Effects of organic removal techniques prior to carbonate stable isotope analysis of lacustrine marls: a case study from palaeo-lake Fucino (central Italy)

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Abstract

RATIONALE: The suitability of organic matter (OM) removal pre-treatments in isotopic studies of lacustrine carbonates is currently under debate. Naturally occurring OM seems to have a negligible effect on the bulk isotopic composition of carbonates compared to changes induced by pre-treatments. This study provides further insights into the possible effects induced by commonly used pre-treatments on natural lacustrine carbonates.

METHODS: Sixteen samples from the Fucino F1-F3 lacustrine succession (Abruzzo, central Italy) were characterised for their mineralogical and geochemical composition and split in three identical aliquots. One aliquot was left untreated while the remaining two were treated with NaOCl and H_2O_2 dilutions. The same treatment was applied to an internal standard constituted of pure Carrara marble. Treated and untreated samples were analysed for their carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope compositions using an

Analytical Precision AP2003 isotope ratio mass spectrometer. RESULTS: The samples had variable proportions of endogenic and detrital components, the detrital portion being more (less) abundant during colder (warmer) climate phases. We observed that neither the NaOCl nor the H_2O_2 treatment were able to completely remove OM and therefore there was selective removal of compounds within the OM pool. A possible effect of pre-treatment is the loss of carbonates intimately interspersed within OM, as suggested by the evolution of isotopic ratios towards the local detrital array.

CONCLUSIONS: Our study highlights sample-specific changes in geochemistry associated with sample pre-treatments, however such changes do not seem to lead to either systematic and/or predictable isotopic shifts. We suggest that the suitability of NaOCl or H_2O_2 pre-treatments for OM removal should be evaluated on a case-by-case basis. In the specific case of lacustrine marls from palaeo-lake Fucino containing relatively low amounts of OM and in which both detrital and endogenic carbonates occur, both pre-treatments should be avoided.

Introduction

Stable carbon and oxygen isotope ($\delta^{13}C$ and $\delta^{18}O$) values of biogenic and endogenic carbonates are commonly used in palaeoenvironmental studies as proxies for hydrological and environmental processes.[1,2] The most common analytical procedure involves digestion of bulk sample material in orthophosphoric acid (H_3PO_4) followed by isotope ratio mass spectrometry on the evolved carbon dioxide (CO_2). Although highly precise, the accuracy of the $\delta^{13}C$ and $\delta^{18}O$ values can be biased by organic matter (OM) naturally occurring in lake sediments. During acid reaction, OM can evolve volatile organic impurities (e.g., C_2H_5OH, CS, BCl_3, NO_2, N_2O) [3,4,5] which have the potential to increase measured $\delta^{13}C$ and $\delta^{18}O$. [4] Furthermore, CO_2 can evolve directly from OM possibly introducing CO_2 with an isotopic value very different to that of the carbonate. [2, 6] For these reasons, various physical and chemical pre-treatment methods have been used to remove OM from geologic materials in palaeoenvironmental and pedological studies. [4, 7] Except for the method recently proposed by Falster and co-authors, [8] there is no universally accepted method for completely removing OM and, at the same time, not changing the isotope composition of the sample to be analysed [7, 9].

Studies on the effects induced by sample pre-treatment on lacustrine carbonates have so far focused on artificial sediments obtained by mixing pure crystalline calcite (e.g., shells, synthetic calcite, ground marble or limestone) with variable amounts of organic compounds which are typically found in lacustrine sediments, [4, 5] or with decarbonated lacustrine sediments. [8] By analysing artificial calcite- OM mixtures with variable total inorganic carbon/total organic carbon (TIC/TOC) ratios, Oehlerich and co-authors observed that $\delta^{13}C$ and $\delta^{18}O$ values are biased only up to TIC/TOC = 0.3, and advised to pre-treat only samples laying below this threshold. [5] However, chemical pre-treatment with sodium hypochlorite (NaOCl) or hydrogen peroxide (H_2O_2) is still routinely applied in palaeolimnological studies as its effect on geologic materials is poorly understood. Here we apply NaOCl and H_2O_2 to natural samples from the palaeo-lake Fucino sedimentary succession in Abruzzo (central Italy) [11] as well as to a reference material. We compare independent measurements on untreated and treated samples to evaluate the effect of the two OM removal methods.

Materials and methods

We analysed 16 samples from the Fucino palaeo-lake sedimentary succession and a single reference material consisting of pure Carrara Marble (Marmo Statuario, labelled as Standard or "STD"). Selected samples lay above the organic-bias threshold proposed by Oehlerich and co-authors [5] and are representative of the whole spectrum of TIC/TOC values of the last two glacial-interglacial cycles. [10] (Supplementary figure SF 1). For each sample,1500 mg of loose material were divided into 3 identical aliquots and placed in three separate vials for isotope and geochemical analyses on untreated sample (specimens labelled "***TQ***") or after reaction with 0.5 M NaOCl (specimens labelled "***N***") or 30% H_2O_2 (specimens labelled "***H***") dilutions. [7]

Sample treatment

Approximately 500 mg of subsamples were placed in 500 mL beakers and mixed with 50 mL of NaOCl or H_2O_2. After reacting for 24 h at room temperature (Pisa, 25°C), each beaker was made up to volume (500 mL) with deionised water. After the sediment had settled, the deionised water was decanted and replenished. This was done three times, using a total of 1500 mL of deionised water per sample. After the final rinse, the beaker was partially emptied and the remaining ca. 50–100 mL were filtered with laboratory paper filters. The filtered material was dried in oven at 50 °C for 72 h, loosened and gently ground in an agate mortar.

Geochemical analyses

Geochemical analyses were conducted at the Institute of Geology and Mineralogy of the University of Cologne (Germany). Total nitrogen (TN) was determined with a Vario Micro cube combustion CNS elemental analyser (Elementar Analysensysteme GmbH, Germany), while TC and TIC were measured with a DIMATOC 200 (Dimatec Analysentechnik GmbH, Germany) according to the method specified by Francke and co-authors. [11] The total organic carbon (TOC) content was calculated from the difference between TC and TIC.

