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Effects of handling, storage, and chemical treatments on $\delta^{13}C$ values of terrestrial fossil organic matter

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[1] With the need to interpret small isotopic variations, $\delta^{13}C$ analyses of sedimentary organic matter are more and more widespread in the field of (paleo)climatology. Recent developments require an evaluation of the reliability and reproducibility of the whole data acquisition chain. Literature abounds in protocols for sediment pretreatment prior to physical measurements. These procedures differ at every step: from sampling, handling, and storage conditions to leaching procedure, without cross evaluation. In this study, we focus on two sediment samples: a modern temperate soil and a 70 ka typical loess. We review different protocols that characterize each step of the sediment pretreatment. Handling and storage conditions are tested, e.g., finger skin contact, mild- to high-temperature oven-dry, and freeze-drying. Likewise, different decarbonation protocols are compared: wet decarbonation under cold 0.6 N HCl, 2 N HCl and boiling 1 N HCl, and acid fuming with 36% HCl. This study identifies up to 1.5‰ isotopic shifts linked to experimental bias. This large bias might be at the origin of erroneous paleoclimatic interpretation. On the basis of these results, we propose specific treatments adapted to the sample type.

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1. Introduction

[2] Organic matter $\delta^{13}C$ is a basic paleoclimatic proxy to reconstruct past climate variability in continental areas. The rationale behind using carbon isotopes for extracting paleo-environmental parameters is that both photosynthetic pathways (C3 and C4-type plant) and external climatic and environmental parameters imprint the isotopic signature of the original plant matter. For a long time, interpretations were based on observed larger than 10‰ isotopic shifts associated to the C4 to C3-type vegetation transition [*de Freitas et al.*, 2001; *Schwartz and Mariotti*, 1998; *Wang and Follmer*, 1998; *Zhang et al.*, 2003]. Recent trends in paleoclimatology use the small amplitude isotopic variability (less than 2‰) to reconstruct past climate properties [e.g., *Hatté and Guiot*, 2005]. In that context, an increasing number of studies question the preservation of the original isotopic signal during diagenesis and fossilization. In several cases, measured $\delta^{13}C$ values are corrected by assuming specific isotopic shifts [e.g., *Rousseau*

et al., 2006]. But quantitative reconstructions demands as prerequisite a detailed study of the reliability and reproducibility of the whole data acquisition chain: from sampling to physical measurement. The main steps to control are (1) collection and proper storage of sediments, (2) elimination of carbonate that might be incorporated in sediment and (3) handling of sediment prior and after carbonate removal. Different protocols are described in literature. For step 1, a sampling without contact with any source of organic matter, and/or immediate drying after sampling at mild temperature and measurements as soon as possible have been proposed [*Hatté et al.*, 1999] but many studies use samples from sediment cores stored in basements for several years. Likewise, carbonate leaching procedures show a wide disparity. These preparation methods differ from mild conditions with low acid concentration at room temperature to drastic protocols with pure and/or boiling acid.

[3] As a result of preferential removal of part of the biochemical components [*Benner et al.*, 1987], microbial reworking of the original substrate [*Fogel and Tuross*, 1999] and/or defunctionalization within biomolecules [*Deleens et al.*, 1984; *van Bergen et al.*, 2000], structure of fossilized organic matter greatly differs from plant organic matter. This mainly depends on the degree of preservation, i.e., heterogeneity of the remaining components and complexity of molecular structures. Thus, according to the degree of maturity, organic matter should not react in the same way to the chemical treatment prior to the targeted physical measurement. Likewise, influence of external contaminant varies greatly depending on the organic carbon content in the sediment (with a range over two orders of magnitude).

[4] We focus here on two extreme cases of sediments commonly used in paleoclimate and environmental studies. We investigate the impact of leaching procedure, storage conditions and handling methods for these sediment types.

2. Samples and Methodology

2.1. Samples

[5] Two different types of sediment are selected to be representative of commonly studied sediments for paleoclimatology: (1) A modern soil (upper 5 cm of the A horizon of a brunisol under *Sequoiadendron Giganteum*) which is typical of early degraded organic matter and high organic carbon content. It has been sampled in Gif-sur-Yvette, France (48°42′11.49″N 2°08′30.34″E, 57 ma.s.l.). (2) A glacial typical loess, which is representative of "mature" organic matter and low organic carbon content: this 70 ka old loess has been sampled in Surduk, Serbia (45°4′N 20°20′E, 111 ma.s.l.) [*Fuchs et al.*, 2008].

