

1  
2 **1 Effects of organic removal techniques prior to carbonate stable isotope analysis of**  
3  
4  
5 **2 lacustrine marls: a case study from palaeo-lake Fucino (central Italy)**  
6  
7  
8  
9

10  
11 **3**  
12  
13 4 G. Mannella\*, G. Zanchetta, E. Regattieri, N. Perchiazzi, N.R. Drysdale, B. Giaccio, M.J.  
14  
15  
16  
17  
18 5 Leng, B. Wagner  
19  
20  
21  
22

23  
24  
25  
26 6  
27  
28 7 \* Author for Correspondence: Giorgio Mannella, Dipartimento di Scienze della Terra,  
29  
30  
31 8 Università di Pisa, Via Santa Maria 53, 56125 Pisa, Italy. Email:  
32  
33 9 [giorgio.mannella@dst.unipi.it](mailto:giorgio.mannella@dst.unipi.it)  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

10  
11 **Abstract**

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

17 METHODS: Sixteen samples from the Fucino F1-F3 lacustrine succession (Abruzzo,  
18 central Italy) were characterised for their mineralogical and geochemical composition and  
19 split in three identical aliquots. One aliquot was left untreated while the remaining two were  
20 treated with NaOCl and H<sub>2</sub>O<sub>2</sub> dilutions. The same treatment was applied to an internal  
21 standard constituted of pure Carrara marble. Treated and untreated samples were  
22 analysed for their carbon ( $\delta^{13}\text{C}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotope compositions using an

1  
2 23 Analytical Precision AP2003 isotope ratio mass spectrometer. RESULTS: The samples  
3  
4  
5 24 had variable proportions of endogenic and detrital components, the detrital portion being  
6  
7  
8 25 more (less) abundant during colder (warmer) climate phases. We observed that neither the  
9  
10  
11 26 NaOCl nor the H<sub>2</sub>O<sub>2</sub> treatment were able to completely remove OM and therefore there  
12  
13 27 was selective removal of compounds within the OM pool. A possible effect of pre-  
14  
15  
16 28 treatment is the loss of carbonates intimately interspersed within OM, as suggested by the  
17  
18 29 evolution of isotopic ratios towards the local detrital array.

20  
21 30 CONCLUSIONS: Our study highlights sample-specific changes in geochemistry  
22  
23 31 associated with sample pre-treatments, however such changes do not seem to lead to  
24  
25  
26 32 either systematic and/or predictable isotopic shifts. We suggest that the suitability of  
27  
28  
29 33 NaOCl or H<sub>2</sub>O<sub>2</sub> pre-treatments for OM removal should be evaluated on a case-by-case  
30  
31 34 basis. In the specific case of lacustrine marls from palaeo-lake Fucino containing relatively  
32  
33  
34 35 low amounts of OM and in which both detrital and endogenic carbonates occur, both pre-  
35  
36 36 treatments should be avoided.

37  
38  
39 37

## 41 38 Introduction

42  
43  
44 39

45  
46 40 Stable carbon and oxygen isotope ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) values of biogenic and endogenic  
47  
48  
49 41 carbonates are commonly used in palaeoenvironmental studies as proxies for hydrological  
50  
51  
52 42 and environmental processes.<sup>[1,2]</sup> The most common analytical procedure involves  
53  
54  
55 43 digestion of bulk sample material in orthophosphoric acid (H<sub>3</sub>PO<sub>4</sub>) followed by isotope ratio  
56  
57 44 mass spectrometry on the evolved carbon dioxide (CO<sub>2</sub>). Although highly precise, the  
58  
59  
60

1  
2 45 accuracy of the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values can be biased by organic matter (OM) naturally  
3  
4  
5 46 occurring in lake sediments. During acid reaction, OM can evolve volatile organic  
6  
7 47 impurities (e.g.,  $\text{C}_2\text{H}_5\text{OH}$ ,  $\text{CS}$ ,  $\text{BCl}_3$ ,  $\text{NO}_2$ ,  $\text{N}_2\text{O}$ ) [3,4,5] which have the potential to increase  
8  
9  
10 48 measured  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . [4] Furthermore,  $\text{CO}_2$  can evolve directly from OM possibly  
11  
12 49 introducing  $\text{CO}_2$  with an isotopic value very different to that of the carbonate. [2, 6] For these  
13  
14  
15 50 reasons, various physical and chemical pre-treatment methods have been used to remove  
16  
17  
18 51 OM from geologic materials in palaeoenvironmental and pedological studies. [4, 7] Except  
19  
20  
21 52 for the method recently proposed by Falster and co-authors, [8] there is no universally  
22  
23 53 accepted method for completely removing OM and, at the same time, not changing the  
24  
25  
26 54 isotope composition of the sample to be analysed [7, 9].  
27

28 55  
29  
30  
31 56 Studies on the effects induced by sample pre-treatment on lacustrine carbonates have so  
32  
33 57 far focused on artificial sediments obtained by mixing pure crystalline calcite (e.g., shells,  
34  
35 58 synthetic calcite, ground marble or limestone) with variable amounts of organic  
36  
37  
38 59 compounds which are typically found in lacustrine sediments, [4, 5] or with decarbonated  
39  
40  
41 60 lacustrine sediments. [8] By analysing artificial calcite- OM mixtures with variable total  
42  
43  
44 61 inorganic carbon/total organic carbon (TIC/TOC) ratios, Oehlerich and co-authors  
45  
46 62 observed that  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values are biased only up to TIC/TOC = 0.3, and advised to  
47  
48  
49 63 pre-treat only samples laying below this threshold. [5] However, chemical pre-treatment  
50  
51  
52 64 with sodium hypochlorite ( $\text{NaOCl}$ ) or hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is still routinely applied in  
53  
54  
55 65 palaeolimnological studies as its effect on geologic materials is poorly understood. Here  
56  
57 66 we apply  $\text{NaOCl}$  and  $\text{H}_2\text{O}_2$  to natural samples from the palaeo-lake Fucino sedimentary  
58  
59  
60

1  
2 67 succession in Abruzzo (central Italy) <sup>[11]</sup> as well as to a reference material. We compare  
3  
4  
5 68 independent measurements on untreated and treated samples to evaluate the effect of the  
6  
7  
8 69 two OM removal methods.

9  
10 70

## 11 12 13 71 **Materials and methods**

14  
15 72

16  
17  
18 73 We analysed 16 samples from the Fucino palaeo-lake sedimentary succession and a  
19  
20  
21 74 single reference material consisting of pure Carrara Marble (Marmo Statuario, labelled as  
22  
23 75 Standard or "STD"). Selected samples lay above the organic-bias threshold proposed by  
24  
25  
26 76 Oehlerich and co-authors <sup>[5]</sup> and are representative of the whole spectrum of TIC/TOC  
27  
28 77 values of the last two glacial-interglacial cycles. <sup>[10]</sup> (Supplementary figure SF 1). For each  
29  
30  
31 78 sample, 1500 mg of loose material were divided into 3 identical aliquots and placed in three  
32  
33  
34 79 separate vials for isotope and geochemical analyses on untreated sample (specimens  
35  
36 80 labelled "*TQ*") or after reaction with 0.5 M NaOCl (specimens labelled "*N*") or 30% H<sub>2</sub>O<sub>2</sub>  
37  
38  
39 81 (specimens labelled "*H*") dilutions. <sup>[7]</sup>

### 40 41 82 Sample treatment

42  
43  
44 83 Approximately 500 mg of subsamples were placed in 500 mL beakers and mixed with 50  
45  
46  
47 84 mL of NaOCl or H<sub>2</sub>O<sub>2</sub>. After reacting for 24 h at room temperature (Pisa, 25°C), each  
48  
49  
50 85 beaker was made up to volume (500 mL) with deionised water. After the sediment had  
51  
52  
53 86 settled, the deionised water was decanted and replenished. This was done three times,  
54  
55  
56 87 using a total of 1500 mL of deionised water per sample. After the final rinse, the beaker  
57  
58  
59 88 was partially emptied and the remaining ca. 50–100 mL were filtered with laboratory paper  
60

1  
2 89 filters. The filtered material was dried in oven at 50 °C for 72 h, loosened and gently  
3  
4  
5 90 ground in an agate mortar.

