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Polyphasic carbonate precipitation in the shallow subsurface: insights from microbially-formed authigenic carbonate beds in upper Miocene sediments of the Tertiary Piedmont Basin (NW Italy)

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ABSTRACT

Eliminato: ¶
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47 Authigenic methane-derived carbonates hosted in upper Miocene slope sediments of the
48 Tertiary Piedmont Basin (NW Italy) are studied by a multidisciplinary approach including
49 petrography, stable oxygen and carbon isotopes of carbonates, as well as lipid biomarkers in order
50 to explore the relationship between microbial activity and carbonate precipitation in the shallow
51 subsurface. The studied rocks show a bed parallel geometry and are characterized by dolomitic
52 intergranular cement, which is typified by positive $\delta^{13}\text{C}$ values as high as +6.2‰ VPDB. A striking
53 feature of some dolomite beds is an intricate network of septarian-like cracks filled with both
54 injected sediments and polyphasic carbonate cements. Prokaryotic molecular fossils in the
55 dolomite beds comprise archaeal ($\delta^{13}\text{C}$: -40‰ VPDB) and various bacterial dialkyl glycerol
56 diethers (DAGEs; $\delta^{13}\text{C}$: -30‰ VPDB), strongly suggesting that dolomite precipitation took place at
57 the interface of the zones of archaeal methanogenesis and bacterial sulphate reduction. In
58 contrast, extremely negative $\delta^{13}\text{C}$ values of carbonate cements (as low as -56.3‰ VPDB) and
59 various archaeal and bacterial molecular fossils (e.g. pentamethylcosane (PMI): -106‰ VPDB)
60 are recorded in the crack-filling carbonate cements. These cements precipitated due to anaerobic
61 oxidation of methane coupled to sulphate reduction. We propose a scenario for the formation of the
62 diagenetic beds, suggesting that carbonate precipitation was the result of three microbially-driven
63 processes (sulphate reduction, methanogenesis, and, finally, anaerobic oxidation of methane).
64 This unusual sequence was a consequence of a dynamic change of environmental geochemical
65 conditions and fluid circulation patterns that prevailed in the ancient subseafloor during early
66 diagenesis of the unconsolidated sediments. Anaerobic oxidation of methane, which usually
67 predates methanogenesis during increasing burial, postdates methanogenesis in case of the
68 septarian-like beds after the beds were affected by crack formation induced by overcritical pore
69 pressure, allowing the ingress of sulphate-rich water from above and methane-rich water from
70 below.

71

72 *Keywords:*

73 Authigenic carbonates

74 Septarian-like cracks

75	Methanogenesis
76	Anaerobic oxidation of methane
77	Stable isotopes
78	Biomarkers

79

80 **1. Introduction**

81

82 Microbially-induced carbonate precipitation occurs within various types of marine
83 sediments, as well as in brackish, and lacustrine sediments. Studies of modern sedimentary
84 environments and culture experiments have shown that the activity of sulphate-reducing bacteria
85 (SRB) can promote the precipitation of carbonate minerals (calcite, aragonite, dolomite) due to an
86 increase in alkalinity related to organic matter degradation (Irwin et al., 1977; Vasconcelos et al.,
87 1995; van Lith et al., 2003; Wright and Oren, 2005; Wacey et al., 2008). Since the metabolism and
88 carbon fixation modes of SRB vary strongly under different environmental conditions (Londry et al.,
89 2004), the resulting fractionation of stable carbon isotopes cannot be easily predicted. Negative
90 $\delta^{13}\text{C}$ values of carbonates are often used as evidence for the activity of SRB, but laboratory
91 experiments (Londry et al., 2004) and environmental studies (Heindel et al., 2010) reveal that SRB
92 cannot always be traced by carbonate carbon isotopes.

93 Below the zone of sulphate reduction, ongoing organic matter degradation is chiefly
94 performed by methanogenic archaea (e.g. Martens and Berner, 1974; Whiticar et al., 1986; Sivan
95 et al., 2007). During methanogenesis, ^{12}C is preferentially incorporated in methane, while the
96 residual pore water becomes enriched in ^{13}C (e.g. Boehme et al., 1996). In these settings,
97 autotrophic methanogenesis is removing CO_2 effectively, which is thought to locally trigger the
98 formation of carbonates by increasing the pH value of pore waters (Budai et al., 2002). These
99 methanogenic carbonates are characterized by positive $\delta^{13}\text{C}$ values due to precipitation from a
100 ^{13}C -enriched carbon pool (e.g. Budai et al., 2002). Another scenario leading to dolomite formation
101 assumes the ascent of ^{13}C -enriched fluids from deeper sediment (Meister et al., 2011). In
102 laboratory experiments, Kenward et al. (2009) observed that methanogenesis is indeed capable to

103 induce dolomite precipitation. Interestingly, ^{13}C -enriched dolomites from the Monterey Formation
104 were found to contain biomarkers of archaea, presumably representing methanogens (Hoffmann-
105 Sell et al., 2011), but an archaeal involvement in dolomite formation is difficult to prove in this case.
106 Similarly as for sulphate-reducing bacteria, it is known for one methanogenic archaeon
107 (*Methanosarcina barker*) that a great variability in the extent of carbon isotope fractionations is
108 possible, which is reflected by a wide range of $\delta^{13}\text{C}$ values of lipid biomarkers produced by
109 methanogens (Londry et al., 2008).

110 A further process triggering precipitation of carbonates in sediments is the anaerobic
111 oxidation of methane (AOM; e.g. Ritger et al., 1987; Ussler and Paull, 2008). During its ascent
112 through the sedimentary column, methane is oxidized anaerobically by consortia of archaea and
113 sulphate-reducing bacteria at the base of the sulphate reduction zone (e.g. Hinrichs et al., 1999;
114 Boetius et al., 2000; Orphan et al., 2001, 2002). AOM generates a local increase in alkalinity, thus
115 promoting the precipitation of carbonates. The shape and appearance of subsurface AOM-induced
116 carbonate precipitates are manifold, including carbonate pavements, massive blocks, friable
117 concretions, oil-filled, porous carbonates, and macrofossil-rich carbonates (e.g. Mazzini et al.,
118 2004; Roberts et al., 2010). Generally, authigenic methane-seep carbonates are composed of
119 various calcitic and aragonitic cements (e.g. Roberts and Aharon, 1994; Peckmann and Thiel,
120 2004; Naehr et al., 2009), and less commonly consist of dolomite (e.g. Peckmann et al., 1999).
121 Most methane-seep carbonates are characterized by extreme ^{13}C -depletions, with $\delta^{13}\text{C}$ values as
122 low as -50‰ VPDB or even lower (Peckmann and Thiel, 2004), but some seep limestones do not
123 show low $\delta^{13}\text{C}$ values either due to (1) substantial admixture of marine carbonate or (2)
124 superimposed carbonate formation driven by methanogenesis (Kuechler et al., 2011). At some
125 sites, the methane flux can be vigorous enough to reach the sediment surface, enabling aerobic
126 methanotrophic bacteria to flourish (e.g. Niemann et al., 2006; Birgel et al., 2011). In modern
127 environments, AOM and its impact on carbonate precipitation was extensively studied using pore-
128 fluid chemistry, microbiology, lipid biomarkers, element patterns, and stable isotopes (e.g. Hinrichs
129 et al., 1999; Pancost et al., 2000; Knittel et al., 2005; Rossel et al., 2011). However, most of the
130 methods used to unravel microbial processes cannot be applied in ancient sedimentary

131 sequences. Microbial processes that prevailed during carbonate formation can still be assessed by
132 studying persistent proxies preserved in ancient seep carbonates. Such methods include
133 petrography, stable isotope geochemistry, and lipid biomarkers and their isotopic composition (e.g.
134 Peckmann et al., 1999; Birgel et al., 2008b).

135 Here we provide a multiproxy data set obtained from authigenic carbonates from upper
136 Miocene deposits of the Tertiary Piedmont Basin. These carbonate rocks belong to a recently
137 identified ancient subsurface seepage system that includes a wide array of carbonate concretions
138 showing different shapes (tubular, cylindrical, ellipsoidal), which formed as a consequence of
139 upward rising methane-rich fluids (Dela Pierre et al., 2010). Among these different types of
140 authigenic methane-derived carbonates, stratiform carbonates are most abundant. In these beds
141 discussed herein not only features typical of methane-seep carbonates are observed, reflecting
142 AOM, but also methanogenesis and sulphate reduction are archived. We particularly focus on the
143 relationship of authigenic carbonate formation and microbial activity as a consequence of the
144 dynamic and changing geochemical conditions and fluid circulation patterns that prevailed in the
145 ancient subseafloor.

146

147 **2. Geological and stratigraphic setting**

148

149 The Tertiary Piedmont Basin (TPB) is located in northwestern Italy and is filled with up to
150 5000 m of upper Eocene to Messinian sediments that unconformably overlie both Alpine
151 metamorphic rocks and Apennine Ligurian Units juxtaposed by the Mesoalpine collision (e.g.
152 Mosca et al., 2009; Fig. 1). The studied area is located in the Borbera-Grue sector in the eastern
153 part of the TPB to the south of the Villalvernia Varzi Line (Fig. 2). The stratigraphic succession of
154 this sector is represented by Oligocene to Pliocene terrigenous sediments deposited
155 unconformably on the Ligurian Unit (Ghibaudo et al., 1985). The upper Miocene part of the
156 succession consists of the Sant'Agata Fossili marls (Tortonian-lower Messinian), the Valle Versa
157 chaotic complex (upper Messinian), and the Cassano Spinola conglomerates (upper Messinian). In
158 the studied sector, (Ripa dello Zolfo area, Fig. 2), the Sant'Agata Fossili marls are further

159 subdivided into two members (Ghibaudo et al., 1985): (1) the lower member (Tortonian), consisting
160 of outer shelf deposits, which are strongly bioturbated and (2) the upper member (lower
161 Messinian), which hosts the studied carbonate rocks (Fig. 3), deposited on the slope. The grey
162 upper member marls are poorly bedded and show a carbonate content of 15 wt % of the total rock
163 on average (Table 1). They contain abundant fossils (planktic and benthic foraminifera, bivalves,
164 gastropods, and land plant debris), and especially in the uppermost part they are cyclically
165 interbedded with laminated euxinic shales. Like in other parts of the Mediterranean, this cyclic
166 stacking pattern reflects precession-controlled climate changes resulting in the deposition of marls
167 during precession maxima (insolation minima) and laminated shales during precession minima
168 (insolation maxima; e.g. Hilgen et al., 1995). The Sant'Agata Fossili marls are truncated by a
169 regional unconformity (Messinian erosional surface) and are overlain by the chaotic sedimentary
170 bodies of the Valle Versa chaotic complex that consists of blocks of various composition and size
171 floating in a poorly exposed clayey matrix. The blocks include shallow-water evaporites, evaporitic
172 vuggy carbonates, bioclastic carbonates, and authigenic methane-derived carbonates. The
173 overlying Cassano Spinola conglomerates, consisting of upper Messinian deltaic to lagoonal
174 brackish water sediments, correlate with the "Lago Mare" interval recognized all over the
175 Mediterranean area (e.g. Orszag Sperber, 2006).

