are translocated into the soil profile.[14,20]

CONCLUSIONS

The evolution of the total C content and ^{13}C enrichment of SOM in the investigated soil profile could be well fitted by the Rayleigh equation. This suggests that the observed trend of ^{13}C enrichment with increasing depth can be largely explained by isotopic discrimination associated with SOM decomposition. The rate of change of the ^{13}C enrichment per depth increment ($\Delta\delta^{13}$C values) showed a strong, positive correlation with the degradability of the SOM (C decomposition rate constants). This suggests that $\Delta\delta^{13}$C values in undisturbed soil profiles under continuous C_3 vegetation may serve as an indicator of the SOM quality at different depths in the profile. As this is a case study performed on a single soil profile, research on the evolution of the δ^{13}C signature in undisturbed soil profiles of different soil types under permanent grassland might further elucidate the relationship between $\Delta\delta^{13}$C values and potential C dynamics at different depths in the profile. As the isotope enrichment factor ε relates the variation of the quantity of C to the variation of its ^{13}C enrichment, which in turn is related to the quality of the C in the investigated soil profile, further research will be developed to evaluate the possible relationship between this ε factor and the total CO_2 fluxes from different soil profiles under permanent grassland.

Acknowledgements

This study was supported by the Belgian Ministry of Small Enterprises, Traders and Agriculture – Directorate of Research and Development.

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