Mineralogical analyses

X-ray powder diffraction (XRPD) analyses were performed at the Department of Earth Sciences, University of Pisa (Italy) using a Bruker D2 Phaser (Bruker Corporation, USA). Details on instrumental settings are provided by Mannella and co-authors. [10] Diffraction patterns were processed with the open source QualX [12] and Fityk [13] softwares to determine sample bulk mineralogy (qualitative analysis) and to estimate the relative abundance of mineral phases based on main peak area ratios. Molar proportions of carbonate minerals were calculated by calibrating measured peak intensity ratios to reference patterns of carbonate minerals mixtures. [14]

Scanning Electron Microscopy

Field Emission Scanning Electron Microscopy (FESEM) imaging of 8 selected glacial, interglacial and transitional samples (Supplementary figure SF 2) was performed at the Department of Civil and Industrial Engineering of the University of Pisa (Italy) using a QUANTA FEG 450 equipped with a QUANTA XFlash 6|10 energy dispersive spectrometer (Thermo Fisher Scientific, USA) and operating at 15 kV accelerating voltage. Each sample was gently loosened, fixed on top of 12 mm ø adhesive carbon discs mounted on aluminium pin stubs and graphitized at the Department of Earth Sciences, University of Pisa.

Stable isotope analyses

Treated and untreated specimens were jointly analysed for their stable isotope composition ($\delta^{13}C$ and δ ^{18}O values) with an Analytical Precision AP2003 continuous-flow isotope-ratio mass spectrometer (Analytical Precision Ltd., Northwich, Cheshire, UK) at the School of Geography, The University of Melbourne, Australia.

Based on specimen TIC content, 0.18 ± 0.01 mg of carbonate equivalent were accurately weighed and sealed in septum vials. Vials containing 0.18 ± 0.01 mg of the NBS-19 (supplier: International Atomic Energy Agency – IAEA, Austria) international standard and of 3 internal standards calibrated against NBS18 and NBS19 (IAEA) using dual-inlet mass spectrometry (Finnigan MAT-251, Thermo Finnigan LLC, USA) at the Research School of Earth Sciences, The Australian National University, Melbourne, Australia (Prof. Russell Drysdale, personal communication, August 7, 2019) were added to the sample pool with a standard to sample ratio of 1:3. For each sample, untreated (***TQ***) and treated (***N*** and ***H***) specimens were placed in consecutive positions in the AP2003 autosampler tray and digested in 105% orthophosphoric (H_3PO_4) acid at 70 °C for 1h. The evolved CO_2 was introduced in the mass spectrometer in a stream of ultra-high purity He during a single acquisition. Measurements were conducted in four consecutive loops which were bracketed by reference gas injections. All the results were normalized and calibrated to the Vienna Pee Dee Belemnite (V-PDB) scale using an internal working standard (NEW1, Carrara Marble), calibrated against the international standards NBS18 and NBS19 (IAEA). Mean analytical precision (1σ) on internal standards was ± 0.05‰ and ± 0.10‰ for $\delta^{13}C$ and $\delta^{18}O$ values, respectively. Average analytical precision (2σ) on specimens was ± 0.13‰ and ± 0.20‰ for $\delta^{13}C$ and $\delta^{18}O$ values, respectively. Specimen analytical precision is reported as well to account for variations from mean uncertainty values (Tab. 1). To better evaluate shifts in isotopic ratio and compare different samples, we set to zero the isotopic ratio of untreated specimens and calculated the difference between treated and untreated specimens. Additionally, 7 samples from a fluvial terrace of the Giovenco River, the main tributary of former Lake Fucino, [10] were analysed according to the aforementioned procedure to characterise the isotopic composition of the local detritus.

Data analysis

For ease of discussion we introduce here some variables derived from the dataset. We calculated the variables ΔTOC and ΔTIC which represent the difference between the organic and inorganic carbon content, respectively, measured in treated ($_{tr.}$ can be $_{H}$ or $_{N}$) and untreated specimens (eq. 1 and 2).

$$\text{(eq. 1) } \Delta TOC = TOC_{tr.} - TOC_{TQ}$$

$$\text{(eq. 2) } \Delta TIC = TIC_{tr.} - TIC_{TQ}$$

As TOC analyses only provide the weight fraction of C bound to organic components and not a measure of the abundance of the organic components themselves, ΔTOC can only provide an approximation of the performance of the OM removal treatment. Similarly, ΔTIC is an approximation of the dissolution of carbonates during sample treatment. Negative ΔTIC values testifying the loss of carbonates from specimens during treatment, while positive ΔTIC values potentially indicate the preservation of carbonates, but could also conceal negative TIC balances. This is due to the fact that we cannot calculate the expected increase of TIC (wt %) related to the removal of OM and, possibly, of detritus. To do so, one should accurately know the masses (in g) of endogenic and allogenic minerals and of OM prior to and after treatment.

Δ(TOC/TN) is defined as the difference between the TOC/TN ratios measured in treated ($_{tr.}$ can be $_{H}$ or $_{N}$) and in the untreated specimens (eq. 3).

$$\text{(eq. 3) } \Delta(TOC/TN) = TOC/TN_{tr.} - TOC/TN_{TQ}$$

Null Δ(TOC/TN) values indicate congruent OM removal, whilst positive (negative) Δ(TOC/TN) values indicate preferential removal of OM sourcing mainly from the lake (catchment basin) during sample treatment.