2.2. Methodology

[6] All our tests are based on published protocols but to highlight problems we voluntarily minimized some of the routine handling and storage precautions. Acid dilution, oven-dry temperature, or storage and handling were considered separately. To make the reading easier, descriptions of the tests are organized by comparison to a defined protocol, further called "protocol 1". All study conditions are gathered in Table 1.

2.2.1. Initial Pretreatment

[7] Dry samples are lightly disaggregated in a glass mortar and sieved with 250 μm mesh to retain the fine fraction. The sieving removes rootlets and pebbles, without interference on sediment organic matter mostly embedded in the clay layers (<20 μm). This ensures that samples are homogenized and representative of the organic carbon fraction of the bulk sediment.

[8] Our loess reference sample, already oven-dried (40°C) and stored (about 1 year) has been rehumidified prior the protocol application to ensure homogeneous treatment.

2.2.2. "Protocol 1"

[9] Samples are collected and inserted into clean plastic Minigrip bags, using clean tools (organic free knives and spatula) avoiding any contact with fingers or any other organic substances (wool, cotton, etc.). Wet samples are oven-dried at 40°C, as soon as possible after sampling.

[10] Decarbonation is performed on approximately 4 g of dry sediment in a 250 mL glass beaker. Samples are acid-leached at room temperature with 0.6 N HCl diluted with ultrapure water (ELGA Maxima). This step lasts 3–4 days, until no further gas bubbling is recognized. Acid supernatant is replaced once a day after particles settling. At completion, samples are washed with ultrapure water until pH = 5 to 6 (evaluation by tipping supernatant water onto a pH paper and not dipping pH paper into beaker!). Sample is then oven-dried at 40°C. Carbonate-free sample is collected out of the beaker with clean stainless tools, crushed in a

Table 1. Analysis Conditions[a]

Handling conditions	clean	**finger-crushed**	**clean**	**clean**	clean	clean	clean	clean	clean	clean	clean	clean	clean
Storage	oven-dry 40°C	oven-dry 40°C	oven-dry 40°C	**wet** **wet**	**oven-dry** **60°C and 100°C**		**freeze-dry** **freeze-dry**	**frozen** **40°C and 60°C and 100°C**	**frozen** **freeze-dry**	oven-dry 40°C	oven-dry 40°C	oven-dry 40°C
Decarbonation	0.6N	0.6N	0.6N	0.6N	0.6N		0.6N	0.6N	0.6N	**2N @ RT**	**1N boiling HCl**	**pure fuming HCl**
Oven-drying	40°C	40°C	40°C	40°C	40°C		40°C	40°C	40°C	40°C	40°C	40°C
Water quality	ultrapure	**ultrapure**	**demineralized**	ultrapure	ultrapure		ultrapure	ultrapure	ultrapure	ultrapure	ultrapure	ultrapure
Handling conditions	clean	**clean**	**dirty tools**	**clean**	clean		clean	clean	clean	clean	clean	clean
Label	protocol 1	fi/40°C/ 0.6N/40°C	40°C/0.6N/ 40°C/dt	40°C/0.6N+ dw/40°C	H/0.6N/ 40°C	60°C or 100°C/0.6N/ 40°C	fd/0.6N/ 40°C	fr/40°C or 60°C or 100°C/0.6N/ 40°C	fr/fd/0.6N/ 40°C	40°C/2N/ 40°C	40°C/boil HCl/40°C	40°C/fum HCl/40°C

[a] The first column specifies protocol 1 in terms of handling, sampling, and chemical conditions. Bold characters highlight deviations to protocol 1. The last line reveals sample labels as used in Tables 2 and 3 and Figures 1 and 2.

clean glass mortar and stored in clean vials. From sampling to final physical measurement, maximum attention is taken to avoid contact between the sample and organic contaminants from, e.g., fingers, wool, and cotton. Samples are handled with stainless instruments, rinsed with CH_2Cl_2 before use. Just prior to utilization, all glass dishes are cleaned in dishwasher containing HCl 10% for final rinsing and then oven-burnt at 450°C for at least 4 hours.

2.2.3. Deviations to "Protocol 1" (Table 1)

[11] The cleanliness conditions are as follows:

[12] 1. Wet samples are crushed with fingers.

[13] 2. Tools for packing tin capsules prior to EA and IRMS measurements are contaminated by immersion in organic oils (sunflower, olive and canola to an approximate δ^{13}C of about −21‰) then wiped with paper tissue.