176

177 **3. Methods**

178

179 Field analyses of the lithology and geometry of carbonate-rich beds were carried out in the
180 Ripa dello Zolfo area. Approximately 30 representative samples of the various carbonate beds
181 were selected for petrography and geochemistry studies. The unconsolidated background
182 sediments were sampled for biostratigraphy, palaeoecology, and stable isotope investigations.
183 Semiquantitative analyses on the mineralogical composition of the carbonate fraction focused on
184 the evaluation of the relative abundances of calcite and dolomite, and were carried out on five
185 samples, including both cemented beds and unconsolidated sediments, in an ICP-OES laboratory
186 (Department of Mineralogical and Petrological Sciences, University of Torino), using an IRIS II

187 Advantage/1000 (Thermo-Jarrel Ash Corporation). Two hundred mg of sample were homogenized
188 and dissolved in 10 ml of acetic acid, in order to remove the carbonates, but preserving the
189 silicates at the same time. The filtered solution was analysed with ICP-OES and the amount of
190 Mg^{2+} and Ca^{2+} was measured. The relative abundance of dolomite in the samples was calculated,
191 assigning the total amount of Mg^{2+} , along with the corresponding amount of Ca^{2+} , to stoichiometric
192 dolomite. The remaining Ca^{2+} was assumed to derive from calcite and was used to calculate the
193 abundance of stoichiometric calcite.

194 After cutting and polishing carbonate samples, 50 standard petrographic thin sections were
195 prepared. Petrographic and cathodoluminescence observations were carried out by plane-
196 polarized and cross-polarized light microscopy using a CITL 8200 MK3 equipment, operating at
197 about 17 kV and 400 mA. Thin sections were further analysed for their UV-fluorescence with a
198 Nikon microscope with a UV-2A filter block, using ultraviolet light (illumination source 450-490 nm).
199 Scanning electron microscopy (SEM) was carried out on slightly etched polished rock surfaces
200 obtained from the same samples used for thin sections, using a SEM Cambridge Instruments
201 Stereoscan 360 equipped with an energy-dispersive (EDS) microprobe Link System Oxford
202 Instruments.

203 Microdrilled and micromilled samples were measured for their carbon and oxygen isotope
204 composition. For microdrilled samples, the carbonate fraction was analysed following the method
205 after McCrea (1950) using Finnigan MAT 251 and 252 mass spectrometers. The isotopic ratios are
206 expressed as $\delta^{13}C$ and $\delta^{18}O$ values relative to the VPDB standard (precision $< \pm 0.05\%$). These
207 isotope analyses were performed in the *ISO4* Laboratory (Turin, Italy) and in the *MARUM* Stable
208 Isotope Laboratory (Bremen, Germany). Micromilling was conducted at 0.1 mm resolution, using a
209 video-controlled New Wave Research instrument. Sample surfaces were polished before analysis.
210 A trench about 2 mm in width and 0.2 to 0.3 mm in depth was micromilled concordantly to the
211 crystal growth directions. Isotope measurements were performed using a continuous-flow isotope
212 ratio mass spectrometer and an automated carbonate preparation system calibrated against NBS
213 and IAEA standard reference materials. These analyses were performed at the Institute of Geology
214 and Palaeontology of the University of Innsbruck, Austria. The isotopic ratios are expressed as

215 $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in per mil versus VPDB. The long-term precision of these analyses is 0.06
216 and 0.08‰, respectively.

217 Lipid biomarkers were extracted from two carbonate samples using the preparation
218 procedure described by Birgel et al. (2006b). The samples (ZF 105: 280 g, DM 115: 326 g) were
219 crushed to small pieces for cleaning and decalcification. After decalcification, a cleaning procedure
220 was applied before the samples were subsequently saponified in 6% KOH in methanol. Lipid
221 biomarker extraction was carried out with a microwave extraction system (CEM MARS X) at 80 °C
222 and 600 W with dichloromethane/methanol (3:1) until the solvents used became colourless. The
223 separation of the resulting extracts into four fractions was achieved by column chromatography
224 (500 mg DSC-NH₂ cartridges, Supelco) using the indicated amount of solvents: hydrocarbons (4
225 ml *n*-hexane), ketones (6 ml *n*-hexane/dichloromethane, 3:1), alcohols (7 ml
226 dichloromethane/acetone, 9:1), carboxylic acid fraction (8 ml of 2% formic acid in
227 dichloromethane). Alcohols and carboxylic acids were measured as their trimethyl-silyl (TMS) and
228 methyl ester (ME) derivatives, respectively. All fractions were measured using a gas
229 chromatography–mass spectrometry system (Thermo Electron Corporation Trace MS) equipped
230 with a 30 m Rxi-5 MS fused silica capillary column (0.32 mm i.d., 0.25 μm film thickness). The
231 carrier gas was He. The gas chromatography (GC) temperature program used was as follows: 60
232 °C (1 min); from 60 to 150°C at 10°C/min then to 320°C at 4°/min; 27 min isothermal for
233 hydrocarbons or 37.5 min for alcohols and carboxylic acids. Identification of compounds was based
234 on GC retention times and comparison of the obtained mass spectra with published ones.
235 Compound-specific carbon isotope analyses were carried out with a Hewlett Packard 5890 gas
236 chromatograph linked to a Thermo Electron GC-combustion-interface and a Finnigan MAT 252
237 mass spectrometer. GC conditions were identical to those described above. Carbon isotopes are
238 expressed as $\delta^{13}\text{C}$ values relative to the VPDB standard. The carbon isotope measurements were
239 corrected for the addition of TMS- and ME-derivatives. Several pulses of CO₂ with known $\delta^{13}\text{C}$
240 values at the beginning and the end of the runs were used for calibration. Instrument precision was
241 checked using a mixture of *n*-alkanes with known isotopic composition. The analytical standard
242 deviation was <0.6‰. For analysis of stable carbon isotopic compositions of glycerol dibiphytanyl

243 glycerol tetraether (GDGT)-derived biphytanes, tetraethers were subjected to ether-cleavage. An
244 aliquot of non-derivatised alcohols was reacted with HI and acetic acid glacial. The resulting
245 iodides were reduced to hydrocarbons with LiAlH₄ in tetrahydrofuran under argon atmosphere. The
246 resulting biphytanes were analysed using GC-FID and GC-MS and subjected to compound-specific
247 carbon isotope analysis as described above.

248

249 **4. Results**

250

251 Nine vertically-stacked carbonate beds have been recognized in the Ripa dello Zolfo area
252 (Fig. 3A). They occur throughout the upper member of the Sant'Agata Fossili marls from the base
253 of this unit up to 5-6 m below the overlying Valle Versa chaotic complex (Dela Pierre et al., 2010).
254 The beds laterally extend for several tens of metres parallel to bedding (Fig. 4). All of them reveal
255 sharp contacts with the enclosing, poorly consolidated marls; locally bed surfaces are wavy. On the
256 basis of different internal features, three types of beds have been recognized: (1) homogeneous
257 beds, (2) septarian-like beds, and (3) brecciated beds (cf. Dela Pierre et al., 2010). Petrographic
258 and isotopic characteristics of these beds have been already described in Dela Pierre et al. (2010).
259 In the following, we will focus only on the septarian-like beds.

260

261 *4.1. Septarian-like beds*

262

263 *4.1.1. Petrography*

264 Three septarian-like beds were found in sections 1 and 2 (Fig. 3A). The most prominent bed,
265 10 to 60 cm in thickness, shows a lateral extension of about 400 m, forming a distinctive marker
266 bed useful for stratigraphic correlation (bed 4 in Figs. 3 and 4). The septarian-like beds reveal the
267 same lithology as the background sediments but are extensively cemented by rhombohedral
268 microcrystals of dolomite (Fig. 5E). Dolomite comprises up to 70 wt.% of the rock (Table 1). An
269 intense fluorescence of the dolomite points to a high content of organic matter (Fig. 5C-D).
270 Sulphide minerals are abundant, chiefly consisting of framboidal pyrite, but many framboids have

271 been oxidized (Fig. 5F). The most striking feature of the studied beds is an intricate network of
272 several mm- to cm-wide fractures, referred to as septarian-like cracks here because of their
273 similarity to fractures of septarian concretions (cf. Dela Pierre et al., 2010). Fractures are orientated
274 predominantly perpendicular or parallel to bedding (Fig. 5A-B) and many of them are filled either
275 with injected sediments (clastic dykes) or polyphasic carbonate cements (Fig. 5A); in the upper
276 part of the beds, fractures are generally empty (Fig. 5B). The injected sediment consists of
277 mudstone clasts of different size floating within a fine-grained matrix (Dela Pierre et al., 2010). The
278 crack-filling cements consist of isopachous, finely to medium crystalline, fibrous dolomite followed
279 by non-drusy limpid sparry low-Mg calcite interlayered with isopachous turbid fibrous high-Mg
280 calcite. Cathodoluminescence allows to distinguish different cement zones and displays unusual
281 growth geometries, referred to as “pinch-out structures”, which have been interpreted to reflect the
282 past occurrence of gas hydrates within the cracks (cf. Martire et al., 2010). Like in the background
283 sediment, partially oxidized pyrite framboids are abundant both in the injected sediments and in the
284 fracture-filling cements.

285 One of the septarian beds is locally characterized by further internal complexity (Figs. 3A and
286 6A). The lower part of this bed was affected by fracturing with fractures oriented at a high angle
287 with respect to the bedding planes, cross-cutting the entire bed from its base to the top. The upper
288 part of the bed is typified by a domal structure, which is approximately 20 cm in height and 40 cm
289 in width. The domal extension of the bed is cemented entirely by dolomite and invaded by a
290 complex network of cm-large empty fractures (Fig. 6A), whereas fractures in the lower part are
291 filled with sediment and carbonate cement (sample DM115; Figs. 3B and 6B). The cement is
292 represented by brownish, fluorescent dolomite crystals that grew directly on the fracture walls. In
293 some fractures different generations of calcite and dolomite spar are arranged to form spherulites
294 up to 3 mm in width (Fig. 6C).