6  
7 91

8  
9  
10 92 Geochemical analyses

11  
12 93 Geochemical analyses were conducted at the Institute of Geology and Mineralogy of the  
13  
14  
15 94 University of Cologne (Germany). Total nitrogen (TN) was determined with a Vario Micro  
16  
17  
18 95 cube combustion CNS elemental analyser (Elementar Analysensysteme GmbH,  
19  
20  
21 96 Germany), while TC and TIC were measured with a DIMATOC 200 (Dimatec  
22  
23 97 Analysentechnik GmbH, Germany) according to the method specified by Francke and co-  
24  
25  
26 98 authors. <sup>[11]</sup> The total organic carbon (TOC) content was calculated from the difference  
27  
28  
29 99 between TC and TIC.

30  
31 100

32  
33 101 Mineralogical analyses

34  
35  
36 102 X-ray powder diffraction (XRPD) analyses were performed at the Department of Earth  
37  
38  
39 103 Sciences, University of Pisa (Italy) using a Bruker D2 Phaser (Bruker Corporation, USA).  
40  
41  
42 104 Details on instrumental settings are provided by Mannella and co-authors. <sup>[10]</sup> Diffraction  
43  
44 105 patterns were processed with the open source QualX <sup>[12]</sup> and Fityk <sup>[13]</sup> softwares to  
45  
46  
47 106 determine sample bulk mineralogy (qualitative analysis) and to estimate the relative  
48  
49  
50 107 abundance of mineral phases based on main peak area ratios. Molar proportions of  
51  
52  
53 108 carbonate minerals were calculated by calibrating measured peak intensity ratios to  
54  
55  
56 109 reference patterns of carbonate minerals mixtures. <sup>[14]</sup>

57 110  
58  
59  
60

1  
2 111 Scanning Electron Microscopy

3  
4  
5 112 Field Emission Scanning Electron Microscopy (FESEM) imaging of 8 selected glacial,

6  
7 113 interglacial and transitional samples (Supplementary figure SF 2) was performed at the

8  
9  
10 114 Department of Civil and Industrial Engineering of the University of Pisa (Italy) using a

11  
12 115 QUANTA FEG 450 equipped with a QUANTA XFlash 6|10 energy dispersive spectrometer

13  
14  
15 116 (Thermo Fisher Scientific, USA) and operating at 15 kV accelerating voltage. Each sample

16  
17 117 was gently loosened, fixed on top of 12 mm  $\varnothing$  adhesive carbon discs mounted on

18  
19  
20 118 aluminium pin stubs and graphitized at the Department of Earth Sciences, University of

21  
22  
23 119 Pisa.

24  
25  
26 120

27  
28 121 Stable isotope analyses

29  
30  
31 122 Treated and untreated specimens were jointly analysed for their stable isotope

32  
33 123 composition ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values) with an Analytical Precision AP2003 continuous-flow

34  
35  
36 124 isotope-ratio mass spectrometer (Analytical Precision Ltd., Northwich, Cheshire, UK) at the

37  
38  
39 125 School of Geography, The University of Melbourne, Australia.

40  
41 126 Based on specimen TIC content,  $0.18 \pm 0.01$  mg of carbonate equivalent were accurately

42  
43  
44 127 weighed and sealed in septum vials. Vials containing  $0.18 \pm 0.01$  mg of the NBS-19

45  
46  
47 128 (supplier: International Atomic Energy Agency – IAEA, Austria) international standard and

48  
49  
50 129 of 3 internal standards calibrated against NBS18 and NBS19 (IAEA) using dual-inlet mass

51  
52 130 spectrometry (Finnigan MAT-251, Thermo Finnigan LLC, USA) at the Research School of

53  
54  
55 131 Earth Sciences, The Australian National University, Melbourne, Australia (Prof. Russell

56  
57 132 Drysdale, personal communication, August 7, 2019) were added to the sample pool with a

1  
2 133 standard to sample ratio of 1:3. For each sample, untreated (*TQ*) and treated (*N* and *H*)  
3  
4  
5 134 specimens were placed in consecutive positions in the AP2003 autosampler tray and  
6  
7  
8 135 digested in 105% orthophosphoric ( $\text{H}_3\text{PO}_4$ ) acid at 70 °C for 1h. The evolved  $\text{CO}_2$  was  
9  
10 136 introduced in the mass spectrometer in a stream of ultra-high purity He during a single  
11  
12  
13 137 acquisition. Measurements were conducted in four consecutive loops which were  
14  
15 138 bracketed by reference gas injections. All the results were normalized and calibrated to the  
16  
17  
18 139 Vienna Pee Dee Belemnite (V-PDB) scale using an internal working standard (NEW1,  
19  
20 140 Carrara Marble), calibrated against the international standards NBS18 and NBS19 (IAEA).  
21  
22  
23 141 Mean analytical precision ( $1\sigma$ ) on internal standards was  $\pm 0.05\text{‰}$  and  $\pm 0.10\text{‰}$  for  $\delta^{13}\text{C}$   
24  
25  
26 142 and  $\delta^{18}\text{O}$  values, respectively. Average analytical precision ( $2\sigma$ ) on specimens was  $\pm$   
27  
28  
29 143  $0.13\text{‰}$  and  $\pm 0.20\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values, respectively. Specimen analytical precision  
30  
31 144 is reported as well to account for variations from mean uncertainty values (Tab. 1). To  
32  
33  
34 145 better evaluate shifts in isotopic ratio and compare different samples, we set to zero the  
35  
36 146 isotopic ratio of untreated specimens and calculated the difference between treated and  
37  
38  
39 147 untreated specimens. Additionally, 7 samples from a fluvial terrace of the Giovenco River,  
40  
41 148 the main tributary of former Lake Fucino, <sup>[10]</sup> were analysed according to the  
42  
43  
44 149 aforementioned procedure to characterise the isotopic composition of the local detritus.  
45  
46  
47 150

#### 49 151 Data analysis

50  
51  
52 152 For ease of discussion we introduce here some variables derived from the dataset. We  
53  
54  
55 153 calculated the variables  $\Delta\text{TOC}$  and  $\Delta\text{TIC}$  which represent the difference between the  
56  
57  
58  
59  
60

1  
2 154 organic and inorganic carbon content, respectively, measured in treated (<sub>tr.</sub> can be <sub>H</sub> or <sub>N</sub>)  
3  
4  
5 155 and untreated specimens (eq. 1 and 2).

6  
7 156

8  
9  
10 157 
$$\text{(eq. 1) } \Delta\text{TOC} = \text{TOC}_{\text{tr.}} - \text{TOC}_{\text{TQ}}$$

11  
12 158 
$$\text{(eq. 2) } \Delta\text{TIC} = \text{TIC}_{\text{tr.}} - \text{TIC}_{\text{TQ}}$$

13  
14  
15 159

16  
17  
18 160 As TOC analyses only provide the weight fraction of C bound to organic components and  
19  
20 161 not a measure of the abundance of the organic components themselves,  $\Delta\text{TOC}$  can only  
21  
22 162 provide an approximation of the performance of the OM removal treatment. Similarly,  $\Delta\text{TIC}$   
23  
24 163 is an approximation of the dissolution of carbonates during sample treatment. Negative  
25  
26 164  $\Delta\text{TIC}$  values testifying the loss of carbonates from specimens during treatment, while  
27  
28 165 positive  $\Delta\text{TIC}$  values potentially indicate the preservation of carbonates, but could also  
29  
30 166 conceal negative TIC balances. This is due to the fact that we cannot calculate the  
31  
32 167 expected increase of TIC (wt %) related to the removal of OM and, possibly, of detritus. To  
33  
34 168 do so, one should accurately know the masses (in g) of endogenic and allogenic minerals  
35  
36 169 and of OM prior to and after treatment.

