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F. Conen, M. Zimmermann, J. Leifeld, B. Seth, C. Alewell. Relative stability of soil carbon revealed by shifts in  $\delta^{15}\text{N}$  and C:N ratio. *Biogeosciences Discussions*, 2007, 4 (4), pp.2915-2928. hal-00297914

**HAL Id: hal-00297914**

**<https://hal.science/hal-00297914>**

Submitted on 18 Jun 2008

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# Relative stability of soil carbon revealed by shifts in $\delta^{15}\text{N}$ and C:N ratio

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Received: 8 August 2007 – Accepted: 22 August 2007 – Published: 27 August 2007

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## Abstract

Life on earth drives a continuous exchange of carbon between soils and the atmosphere. Some forms of soil carbon, or organic matter, are more stable and have a longer residence time in soil than others. Relative differences in stability have often been derived from shifts in  $\delta^{13}\text{C}$  (which is bound to a vegetation change from C3 to C4 type) or through  $^{14}\text{C}$ -dating (which is bound to small sample numbers because of high measurement costs). Here, we propose a new concept based on the increase in  $\delta^{15}\text{N}$  and the decrease in C:N ratio with increasing stability. We tested the concept on grasslands at different elevations in the Swiss Alps. Depending on elevation and soil depth, it predicted mineral-associated organic carbon to be 3 to 73 times more stable than particulate organic carbon. Analysis of  $^{14}\text{C}$ -ages generally endorsed these predictions.

## 1 Introduction

Soils store about twice as much carbon in form of organic matter (OM) as the atmosphere currently contains as  $\text{CO}_2$ . As a result of increasing temperatures, soils may globally turn into a net source of  $\text{CO}_2$  and further accelerate climate change (e.g. Jones et al., 2005). Hereby, a critical factor is the stability of OM, which has gained increasing attention (Krull et al., 2003; Knorr et al., 2005; Davidson and Janssens, 2006). Stability depends on several factors, such as selective preservation due to chemical recalcitrance, spatial inaccessibility of OM to decomposers and stabilisation by interaction with mineral surfaces (v. Lützow et al., 2006). A boundless variety of chemical forms of OM exists in soils. Furthermore, microbial diversity in soils is huge and trophic interactions are complex (e.g. Nannipieri et al., 2003). The multifaceted structure of natural soils and multiple interactions with mineral surfaces make it difficult to derive stability of OM from first order principles. Inversely, stability of OM can be determined through  $^{14}\text{C}$ -age measurements, which are relatively expensive and has thus to rely on

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few measurement points. An alternative method is to determine changes in  $\delta^{13}\text{C}$  after a vegetation change from C3 to C4 type of photosynthesis, a method first described by Balesdent et al. (1987) and extensively applied since. However, the latter vegetation change is only found in few, mostly ploughed or otherwise disturbed ecosystems, where OM may not be in a state of equilibrium. Here, we propose a new concept to make out changes in OM stability which differs from previous approaches by focussing on changes in  $\delta^{15}\text{N}$  and C:N ratio.

## 2 Concept

Decomposition of unprotected soil organic matter and its re-synthesis by soil organisms leads to stabilised OM residuals (Gleixner et al., 2002; Lützwow et al., 2006) while simultaneously their C:N ratios decrease (Kramer et al., 2003). With decreasing C:N ratio, N-rich organic compounds are increasingly utilised as a C source, while excess N is mineralised, resulting in  $^{15}\text{N}$  enrichment of the remaining substrate (Snider et al., 2002; Dijkstra et al., 2006), which is increasingly associated with mineral particles. Thus, mineral-associated organic matter (mOM) has generally lower C:N ratios, is enriched in  $^{15}\text{N}$  and more stable than the particulate organic matter (POM) it derived from (Baisden et al., 2002; Liao et al., 2006). These insights are well founded but no general concept exists so far to quantify changes in OM stability as a function of changes in C:N ratio and  $\delta^{15}\text{N}$ . We do not consider changes in C:N ratio or  $\delta^{15}\text{N}$  as the cause for altered stability but we hypothesize that both parameters can be used as an indicator of increasing OM stability. Our objective is to describe the relative increase in the stability of OM when transformed from POM to mOM (Fig. 1) as a function of these parameters. Our reference measure of stability is  $^{14}\text{C}$ -age.