295

296 4.1.2 *Stable carbon and oxygen isotopes*

297 Several carbonate phases of the septarian-like beds, including the microcrystalline
298 intergranular cement and the fracture-filling cements, have been analysed for their carbon and

299 oxygen stable isotope signatures. The results of more than 80 analyses are reported in Table 2. In
300 particular for crack-filling cements, the micromill technique was used in order to minimize the errors
301 resulting from the mixing of different carbonate phases. In fact, micromill sampling allowed a
302 separation of cements pertaining to the different diagenetic phases recognized by petrographic and
303 cathodoluminescence analyses. The unconsolidated background sediment, whose carbonate
304 contents reach up to 15 wt.% (see Table 1), was also analyzed for its isotope signature, revealing
305 $\delta^{13}\text{C}$ values ranging from -4.1 to -1.1‰ and $\delta^{18}\text{O}$ values ranging from -3.0 to -1.5‰ (Fig. 7).

306 The intergranular dolomite cement mostly yielded positive $\delta^{13}\text{C}$ values (as high as $+6.2\text{‰}$,
307 sample ZF106; see Fig. 3B). Interestingly, a sharp trend to lower $\delta^{13}\text{C}$ values is observed very
308 close to the cracks of the domal structure (as low as -48‰ , samples DM115 and DM142; Fig. 3B).
309 The intergranular cement is characterized by positive $\delta^{18}\text{O}$ values ($+6$ to $+7\text{‰}$). The fracture-filling
310 sediments (clastic dykes) show variable $\delta^{13}\text{C}$ values ranging from -32.2 to $+3.5\text{‰}$, $\delta^{18}\text{O}$ values
311 vary from $+6$ to $+7\text{‰}$. Fracture-filling cements were studied in more detail. In the polyphasic
312 cements $\delta^{13}\text{C}$ values range from -56.3 to -16.6‰ ; $\delta^{18}\text{O}$ values vary from -6.3 to $+6.6\text{‰}$. Micromill
313 analyses were performed on a 5 mm-wide cement-filled fracture from below the domal structure
314 (Fig. 8; sample DM115), revealing a significant variation of the isotopic composition. The $\delta^{13}\text{C}$
315 values are strongly negative (as low as -56.3‰) with a trend toward higher values in the centre of
316 the fracture (-41‰). The $\delta^{18}\text{O}$ values decrease toward the centre, with positive values (as high as
317 $+6.6\text{‰}$) close to the fracture walls and negative values (as low as -6.2‰) in the axial part.

318

319 *4.1.3 Lipid biomarkers*

320 A molecular fossil (lipid biomarker) approach was applied in order to look into the role of
321 microbial activity in carbonate precipitation (cf. Birgel et al., 2008b). Gas-chromatography
322 amenable molecular fossils were extracted from two different portions of the most prominent
323 septarian-like bed (Fig. 3B). Sample ZF105 derives from the lower portion of this bed; its carbonate
324 component is representative of the intergranular cement. Sample DM115 was taken close to the
325 domal structure and is representative of the fracture-filling cement (Fig. 9).

326

327 4.1.3.1. *Intergranular cement (sample ZF105)*. The head-to-tail linked C₂₀ isoprenoid phytane was
328 identified in trace amounts in the hydrocarbon fraction. In the alcohol fraction, low amounts of the
329 archaeal diether-bound isoprenoids archaeol and *sn*-2 hydroxyarchaeol were detected (Fig. 9).
330 The content of archaeol is 2-fold higher than that of *sn*-2 hydroxyarchaeol. Both compounds
331 represent membrane lipids of various archaea, including methanogens, halophiles, as well as
332 methanotrophs (e.g. de Rosa and Gambacorta, 1988; Teixidor et al., 1993; Koga et al., 1993,
333 1998; Hinrichs et al., 1999), whereas phytane can be a degradation product of (1) archaeal
334 membrane lipids (e.g. Birgel et al., 2006b) or (2) chlorophyll (e.g. Goossens et al., 1984). Glycerol
335 dibiphytanyl glycerol tetraethers (GDGTs) were identified as their ether-cleaved biphytanes.
336 GDGTs are sourced by various archaea, for example planktic thaumarchaea and benthic
337 euryarchaea including methanogens and methanotrophs (e.g. DeLong et al., 1998; King et al.,
338 1998; Schouten et al., 2000; Lipp and Hinrichs, 2009). Ether-cleaved biphytanes were not
339 quantified, but a relative distribution of the biphytane chains was determined. The acyclic biphytane
340 is the predominant biphytane with 57% of all biphytanes (Fig. 9), whereas all cyclic biphytanes
341 range between 11 to 16%, with the monocyclic biphytane being least abundant. The $\delta^{13}\text{C}$ value
342 found for archaeol is -40‰ , that of *sn*-2 hydroxyarchaeol is -43‰ , whereas the content of phytane
343 was too low to measure its isotopic composition. Similarly, the overall contents of ether-cleaved
344 biphytanes were too low for isotope analysis, with the exception of acyclic biphytane, which yielded
345 a value of -22‰ .

346 Bacterial compounds are present in the alcohol and carboxylic acid fractions, comprising
347 terminally-branched C₁₅ fatty acids (*iso* and *anteiso* fatty acids), with a slight preponderance of the
348 *anteiso* over *iso* fatty acid. Non-isoprenoidal dialkyl glycerol diethers (DAGEs) are dominated by
349 C₃₀ DAGEs. Contents of the identified DAGEs are somewhat higher than those of the archaeal
350 isoprenoid diethers archaeol and *sn*-2 hydroxyarchaeol, but lower than those of fatty acids (Fig. 9).
351 Further bacterial molecular fossils are hopanoic acids with 17 β (H),21 β (H)-32-hopanoic acid being
352 most abundant. The $\delta^{13}\text{C}$ values of DAGEs are -30‰ on average. The $\delta^{13}\text{C}$ values of bacterial
353 fatty acids could only be measured for the *anteiso*-C₁₅ fatty acid (-29‰). The $\delta^{13}\text{C}$ values of the
354 17 β (H),21 β (H)-32-hopanoic acid was found to be -26‰ .

355

356 4.1.3.2. *Fracture-filling cement (sample DM115)*. The molecular fossil content of the fracture-filling
357 carbonate cements (sample DM115) differs from that of the intergranular cement. The overall
358 biomarker contents are significantly higher compared to those of the intergranular cement (Fig. 9).
359 The isoprenoid phytane is abundant, the tail-to-tail linked isoprenoid 2,6,10,15,19-
360 pentamethylcosane (PMI) is less abundant. PMI was not observed in sample ZF105. The content
361 of archaeol is slightly higher than in sample ZF105. Interestingly, *sn*-3 hydroxyarchaeol was
362 detected, but not *sn*-2 hydroxyarchaeol. The content of *sn*-3 hydroxyarchaeol was 2.6-fold higher
363 than that of archaeol. Biphytanes were detected after ether-cleavage of GDGTs, but only in minor
364 amounts. However, the distribution of biphytane chains is much different than that of sample
365 ZF105. The content decreases from acyclic, over monocyclic to bicyclic biphytane; tricyclic
366 biphytane is not present. Biphytanic diacids were only identified in this sample. Interestingly, $\delta^{13}\text{C}$
367 values of almost all isoprenoids range from -106‰ (PMI) to -101‰ (*sn*-3 hydroxyarchaeol), with
368 the exception of phytane, which is less ^{13}C -depleted, yielding a value of -70‰ .

369 Compared to the contents of archaeal lipids, the bacterial terminally-branched fatty acids
370 are only present in low amounts, but the mid-chain branched 10Me-C₁₆ fatty acid is abundant (Fig.
371 10). DAGEs were not identified, but two bacterial monoalkyl glycerol monoethers (MAGEs),
372 MAGE-C₁₆ and MAGE-10Me-C₁₆, were recognised. 17 β (H),21 β (H)-hopanoic acids with 31 to 33
373 carbons were found, with the C₃₂ homologue being most abundant. The $\delta^{13}\text{C}$ values of all bacterial
374 biomarkers are very similar, ranging from -78 (10Me-C₁₆ fatty acid) to -74‰ (hopanoic acids).
375 Only the MAGE-C₁₆ is significantly more ^{13}C -depleted (-95‰).

376

377 5. Discussion

378

379 5.1. *Stable isotope and lipid biomarker signatures of the septarian-like beds: evaluating the role of*
380 *microorganisms in carbonate authigenesis*

381

382 5.1.1. *Intergranular cement*

383 Differently from the narrow range of carbon isotope signals found for the Sant'Agata Fossili
384 marls (−4.1 to −1.1‰), a wide range of $\delta^{13}\text{C}$ values (−0.4 to +6.2‰), has been observed for the
385 intergranular dolomite cement of the lower part of the septarian-like beds. The exact formation
386 mechanisms and the possible contribution of microorganisms in diagenetic dolomite formation with
387 positive carbon isotope values is still not well understood, although there is growing evidence that
388 ^{13}C -enriched dolomite indeed forms in the zone of methanogenesis (Mazzullo, 2000; Warren,
389 2000; Meister et al., 2011). Interestingly, ^{13}C -enriched dolomites of the Miocene Monterey
390 Formation contain abundant molecular fossils of archaea, some of which apparently represented
391 methanogens (Hoffmann-Sell et al., 2011). Carbonates precipitated from an ^{13}C -enriched carbon
392 pool affected by methanogenesis show values as high as +34‰ (Boehme et al., 1996; Greinert et
393 al., 2001; Budai et al., 2002; Meister et al., 2011; Hoffmann-Sell et al., 2011).

394 A completely different signature characterizes the domal structure of the most prominent
395 septarian-like bed where strongly ^{13}C -depleted intergranular dolomite cement (−48 to −35‰)
396 occurs. This locally confined ^{13}C depletion suggests a causal relationship between the genesis of
397 the fractures in the domal structure and carbonate formation resulting from methane oxidation (cf.
398 Peckmann and Thiel, 2004). This is supported by the strongly negative $\delta^{13}\text{C}$ values of the fracture-
399 filling cements (see paragraph 5.1.2.).