37  
38  
39 170  $\Delta(\text{TOC}/\text{TN})$  is defined as the difference between the TOC/TN ratios measured in treated (<sub>tr.</sub>  
40  
41 171 can be <sub>H</sub> or <sub>N</sub>) and in the untreated specimens (eq. 3).

42  
43  
44 172

45  
46 173 
$$\text{(eq. 3) } \Delta(\text{TOC}/\text{TN}) = \text{TOC}/\text{TN}_{\text{tr.}} - \text{TOC}/\text{TN}_{\text{TQ}}$$

47  
48  
49 174



175 Null  $\Delta(\text{TOC}/\text{TN})$  values indicate congruent OM removal, whilst positive (negative)

176  $\Delta(\text{TOC}/\text{TN})$  values indicate preferential removal of OM sourcing mainly from the lake

177 (catchment basin) during sample treatment.

178  $\Delta\delta^{13}\text{C}_{\text{tr.}}$  and  $\Delta\delta^{18}\text{O}_{\text{tr.}}$  are the differences between the isotopic values of untreated and

179 treated ( $\text{tr.}$  can be  $\text{H}$  or  $\text{N}$ ) samples after adding a large positive constant to operate with

180 positive values only (eq. 4 and 5).

181

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

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

184

185  $\Delta\delta_{\text{G.tr.}}$  is the geometric isotopic distance of the treated ( $\text{tr.}$  can be  $\text{H}$  or  $\text{N}$ ) specimen from the

186 untreated specimen (eq. 6).

187

188 (eq. 6)  $\Delta\delta_{\text{G.tr.}} = (\Delta\delta^{13}\text{C}_{\text{tr.}}^2 + \Delta\delta^{18}\text{O}_{\text{tr.}}^2)^{1/2}$

189

190 In the  $\delta^{13}\text{C}$ - $\delta^{18}\text{O}$  Cartesian plane,  $\Delta\delta_{\text{G.tr.}}$  represents the modulus of the vector conjoining

191 the isotopic composition of the untreated specimen to that of treated ones.

192

## 193 Results

194

195 Sample properties are given in Table 1 and their main features are introduced hereafter.

196 Samples were characterised by large variations in relative abundance and composition of

1  
2 197 the endogenic and detrital fractions. The endogenic fraction was made of variable  
3  
4  
5 198 proportions of polycrystalline aggregates of small, euhedral prisms of calcite, diatoms,  
6  
7  
8 199 sparse shell fragments (Supplementary figure SF 2) and OM remnants from lower aquatic  
9  
10 200 plants (TOC/TN < 10). The detrital fraction can consist of multiple components, including  
11  
12  
13 201 OM from higher plants living in the catchment basin (TOC/TN > 40), volcanoclastic  
14  
15 202 material, aeolian quartz, clay minerals, dolomite and, possibly, calcite. Calcite was the  
16  
17  
18 203 main mineralogical phase, except for sample 12, where the quartz/calcite (Qtz/Cal) ratio  
19  
20  
21 204 was >1 (Tab. 1). Dolomite-calcite molar proportions showed a large variety and range from  
22  
23 205 values below detection limit (< 1 %) up to 8.7 % of the carbonate pool (Tab. 1: Dolomite  
24  
25  
26 206 mol<sub>carb.</sub> %).

27  
28 207

29  
30  
31 208 Although the *H* and *N* treatments consistently reduced the OM content of the material,  
32  
33  
34 209 neither treatment was able to completely remove OM (Tab. 1). The TOC/TN ratios of  
35  
36 210 untreated and treated specimens indicated that, compared to the *H* treatment, the *N*  
37  
38  
39 211 treatment was more effective in decomposing OM of lacustrine origin rather than OM from  
40  
41 212 the catchment basin (Tab. 1). However, there was no clear correlation between the  
42  
43  
44 213 efficiency of OM removal ( $\Delta$ TOC) and changes in TOC/TN values, neither for the *H*, nor  
45  
46  
47 214 the *N* treatment. The  $\Delta$ TIC values of *H* and *N* specimens (Tab. 1) suggest that 7 and 11  
48  
49 215 specimens unequivocally experienced a TIC loss during the respective treatments and that  
50  
51  
52 216 the *H* treatment produced greater TIC losses with respect to the *N* one.

1  
2 217 Despite the incomplete OM removal and partial carbonate dissolution, both treatments  
3  
4  
5 218 increased the TIC/TOC ratio of all samples except for sample 16, which, during the *H*  
6  
7  
8 219 treatment, experienced the most severe carbonate loss (Tab. 1).  
9

10 220  
11  
12  
13 221 C and O isotope ratios showed large variations, with more negative (positive)  $\delta^{13}\text{C}$  and  
14  
15 222 more positive (negative)  $\delta^{18}\text{O}$  values occurring during colder (warmer) periods.  
16  
17

18 223

19  
20  
21 224 In figure 1 we evaluated the effect of OM removal treatments on the isotopic composition  
22  
23 225 of our samples by plotting the isotopic shift of treated specimens relative to untreated  
24  
25  
26 226 specimens. We also plotted confidence intervals relative to measurements on untreated  
27  
28  
29 227 and treated material. The isotope composition of 6 and 8 specimens out of the 16 *N* and *H*  
30  
31 228 ones, respectively, was not statistically consistent with that of untreated specimens (Fig.  
32  
33  
34 229 1), either for  $\delta^{13}\text{C}$  and/or  $\delta^{18}\text{O}$  values (<sup>car.</sup> and <sup>ox.</sup> superscripts hereafter). We observed  
35  
36 230 large isotopic shifts also for treated standards, which became significant for the  $\delta^{18}\text{O}$   
37  
38  
39 231 isotopic value of the *H* specimen (Fig. 1), unambiguously indicating a treatment-induced  
40  
41 232 bias.

42  
43  
44 233 Sample specimens showing significant isotopic shifts were 2<sup>ox.</sup>, 5<sup>car., ox.</sup>, 6<sup>car.</sup>, 10<sup>ox.</sup>, 11<sup>car.</sup>,

45  
46 234 13<sup>car., ox.</sup> for the *N* series and 1<sup>car.</sup>, 3<sup>ox.</sup>, 5<sup>car.</sup>, 9<sup>ox.</sup>, 10<sup>ox.</sup>, 13<sup>car., ox.</sup>, 14<sup>car.</sup>, 16<sup>ox.</sup> for the *H* series

47  
48  
49 235 (Fig. 1). Significant isotopic shifts verified in spite of different sample characteristics and of  
50  
51  
52 236 selective carbonate and OM removal.  
53

54 237  
55  
56  
57  
58  
59  
60

1  
2 238 In figure 2 we evaluated the effect of dolomite content and changes in acid reaction times  
3  
4  
5 239 throughout mass spectrometric analysis on the reproducibility of samples. We did not  
6  
7  
8 240 observe any particular relationship between calcite-dolomite molar proportions, acid  
9  
10 241 reaction times and isotope geometric distances. In our experimental setting small  
11  
12  
13 242 variations in reaction times did not affect sample reproducibility, regardless of the calcite-  
14  
15 243 dolomite molar proportions.