$\Delta\delta^{13}C_{tr.}$ and $\Delta\delta^{18}O_{tr.}$ are the differences between the isotopic values of untreated and treated ($_{tr.}$ can be $_{H}$ or $_{N}$) samples after adding a large positive constant to operate with positive values only (eq. 4 and 5).

$$\text{(eq. 4) } \Delta\delta^{13}C = \delta^{13}C_{tr.}^{+} - \delta^{13}C_{TQ}^{+}$$

$$\text{(eq. 5) } \Delta\delta^{18}O = \delta^{18}O_{tr.}^{+} - \delta^{18}O_{TQ}^{+}$$

$\Delta\delta_{G\_tr.}$ is the geometric isotopic distance of the treated ($_{tr.}$ can be $_{H}$ or $_{N}$) specimen from the untreated specimen (eq. 6).

$$\text{(eq. 6) } \Delta\delta_{G\_tr.} = (\Delta\delta^{13}C_{tr.}{}^{\wedge 2} + \Delta\delta^{18}O_{tr.}{}^{\wedge 2})^{\wedge 1/2}$$

In the $\delta^{13}C$-$\delta^{18}O$ Cartesian plane, $\Delta\delta_{G\_tr.}$ represents the modulus of the vector conjoining the isotopic composition of the untreated specimen to that of treated ones.

Results

Sample properties are given in Table 1 and their main features are introduced hereafter. Samples were characterised by large variations in relative abundance and composition of the endogenic and detrital fractions. The endogenic fraction was made of variable proportions of polycrystalline aggregates of small, euhedral prisms of calcite, diatoms, sparse shell fragments (Supplementary figure SF 2) and OM remnants from lower aquatic plants (TOC/TN < 10). The detrital fraction can consist of multiple components, including OM from higher plants living in the catchment basin (TOC/TN > 40), volcanoclastic material, aeolian quartz, clay minerals, dolomite and, possibly, calcite. Calcite was the main mineralogical phase, except for sample 12, where the quartz/calcite (Qtz/Cal) ratio was >1 (Tab. 1). Dolomite-calcite molar proportions showed a large variety and range from values below detection limit (< 1 %) up to 8.7 % of the carbonate pool (Tab. 1: Dolomite $\mathrm{mol_{carb.}}$%).

Although the ***H*** and ***N*** treatments consistently reduced the OM content of the material, neither treatment was able to completely remove OM (Tab. 1). The TOC/TN ratios of untreated and treated specimens indicated that, compared to the ***H*** treatment, the ***N*** treatment was more effective in decomposing OM of lacustrine origin rather than OM from the catchment basin (Tab. 1). However, there was no clear correlation between the efficiency of OM removal (ΔTOC) and changes in TOC/TN values, neither for the ***H***, nor the ***N*** treatment. The ΔTIC values of ***H*** and ***N*** specimens (Tab. 1) suggest that 7 and 11 specimens unequivocally experienced a TIC loss during the respective treatments and that the ***H*** treatment produced greater TIC losses with respect to the ***N*** one.

Despite the incomplete OM removal and partial carbonate dissolution, both treatments increased the TIC/TOC ratio of all samples except for sample 16, which, during the ***H*** treatment, experienced the most severe carbonate loss (Tab. 1).

C and O isotope ratios showed large variations, with more negative (positive) δ^{13}C and more positive (negative) δ^{18}O values occurring during colder (warmer) periods.

In figure 1 we evaluated the effect of OM removal treatments on the isotopic composition of our samples by plotting the isotopic shift of treated specimens relatively to untreated specimens. We also plotted confidence intervals relative to measurements on untreated and treated material. The isotope composition of 6 and 8 specimens out of the 16 ***N*** and ***H*** ones, respectively, was not statistically consistent with that of untreated specimens (Fig. 1), either for δ^{13}C and/or δ^{18}O values ($^{\text{car.}}$ and $^{\text{ox.}}$ superscripts hereafter). We observed large isotopic shifts also for treated standards, which became significant for the δ^{18}O isotopic value of the ***H*** specimen (Fig. 1), unambiguously indicating a treatment-induced bias.

Sample specimens showing significant isotopic shifts were $2^{\text{ox.}}$, $5^{\text{car., ox.}}$, $6^{\text{car.}}$,$10^{\text{ox.}}$, $11^{\text{car.}}$, $13^{\text{car., ox.}}$ for the ***N*** series and $1^{\text{car.}}$, $3^{\text{ox.}}$, $5^{\text{car.}}$, $9^{\text{ox.}}$, $10^{\text{ox.}}$, $13^{\text{car., ox.}}$, $14^{\text{car.}}$, $16^{\text{ox.}}$ for the ***H*** series (Fig. 1). Significant isotopic shifts verified in spite of different sample characteristics and of selective carbonate and OM removal.

In figure 2 we evaluated the effect of dolomite content and changes in acid reaction times throughout mass spectrometric analysis on the reproducibility of samples. We did not observe any particular relationship between calcite-dolomite molar proportions, acid reaction times and isotope geometric distances. In our experimental setting small variations in reaction times did not affect sample reproducibility, regardless of the calcite-dolomite molar proportions.

In figure 3 we evaluated the possibility of a differential response of endogenic and detrital carbonates to pre-treatment techniques. We plotted the isotopic geometric distances of treated samples relatively to the untreated counterpart together with the isotopic composition of the local detrital array. The local detrital array is defined through isotopic analyses on limestone gravels, weathered carbonates and silt loam transported by Giovenco River. Shifts both in the $\delta^{13}C$ and $\delta^{18}O$ values detected in treated specimens, though not always statistically significant, define vectors of isotope geometric distance aligned onto common patterns pointing towards the isotopic composition of the local detrital array (Fig. 3). This is particularly the case for samples rich in detrital carbonate, while samples higher in endogenic carbonate do not show such a feature and evolve towards more negative $\delta^{13}C$ and/or $\delta^{18}O$ values.

Discussion

Observed changes in geochemical and mineralogical sediment characteristics reflect the glacial-interglacial cyclicity (Saalian-Eemian and Weichselian-Holocene): samples from warmer climate phases are characterised by the highest abundance of endogenic calcite and OM (probably from higher plants in the catchment basin), while samples from colder climate phases show larger proportions of detrital minerals (quartz and dolomite) as well as lower percentages of both endogenic and detrital OM. Consequently, samples from warmer (colder) climate phases lay closer to (further from) Oehlerich's TIC/TOC threshold.