[14] 3. Demineralized water (ions exchange resin under 37 MΩ resistivity) is used instead of ultrapure water for both diluting acid and rinsing samples.

[15] Storage conditions are as follows:

[16] 1. Wet samples are enclosed in a bag laid on a desk under fluctuating temperatures (15 to 28°C) for 3 months.

[17] 2. Wet samples are either dried in a dedicated oven at 60°C and 100°C or freeze-dried.

[18] 3. Wet samples are frozen at −18°C for 2 months and then defrosted at 40°C, 60°C or 100°C in an oven or freeze-dried.

[19] Leaching conditions are as follows:

[20] 1. Aqueous acid leaching at 2 N HCl at room temperature.

[21] 2. Aqueous acid leaching with boiling 1 N HCl.

[22] 3. Pure fuming HCl (37%wt) leaching.

[23] Protocol decarbonation using pure acid drops within tin capsules was not tested to protect EA quartz columns.

2.3. Measurements

2.3.1. Organic Carbon Content

[24] Two different carbon measurements are done on each sediment sample: total carbon on bulk

Table 2. Organic Carbon Content and Carbon Isotopic Composition for Modern Soil Sample[a]

Experiments		Organic Carbon Content		$\delta^{13}C$	
Description	Label	%wt	±	‰	±
Protocol 1	40°C/0.6N/40°C	0.71	0.03	−26.77	0.06
Handling					
Fingers	fi/40°C/0.6N/40°C	0.66	0.03	−26.71	0.06
Dirty tools	40°C/0.6N/40°C/dt	0.76	0.04	−26.48	0.04
Demineralized water	40°C/0.6N+dw/40°C	0.71	0.03	−26.75	0.06
Storage					
Wet storage	H/0.6N/40°C	0.67	0.01	−26.51	0.06
60°C oven-drying	60°C/0.6N/40°C	0.77	0.04	−26.74	0.07
100°C oven-drying	100°C/0.6N/40°C	0.69	0.03	−26.73	0.09
Freeze-drying	fd/0.6N/40°C	0.71	0.01	−26.75	0.06
−18°C+ 40°C defrost	fr/40°C/0.6N/40°C	0.81	0.01	−26.73	0.06
−18°C+ 60°C defrost	fr/60°C/0.6N/40°C	0.66	0.02	−26.81	0.06
−18°C+ 100°C defrost	fr/100°C/0.6N/40°C	0.75	0.01	−26.88	0.06
−18°C+ freeze-drying defrost	fr/fd/0.6N/40°C	0.58	0.01	−26.76	0.06
Leaching					
2N HCl	40°C/2N/40°C	0.74	0.04	−26.77	0.06
Boiling HCl	40°C/boil HCl/40°C	0.52	0.01	−27.45	0.07
Fuming HCl	40°C/fum HCl/40°C	0.68	0.03	−26.79	0.06

[a] The first column characterizes the experiment type. The second column shows the label defining the experiment as in Figure 1. The last four columns are for organic carbon content and carbon isotopic composition with their incertitude ranges.

sediment and organic carbon after sediment leaching. About 15–20 mg of sediment is weighed using tin cups for measurement (with a precision of 1 μg). The sample is combusted in a Fisons Instrument NA 1500 Element Analyzer and carbon content determined by the Eager software. An acetanilide standard (71.07%wt of carbon) is inserted every 10 samples. Organic carbon content in bulk sediment is deduced assuming that mineral carbon exists only as $CaCO_3$. Results are reported in %weight of organic carbon/bulk sediment.

2.3.2. Carbon Isotopic Composition

[25] Analysis is performed online with a continuous flow EA-IRMS coupling: Fisons Instrument

Table 3. Organic Carbon Content and Carbon Isotopic Composition for Typical Loess Sample[a]