16  
17  
18 244  
19  
20 245 In figure 3 we evaluated the possibility of a differential response of endogenic and detrital  
21  
22  
23 246 carbonates to pre-treatment techniques. We plotted the isotopic geometric distances of  
24  
25  
26 247 treated samples relatively to the untreated counterpart together with the isotopic  
27  
28  
29 248 composition of the local detrital array. The local detrital array is defined through isotopic  
30  
31 249 analyses on limestone gravels, weathered carbonates and silt loam transported by  
32  
33  
34 250 Giovenco River. Shifts both in the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values detected in treated specimens,  
35  
36 251 though not always statistically significant, define vectors of isotope geometric distance  
37  
38  
39 252 aligned onto common patterns pointing towards the isotopic composition of the local  
40  
41 253 detrital array (Fig. 3). This is particularly the case for samples rich in detrital carbonate,  
42  
43  
44 254 while samples higher in endogenic carbonate do not show such a feature and evolve  
45  
46 255 towards more negative  $\delta^{13}\text{C}$  and/or  $\delta^{18}\text{O}$  values.

47  
48  
49 256

50  
51  
52 257 **Discussion**

53  
54 258  
55  
56  
57  
58  
59  
60

1  
2 259 Observed changes in geochemical and mineralogical sediment characteristics reflect the  
3  
4  
5 260 glacial-interglacial cyclicity (Saalian-Eemian and Weichselian-Holocene): samples from  
6  
7  
8 261 warmer climate phases are characterised by the highest abundance of endogenic calcite  
9  
10 262 and OM (probably from higher plants in the catchment basin), while samples from colder  
11  
12  
13 263 climate phases show larger proportions of detrital minerals (quartz and dolomite) as well  
14  
15 264 as lower percentages of both endogenic and detrital OM. Consequently, samples from  
16  
17  
18 265 warmer (colder) climate phases lay closer to (further from) Oehlerich's TIC/TOC threshold.  
19  
20  
21 266 Our study confirms previous findings about the limited effectiveness of the *H* and *N* pre-  
22  
23 267 treatments in removing OM from geologic materials. <sup>[7]</sup> Furthermore, it provides the first  
24  
25  
26 268 evidence for incongruent OM removal during chemical pre-treatment: in general, we  
27  
28  
29 269 observed an increase in the TOC/TN ratio of the pre-treated material which indicates the  
30  
31  
32 270 preferential removal of low-nitrogen organic compounds typically found in aquatic and  
33  
34 271 bacterial sources. <sup>[15]</sup> Compared to the *H* pre-treatment, the *N* pre-treatment produced a  
35  
36 272 more pronounced increase in the TOC/TN ratio which could be explained by a different  
37  
38  
39 273 extent in the removal of readily movable inter-crystalline and strongly bound intra-  
40  
41  
42 274 crystalline OM.  
43  
44 275 The selective removal of different OM fractions during the *H* and *N* treatment could be  
45  
46  
47 276 explained by the extent of concomitant dissolution of biogenic carbonates. At a first glance  
48  
49  
50 277 it would appear that samples from the Eemian and late Saalian (2, 4, 5 and 7) with high  
51  
52 278 TIC and TOC percentages preserved calcite, while samples from intermediate-glacial  
53  
54  
55 279 climate phases and the Holocene with overall smaller TIC percentages and higher content  
56  
57 280 in detrital carbonate underwent carbonate loss. However, this could be an artefact due to  
58  
59  
60

1  
2 281 the concomitant removal of larger amounts of OM in samples 2,4, 5 and 7. Previous  
3  
4  
5 282 studies have shown that H<sub>2</sub>O<sub>2</sub> tends to dissolve carbonates [4, 6, 9, 17, 18], especially in its  
6  
7  
8 283 acidic form, [8] while NaOCl has little or no interaction with carbonates. [18] However, our  
9  
10 284 data indicate that carbonates undergo dissolution also during the *N*pre-treatment, even  
11  
12  
13 285 though the extent of dissolution and the number of samples interested is smaller compared  
14  
15 286 to the *H*pre-treatment. This can be explained by taking into account the substantial  
16  
17  
18 287 difference between natural and artificial lacustrine sediments. Differently from artificial  
19  
20 288 mixtures of carbonates and OM, in natural lacustrine sediments, endogenic carbonates are  
21  
22  
23 289 intimately interspersed with lacustrine OM; we propose that oxidation of OM during sample  
24  
25  
26 290 pre-treatment could lead to a localised reduction in pH at the OM-carbonate interface thus  
27  
28  
29 291 promoting dissolution of endogenic carbonates.  
30  
31 292 Moreover, carbonate loss during pre-treatment could be induced by the large volumes of  
32  
33  
34 293 deionised water used to rinse the samples in our experiment (at least 1500 mL of  
35  
36 294 deionised water per 500 mg of sample was used). Even if weakly acidic, deionised water  
37  
38  
39 295 has the potential to dissolve carbonates. [18, 19]  
40  
41 296  
42  
43  
44 297 Previous studies have shown that both the *H* and *N*pre-treatments bias measurements  
45  
46  
47 298 towards lower isotopic values. [4,17] However, our data do not fully support this general rule.  
48  
49 299 While the standard pre-treated with H<sub>2</sub>O<sub>2</sub> clearly shows a depletion in the δ<sup>18</sup>O value,  
50  
51  
52 300 isotopic values of treated samples can also be more positive than their untreated  
53  
54  
55 301 equivalents either for δ<sup>13</sup>C and/or δ<sup>18</sup>O (Fig. 1).  
56  
57 302  
58  
59  
60

1  
2 303 This discrepancy could be explained by the intrinsic difficulty of accurately measuring  
3  
4  
5 304 complex lacustrine sediments where carbonates of detrital and endogenic origin coexist. In  
6  
7  
8 305 our specific case, owing to slower reaction kinetics of dolomite compared to calcite during  
9  
10 306  $\text{H}_3\text{PO}_4$  digestion, <sup>[20]</sup> progressively larger proportions of dolomite may have evolved  $\text{CO}_2$   
11  
12  
13 307 throughout sample analysis affecting the reproducibility of measurements among  
14  
15 308 untreated and treated specimens. However, our data did not provide any evidence for a  
16  
17  
18 309 larger dolomite bias for those specimens that reacted for longer times (Fig. 2). This could  
19  
20  
21 310 be explained by the fact that all samples were reacted with  $\text{H}_3\text{PO}_4$  at  $70^\circ\text{C}$  for the same  
22  
23 311 amount of time and that further reaction pending mass spectrometric analysis occurred at  
24  
25  
26 312 progressively colder temperatures (down to room temperature, Melbourne  $\approx 20^\circ\text{C}$ ). We  
27  
28 313 propose that, owing to the strong and direct dependence of acid reaction rates of  
29  
30  
31 314 carbonates on temperature, <sup>[20]</sup> specimens of the same sample evolved comparable  
32  
33  
34 315 amounts of  $\text{CO}_2$  from dolomite during acid digestion/reaction.