Decomposition of POM and re-synthesis as mOM result in mineralization and loss of N during the transformation process. Fractionation of N occurs with the fractionation factor  $\varepsilon$  [‰]. Knowledge of  $\varepsilon$  and the difference in  $\delta^{15}\text{N}$  between POM and mOM

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indicate the proportion of N lost during the transformation ( $f_N$ ) (Robinson, 2001).

$$f_N = 1 - e^{\left(\frac{\delta_m - \delta_p}{\varepsilon}\right)} \quad (1)$$

where  $\delta_p$  is the  $\delta^{15}\text{N}$  value of POM and  $\delta_m$  is the  $\delta^{15}\text{N}$  value of mOM [‰].

The proportion of C lost ( $f_C$ ) can be stoichiometrically derived from the loss of N ( $f_N$ ) and the C:N ratios of POM ( $r_p$ ) and mOM ( $r_m$ ).

$$f_C = f_N + (1 - f_N) \cdot \left(1 - \left(\frac{r_m}{r_p}\right)\right) \quad (2)$$

Together with the masses of POM-C ( $C_p$ ) and mOM-C ( $C_m$ ) per unit mass of bulk soil [ $\text{mg g}^{-1}$ ], we can now estimate a factor [ $n$ ], by which the age of mOM exceeds the age of POM under steady state conditions.

$$n = \frac{C_m}{C_p \cdot (1 - f_C)} \quad (3)$$

Thus, the relative increase in age ( $n$ ), or stability, of organic matter transformed from POM to mOM is described by  $\varepsilon$  and shifts in  $\delta^{15}\text{N}$ , C:N ratio and the concentrations of C in POM and mOM. Independent validation of our concept is possible by determining values of  $n$  for a wide range of soil materials and comparing them to values obtained by  $^{14}\text{C}$ -dating ( $n_i$ ).

$$n_i = \frac{a_m}{a_p} \quad (4)$$

where  $a_m$  and  $a_p$  are the  $^{14}\text{C}$ -ages of mOM and POM, respectively.

### 3 Material and methods

Soil samples were taken in 2005 from two locations in the Swiss Alps, in the cantons of Valais and Grisons. In Valais, we chose permanent grasslands along an elevation gradient with constant geology (gneiss and chloride-schist) in the Swiss Alps

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(46°15' N/7°55' E) at 990 m, 1410 m, and 1795 m a.s.l. Mean annual temperature decreases from +7.8°C at 990 m to +3.9°C at 1795 m a.s.l. Annual precipitation increases with altitude from 658 mm to 1040 mm, and all sites are irrigated with glacial meltwater during summer. Six cores (5.5 cm diameter) of the top 20 cm (three increments 0–5, 5–10 and 10–20 cm) were taken at each altitude along a horizontal transect of 15 m. In Grisons, we chose a long term meadow and a pasture, both at an elevation of 2020 between 2045 m a.s.l. (46°32' N/9°38' E), with a mean annual temperature of +2.2°C and an annual precipitation of 1050 mm. The bedrock at that site is serpentine. In Grisons, we sampled the depth intervals 0–4 cm and 4–20 cm in eight replicates.