Experiments		Organic Carbon Content		$\delta^{13}C$	
Description	Label	%wt	±	‰	±
Protocol 1	40°C/0.6N/40°C	0.095	0.006	−23.25	0.10
Handling					
Fingers	fi/40°C/0.6N/40°C	0.100	0.006	−23.99	0.06
Dirty tools	40°C/0.6N/40°C/dt	0.095	0.006	−23.38	0.05
Demineralized water	40°C/0.6N+dw/40°C	0.102	0.006	−23.50	0.09
Storage					
Wet storage	H/0.6N/40°C	0.108	0.003	−24.04	0.07
60°C oven-drying	60°C/0.6N/40°C	0.098	0.006	−23.13	0.07
100°C oven-drying	100°C/0.6N/40°C	0.094	0.006	−23.51	0.06
Freeze-drying	fd/0.6N/40°C	0.092	0.005	−23.12	0.07
−18°C+ 40°C defrost	fr/40°C/0.6N/40°C	0.099	0.002	−23.37	0.08
−18°C+ 60°C defrost	fr/60°C/0.6N/40°C	0.094	0.003	−23.32	0.06
−18°C+ 100°C defrost	fr/100°C/0.6N/40°C	0.093	0.003	−23.52	0.05
−18°C+ freeze-drying defrost	fr/fd/0.6N/40°C	0.092	0.003	−23.44	0.07
Leaching					
2N HCl	40°C/2N/40°C	0.090	0.005	−23.04	0.06
Boiling HCl	40°C/boil HCl/40°C	0.089	0.004	−23.55	0.07
Fume HCl	40°C/fum HCl/40°C	0.086	0.003	−22.54	0.06

[a] As for Table 2.

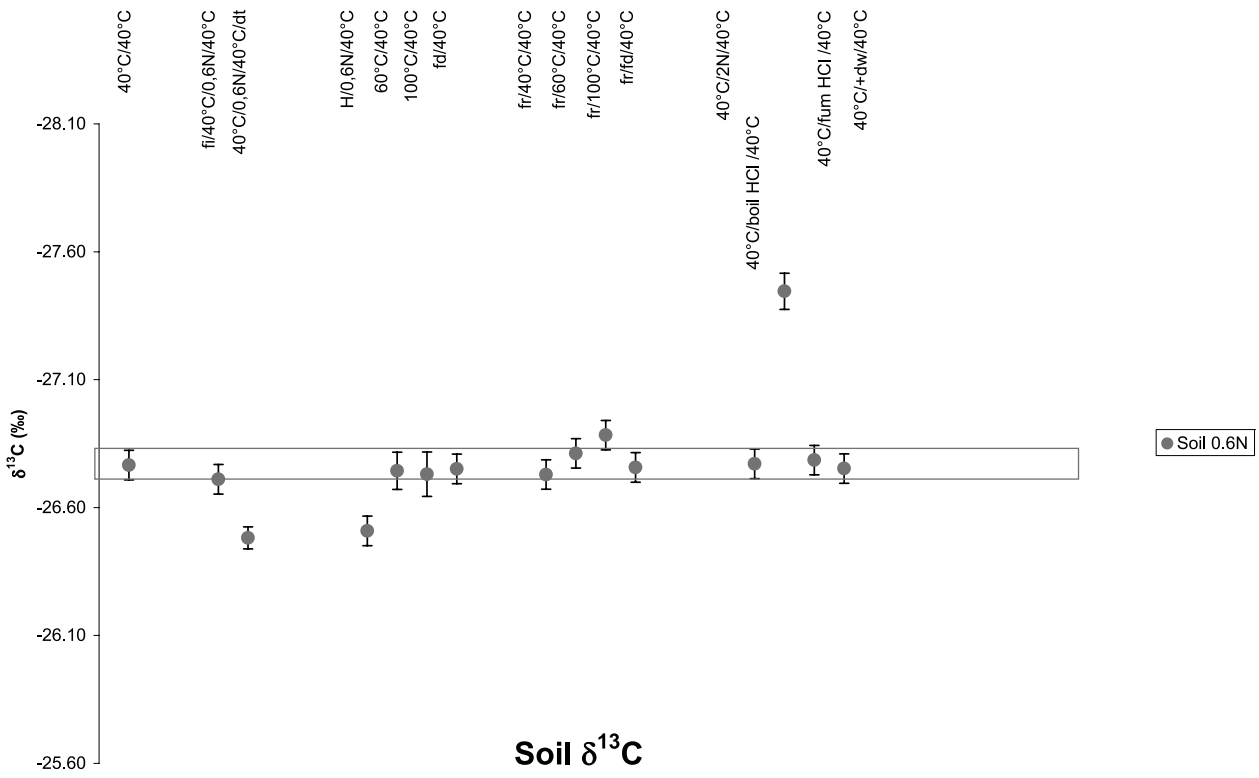

Figure 1. Modern soil carbon isotopic composition. The open rectangle marks the isotopic range ($\pm 1\sigma$) corresponding to "protocol 1". Data are shown with uncertainties range.