Our study confirms previous findings about the limited effectiveness of the ***H*** and ***N*** pre-treatments in removing OM from geologic materials. [7] Furthermore, it provides the first evidence for incongruent OM removal during chemical pre-treatment: in general, we observed an increase in the TOC/TN ratio of the pre-treated material which indicates the preferential removal of low-nitrogen organic compounds typically found in aquatic and bacterial sources. [15] Compared to the ***H*** pre-treatment, the ***N*** pre-treatment produced a more pronounced increase in the TOC/TN ratio which could be explained by a different extent in the removal of readily movable inter-crystalline and strongly bound intra-crystalline OM.

The selective removal of different OM fractions during the ***H*** and ***N*** treatment could be explained by the extent of concomitant dissolution of biogenic carbonates. At a first glance it would appear that samples from the Eemian and late Saalian (2, 4, 5 and 7) with high TIC and TOC percentages preserved calcite, while samples from intermediate-glacial climate phases and the Holocene with overall smaller TIC percentages and higher content in detrital carbonate underwent carbonate loss. However, this could be an artefact due to the concomitant removal of larger amounts of OM in samples 2,4, 5 and 7. Previous studies have shown that H_2O_2 tends to dissolve carbonates [4, 6, 9, 17, 18], especially in its acidic form, [8] while NaOCl has little or no interaction with carbonates. [18] However, our data indicate that carbonates undergo dissolution also during the ***N*** pre-treatment, even though the extent of dissolution and the number of samples interested is smaller compared to the ***H*** pre-treatment. This can be explained by taking into account the substantial difference between natural and artificial lacustrine sediments. Differently from artificial mixtures of carbonates and OM, in natural lacustrine sediments, endogenic carbonates are intimately interspersed with lacustrine OM; we propose that oxidation of OM during sample pre-treatment could lead to a localised reduction in pH at the OM-carbonate interface thus promoting dissolution of endogenic carbonates.

Moreover, carbonate loss during pre-treatment could be induced by the large volumes of deionised water used to rinse the samples in our experiment (at least 1500 mL of deionised water per 500 mg of sample was used). Even if weakly acidic, deionised water has the potential to dissolve carbonates. [18, 19]

Previous studies have shown that both the ***H*** and ***N*** pre-treatments bias measurements towards lower isotopic values. [4,17] However, our data do not fully support this general rule. While the standard pre-treated with H_2O_2 clearly shows a depletion in the $\delta^{18}O$ value, isotopic values of treated samples can also be more positive than their untreated equivalents either for $\delta^{13}C$ and/or $\delta^{18}O$ (Fig. 1).

This discrepancy could be explained by the intrinsic difficulty of accurately measuring complex lacustrine sediments where carbonates of detrital and endogenic origin coexist. In our specific case, owing to slower reaction kinetics of dolomite compared to calcite during H_3PO_4 digestion, [20] progressively larger proportions of dolomite may have evolved CO_2 throughout sample analysis affecting the reproducibility of measurements among untreated and treated specimens. However, our data did not provide any evidence for a larger dolomite bias for those specimens that reacted for longer times (Fig. 2). This could be explained by the fact that all samples were reacted with H_3PO_4 at 70°C for the same amount of time and that further reaction pending mass spectrometric analysis occurred at progressively colder temperatures (down to room temperature, Melbourne $\approx$ 20°C). We propose that, owing to the strong and direct dependence of acid reaction rates of carbonates on temperature, [20] specimens of the same sample evolved comparable amounts of CO_2 from dolomite during acid digestion/reaction.

The measured $\delta^{13}C$ and $\delta^{18}O$ values are in accordance with changes in soil productivity and spatial extent as well as in the local hydrological regime in response to the glacial-interglacial cyclicity. The fact that vectors of isotope geometric distance of samples rich in detrital carbonate tend to align onto common patterns pointing towards the isotopic composition of the local detrital array (Fig. 3) provides an independent indication for the preferential dissolution of endogenic carbonates during the ***H*** and ***N*** sample treatments. This can be explained by the relatively small dimension of crystals and polycrystalline aggregates of endogenic calcite with respect to larger clasts of detrital calcite and dolomite. Conversely, the shift towards more negative δ^{13}C and/or δ^{18}O values of samples higher in endogenic carbonate (Fig. 3) is coherent with previous findings on biases induced by the ***H*** and ***N*** sample pre-treatments. [4,18]

Conclusions

Our study confirms that neither the NaOCl nor the H_2O_2 pre-treatment is capable of fully removing OM from lacustrine marls, and demonstrates that, when applied to complex lacustrine sediments made of detrital and endogenic carbonates, both treatments induce a preferential loss of endogenic carbonates. The interplay of biases introduced by pre-treatments themselves and by the differential dissolution of endogenic and detrital carbonates produces an unpredictable change in the isotopic composition of natural lacustrine samples. We suggest to evaluate on a case-by-case basis the suitability of NaOCl and H_2O_2 pre-treatments for OM removal from lacustrine marls and discourage their application in cases similar to the one presented here, where TIC/TOC ratios lay above Ohelerich's threshold and in which both endogenic and detrital (even if in negligible amounts) carbonates occur.