All cores were dried at 40°C, crushed, sieved to <2 mm and mixed to obtain one composite sample per depth for each site. A light (density <1.8 g cm<sup>-3</sup>) particulate organic matter fraction (POM) >63 μm was obtained by combined ultrasonic dispersion applying an energy of 22 J ml<sup>-1</sup> suspension, sieving, and density separation. The difference to the bulk soil organic matter is regarded as mineral-associated (mOM). Carbon and nitrogen contents of all samples were measured after combustion with an elemental analyzer. Stable N isotope ratios were measured on POM and bulk soils by ion ratio mass spectrometry (Thermo Finnigan Delta plus XP coupled with an elemental analyzer Flash EA 1112 Series) in Basel. The <sup>15</sup>N contents of mOM were calculated by difference. To calculate the factor by which age of mOM exceeds age of POM (*n*) we applied above Eqs. (1) to (3), using a value of -2.0‰ for the fractionation factor  $\epsilon$ , derived from literature discussed in the next section.

Radiocarbon measurements in composite bulk soil samples and in POM were performed at the accelerator mass spectrometry laboratory of ETH Zurich. The <sup>14</sup>C contents of mOM were calculated by difference. A <sup>14</sup>C model accounting for “bomb” <sup>14</sup>C and radioactive decay was applied to calculate mean age of the respective fractions. Based on the approach of Harkness et al. (1986), the <sup>14</sup>C activity of the soil organic carbon (SOC) can be expressed as

$$A_t = A_{(t-1)} \cdot e^{-k} + (1 - e^{-k}) \cdot A_i - A_{(t-1)} \cdot \lambda \quad (5)$$

where  $A_{(t)}$  is the  $^{14}\text{C}$  activity of SOC at time  $t$ ,  $A_{(t-1)}$  the  $^{14}\text{C}$  activity of the previous year;  $A_i$  the input  $^{14}\text{C}$  activity of the plant residue,  $k$  the exchange rate constant of the SOC pool, and  $\lambda$  the  $^{14}\text{C}$  decay constant ( $1/8268 \text{ a}^{-1}$ ). The atmospheric  $^{14}\text{C}$  activity, expressed as percent modern carbon (% modern) was compiled using records from 5 Stuiver et al. (1998) for the period from year 1511 to 1954, from Levin et al. (1994) for 1959 to 1983 (site “Vermunt”), and from Levin and Kromer (1997) for the period 1977 to 1997 (site “Schauinsland”). The period between 1954 and 1959 was linearly interpolated. Mean ages were calculated according to Eq. (5) by iteratively varying the value for age until it matched the measured  $^{14}\text{C}$  activity of the sample. This was done 10 separately for POM and mOM and gave us the values for  $n_i$  in Eq. (4).

## 4 Results and discussion

In Valais, concentrations of POM near the surface (0–5 cm) increased six-fold from 990 m to 1795 m a.s.l. (Table 1). Deeper soil layers had lower POM concentrations which did not change with elevation. Little change with elevation was found in all depths 15 for mOM, which was generally the larger C-pool (with the exception of the 0–5 cm layer at the highest elevation). Values of  $\delta^{15}\text{N}$  increased with depth and were on average 1.7‰ (s.d.  $\pm 0.8\%$ ) larger in the mOM fraction. The C:N ratio was on average 8.8 (s.d.  $\pm 0.6$ ) in mOM and 15.3 (s.d.  $\pm 2.3$ ) in POM. With increasing depth we found consistently smaller C:N ratios in mOM. The age of POM according to  $^{14}\text{C}$ -dating ranged from 6 to 20 22 years, except for the deepest sample at the highest elevation, where it was 220 years. C-14 age of mOM increased with depth, both in absolute terms and relative to the age of POM. Systematically higher ages for mOM relative to POM and increasing ages of organic matter with depth are in line with previous findings from temperate soils (Tumbore et al., 1990; Trumbore, 1993). Overall, mOM was between 2 and 67 25 times older than POM, a wide range despite the small geographical area which we had sampled and likely to be caused by different environmental conditions along the

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elevation gradient. In Grisons, concentrations of POM in the surface layer were lower than expected from the experience in Valais which may have to do with more intensive utilisation of above ground biomass and therefore smaller inputs of litter. Ratios of C:N in POM and mOM were larger than in comparable depths at the highest elevation in Valais. C-14 ages of organic carbon were substantially larger in the pasture than in the meadow soil, probably reflecting the incorporation and stabilization of residues in soil by trampling which is also indicated by larger POM contents. Larger amounts of sand in the meadow soil (43% compared to 34% under pasture) may have fostered a more rapid turnover due to a lack of physical protection.