NA 1500 Element Analyzer coupled to a Thermo-Finigan Delta+XP Isotope-Ratio Mass Spectrometer. Two internal standards (oxalic acid, $\delta^{13}C = -19.3‰$ and GCL, $\delta^{13}C = -26.7‰$) are inserted every five samples. Results are reported in the δ notation:

$$\delta^{13}C = \left[{}^{R_{sample}}/_{R_{standard}} - 1 \right] \times 1000$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and international standard VPDB, respectively. Measurements are at least triplicated to ensure measurements are representative. The external reproducibility of analysis is better than 0.1‰, typically 0.06‰.

3. Results and Discussion

[26] All results are presented in Tables 2 and 3 and shown in Figures 1 and 2. Mean organic carbon content is 0.67%wt ($\sigma = 0.07$, n = 28) for soil and 0.095%wt ($\sigma = 0.005$, n = 17) for loess; measured isotopic values range between $-27.45 \pm 0.07‰$ and $-26.48 \pm 0.04‰$ for soil and $-24.04 \pm 0.07‰$ and $-22.54 \pm 0.06‰$ for loess. Organic carbon content and $\delta^{13}C$ obtained for the defined "protocol 1" are 0.71 ± 0.03%wt and $-26.77 \pm 0.06‰$ for the modern temperate soil, and 0.095 ± 0.006%wt and $-23.25 \pm 0.10‰$ for loess (Tables 2 and 3).

[27] The isotopic data suggests a C3-type vegetation derived organic matter. Lightest $\delta^{13}C$ values are measured in the modern soil, whereas heaviest $\delta^{13}C$ values are obtained for the glacial loess. These results are typical and derive from Glacial to modern changes in factors impacting C3-type plant isotopic signature [*Hatté et al.*, 2001]. However, the high analytical dispersion for the same sample, 1‰ ($-27.45 \pm 0.07‰$ to $-26.48 \pm 0.04‰$ respectively) for the modern soil and 1.5‰ ($-24.04 \pm 0.07‰$ to $-22.54 \pm 0.06‰$) for the 70 ka loess, underlines the importance of the protocol quality to get reliable isotopic records. Such large isotopic shift, induced by inadequate laboratory work, might be interpreted, considering recent paleoclimatic reconstructions, as a 75 ppm change of the past CO_2 concentration [*Feng and Epstein*, 1995], or a 450 mm a^{-1} variation of the mean annual precipitation [*Stewart et al.*, 1995].

3.1. Impact of Sampling and Handling Conditions

[28] For loess, the isotopic effect of skin contact on samples is obvious: $\delta^{13}C$ shift by approximately