400 Despite this exception, intergranular cement is remarkably enriched in ^{13}C , suggesting that
401 archaeal methanogenesis was a prominent process during the formation of septarian-like beds.
402 Their inventory of molecular fossils confirms that archaea strongly imprinted the early diagenetic
403 environment. The archaeal lipids archaeol and *sn*-2 hydroxyarchaeol occur in many archaeal
404 groups, including various methanogens (e.g. Koga et al., 1998). Their $\delta^{13}\text{C}$ values of −40 and
405 −43‰, respectively, do not provide undisputable evidence to constrain the metabolism of the
406 source organisms. These values alone do not allow to judge with certainty if the archaea were
407 methanotrophs, methanogens, or heterotrophic sedimentary archaea, although the latter group
408 appears to synthesize archaeal diethers only in minor amounts (Lipp and Hinrichs, 2009). Other
409 archaeal biomarkers found in the intergranular cement of the septarian-like bed, as for example
410 GDGTs, measured as ether-cleaved biphytanes in this study, help to identify the affiliation of the

411 source organisms and point in another direction than the diethers. The relatively higher content of
412 monocyclic biphytane, as well as a lower relative abundance of the tricyclic biphytane in the
413 intergranular cement compared to fracture-filling cement typifies heterotrophic archaea thriving in
414 the deep biosphere (cf. Biddle et al., 2006; Lipp and Hinrichs, 2009). The observed pattern of
415 archaeal lipids renders unlikely a major contribution of planktic thaumarchaea. Interestingly, an
416 increased relative abundance of acyclic biphytane is also typical for methanogenic archaea
417 (Pancost et al., 2008). A derivation of biphytanes from methanotrophic archaea, on the other hand,
418 is very unlikely, since biphytane patterns of methanotrophic archaea are different (Birgel et al.,
419 2008a). Moreover, other biomarkers of methanotrophic archaea such as PMI and crocetane
420 (Peckmann and Thiel, 2004) were not detected in the intergranular cement. Biomarkers of
421 sulphate-reducing bacteria (SRB), the syntrophic partners of methanotrophic archaea in AOM, are
422 present (*iso* and *anteiso* fatty acids, DAGEs), but their relatively high $\delta^{13}\text{C}$ values do not reflect the
423 incorporation of methane-derived carbon, revealing that the source organisms were not involved in
424 AOM. For example, $\delta^{13}\text{C}$ values of DAGEs and *anteiso*-C₁₅ fatty acid are approximately 10‰
425 higher than those of archaeol and *sn*-2 hydroxyarchaeol.

426 Based on their isotopic composition, biphytanes (acyclic biphytane: -22‰) and archaeal
427 diethers (average -41‰) are apparently not derived from the same source organisms, although
428 the reasoning above suggests that both groups of compounds derive from sedimentary archaea.
429 Heterotrophic sedimentary archaea typically produce lipids with intermediate $\delta^{13}\text{C}$ values (Biddle et
430 al., 2006), whereas the isotopic composition of lipids of methanogenic archaea can vary
431 significantly in culture and the environment depending on substrates and conditions (Londry et al.,
432 2008; Hoffmann-Sell et al., 2011). The observed biphytane distributions resemble those of
433 heterotrophic archaea (cf. Biddle et al., 2006), whereas archaeol and *sn*-2 hydroxyarchaeol most
434 likely reflect archaeal methanogenesis. Overall, the biomarker pattern and isotopic signatures of
435 lipids in the ^{13}C -enriched intergranular cements point to dolomite precipitation close to the interface
436 of the zones of methanogenesis (archaeal diethers) and sulphate reduction (terminally-branched
437 fatty acids, bacterial diethers).

438

439 5.1.2. *Fracture-filling cements*

440 In contrast to the intergranular cement, the cement filling cracks shows more negative $\delta^{13}\text{C}$
441 values (-56 to -25%). Such values typify modern (e.g. Aloisi et al., 2000; Ussler and Paull, 2008;
442 Bahr et al., 2010) and ancient seep carbonates (e.g. Peckmann et al., 1999; Campbell et al., 2006;
443 Himmler et al., 2008; Clari et al., 2009). Therefore, these values reflect incorporation of methane-
444 derived carbon, pointing to AOM (e.g. Ritger et al., 1987). However, the less negative values may
445 also point to other sources or a mixture of various sources. Possible carbon sources of the
446 fracture-filling cement include (1) thermogenic methane (cf. Whiticar, 1999), deriving from the
447 Mesozoic sequence underlying the Cenozoic succession, (2) contributions of fluids enriched in
448 heavier hydrocarbons, such as ethane, propane or crude oil (cf. Roberts and Aharon, 1994), (3) a
449 contribution from a carbonate pool affected by methanogenesis, (4) organic matter degradation
450 (Moozley and Burn, 1993; Raiswell et al, 2002), or (5) the contribution of marine dissolved
451 inorganic carbon and skeletal material of marine organisms.

452 The former occurrence of AOM is confirmed by the lipid biomarker data that resemble those
453 of ancient seep carbonates. One of the most abundant and persistent AOM biomarkers is PMI (e.g.
454 Elvert et al., 1999; Peckmann and Thiel, 2004), showing a $\delta^{13}\text{C}$ value of -106% in the fracture-
455 filling cement. It is accompanied by ^{13}C -depleted archaeol (-102%) and abundant biphytanic
456 diacids (-105%) containing 0, 1, and 2 cyclopentane rings. The latter compounds have been
457 described from ancient seep carbonates and have been suggested to represent particularly reliable
458 AOM biomarkers, often revealing the lowest $\delta^{13}\text{C}$ values among all AOM-lipids (Birgel et al.,
459 2008a). The *sn*-3 hydroxyarchaeol (-101%) detected in the fracture-filling cement has been
460 recognized at some seeps, but this compound is rather uncommon and it is unknown by which
461 type of methanotrophic archaea it is produced (Pancost et al., 2001b; De Boever et al., 2009).

462 A further difference from the intergranular cement is the presence of ^{13}C -depleted
463 biomarkers of SRB. However, biomarkers of SRB (10Me- $\text{C}_{16:0}$ fatty acid: -78% ; *anteiso*- $\text{C}_{15:0}$ fatty
464 acid: -75%) show very low contents compared to archaeal biomarkers. *Anteiso*- $\text{C}_{15:0}$ fatty acid is a
465 biomarker of SRB involved in AOM of rather high specificity (Elvert et al., 2003; Birgel et al.,
466 2006b). 10Me- $\text{C}_{16:0}$ is a well known biomarker of SRB, but this compound does not typically occur

467 in SRB involved in AOM. The two non-isoprenoidal MAGEs detected belong to a group of
468 compounds known to be synthesized by mesophilic SRB (Rütters et al., 2001) and have been
469 identified at modern seeps, revealing extreme ^{13}C -depletions (e.g. Hinrichs et al., 2000; Pancost et
470 al. 2001a). In ancient methane-seep limestones, MAGEs were never identified before, suggesting
471 an exceptional preservation of molecular fossils in the fracture-filling cement. The observed
472 hopanoic acids, exhibiting very similar isotopic composition like the other SRB-derived compounds,
473 probably derive from SRB as well, since no molecular fossils of aerobic methanotrophic bacteria
474 were identified such as 3-methylated hopanoids or lanostanes (cf. Birgel and Peckmann, 2008). In
475 summary, the low $\delta^{13}\text{C}_{\text{carbonate}}$ values as well as molecular fossils and their isotopic compositions
476 reveal that AOM occurred in the fractures of the septarian-like bed and confirm that carbonate
477 precipitation resulted from AOM.

478

479 5.2. Gas hydrates in the shallow subsurface: insights from oxygen isotopes

480

481 Apart from carbon isotopes, oxygen isotopes are commonly used to characterize the
482 composition and temperature of pore fluids at the time of carbonate precipitation (e.g. Fritz and
483 Smith, 1970; Vasconcelos et al., 2005). The oxygen isotope composition of carbonates precipitated
484 in equilibrium with early Messinian seawater averages around -2.0‰ for calcite and $+2.0\text{‰}$ for
485 dolomite (e.g. Pierre et al., 1998; Pierre and Rouchy, 2004). The $\delta^{18}\text{O}$ values of the studied
486 carbonates show a wide range of both extremely positive values (as high as $+7.7\text{‰}$) as well as
487 negative values (as low as to -6.3‰). The excellent preservation of molecular fossils rules out
488 substantial late diagenetic alteration. The oxygen isotope pattern consequently suggests that the
489 Ripa dello Zolfo authigenic carbonates precipitated from fluids with $\delta^{18}\text{O}$ values different from those
490 of normal marine Messinian seawater. Processes that can produce ^{18}O -enrichment include
491 evaporation of sea water (e.g. McKenzie et al., 1979), dehydration of smectite clay minerals
492 (Dählmann and de Lange, 2003), and gas hydrate destabilization (e.g. Aloisi et al., 2000; Pierre
493 and Rouchy, 2004). With respect to the positive $\delta^{18}\text{O}$ values of the Ripa dello Zolfo carbonates,
494 Dela Pierre et al. (2010) concluded that the coincidence of positive $\delta^{18}\text{O}$ values with negative $\delta^{13}\text{C}$

495 values agrees best with gas hydrate destabilization. This interpretation is further supported by the
496 occurrence of unusual cements within the septarian-like fractures, characterized by pinch-out
497 structures, which have been interpreted as cements resulting from gas hydrate decomposition
498 (Martire et al., 2010).

499 Negative $\delta^{18}\text{O}$ values were not observed in the Ripa dello Zolfo carbonates before (Dela
500 Pierre et al., 2010; Martire et al., 2010). Such values are only found in the fracture-filling cement
501 (Fig. 9) and, in particular, within the last carbonate phase sealing the fractures. These values
502 consequently recorded the isotopic composition of the latest fluids circulating in the cracks. The
503 explanations most commonly brought forward for anomalously low oxygen values are (1) dilution of
504 parent fluids by meteoric waters (Mozley and Burns, 1993), (2) low-temperature alteration of
505 volcanic material (Gieskes and Lawrence, 1981), (3) upward migration of high-temperature fluids
506 (Sample and Kopf, 1995), and (4) formation of gas hydrates (e.g. Pierre and Rouchy, 2004). The
507 absence of volcanoclastic layers in the stratigraphic succession allow to exclude the alteration of
508 volcanic glass. The negative oxygen isotope signatures of the Ripa dello Zolfo carbonates coupled
509 with strongly negative $\delta^{13}\text{C}$ values ($\delta^{13}\text{C} < -50\text{‰}$), also make meteoric fluids an unlikely
510 explanation. Moreover, the severely ^{13}C -depleted carbonates, more consistent with biogenic
511 methane produced in the shallow subsurface than with thermogenic gas, allow to exclude hot,
512 deeply sourced fluids. Finally, structures believed to be related to the former presence of gas
513 hydrates in the sediments suggests that the observed negative $\delta^{18}\text{O}$ values in the fracture fillings
514 indeed reflect gas hydrate formation, with the low values resulting from the incorporation of ^{18}O -
515 enriched water in the gas hydrates structure (cf. Ussler and Paull, 1995).