The relative age of mOM estimated with Eqs. (1) to (3) was generally congruent to  $^{14}\text{C}$  dating. A substantial divergence was only observed for two samples at the greatest depth at the highest elevation in Valais and at the even greater elevation at Grisons. Maybe, conditions at these points fell outside the limits of our concept, which assumes (a) a steady state in pool-sizes of POM and mOM, (b) no backward flux of mOM into the pool of POM, (c) no preservation of POM as charcoal, and (d) no vertical translocation of POM or mOM between the depth intervals of sampling. It is possible that assumption (d) might be violated because of incipient podsolisation at these elevations. Within the scope of our study, we therefore consider the two points (Valais, 1795 m a.s.l., 10–20 cm depth; Grisons, pasture, 4–20 cm depth) as outliers and exclude them from further analysis; although it is certainly worth investigating the issue in an other study.

A critical parameter in our concept is the value of  $\varepsilon$  (Fig. 1). Robinson (2001) gained a range of 0‰ to –5‰ from the review of earlier studies. More recently, Varvaet et al. (2002) estimated elegantly the fractionation factor for N mineralization from depth profiles of  $\delta^{15}\text{N}$  and total nitrogen, assuming that net N mineralization was the only major process responsible for shifts in  $\delta^{15}\text{N}$  with depth. Fractionation factors ranged from –1.8‰ to –6.6‰. They fell into two groups, one with a mean of –2.4‰ (n=6) and one with a mean of –5.2‰ (n=3). The larger absolute values may result from a violation of above assumption. At the sites with large absolute values for estimates of

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$\varepsilon$ , processes other than N mineralization probably had a non-negligible influence on the  $\delta^{15}\text{N}$  profile. Such processes could include the fractionating loss of  $\text{NO}_3^-$  through leaching or a significant loss of gaseous forms of N via nitrification or denitrification. Therefore, the smaller absolute values are certainly closer to the fractionation factor for N mineralization only, and it seems justified that we can apply a value for  $\varepsilon$  of  $-2.0\text{‰}$  in our study. The sensitivity of our estimates for  $n$  to the value for  $\varepsilon$  can be assessed by a re-analysis of our data. Compared to the  $^{14}\text{C}$  based estimates ( $n_i$ ), a value of  $-3.2\text{‰}$  would have resulted in an under-estimation of  $n$  by 29%, whereas a value of  $-1.6\text{‰}$  would have resulted in a comparable over-estimation (Fig. 2). No bias was found for  $\varepsilon = -2.0\text{‰}$ . Apart from  $\varepsilon$ , there is also uncertainty associated with the directly measured parameters. Say, the uncertainty in the C and N analysis is  $\pm 5\%$  of the mean and the absolute uncertainty in the  $\delta^{15}\text{N}$  measurement is  $\pm 0.2\text{‰}$ . Applying the rules of error propagation to our data, we get an overall uncertainty of  $\pm 15\%$  for the value of  $n$  associated with analytical errors.