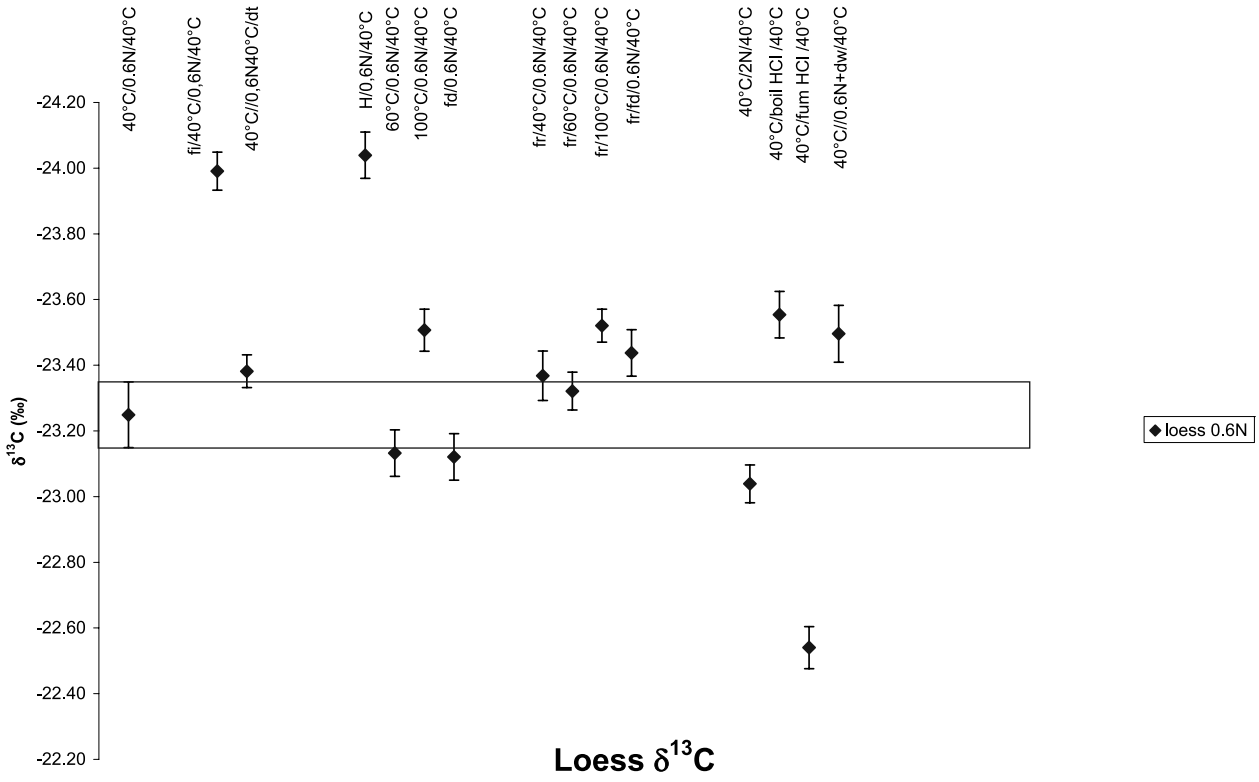

Figure 2. Typical loess carbon isotopic composition (as Figure 1).

−0.75‰ compared to "protocol 1". In contrast, use of dirty tools for loess treatment only induces a slight change toward more depleted values and richer organic carbon content. For soil, skin contact does not significantly change the original soil isotopic signature that remains within the range −26.7‰ to −26.8‰, but dirty tools induce a large shift toward enriched values by about 0.3‰. Both, the difference of δ^{13}C and organic carbon concentration between sample and potential contaminant may explain these results. Finger exudates, ceramides and sebum, are lipids with light δ^{13}C value. Both dilution effect and a close δ^{13}C value may be invoked to explain respectively the lack of impact on soil and the strong influence on loess. Tools were dirtied in successive oils, from C3 and C4-types plant origin. The resulting δ^{13}C (about −21‰) is closer to loess δ^{13}C than to soil δ^{13}C and thus does not significantly bias loess original isotopic signature but clearly enrich soil δ^{13}C.

[29] Loess δ^{13}C is affected by the use of demineralized water instead of ultrapure water during rinsing processes. This results in ∼0.25‰ lighter δ^{13}C values compared to "protocol 1" (Table 3). In contrast, for soil, the use of demineralized water does not impact its organic content or δ^{13}C (Table 2). Water demineralization does not remove organic components and consequently small amount of water dissolved organic compounds can be found within the sample. Owing to the dilution effect, this organic adjunction might be visible in loess and is not likely to be noticed in soil.

[30] In brief, we advocate the use of ultraclean tools from the sampling to the last stage of sample handling prior to measurement and to avoid any contact with any source of external organic contaminants. Likewise we recommend the use of ultrapure water for rinsing step and dilution.

3.2. Impact of Storage Conditions

[31] As shown in Figures 1 and 2 and Tables 2 and 3, impact of storage conditions on both organic carbon content and stable isotope composition greatly differs with the type of sample.

[32] Storage of wet sediments under fluctuating temperature for extended period must be avoided because it helps in the production of microorganisms [*Wohlfarth et al.*, 1998]. In the low organic content loess, microorganisms development results in a shift toward depleted values (∼0.8‰, Table 3).

This is consistent with microbial organic matter δ^{13}C, depleted compared to its carbon source [*Fogel and Tuross*, 1999; *Petsch et al.*, 2001]. The organic carbon content after this treatment is the highest within all experiments. Although barely significant it may indicate synthesis of organic carbon from diffusion of atmospheric CO_2 either by microbial chemical pathway or by photosynthesis. For soil, impact on δ^{13}C is less because of its high initial organic carbon content.

[33] Under 100°C oven-drying, loess δ^{13}C decreases by ~0.25‰ compared to the "protocol 1" but does not change if temperature is kept under 60°C (Table 3 and Figure 2). For soil, no significant change in δ^{13}C is noticed for different drying temperatures in the oven (Table 2 and Figure 1). Similar results are noticed for both direct oven-drying and oven-drying that follows a freezing procedure. We are likely to face a partial combustion of labile organic components that occurs with a 100°C oven drying. This effect is not significant for soil because of the high initial organic carbon content.