516

517 *5.3. Genesis of the carbonate-rich beds*

518

519 A new scenario for the formation of stratiform concretions is proposed here that is based on
520 petrographic and geochemical results, but also builds on data and interpretations of previous
521 studies (Dela Pierre et al., 2010; Martire et al., 2010). The proposed scenario considers the
522 contrasting isotope values and biomarker patterns recognized in the septarian-like beds,

523 suggesting that different types of microorganisms thrived under varying environmental conditions.
524 The high carbonate content of 47 to 95 wt.% of the studied beds compared to the host marls
525 (<15%) reveals that bed formation occurred during an early diagenetic stage within still soft and
526 porous Messinian muds at shallow depths. The lack of sediment compaction and the absence of
527 chemosymbiotic macrofossils, which are commonly associated with methane seeps at the seafloor,
528 point to a formation in the shallow subsurface. Unlike typical sub-spherical or ellipsoidal
529 concretions, whose formation occurs around a nucleus (e.g. Sellés-Martinez, 1996; Raiswell and
530 Fisher, 2000), or cylindrical concretions where carbonate precipitation follows the pathway of fluid
531 flow through the sedimentary column (e.g. Clari et al., 2004; De Boever et al., 2009; Nyman et al.
532 2010), the bedding-parallel geometry of the studied examples points to formation at a geochemical
533 interface parallel to the seafloor (cf. Meister et al., 2008; Dela Pierre et al., 2010).

534 Three main stages, each of them characterized by different environmental conditions, are
535 purported to explain the genesis of the Ripa dello Zolfo beds (Fig. 10).

536 537 *5.3.1. Microbial sulphate reduction close to the sediment-water interface*

538 Studies on modern marine sediments demonstrated that bacterial sulphate reduction, i.e.
539 the most common process degrading organic matter apart from aerobic degradation in the marine
540 subsurface, is capable of inducing carbonate precipitation (e.g. Mozley and Burns, 1993). In all
541 Ripa dello Zolfo cemented beds the former occurrence of sulphate reduction is documented by
542 abundant pyrite framboids (Fig. 5F). Moreover, abundant and well-preserved biomarkers of SRB
543 suggest that these bacteria favoured carbonate precipitation (Fig. 10A). However, although
544 sulphate-reduction is usually a dominant biogeochemical process in marine sediments, the lack of
545 moderately low $\delta^{13}\text{C}$ values (Fig. 7) typifying this process suggests that only a small amount of
546 carbonate was produced by sulphate reduction in this case. Accordingly, Raiswell and Fisher
547 (2000, 2004) suggested that carbonate precipitation via sulphate reduction is commonly not
548 significant enough to produce extensive cementation in concretions, calling for other processes to
549 contribute to the formation of concretions and diagenetic beds.

550

551 5.3.2. *Methanogenesis*

552 Initial carbonate precipitation driven by sulphate reduction reduced the sediment pore
553 space, causing a slight reduction of permeability (Fig. 10A). Ongoing sedimentation led to
554 progressive burial and to a relative downward movement of the semi-lithified beds, which were now
555 placed in the zone of archaeal methanogenesis. Here, the residual organic matter not degraded by
556 sulphate-reducing bacteria was decomposed by archaea, as recorded by ^{13}C -enriched dolomite
557 microcrystals (values as high as +6‰; Fig. 10B). *In situ* methanogenesis is further supported by
558 the prominent occurrence of archaeal molecular fossils in the Ripa dello Zolfo beds. These
559 molecules have $\delta^{13}\text{C}$ values as low as -43‰, falling between the values typically found for
560 methanogenic and methanotrophic archaea. However, the range of $\Delta_{\text{substrate-archaeol}}$ in methanogens
561 is extremely large (Londry et al., 2008). In laboratory experiments, Londry et al. (2008) found
562 $\Delta_{\text{substrate-archaeol}}$ in autotrophic methanogenic archaea varying from 11 to 43‰, depending on the
563 experimental conditions. In dolomites from the Monterey Formation, a $\Delta_{\text{substrate-archaeol}}$ of 33‰ ($n = 4$)
564 was reconstructed and interpreted as a signature of methanogenic archaea. In the sample of
565 intergranular cement, the $\Delta_{\text{substrate-archaeol}}$ would have been 46‰, if one uses the mean of the
566 $\delta^{13}\text{C}_{\text{dolomite}}$ values to assess the stable carbon isotopic composition of CO_2 consumed by
567 methanogens. This fractionation is even larger than the largest fractionation found in the laboratory
568 experiments of Londry et al. (2008). However, based on the $\delta^{13}\text{C}_{\text{dolomite}}$ values and since the
569 biomarker inventory of the intergranular cement is more typical of methanogenic rather than
570 methanotrophic archaea and very different from the inventory typifying methanotrophic archaea in
571 the fracture-filling cement, it is most likely that methanogenesis was a prominent process.

572

573 5.3.3. *Anaerobic oxidation of methane in newly generated cracks*

574 The continuing cementation of the Ripa dello Zolfo beds generated a permeability barrier
575 that hampered the rise of fluids, which presumably increased the pore pressure. High pore
576 pressure probably induced the opening of septarian-like fractures within those portions of the beds
577 not yet affected by complete cementation (Fig. 10C). Similarly, an increase in pore fluid pressure
578 favoured by long-lasting seepage activity has been considered as a possible mechanism for crack

579 formation and the injection of mud in laterally extensive seep deposits (Peckmann et al., 2011).
580 The sediment injection, the unusual lobed shape of the domal structure, and the associated
581 fracture system placed at high angle to the bedding plane suggest that fluid overpressure, probably
582 related to supply of methane-rich fluid from deeper stratigraphic levels, is responsible for the
583 opening of a set of septarian-like cracks subsequently infilled by sediment. Similar mechanisms
584 have already been inferred for septarian concretions (e.g. Hounslow, 1997; Astin and Scotchman,
585 1988; Pratt, 2001; Scotchman et al., 2002; Bojanowski, 2007). The Ripa dello Zolfo domal
586 structure resembles a cavernous carbonate crusts from the Black Sea, also suggested to reflect
587 deformation of sediments in the very shallow subsurface induced by overpressure (Mazzini et al.,
588 2008). However, shrinkage processes induced by syneresis, and enhanced by the decay of
589 extracellular polymeric substances can also be responsible for the opening of these fractures (cf.
590 Hendry et al., 2006; Dela Pierre et al., 2010).

591 The $\delta^{13}\text{C}$ values and biomarker patterns of dolomite and calcite cement filling the septarian
592 cracks confirm that AOM was the dominant biogeochemical process in these cavities. At this stage,
593 the sulphate-methane transition zone was shifted to greater sediment depths. This shift probably
594 resulted from the fracturing itself, which allowed a downward flux of sulphate-rich fluids into the
595 newly formed cracks. With methane coming from below, now penetrating the Ripa dello Zolfo bed
596 after fracturing, AOM proceeded and induced cement formation. The intergranular cements
597 surrounding the cracks of the domal structure, marked by negative $\delta^{13}\text{C}$ values, also reflect AOM
598 (Fig.10C), revealing that the matrix of the carbonate bed was still permeable enough to be affected
599 by diagenetic processes. On the basis of the pinch-out geometry of some cements and oxygen
600 isotope values of the crack-filling cements, it has been suggested that methane-rich fluids resulted
601 from gas hydrate decomposition during this stage (Dela Pierre et al., 2010; Martire et al., 2010).

602

603 **6. Conclusions**

604

605 The petrographical, stable isotope, and biomarker data reveal that the upper Miocene Ripa
606 dello Zolfo beds are the product of different biogeochemical processes that induced carbonate

607 precipitation in the shallow subsurface. The most intriguing feature of the septarian-like beds is the
608 concomitant presence of a wide array of stable isotope compositions typifying different generations
609 of carbonate cements. Strongly variable carbon isotope signatures ($\delta^{13}\text{C}$: -56 to $+6\%$) and
610 molecular fossils reveal that sulphate reduction, methanogenesis, and finally anaerobic oxidation of
611 methane occurred within the same sediment volume and resulted in the formation of the Ripa dello
612 Zolfo septarian-like beds. Whereas first sulphate reduction and then methanogenesis occurred in
613 the pore space of sediments, anaerobic oxidation of methane occurred only later within cracks
614 after the beds had been fractured by overcritical pore pressures. Pore fluid overpressure is further
615 supported by the occurrence of abundant sediment injections, suggesting that opening of cracks
616 and injection of liquefied mud were triggered by seepage activity. This study represents an
617 interesting example for the impact of biogeochemical processes on organic-rich sedimentary
618 strata. It reveals that the sequence of biogeochemical processes – which is governed by the
619 energy yield of the respective processes – can be modified by synsedimentary events. In this case,
620 fracturing of semi-lithified beds allowed anaerobic oxidation of methane to occur after
621 methanogenesis, which is usually the terminal process in the remineralisation of organic matter.

622

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624

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632

633

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1032 **FIGURE CAPTIONS**

1033

1034 **Fig. 1** Structural sketch map of northwestern Italy (modified from Bigi et al., 1990). VVL:

1035 Villalvernia Varzi Line; SVZ: Sestri Voltaggio Zone

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1037 **Fig. 2** Simplified geological sketch of the studied area (modified from Ghibaudo et al., 1985)

1038 showing the location of the measured stratigraphic sections.

1039

1040 **Fig. 3** (A) The stacking pattern of the carbonate-rich beds recognised in the upper member of

1041 SAF, reconstructed after the measurement of three stratigraphic sections. (B) Location of the

1042 studied samples in the septarian-like bed and their isotopic signature. VVC: Valle Versa chaotic

1043 complex; SAF: Sant'Agata Fossili marls

1044

1045 **Fig. 4** (A) Outcrop view of the septarian-like bed (bed 4 in Fig.3). (B) Close-up of bed 4; note the

1046 sharp contact with the enclosing poorly consolidated sediments and the lower and upper wavy

1047 surface.