Despite these uncertainties, our approach based on changes in  $\delta^{15}\text{N}$ , C:N ratio and the concentrations of POM and mOM comes to the same conclusions regarding the relative stabilities as does  $^{14}\text{C}$ -dating. For the altitudinal gradient in Valais, these conclusions are a general increase in stability of mOM relative to POM with increasing soil depth at all altitudes and the largest relative differences in stability between mOM and POM for all soil depths at the lowest altitude. With constant geology and land-use along the altitudinal gradient we consider site temperature to be among the most important factors determining organic matter turnover (Davidson and Janssens, 2006). Our data refer to a differential response of POM and mOM to altitude-induced average site temperature. Only the  $^{14}\text{C}$ -age of POM in the upper soil (0–5 cm) increased steadily with altitude, while that of POM in deeper layers and of mOM did not. In contrast,  $^{14}\text{C}$ -ages of mOM (0–5 cm) decreased with altitude. We suggest decomposition rates of mOM and of deeper POM to be dependent on factors such as available surfaces for organo-mineral interactions or aggregation in addition to temperature, thereby “masking” the temperature effect. Together, the results imply stabilisation by mineral association to be

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more important in warmer than in colder soils, probably because microbial processing and thus production of  $^{15}\text{N}$  enriched compounds is faster at warmer sites (Amundson et al., 2003). The same implication is derived from the relative ages of mOM and POM as determined by our concept based on changes in  $\delta^{15}\text{N}$  and C:N ratio. It would be pretentious to draw any general conclusions from the few observations in Grisons, say regarding the effect of grazing on OM stability. Nevertheless, these observations support the notion of our concept as a useful tool that is probably transferable to other sites. This concept of relative stabilities may help for a better quantitative understanding of the several stabilization mechanisms in soil. Where  $n$  is small, accumulation of SOM is not strongly related to mineral interactions of microbial-derived products but due to incomplete transformation of plant residues. SOM in such sites will likely be more vulnerable to environmental changes such as warming or land-use change than at sites where a large  $n$  refers to stabilization of microbial residues.

*Acknowledgements.* This study was supported by the Swiss Federal Office for the Environment and contributes to the National Climate Change Research Program (NCCR Climate) project GRASS. We would like to thank P. Calanca for helping to calculate the climatic parameters, and S. Fenner and A. Bonvicini for assistance in the laboratory. We thank G. Bonani, ETH Zürich, for the  $^{14}\text{C}$  analyses of our samples.

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**Table 1.** Parameters of soil samples from permanent grassland in the Swiss Alps. Directly measured were parameters relating to particulate organic matter (POM); parameters relating to mineral-associated organic matter (mOM) were calculated by difference from measurements of POM and bulk soil. The age of mOM relative to that of POM was estimated using Eqs. (1–3) in the text. Independently, it was also determined by <sup>14</sup>C dating.

altitude [m]	depth [cm]	Concentration [mg C g <sup>-1</sup> soil]		$\delta^{15}\text{N}$ [‰]		C:N		<sup>14</sup> C age [years]		Relative age (mOM:POM)	
		POM	mOM	POM	mOM	POM	mOM	POM	mOM	estimated	<sup>14</sup> C dated
Valais (meadows)											
990	0–5	13.6	42.2	2.3	4.6	15.6	9.2	7	165	17	24
990	5–10	3.4	31.9	3.8	5.9	15.4	9.1	6	199	46	33
990	10–20	2.0	24.6	4.7	6.9	16.7	9.0	7	488	73	67
1410	0–5	26.2	49.9	3.7	4.3	13.8	9.2	11	81	4	8
1410	5–10	10.4	47.0	4.4	5.2	13.1	8.9	11	123	10	11
1410	10–20	4.4	40.2	5.4	6.0	14.9	8.6	6	221	22	37
1795	0–5	80.3	58.2	2.4	4.4	13.9	9.3	22	54	3	2
1795	5–10	7.4	44.9	4.9	6.2	13.4	8.3	11	197	19	19
1795	10–20	1.6	20.5	4.1	6.9	20.5	7.6	220	595	147	3
Grisons (meadow)											
2020	0–4	10.6	53.4	3.3	4.7	17.9	10.4	10	166	18	17
2020	4–20	4.0	47.3	3.9	4.5	19.5	10.9	12	286	28	25
Grisons (pasture)											
2045	0–4	23.7	71.9	1.6	2.1	17.0	10.9	86	299	6	3
2045	4–20	5.7	43.6	3.0	3.9	22.0	10.1	112	488	26	4