35  
36 316

37  
38  
39 317 The measured  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values are in accordance with changes in soil productivity  
40  
41 318 and spatial extent as well as in the local hydrological regime in response to the glacial-  
42  
43  
44 319 interglacial cyclicity. The fact that vectors of isotope geometric distance of samples rich in  
45  
46  
47 320 detrital carbonate tend to align onto common patterns pointing towards the isotopic  
48  
49 321 composition of the local detrital array (Fig. 3) provides an independent indication for the  
50  
51  
52 322 preferential dissolution of endogenic carbonates during the *H* and *N* sample treatments.  
53  
54  
55 323 This can be explained by the relatively small dimension of crystals and polycrystalline  
56  
57 324 aggregates of endogenic calcite with respect to larger clasts of detrital calcite and  
58  
59  
60

1  
2 325 dolomite. Conversely, the shift towards more negative  $\delta^{13}\text{C}$  and/or  $\delta^{18}\text{O}$  values of samples  
3  
4  
5 326 higher in endogenic carbonate (Fig. 3) is coherent with previous findings on biases  
6  
7  
8 327 induced by the *H* and *N* sample pre-treatments. [4,18]  
9

10 328

## 11 12 13 329 **Conclusions**

14  
15 330 Our study confirms that neither the NaOCl nor the H<sub>2</sub>O<sub>2</sub> pre-treatment is capable of fully  
16  
17  
18 331 removing OM from lacustrine marls, and demonstrates that, when applied to complex  
19  
20  
21 332 lacustrine sediments made of detrital and endogenic carbonates, both treatments induce a  
22  
23 333 preferential loss of endogenic carbonates. The interplay of biases introduced by pre-  
24  
25  
26 334 treatments themselves and by the differential dissolution of endogenic and detrital  
27  
28  
29 335 carbonates produces an unpredictable change in the isotopic composition of natural  
30  
31 336 lacustrine samples. We suggest to evaluate on a case-by-case basis the suitability of  
32  
33  
34 337 NaOCl and H<sub>2</sub>O<sub>2</sub> pre-treatments for OM removal from lacustrine marls and discourage  
35  
36 338 their application in cases similar to the one presented here, where TIC/TOC ratios lay  
37  
38  
39 339 above Ohelerich's threshold and in which both endogenic and detrital (even if in negligible  
40  
41  
42 340 amounts) carbonates occur.

43  
44 341

## 45 46 47 342 **Acknowledgements**

48  
49 343 This work was developed in the frame of the PhD project of Giorgio Mannella (XXXII cycle  
50  
51  
52 344 of the *Dottorato Regionale Pegaso* in Earth Sciences) and was funded by the University of  
53  
54  
55 345 Pisa (*Progetto Ateneo 2017*, leader G. Zanchetta) and MIUR (PRIN-2017, "FUTURE"  
56  
57 346 project, leader G. Zanchetta). Authors are thankful to Dr.ss R. Anis Ishak Nakhla for  
58  
59  
60



1  
2 347 FESEM investigations. This work greatly benefited from discussion with Dr. Alberto  
3  
4  
5 348 Collareta and three anonymous reviewers.

6  
7 349

8  
9  
10 350 **References**

11  
12  
13 351

14  
15 352 [1] Leng MJ, Marshall JD. Palaeoclimate interpretation of stable isotope data from lake  
16  
17  
18 353 sediment archives. *Quat. Sci. Rev.* 2004, 23, 811-831.

19  
20 354

21  
22  
23 355 [2] Lebeau O, Busigny V, Chaduteau C, Ader M. Organic matter removal for the analysis of  
24  
25  
26 356 carbon and oxygen isotope compositions of siderite. *Chem. Geo.* 2014, 372, 54–61

27  
28 357

29  
30  
31 358 [3] Charef A, Sheppard SMF. Carbon and oxygen isotope analysis of calcite or dolomite  
32  
33  
34 359 associated with organic matter. *Isot. Geosci.* 1984, 2, 325-333.

35  
36 360

37  
38  
39 361 [4] Wierzbowski H. Effects of pre-treatments and organic matter on oxygen and carbon  
40  
41  
42 362 isotope analyses of skeletal and inorganic calcium carbonate. *Int. J. Mass Spectrom.*  
43  
44 363 2007, 268, 16-29.

45  
46  
47 364

48  
49 365 [5] Oehlerich M, Braumer M, Lücke A, Mayr C. Effects of organic matter on carbonate  
50  
51  
52 366 stable isotope ratios ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  values) – implication for analyses of bulk sediments.

53  
54 367 *Rapid Commun. Mass Spectrom.* 2013, 27, 707-712.

55  
56  
57 368

- 1  
2 369 [6] Grottoli AG, Rodrigues LJ, Matthews KA, Palardy JE, Gribb OT. Pre-treatment effects  
3  
4  
5 370 on coral skeletal  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . *Chem. Geo.* 2005, 221, 225-242.  
6  
7  
8 371
- 9  
10 372 [7] Mikutta R, Kleber M, Kaiser K, Jahn R. Organic matter removal from soils using  
11  
12  
13 373 hydrogen peroxide, sodium hypochlorite, and disodium peroxodisulfate. *Soil Sci. Soc. Am.*  
14  
15 374 *J.* 2005, 69, 120-135.  
16  
17  
18 375
- 19  
20 376 [8] Falster G, Delean S, Tyler J. Hydrogen Peroxide Treatment of Natural Lake Sediment  
21  
22  
23 377 Prior to Carbon and Oxygen Stable Isotope Analysis of Calcium Carbonate. *Geochem.*  
24  
25  
26 378 *Geoph. Geosyst.* 2018, 19, 3583-3595.  
27  
28  
29 379
- 30  
31 380 [9] Roberts LR, Holmes J A, Leng MJ, Sloane HJ, Horne DJ. Effects of cleaning methods  
32  
33  
34 381 upon preservation of stable isotopes and trace elements in shells of *Cyprideis torosa*  
35  
36 382 (Crustacea, Ostracoda): Implications for palaeoenvironmental reconstruction. *Quat. Sci.*  
37  
38  
39 383 *Rev.* 2018, 189, 197-209.  
40  
41  
42 384
- 43  
44 385 [10] Mannella G, Giaccio B, Zanchetta G et al.. Palaeoenvironmental and  
45  
46  
47 386 palaeohydrological variability of mountain areas in the central Mediterranean region: A 190  
48  
49 387 ka-long chronicle from the independently dated Fucino palaeolake record (central Italy).  
50  
51  
52 388 *Quat. Sci. Rev.* 2019, 210, 190-210.  
53  
54  
55 389

- 1  
2 390 [11] Francke A, Wagner B, Just J et al. Sedimentological processes and environmental  
3  
4  
5 391 variability at Lake Ohrid (Macedonia, Albania) between 637 ka and the present. *Biogeosci.*  
6  
7 392 2016, 13, 1179-1196.  
8  
9  
10 393  
11  
12 394 [12] Altomare A, Corriero N, Cuocci C, Falcicchio A, Moliterni A, Rizzi R. QUALX2.0: a  
13  
14  
15 395 qualitative phase analysis software using the freely available database POW\_COD. *J.*  
16  
17  
18 396 *App. Cryst.* 2015, 48, 598-603.  
19  
20 397  
21  
22 398 [13] Wojdyr M. Fityk: a general - purpose peak fitting program. *J. App. Cryst.* 2010, 43,  
23  
24  
25 399 1126-1128.  
26  
27  
28 400  
29  
30 401 [14] University of Alberta, Department of Earth & Atmospheric Sciences. Internal Report:  
31  
32  
33 402 Recommended Method for Semi-quantitative Measurement of Aragonite – Calcite –  
34  
35  
36 403 Dolomite Mixtures by Powder X-ray Diffraction.  
37  
38  
39 404 <https://www.eas.ualberta.ca/eml/files/Carbonate-quantitation-2018-with-addendum.pdf>.  
40  
41 405 Accessed on February 19, 2019.  
42  
43  
44 406  
45  
46 407 [15] Meyers PA, Ishiwatari R. Lacustrine organic geochemistry – an overview of indicators of  
47  
48  
49 408 organic matter sources and diagenesis in lake sediments. *Org, Geochem.* 1993, 20, 867-  
50  
51  
52 409 900.  
53  
54 410 [16] Sykes GA, Collins MJ, Walton DI. The significance of a geochemically isolated  
55  
56  
57 411 intracrystalline organic fraction within biominerals. *Org. Geochem.* 1995, 23, 1059-1065.  
58  
59  
60