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Sample	Chronology		XRPD (semi)quantitative analysis		Specim.	Geochemistry				Treatment evaluation			Isotope ratios (‰ vs. V-PDB)			
	Age (ka)	Period	Qtz/Cal	Dolomite mol$_{carb.}$%		TIC %	TOC %	TN %	TOC/TN$_{atom}$	TIC/TOC	ΔTOC %	ΔTIC %	δ^{13}C	2σ	δ^{18}O	2σ
1	2.7	Holocene	0.37	2.7	***TQ***	3.19	5.63	0.67	7.31	0.57			2.32	0.09	-4.64	0.35
					H	2.53	2.54	0.20	11.17	1.00	-3.10	-0.67	1.77	0.15	-4.41	0.15
					N	4.20	2.64	0.10	23.64	1.59	-3.00	1.00	2.33	0.08	-4.55	0.14
2	117.2	Eemian	0.08	1.1	***TQ***	6.47	10.09	0.69	12.81	0.64			3.78	0.11	-4.50	0.07
					H	9.04	3.36	0.23	12.96	2.69	-6.73	2.57	3.74	0.15	-4.45	0.15
					N	8.85	1.53	0.06	21.61	5.79	-8.56	2.38	3.62	0.14	-4.35	0.05
3	64.5	Weichselian	0.54	3.9	***TQ***	2.73	3.58	0.37	8.45	0.76			1.47	0.09	-3.72	0.17
					H	3.75	0.87	0.10	7.96	4.33	-2.72	1.02	1.48	0.08	-3.40	0.13
					N	7.28	0.93	0.06	13.78	7.82	-2.65	4.55	1.25	0.14	-3.74	0.13
4	131.2	Saalian (T II)	0.32	3.6	***TQ***	6.17	7.41	0.48	13.51	0.83			1.00	0.08	-3.31	0.22
					H	6.59	3.38	0.18	16.62	1.95	-4.03	0.43	0.91	0.09	-3.57	0.27
					N	6.95	2.29	0.10	20.06	3.04	-5.12	0.79	0.98	0.11	-3.34	0.21
5	123.8	Eemian (LIG)	0.05	0.7	***TQ***	7.27	7.71	0.59	11.43	0.94			4.29	0.09	-5.53	0.21
					H	8.86	4.09	0.28	12.71	2.17	-3.62	1.60	5.07	0.04	-5.80	0.32
					N	8.68	2.73	0.11	22.09	3.18	-4.98	1.41	3.15	0.11	-5.48	0.07
6	181.3	Saalian (early)	0.53	3.9	***TQ***	3.67	3.48	0.38	8.12	1.06			2.32	0.04	-4.51	0.16
					H	3.04	0.66	0.11	5.37	4.62	-2.82	-0.63	2.40	0.12	-4.71	0.19
					N	3.82	1.36	0.12	9.92	2.82	-2.12	0.14	2.51	0.09	-4.41	0.22
7	121.1	Eemian (late LIG)	0.03	0.6	***TQ***	8.65	5.70	0.40	12.52	1.52			3.80	0.11	-4.90	0.08
					H	9.77	2.21	0.14	14.06	4.42	-3.49	1.12	3.68	0.04	-4.75	0.22
					N	9.78	2.04	0.10	18.24	4.79	-3.66	1.13	3.75	0.13	-4.78	0.23
8	68.0	Weichselian	0.55	3.1	***TQ***	3.19	1.59	0.28	4.99	2.00			1.44	0.06	-4.02	0.25
					H	3.24	0.61	0.08	7.01	4.28	-0.98	-0.57	1.65	0.29	-4.01	0.11
					N	4.96	1.30	0.09	12.38	2.81	-0.29	0.47	1.28	0.18	-4.15	0.22
9	5.7	Holocene	0.09	0.7	***TQ***	8.12	3.23	0.35	8.20	2.51			1.20	0.09	-5.63	0.10
					H	7.27	1.06	0.14	6.77	6.82	-2.17	-0.85	0.97	0.14	-5.70	0.05
					N	7.79	1.13	0.06	17.23	6.90	-2.10	-0.33	1.08	0.09	-5.86	0.25
10	25.1	Weichselian (LGM)	0.39	3.3	***TQ***	2.68	0.88	0.14	5.51	3.05			0.67	0.12	-2.42	0.21
					H	2.08	0.33	0.05	5.66	6.24	-0.55	-0.60	0.83	0.12	-3.25	0.22
					N	2.49	0.50	0.06	6.96	4.96	-0.38	-0.19	0.63	0.14	-3.01	0.10
11	162.7	Saalian	0.84	5.2	***TQ***	3.05	0.85	0.28	2.69	3.57			1.15	0.14	-3.67	0.14
					H	1.92	0.36	0.07	4.66	5.32	-0.49	-1.12	1.16	0.15	-3.95	0.26
					N	2.52	0.42	0.08	4.75	6.02	-0.43	-0.53	0.73	0.06	-4.04	0.40

12	25.6	Weichselian (LGM)	1.23	4.2	***TQ***	2.80	0.68	0.17	3.50	4.12			0.22	0.38	-2.83	0.17
					H	1.99	0.39	0.07	4.61	5.17	-0.29	-0.81	0.66	0.26	-2.90	0.05
					N	2.73	0.34	0.06	4.56	8.08	-0.34	-0.07	0.59	0.11	-3.32	0.43
13	20.1	Weichselian	0.88	8.7	***TQ***	3.29	0.73	0.18	3.55	4.51			0.57	0.21	-2.96	0.08
					H	2.60	0.41	0.05	7.22	6.32	-0.32	-0.69	0.89	0.06	-3.30	0.22
					N	3.46	0.62	0.05	10.11	5.58	-0.11	0.17	0.96	0.18	-3.32	0.29
14	150.1	Saalian	0.57	5.0	***TQ***	3.64	0.72	0.25	2.52	5.06			0.86	0.19	-3.05	0.26
					H	3.43	0.16	0.08	1.79	21.00	-0.56	-0.21	1.23	0.08	-3.37	0.48
					N	3.64	0.27	0.06	3.93	13.43	-0.45	-0.01	1.10	0.09	-3.11	0.16
15	139.3	Saalian	0.34	3.8	***TQ***	5.68	0.80	0.19	3.69	7.10			1.37	0.12	-2.63	0.28
					H	5.21	0.17	0.06	2.34	30.62	-0.63	-0.47	1.22	0.12	-2.97	0.43
					N	5.17	0.40	0.05	6.40	12.88	-0.40	-0.51	1.30	0.18	-2.75	0.17
16	144.3	Saalian	0.38	4.0	***TQ***	5.37	0.48	0.20	2.10	11.19			1.13	0.12	-2.63	0.12
					H	1.08	0.17	0.04	4.11	6.20	-0.31	-4.29	1.09	0.17	-2.90	0.27
					N	5.27	0.36	0.05	6.94	14.56	-0.12	-0.10	1.05	0.07	-2.84	0.27
STD	Carrara marble		-	-	***TQ***	-	-	-	-	-	-	-	2.13	0.10	-1.73	0.14
					H								1.88	0.22	-2.19	0.24
					N								2.23	0.17	-2.14	0.30