1048

1049 **Fig. 5** (A) Polished slab of a septarian-like bed cut perpendicular to bedding, showing an intricate

1050 network of fractures filled with carbonate cements, sediments or still empty (sample DM136); the

1051 isotope signatures of the intergranular dolomite cement of both the concretion body and the clastic

1052 dykes are indicated. (B) Polished slab of the upper part of the septarian-like bed (sample ZF114)

1053 characterized by empty septarian cracks. (C-D) Photomicrographs in transmitted light (C) and in

1054 epi-fluorescence (D) of a septarian-like fracture. Note in (D) the intense autofluorescence of the

1055 dolomite-rich muddy sediments; the white arrow indicates an empty cavity. (E) Large, euhedral

1056 dolomite crystals, showing an interpenetration twinning of rhombohedral crystals. (F) Pyrite

1057 framboid composed of aggregates of hypidiomorphic cubes.

1058

1059 **Fig. 6** (A) Unusual domal structure observed in a septarian-like bed. The lower part of the bed is
1060 affected by a major fracture system, oriented at high angle to the bedding planes; the upper part is
1061 characterized by a lobed shape and by empty septarian cracks. (B) Polished slab (sample DM115)
1062 perpendicular to bedding, revealing the high-angle fracture system, with fractures mainly filled with
1063 polyphasic carbonate cements (see Fig. 6A for location). (C) Photomicrograph showing spherulites
1064 (S) made up of different generation of carbonate cements, growing directly on the fracture walls
1065 and on clasts within the cracks.

1066
1067 **Fig. 7** Cross-plot of the stable isotope data of carbonate phases making up the septarian-like beds
1068 and the unconsolidated marls. SAF: Sant'Agata Fossili marls.

1069
1070 **Fig. 8** Septarian-like bed with a 5 mm wide fracture filled with polyphasic carbonate cements. The
1071 black square indicates a high-resolution transect of isotope analyses performed with the micromill
1072 technique.

1073
1074 **Fig. 9** Synthesis of carbon and oxygen stable isotope values and biomarker results of the two
1075 types of samples analysed.

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1077 **Fig. 10** Scenario showing the genesis of the septarian-like beds. See text for further details.

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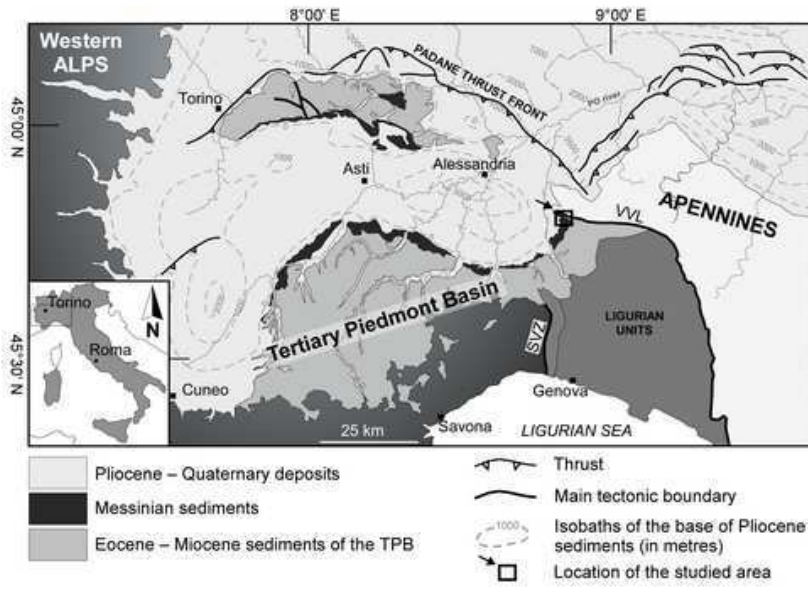
1082 **TABLES**

1083

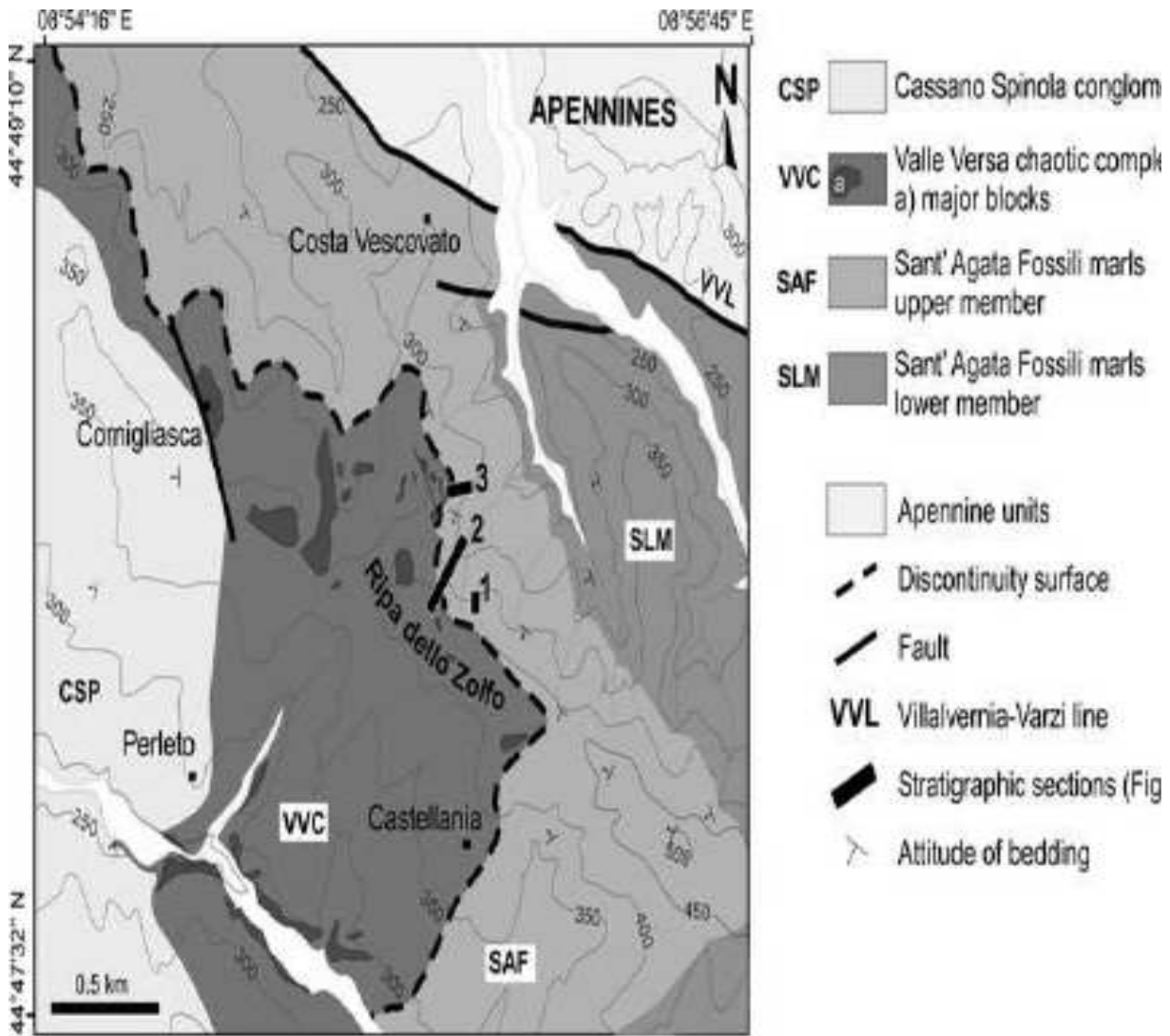
1084 **Table 1** Total carbonate content and relative abundance of dolomite and calcite for the
1085 septarian-like beds and the unconsolidated marls (ICP analyses).

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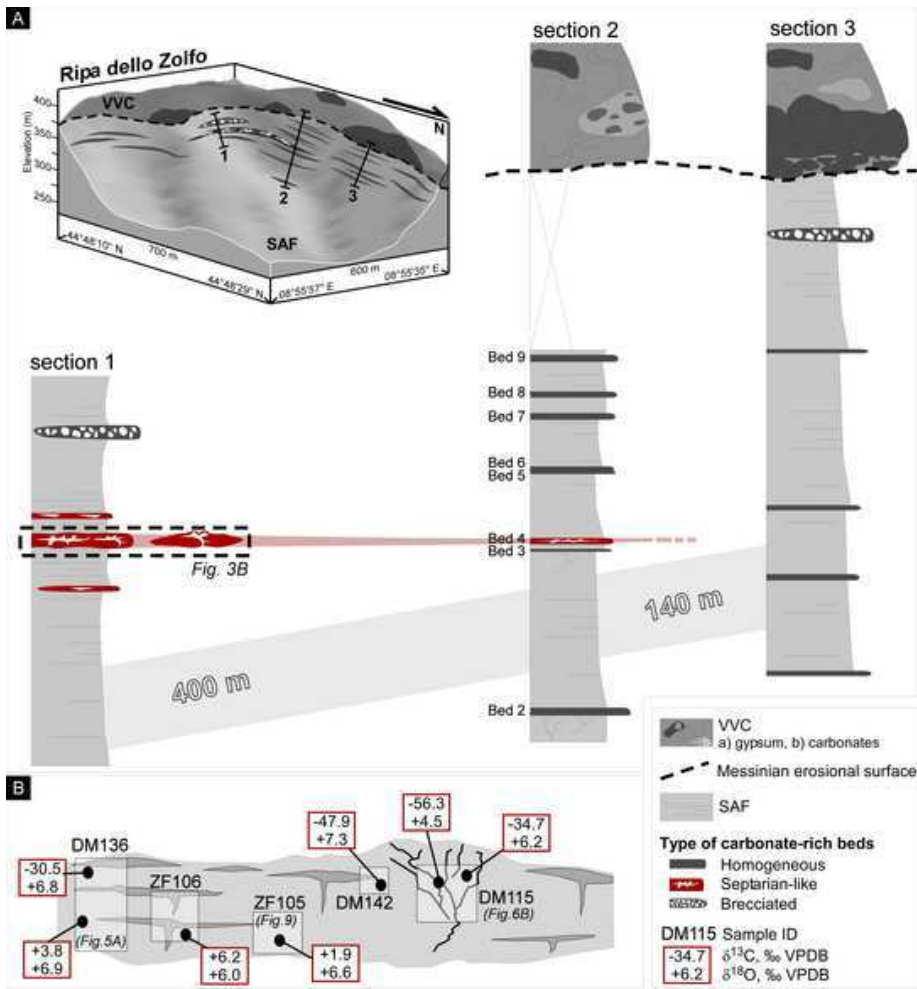
1087 **Table 2** Carbon and oxygen isotope composition of the septarian-like beds and of the
1088 unconsolidated marls; samples from the same fracture analysed by micromilling are marked by an
1089 asterisk (see also Fig. 7)