1  
2 412  
3

4  
5 413 <sup>[17]</sup> Serrano O, Serrano L, Mateo MA. Effects of sample pre-treatment on the  $\delta^{13}\text{C}$  and

6  
7 414  $\delta^{18}\text{O}$  values of living benthic foraminifera. *Chem. Geo.* 2008, 257, 218-220.  
8  
9

10 415  
11

12  
13 416 <sup>[18]</sup> Pingitore NE, Fretzdorff SB, Seitz BP et al. Dissolution kinetics of  $\text{CaCO}_3$  in common

14  
15 417 laboratory solvents. *J. Sed. Res.* 1993, 63, 641-645.  
16  
17

18 418  
19

20  
21 419 <sup>[19]</sup> Loxton J, Najorka J, Humphreys-Williams E et al. The forgotten variable: Impact of

22  
23 420 cleaning on the skeletal composition of a marine invertebrate. *Chem. Geo.* 2017, 474, 45-

24  
25 421 57.  
26  
27

28 422  
29

30  
31 423 <sup>[20]</sup> Al-Aasm IS, Taylor BE, South B. Stable isotope analysis of multiple carbonate samples

32  
33 424 using selective acid extraction. *Chem. Geol.* 1990, 80, 119-125.  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Sample	Chronology		XRPD (semi)quantitative analysis		Specim.	Geochemistry				Treatment evaluation			Isotope ratios (‰ vs. V-PDB)			
	Age (ka)	Period	Qtz/Cal	Dolomite mol <sub>carb.</sub> %		TIC %	TOC %	TN %	TOC/TN <sub>atom</sub>	TIC/ TOC	ΔTOC %	ΔTIC %	δ <sup>13</sup> C	2σ	δ <sup>18</sup> O	2σ
1	2.7	Holocene	0.37	2.7	<i>TQ</i>	3.19	5.63	0.67	7.31	0.57			2.32	0.09	-4.64	0.35
					<i>H</i>	2.53	2.54	0.20	11.17	1.00	-3.10	-0.67	1.77	0.15	-4.41	0.15
					<i>N</i>	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	<i>TQ</i>	6.47	10.09	0.69	12.81	0.64			3.78	0.11	-4.50	0.07
					<i>H</i>	9.04	3.36	0.23	12.96	2.69	-6.73	2.57	3.74	0.15	-4.45	0.15
					<i>N</i>	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	<i>TQ</i>	2.73	3.58	0.37	8.45	0.76			1.47	0.09	-3.72	0.17
					<i>H</i>	3.75	0.87	0.10	7.96	4.33	-2.72	1.02	1.48	0.08	-3.40	0.13
					<i>N</i>	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	<i>TQ</i>	6.17	7.41	0.48	13.51	0.83			1.00	0.08	-3.31	0.22
					<i>H</i>	6.59	3.38	0.18	16.62	1.95	-4.03	0.43	0.91	0.09	-3.57	0.27
					<i>N</i>	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	<i>TQ</i>	7.27	7.71	0.59	11.43	0.94			4.29	0.09	-5.53	0.21
					<i>H</i>	8.86	4.09	0.28	12.71	2.17	-3.62	1.60	5.07	0.04	-5.80	0.32
					<i>N</i>	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	<i>TQ</i>	3.67	3.48	0.38	8.12	1.06			2.32	0.04	-4.51	0.16
					<i>H</i>	3.04	0.66	0.11	5.37	4.62	-2.82	-0.63	2.40	0.12	-4.71	0.19
					<i>N</i>	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	<i>TQ</i>	8.65	5.70	0.40	12.52	1.52			3.80	0.11	-4.90	0.08
					<i>H</i>	9.77	2.21	0.14	14.06	4.42	-3.49	1.12	3.68	0.04	-4.75	0.22
					<i>N</i>	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	<i>TQ</i>	3.19	1.59	0.28	4.99	2.00			1.44	0.06	-4.02	0.25
					<i>H</i>	3.24	0.61	0.08	7.01	4.28	-0.98	-0.57	1.65	0.29	-4.01	0.11
					<i>N</i>	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	<i>TQ</i>	8.12	3.23	0.35	8.20	2.51			1.20	0.09	-5.63	0.10
					<i>H</i>	7.27	1.06	0.14	6.77	6.82	-2.17	-0.85	0.97	0.14	-5.70	0.05
					<i>N</i>	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	<i>TQ</i>	2.68	0.88	0.14	5.51	3.05			0.67	0.12	-2.42	0.21
					<i>H</i>	2.08	0.33	0.05	5.66	6.24	-0.55	-0.60	0.83	0.12	-3.25	0.22
					<i>N</i>	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	<i>TQ</i>	3.05	0.85	0.28	2.69	3.57			1.15	0.14	-3.67	0.14
					<i>H</i>	1.92	0.36	0.07	4.66	5.32	-0.49	-1.12	1.16	0.15	-3.95	0.26
					<i>N</i>	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	<i>TQ</i>	2.80	0.68	0.17	3.50	4.12	-0.29	-0.81	0.22	0.38	-2.83	0.17
					<i>H</i>	1.99	0.39	0.07	4.61	5.17			0.66	0.26	-2.90	0.05
					<i>N</i>	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	<i>TQ</i>	3.29	0.73	0.18	3.55	4.51			0.57	0.21	-2.96	0.08
					<i>H</i>	2.60	0.41	0.05	7.22	6.32	-0.32	-0.69	0.89	0.06	-3.30	0.22
					<i>N</i>	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	<i>TQ</i>	3.64	0.72	0.25	2.52	5.06			0.86	0.19	-3.05	0.26
					<i>H</i>	3.43	0.16	0.08	1.79	21.00	-0.56	-0.21	1.23	0.08	-3.37	0.48
					<i>N</i>	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	<i>TQ</i>	5.68	0.80	0.19	3.69	7.10			1.37	0.12	-2.63	0.28
					<i>H</i>	5.21	0.17	0.06	2.34	30.62	-0.63	-0.47	1.22	0.12	-2.97	0.43
					<i>N</i>	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	<i>TQ</i>	5.37	0.48	0.20	2.10	11.19			1.13	0.12	-2.63	0.12
					<i>H</i>	1.08	0.17	0.04	4.11	6.20	-0.31	-4.29	1.09	0.17	-2.90	0.27
					<i>N</i>	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		-	-	<i>TQ</i>	-	-	-	-	-	-	-	2.13	0.10	-1.73	0.14
					<i>H</i>	-	-	-	-	-	-	-	1.88	0.22	-2.19	0.24
					<i>N</i>	-	-	-	-	-	-	-	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 <sup>[10]</sup>, relative mineral abundance and analyses on untreated (*TQ*) and H<sub>2</sub>O<sub>2</sub> or NaOCl-treated specimens (*H* and *N*, respectively). Total inorganic and organic carbon (TIC and TOC), total nitrogen (TN) weight percentages 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  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  (‰ vs. V-PDB) values with relative confidence intervals are shown.