Tab. 1 - Sample characteristics and details. From left to right: sample number, chronological position in the context of past glacial-interglacial cycles as defined by Mannella and co-authors [10], relative mineral abundance and analyses on untreated (***TQ***) and H_2O_2 or NaOCl-treated specimens (***H*** and ***N***, respectively). Total inorganic and organic carbon (TIC and TOC), total nitrogen (TN) weight precentages are used to derive the TOC/TN ratio, the TIC/TOC ratio and relative changes in TIC and TOC associated with the ***H*** and ***N*** pre-treatments. Numbers in red highlight those cases in which pre-treatments clearly failed to remove TOC while preserving TIC and thus increasing the TIC/TOC ratio. In the last four columns $\delta^{13}C$ and $\delta^{18}O$ (‰ vs. V-PDB) values with relative confidence intervals are shown.

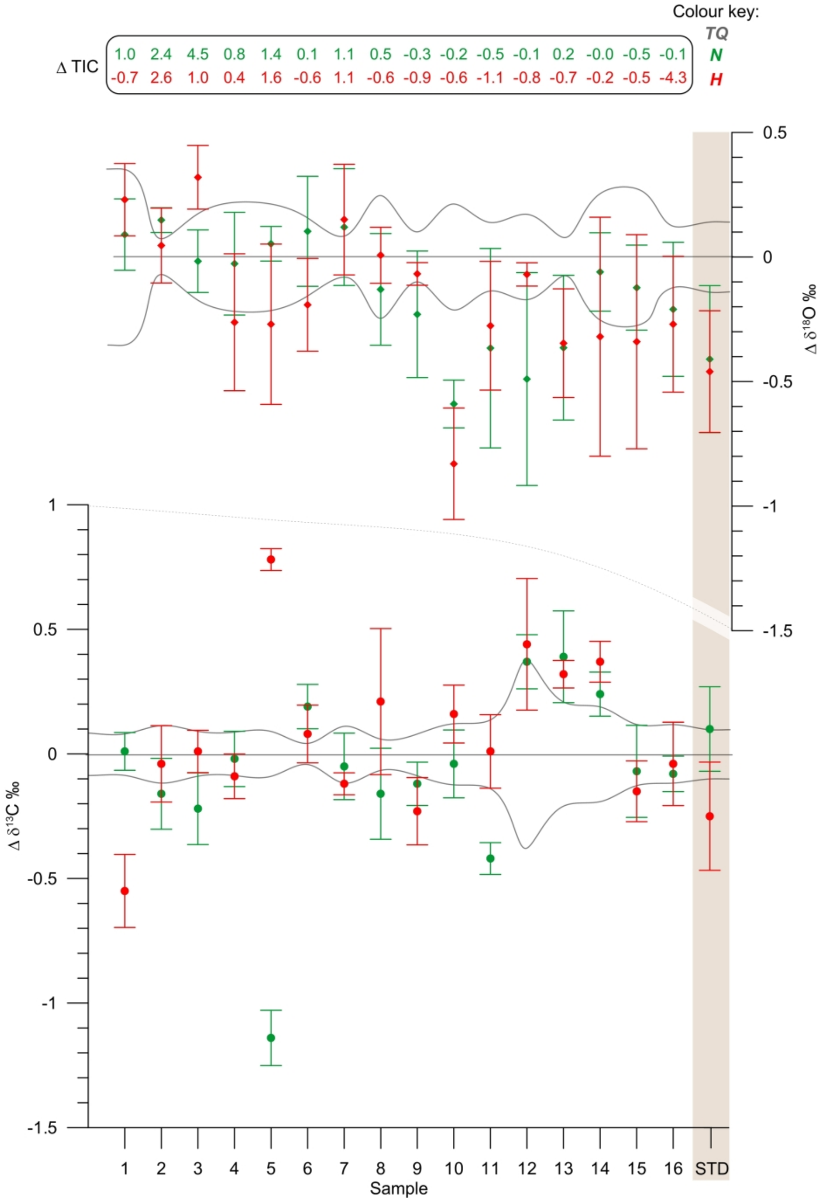


Fig. 1 Whisker plot showing isotopic composition of untreated specimens set to zero and the relative isotopic shifts of treated specimens. 2σ confidence levels for $\delta^{13}C$ and $\delta^{18}O$ isotope values are displayed as black line envelope for untreated specimens and as red and green bars for treated specimens.

200x293mm (300 x 300 DPI)