Natalicchio et al. Fig.1



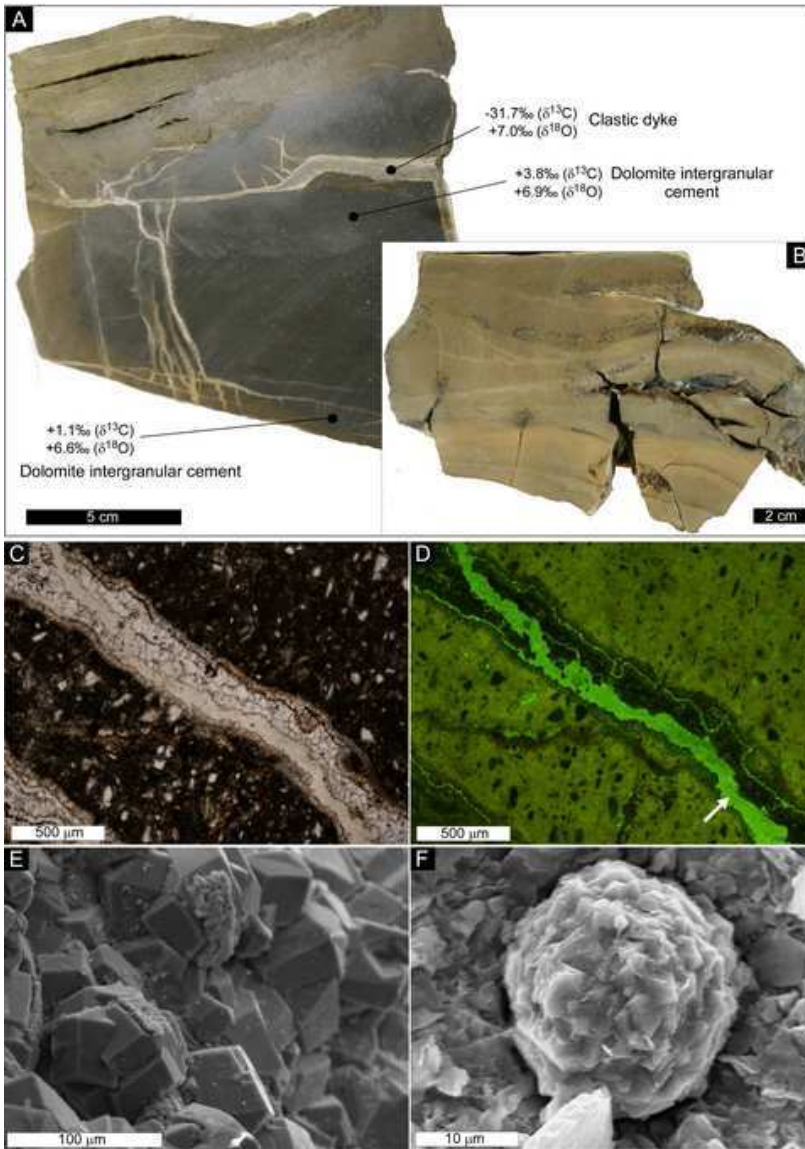
Natalicchio et al. Fig.2



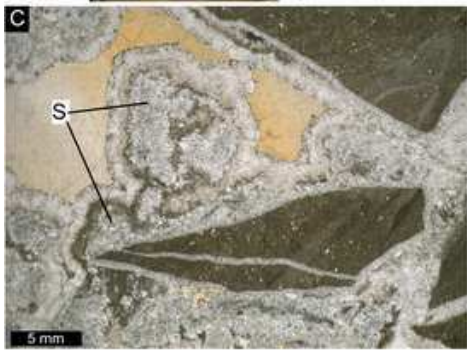
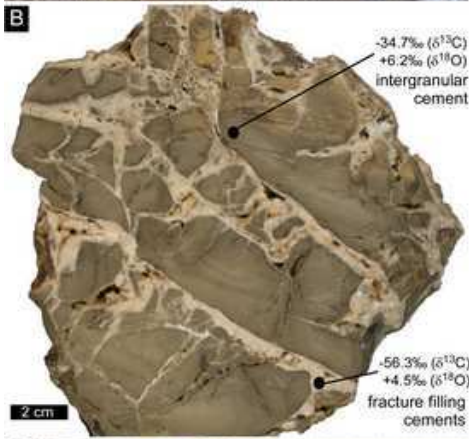
1095 Natalicchio et al. Fig.3.