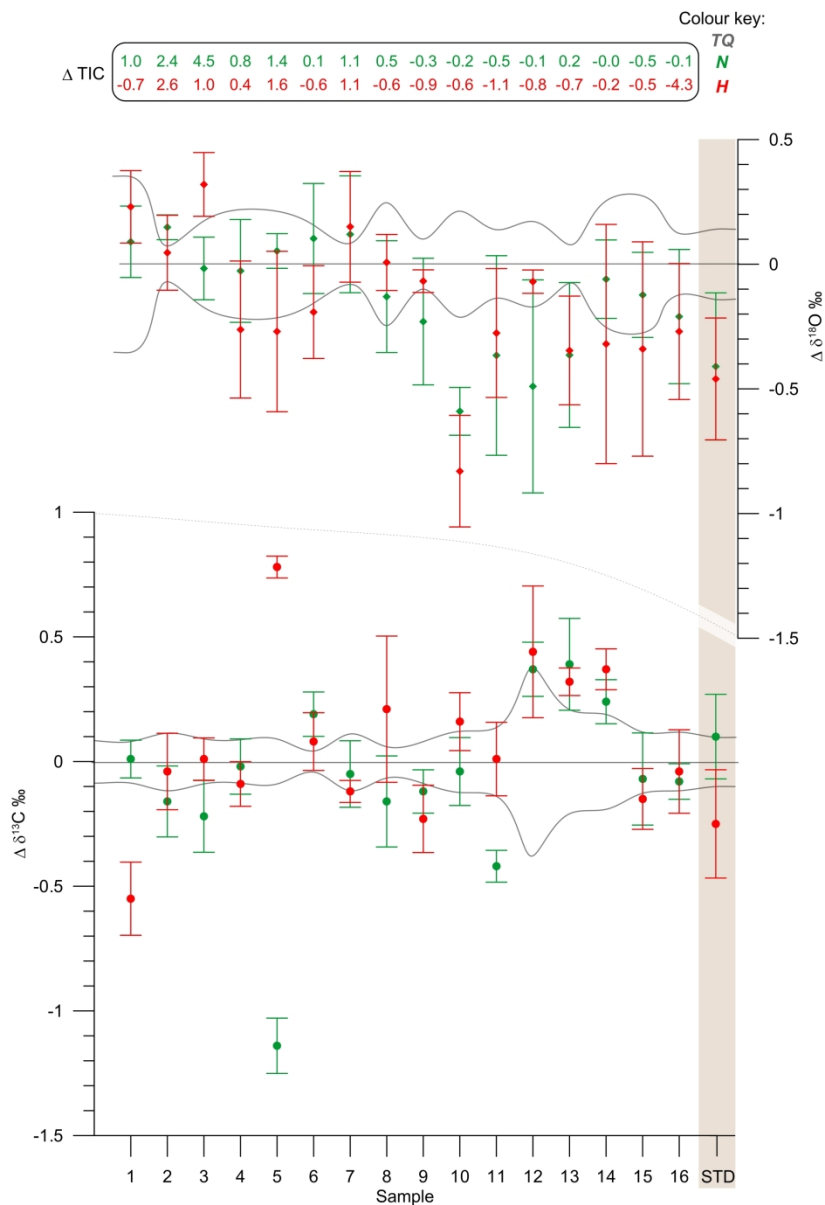


Fig. 1 Whisker plot showing isotopic composition of untreated specimens set to zero and the relative isotopic shifts of treated specimens. 2σ confidence levels for δ<sup>13</sup>C and δ<sup>18</sup>O isotope values are displayed as black line envelope for untreated specimens and as red and green bars for treated specimens.

200x293mm (300 x 300 DPI)

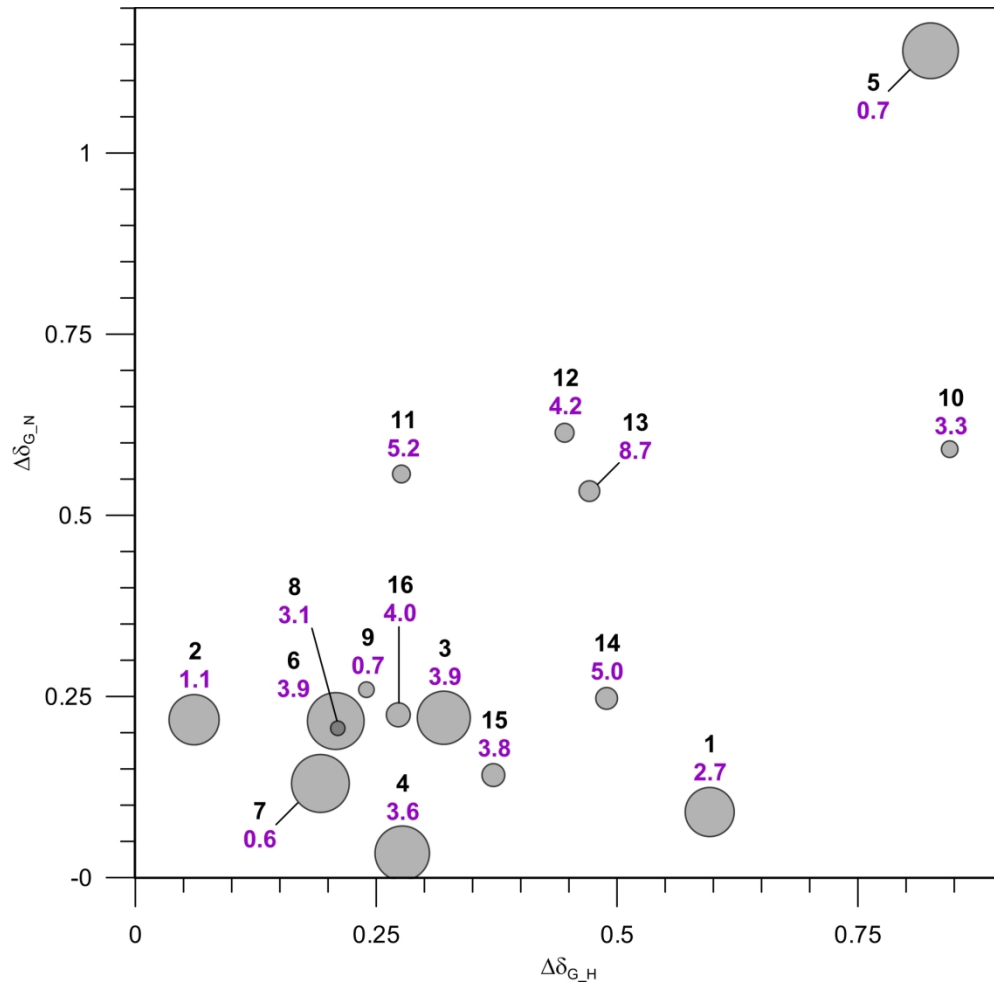


Fig. 2 Isotopic geometric distances of **H** and **N** specimens. Black labels refer to sample number, magenta labels refer to content in dolomite (molar percentages of dolomite in the carbonate pool). Bubble size refers to reaction times during sample awaiting for MS analysis, respectively. The size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

175x170mm (300 x 300 DPI)