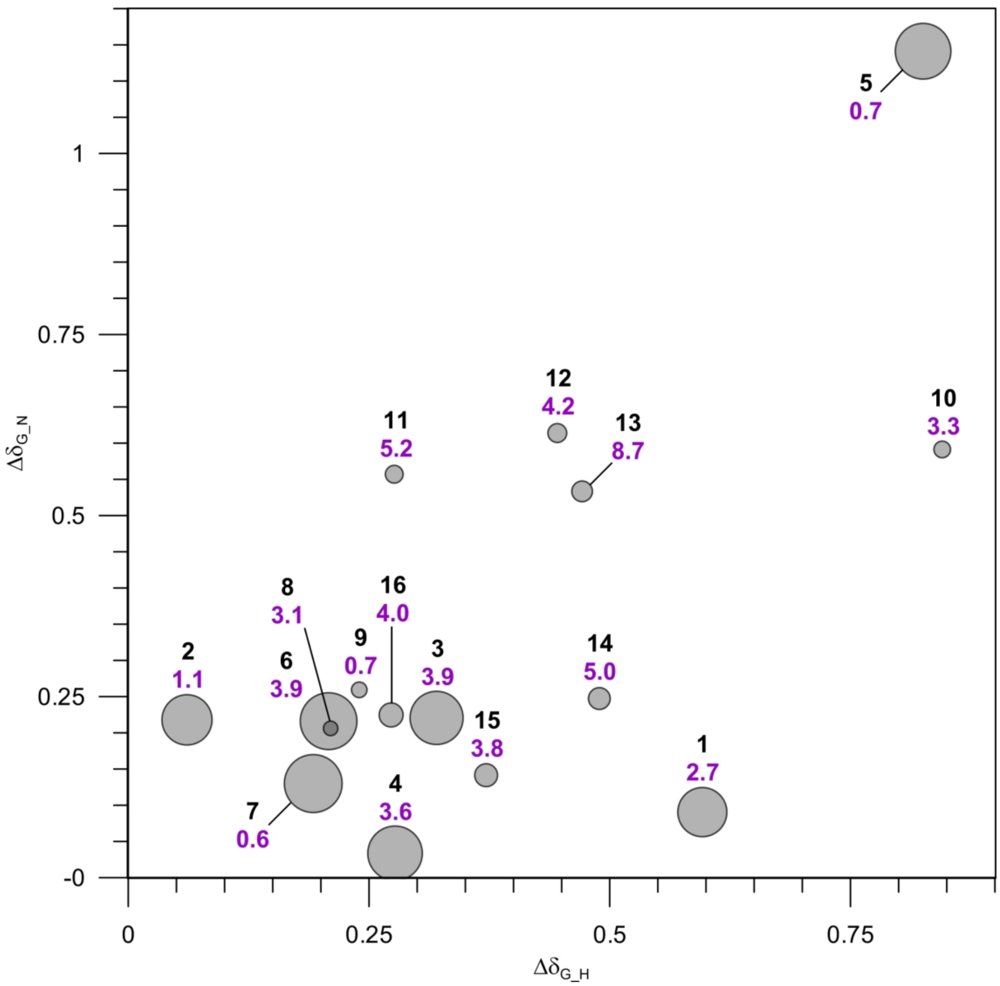


Fig. 2 Isotopic geometric distances of ***H*** and ***N*** specimens. Black labels refer to sample number, magenta labels refer to content in dolomite (molar percentages of dolomite in the carbonate pool). Bubble size refers to reaction times during sample awaiting for MS analysis, respectively. The size of the bubble is proportional to the time of reaction.

175x170mm (300 x 300 DPI)

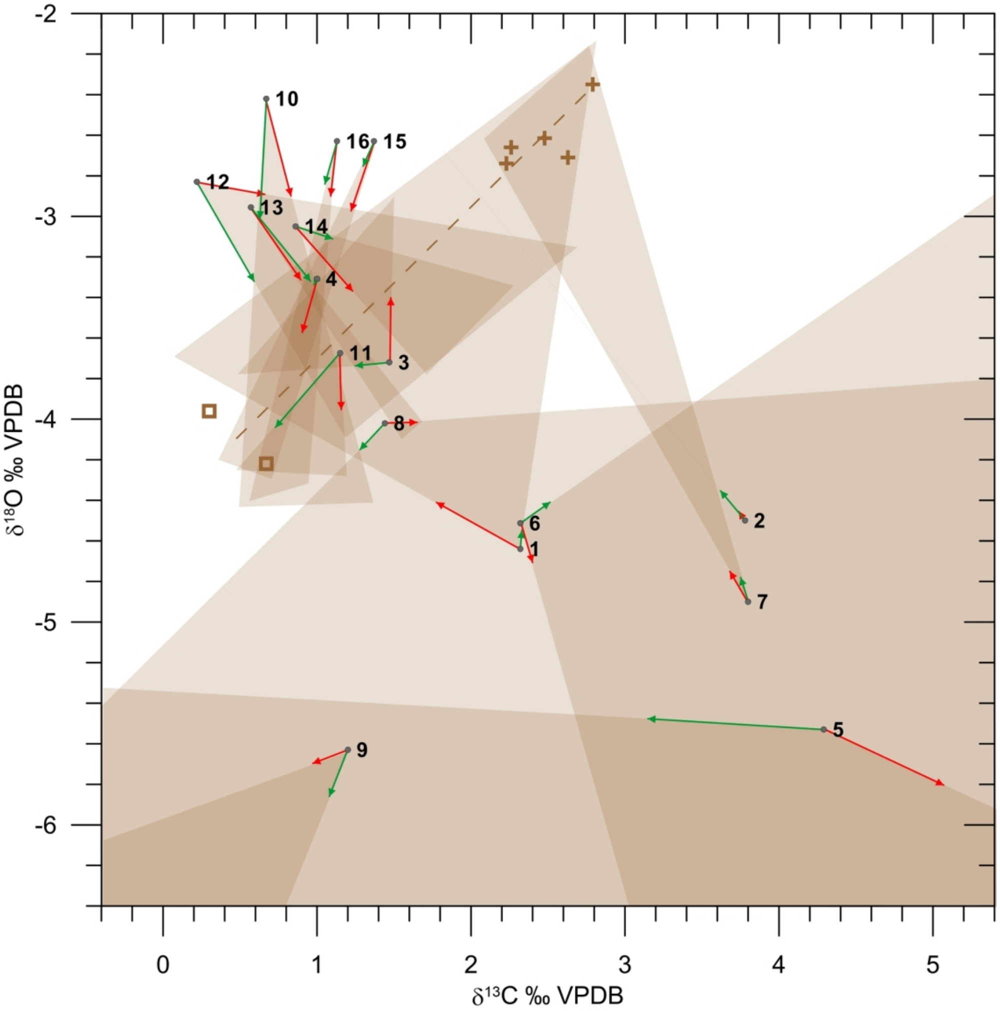
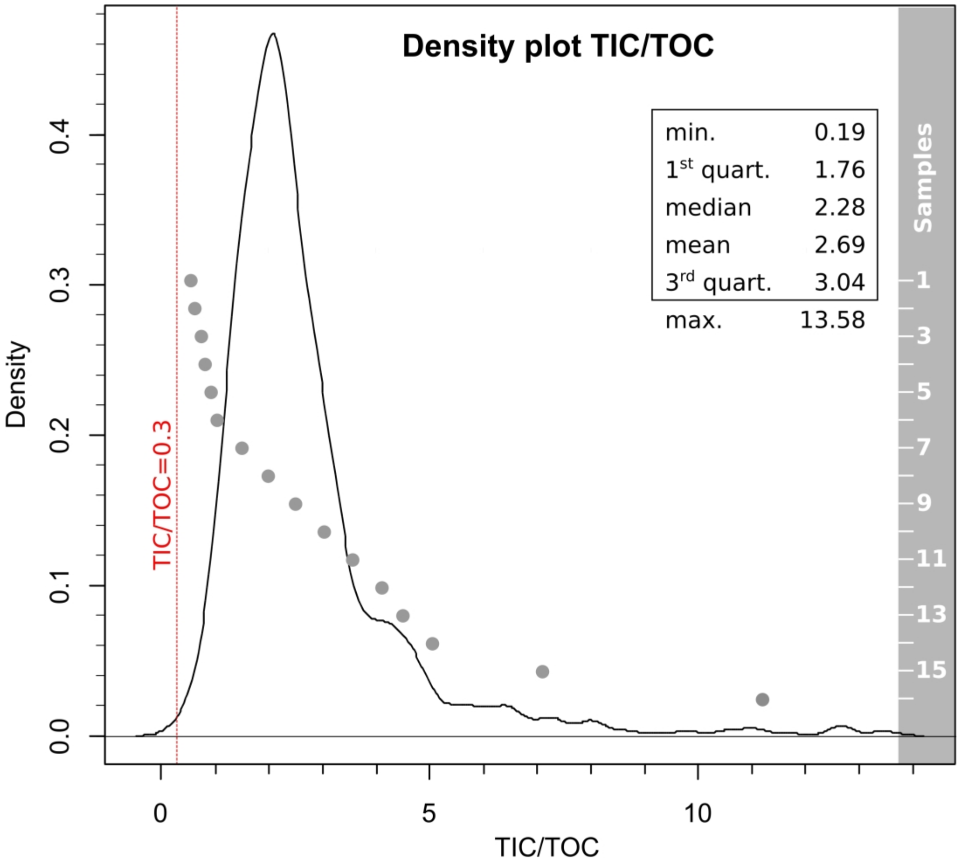