[34] The direct freeze-drying protocol does not impact either soil and loess isotopic composition or organic carbon content (Figures 1 and 2 and Tables 2 and 3) and provides comparable result than "protocol 1". However, the isotopic ratio is significantly altered if freeze-drying is done after the −18°C freezing. In loess this induces a ~0.2‰ isotopic depletion compared to "protocol 1". This may be attributed to a loss of functional groups out in the resistant organic molecules (mostly structural lipid). *Deleens et al.* [1984] highlighted that the δ^{13}C values in lipid moieties were higher than whole molecules; the removal of these groups leave depleted fossilized organic matter in the sample.

[35] To sum up, original isotopic composition in modern soil is not notably influenced by storage methods, whereas the low organic carbon loess δ^{13}C is. Significant isotopic shifts are observed after (1) wet storage, (2) high drying temperature and (3) freezing followed by freeze-drying. We recommend a dry storage of sediment according to either direct freeze-dry protocol or 40°C or 60°C oven-drying.

3.3. Impact of Leaching Procedure

[36] For soil, a loss of organic carbon of 0.19%wt is noticed with 1N acid leaching at boiling temperature. This is coupled to a −0.7‰ δ^{13}C shift compared to "protocol 1" (Table 2 and Figure 1). At room temperature, soil samples do not show significant difference in organic carbon content and isotopic composition for acid decarbonation at different acid concentration ("protocol 1", 2 N HCl, or fuming acid) with a mean organic carbon content of 0.71%wt (σ = 0.03, n = 3) and δ^{13}C of −26.77‰ (σ = 0.01, n = 3). This difference is likely to be associated with hydrolysis of labile organic compounds in the sample. Boiling may accelerate the degradation process and the solution of isotopically heavy cellulose (approximately −23‰) and/or hemicellulose (approximately −25‰) leaving lignin (approximately −28‰) enriched organic matter [*Benner et al.*, 1987].

[37] The loess sample presents a more systematic isotopic dependence with the leaching procedure (Table 3 and Figure 2), from −23.25 ± 0.10‰ with 0.6 N HCl ("protocol 1") to −22.54 ± 0.06‰ with fuming HCl. But there is no significant changes in organic carbon content (~0.09%wt). A differential hydrolysis efficiency of the leaching procedure has to be invoked to explain the δ^{13}C variations (~0.7‰). The explanation favored by *Schubert and Nielsen* [2000], i.e., loss of labile organic matter during rinsing steps, could be invoked for modern soil but not for old loess, except if some of it was embedded within carbonate pebbles and released during leaching. This would leave enriched δ^{13}C fossilized organic matter.

[38] To summarize, we advocate taking sediment specificities into consideration prior to applying any leaching protocol. We recommend avoiding the boiling acid treatment for soil sample. All widespread cool leaching procedures result in consistent δ^{13}C for soil. Loess requires exclusively the mildest pretreatment: the cool wet 0.6 N HCl decarbonation.

4. Conclusions

[39] Laboratory work prior to δ^{13}C measurement is not trivial. We show here that inadequate work conditions might induce a 1.5‰ isotopic shift of the original organic matter isotopic signature. This range is larger than natural variability and will interfere with paleoclimatic interpretation.

[40] Thus to preserve the original δ^{13}C organic matter during lab work, we advocate these simple precautions:

[41] 1. Use ultraclean tools.

[42] 2. Avoid any contact between sediment and any source of organic material.

[43] 3. Avoid decarbonation with boiling acid.

[44] For loess characterized in particular by low organic carbon further caution is needed:

[45] 1. Dry storage (low-temperature oven-drying, freeze-drying) or freeze storage (low-temperature defrost).

[46] 2. Cool wet 0.6 N HCl leaching procedure.

[47] 3. Use of ultrapure water for dilution and rinsing.

[48] 4. Low-temperature oven-drying step after decarbonation step.

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References

Benner, R., M. L. Fogel, K. E. Sprague, and R. E. Hodson (1987), Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies, *Nature*, *329*, 708–710, doi:10.1038/329708a0.

de Freitas, H. A., L. C. R. Pessenda, R. Aravena, S. E. M. Gouveia, A. S. Ribeiro, and R. Boulet (2001), Late Quaternary vegetation dynamics in the southern Amazon basin inferred form carbon isotopes in soil organic matter, *Quat. Res.*, *55*, 39–46, doi:10.1006/qres.2000.2192.

Deleens, E., N. Schwebel-Dugue, and A. Trémolières (1984), Carbon isotope composition of lipidic classes isolated from tobacco leaves, *FEBS Lett.*, *178*(1), 55–58, doi:10.1016/0014-5793(84)81239-9.