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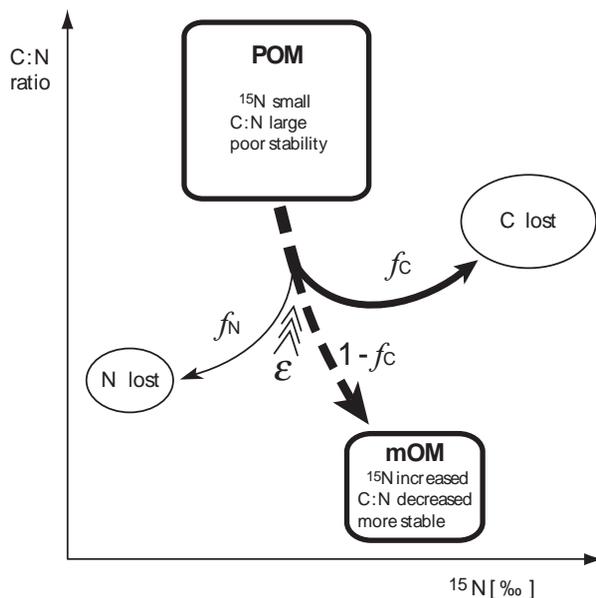
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Interactive Discussion

## Relative stability of soil carbon

F. Conen et al.



**Fig. 1.** Conceptual model of the transition of organic matter from particulate organic matter (POM) to mineral-associated organic matter (mOM). Knowledge of the fractionation factor  $\epsilon$  and differences in  $\delta^{15}\text{N}$  provide information on the fraction of N lost in the process ( $f_N$ ). In addition to changes in C:N ratio, the fraction of lost C ( $f_C$ ) can be calculated. The fraction of C turning into mOM ( $1-f_C$ ) and the concentrations of POM and mOM provide a measure of the stability of mOM in relation to the stability of POM (Eq. 3 in text).

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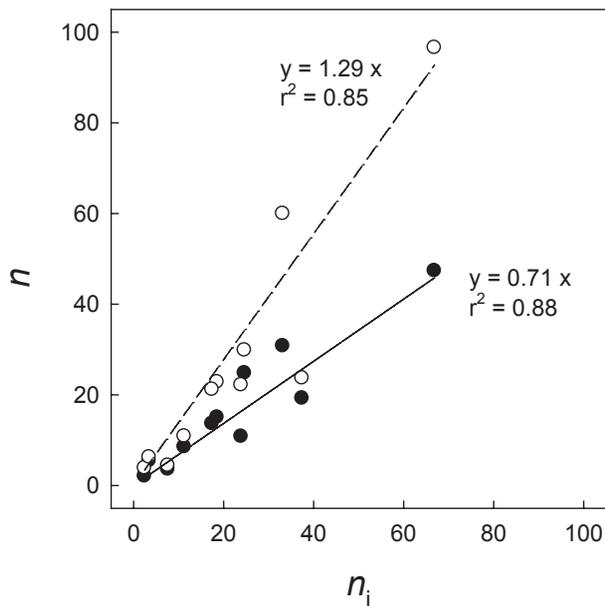
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## Relative stability of soil carbon

F. Conen et al.



**Fig. 2.** Ratio of the age of mineral-associated organic matter (mOM) to the age of particulate organic matter (POM) as predicted by Eqs. (1) to (3), ( $n$ ), against the same ratio based on measured  $^{14}\text{C}$ -ages of mOM and POM, ( $n_i$ ). Open circles show estimates based on a fractionation factor ( $\varepsilon$ ) of  $-1.6\text{‰}$ , closed circles show estimates based on  $\varepsilon = -3.2\text{‰}$ . The regression equation for our best estimate of  $\varepsilon$  ( $-2.0\text{‰}$ ) is:  $n = 1.01 n_i$  ( $r^2 = 0.88$ ).

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