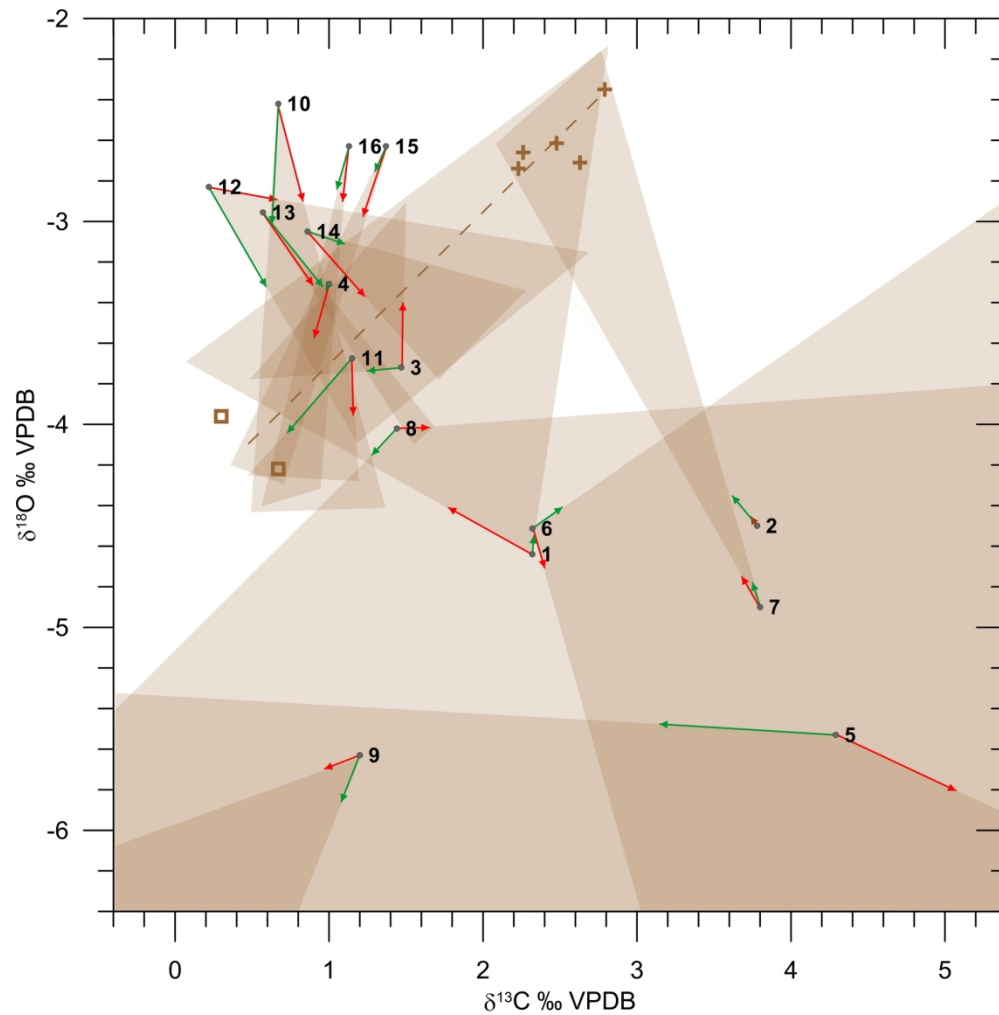
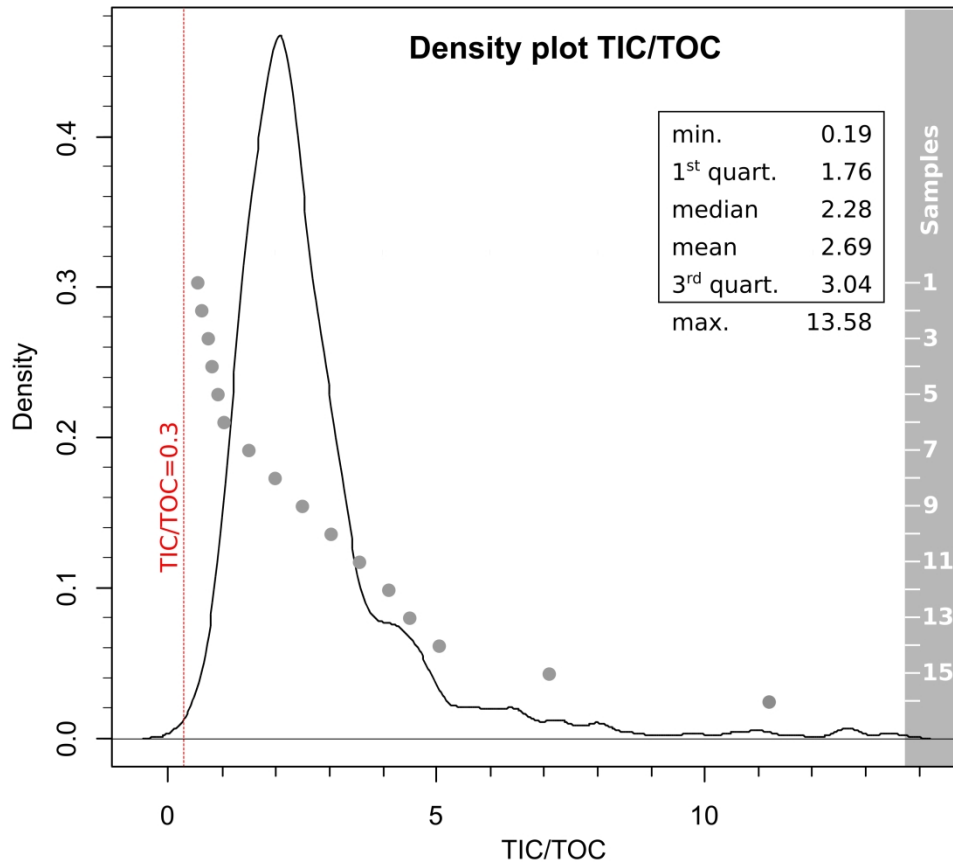


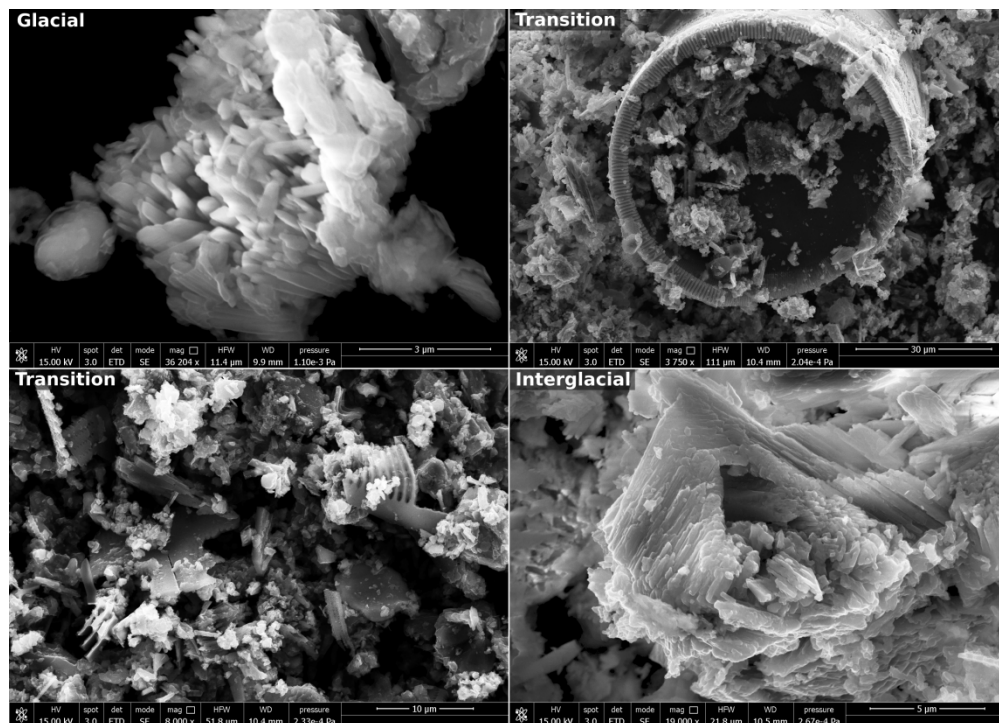
Fig. 3: Brown crosses and squares indicate the isotopic value of limestone gravels and of weathered limestone gravels transported by the Giovenco River, respectively. The dashed brown line represents the local isotopic detrital array (different proportions of unaltered and weathered limestone will result 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<sub>2</sub>O<sub>2</sub>) 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<sup>[5]</sup> 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)