Feng, X., and S. Epstein (1995), Carbon isotopes of trees from arid environments and implications for reconstructing atmospheric CO_2 concentration, *Geochim. Cosmochim. Acta*, *59*(12), 2599–2608, doi:10.1016/0016-7037(95)00152-2.

Fogel, M. L., and N. Tuross (1999), Transformations of plant biochemicals to geological macromolecules during early diagenesis, *Oecologia*, *120*, 336–346, doi:10.1007/s004420050867.

Fuchs, M., D.-D. Rousseau, P. Antoine, C. Hatté, C. Gauthier, S. Markovic, and L. Zöller (2008), Chronology of the last climatic cycle (Upper Pleistocene) of the Surduk loess sequence, Vojvodina, Serbia, *Boreas*, *37*, 66–73.

Hatté, C., and J. Guiot (2005), Paleoprecipitation reconstruction by inverse modeling using the isotopic signal of loess organic matter: Application to the Nu(loch loess sequence (Rhine Valley, Germany), *Clim. Dyn.*, *25*(2–3), 315–327, doi:10.1007/s00382-005-0034-3.

Hatté, C., P. Antoine, M. R. Fontugne, D.-D. Rousseau, N. Tisnérat-Laborde, and L. Zöller (1999), New chronology and organic matter $\delta^{13}C$ paleoclimatic significance of Nußloch loess sequence (Rhine Valley, Germany), *Quat. Int.*, *62*(1), 85–91.

Hatté, C., P. Antoine, M. R. Fontugne, A. Lang, D.-D. Rousseau, and L. Zöller (2001), $\delta^{13}C$ variation of loess organic matter as a potential proxy for paleoprecipitation, *Quat. Res.*, *55*, 33–38, doi:10.1006/qres.2000.2191.

Petsch, S. T., T. I. Eglinton, and K. J. Edwards (2001), ^{14}C-dead living biomass: Evidence for microbial assimilation of ancient organic carbon during shale weathering, *Nature*, *292*, 1127–1131.

Rousseau, D.-D., C. Hatté, J. Guiot, D. Duzer, P. Schevin, and G. Kukla (2006), Reconstruction of the Grande Pile Eemian using inverse modeling of biomes and $\delta^{13}C$, *Quat. Sci. Rev.*, *25*, 2806–2819, doi:10.1016/j.quascirev.2006.06.011.

Schubert, C. J., and B. Nielsen (2000), Effects of decarbonation treatments on $\delta^{13}C$ values in marine sediments, *Mar. Chem.*, *72*(1), 55–59, doi:10.1016/S0304-4203(00)00066-9.

Schwartz, D., and A. Mariotti (1998), $\delta^{13}C$ profiles of ferrasoils and vegetation changes in Congo: Modelling and palaeoecological implications, paper presented at 16th World Congress of Soil Science, Int. Union of Soil Sci., Montpellier, France.

Stewart, G. R., M. H. Turnbull, S. Schmidt, and P. D. Reskine (1995), ^{13}C natural abundance in plant communities along a rainfall gradient: A biological integrator of water availability, *Aust. J. Plant Physiol.*, *22*, 51–55.

van Bergen, P. F., I. Poole, T. M. A. Ogilvie, C. Caple, and R. P. Evershed (2000), Evidence for demethylation of syringil moieties in archeological wood using pyrolysis-gas chromatography/mass spectrometry, *Rapid Commun. Mass Spectrom.*, *14*, 71–79, doi:10.1002/(SICI)1097-0231(20000130)14:2<71::AID-RCM837>3.0.CO;2-9.

Wang, H., and L. R. Follmer (1998), Proxy of monsoon seasonality in carbon isotopes from paleosols of the southern Chinese Loess Plateau, *Geology*, *26*(11), 987–990, doi:10.1130/0091-7613(1998)026<0987:POMSIC>2.3.CO;2.

Wohlfarth, B., G. Skog, G. Possnert, and B. H. Holmqvist (1998), Pitfalls in the AMS radiocarbon-dating of terrestrial macrofossils, *J. Quat. Sci.*, *13*(2), 137–145, doi:10.1002/(SICI)1099-1417(199803/04)13:2<137::AID-JQS352>3.0.CO;2-6.

Zhang, Z., M. Zhao, H. Lu, and A. M. Faiia (2003), Lower temperature as the main cause of C_4 plant declines during glacial periods on the Chinese Loess Plateau, *Earth Planet. Sci. Lett.*, *214*, 467–481, doi:10.1016/S0012-821X(03)00387-X.