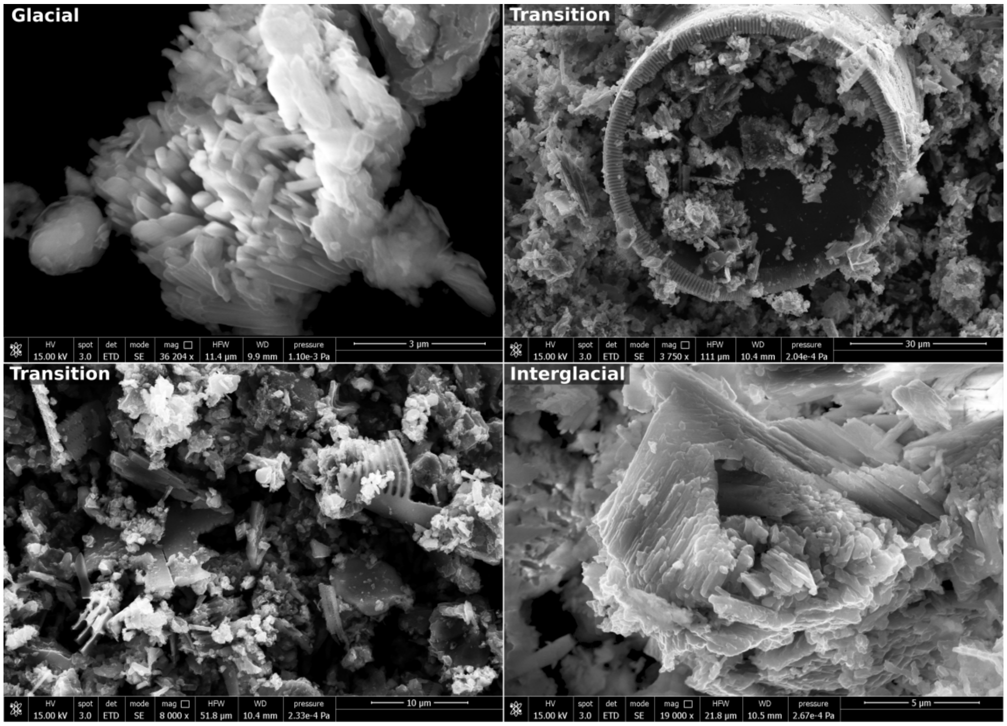
Fig. 3: Brown crosses and squares indicate the isotopic value of limestone gravels and of weathered limestone gravels transported by the Giovenco River, respectively. The dashed brown line represents the local isotopic detrital array (different proportions of unaltered and weathered limestone will result in a detritus having an isotopic compositions laying on this line). Vectors of isotopic geometric distance (untreated-treated specimen: green treatment with NaOCl, red treatment with H_2O_2) are plotted for each sample. These vectors tend to align on common patterns which define cones of evolution (brown shaded areas) of isotopic composition. Cones of evolution point towards the local isotopic detrital array and precisely enclose measured values.

170x172mm (300 x 300 DPI)



Supplementary figure SF 1: Density plot of TIC/TOC values of the F1-F3 sedimentary record (black line) during the last two glacial-interglacial cycles superposed with TIC/TOC values of selected samples (grey dots). The threshold for organic-biased/unbiased analyses by Oehlerich and coauthors[5] is also shown (red line). The diagram clearly shows that, for most part of its history, palaeo-lake Fucino has laid sediments with TIC/TOC ratios well above the 0.3 threshold.

297x268mm (300 x 300 DPI)



Supplementary figure SF 2: FESEM images of sediments laid down by palaeolake Fucino during glacial, transitional and interglacial phases. Endogenic calcite tends to form polycrystalline aggregates of small, euhedral prisms. Remnants from sponges and lacustrine algae are a common feature of warmer phases.

299x215mm (300 x 300 DPI)