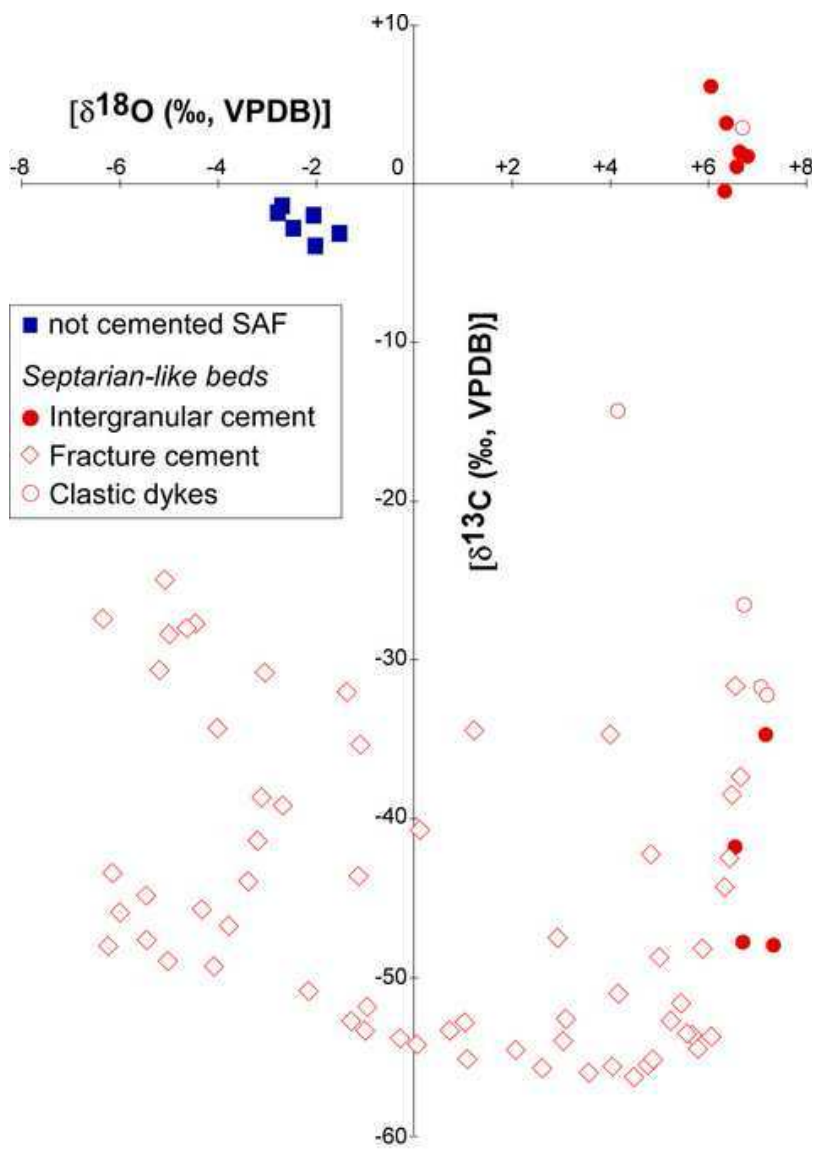
1096 Natalicchio et al. Fig.4



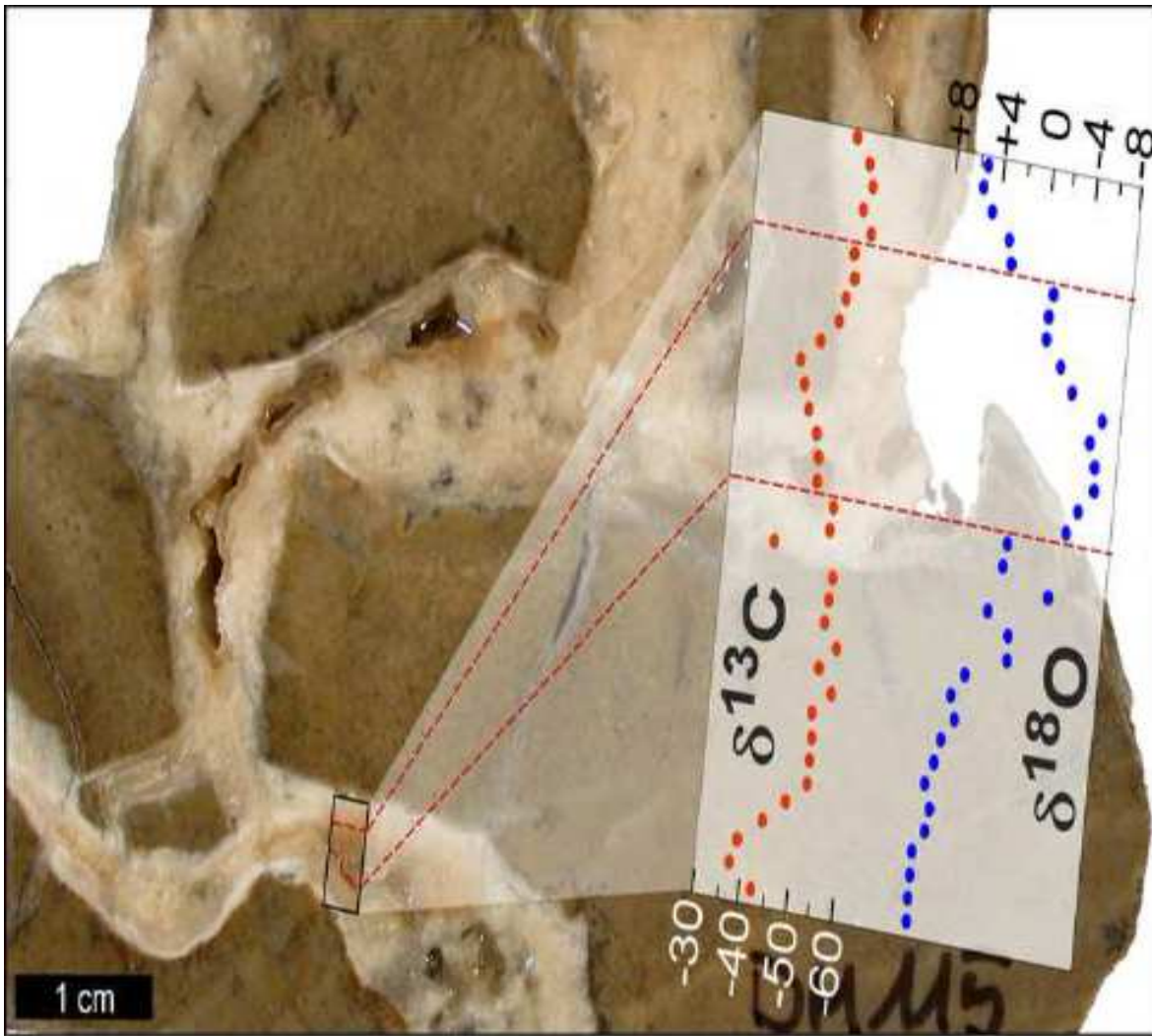
1097 Natalicchio et al. Fig.5



1098 Natalicchio et al. Fig.6


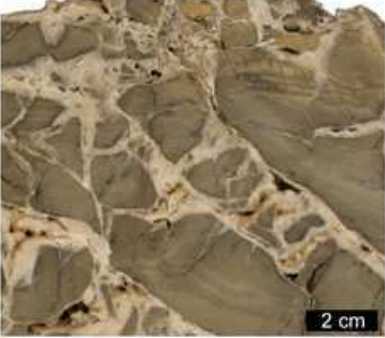


1099 Natalicchio et al. Fig.7

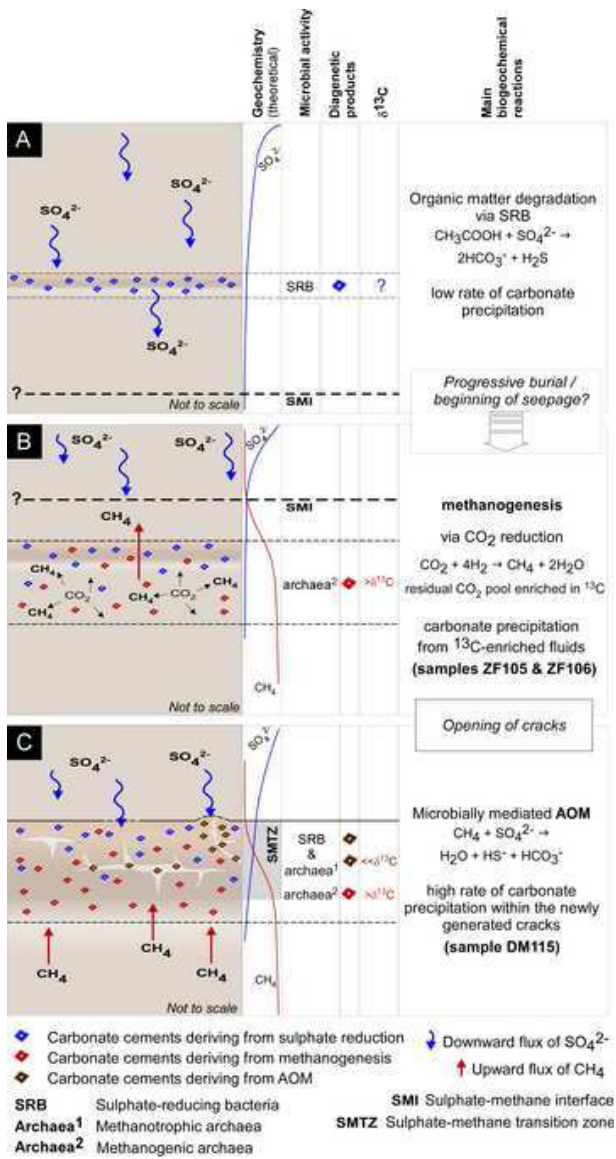


Natalicchio et al. Fig.8

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Sample ZF105			Sample DM115			
						
Type of bed: Septarian-like Type of analysed cement: intergranular			Type of bed: Septarian-like Type of analysed cement: fracture filling			
$\delta^{13}\text{C}$: 1.9‰, VPDB $\delta^{18}\text{O}$: +6.6‰, VPDB			$\delta^{13}\text{C}$: from -56.3 to -24.9‰, VPDB $\delta^{18}\text{O}$: from -6.2 to +6.5‰, VPDB			
Archaeal biomarkers		$\mu\text{g/g}$ rock	$\delta^{13}\text{C}$ ‰ VPDB		$\mu\text{g/g}$ rock	$\delta^{13}\text{C}$ ‰ VPDB
	phytane	tr	tr	phytane	44.0	-70
	archaeol	0.8	-40	PMI	23.3	-106
	<i>sn</i> -2 hydroxyarchaeol	0.4	-43	archaeol	5.4	-102
	acyclic biphytane#	57%	-22	<i>sn</i> -3 hydroxyarchaeol	13.8	-101
	monocyclic biphytane#	11%	nd	acyclic biphytanic diacid	37.4	-104
	bicyclic biphytane#	16%	nd	monocyclic biphytanic diacid	37.3	-105
	tricyclic biphytane#	16%	nd	bicyclic biphytanic diacid	17.5	-105
				acyclic biphytane#	46%	nd
				monocyclic biphytane#	33%	nd
				bicyclic biphytane#	21%	nd
Bacterial biomarkers	<i>iso</i> -C _{15,0} fatty acid	0.9	nd	<i>iso</i> -C _{15,0} fatty acid	2.3	nd
	<i>anteiso</i> -C _{15,0} fatty acid	1.0	-29	<i>anteiso</i> -C _{15,0} fatty acid	3.8	-75
	DAGE C _{30a}	1.5	-30	10MeC _{16,0} fatty acid	10.8	-78
	DAGE C _{30b}	0.9	nd	MAGE C _{16,0}	4.0	-95
	DAGE C _{30c}	1.5	-30	MAGE 10MeC _{16,0}	2.0	nd
	17 β (H),21 β (H)-32-hopanoic acid	1.8	-26	17 β (H),21 β (H)-31-hopanoic acid	2.3	nd
				17 β (H),21 β (H)-32-hopanoic acid	14.8	-74
				17 β (H),21 β (H)-33-hopanoic acid	3.0	-74

DAGE: dialkyl glycerol ether; MAGE: monoalkyl glycerol ether; tr: traces; nd: not determined due to low contents or co-elution; #: no concentrations are available for GDGT-cleaved biphytanes, only relative proportions of the various biphytanes were calculated.



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Sample	Carbonate [wt.%]	Dolomite [%]	Calcite [%]
<i>Septarian-like beds</i>			
DM136T	59.5	53.5	6.0
DM136B	72.1	70.0	2.1
DM75	91.7	41.5	50.2
<i>Maris</i>			
OM5	13.3	5.2	8.1
OM3	15.0	5.2	9.8

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Sample	Cement type	$\delta^{13}\text{C}$ [‰]	$\delta^{18}\text{O}$ [‰]
<i>Septarian-like beds</i>			
ZF105	Intergranular cement	-0.4	+6.3
ZF105-b	Intergranular cement	+1.9	+6.6
ZF106C	Intergranular cement	+6.2	+6.0
ZF106E	Intergranular cement	+1.8	+6.8
DM115-1	Intergranular cement	-34.7	+7.2
DM136-1	Intergranular cement	+3.8	+6.4
DM136-4	Intergranular cement	+1.1	+6.6
DM142 7.3	Intergranular cement	-47.7	+6.7
DM142 7.7	Intergranular cement	-41.8	+6.5
DM142-b	Intergranular cement	-47.9	+7.3
ZF103B	carbonate vein	-34.4	+1.2
DM115-2	carbonate vein	-52.6	+3.1
DM136-5	carbonate vein	-38.7	-3.1
DM136-6	carbonate vein	-28.0	-4.6
DM137	carbonate vein	-39.2	-2.7
FM5-1	carbonate vein	-35.3	-1.1
ZF105-c	carbonate vein	-43.9	-3.4
DM142-a	carbonate vein	-30.6	-5.2
FM5-2 1.2	carbonate vein	-28.3	-5.0
FM5-2 1.3	carbonate vein	-34.7	+4.0
FM5-2 1.4	carbonate vein	-31.6	+6.5
FM5-2 1.6	carbonate vein	-30.8	-3.0
FM5-4 2.1	carbonate vein	-40.7	+0.1
FM5-4 2.2	carbonate vein	-43.6	-1.1
FM5-4 2.3	carbonate vein	-34.3	-4.0
DM115 3.1	carbonate vein	-55.2	+1.1
DM115 3.2	carbonate vein	-27.7	-4.5
DM115 3.3	carbonate vein	-55.6	+4.7
DM115 3.5	carbonate vein	-24.9	-5.1
DM 115 5	carbonate vein*	-51.6	+5.4
DM 115 6	carbonate vein*	-54.4	+5.8
DM 115 7	carbonate vein*	-55.1	+4.8
DM 115 8	carbonate vein*	-53.9	+3.0
DM 115 9	carbonate vein*	-55.7	+2.6
DM 115 10	carbonate vein*	-52.8	-1.3
DM 115 11	carbonate vein*	-53.3	-1.0
DM 115 12	carbonate vein*	-51.9	-1.0
DM 115 13	carbonate vein*	-50.8	-2.2
DM 115 14	carbonate vein*	-46.8	-3.8
DM 115 15	carbonate vein*	-43.5	-6.2
DM 115 16	carbonate vein*	-44.8	-5.5
DM 115 17	carbonate vein*	-45.8	-6.0
DM 115 18	carbonate vein*	-48.0	-6.2
DM 115 19	carbonate vein*	-49.0	-5.0
DM 115 20	carbonate vein*	-49.3	-4.1
DM 115 22	carbonate vein*	-53.3	+0.7
DM 115 23	carbonate vein*	-52.8	+1.0
DM 115 24	carbonate vein*	-41.4	-3.2
DM 115 25	carbonate vein*	-54.5	+2.0
DM 115 26	carbonate vein*	-54.1	+0.0
DM 115 27	carbonate vein*	-53.9	-0.3
DM 115 28	carbonate vein*	-56.0	+3.5
DM 115 29	carbonate vein*	-56.3	+4.5
DM 115 30	carbonate vein*	-55.6	+4.0
DM 115 31	carbonate vein*	-52.7	+5.2
DM 115 32	carbonate vein*	-53.6	+5.6
DM 115 33	carbonate vein*	-53.7	+6.0
DM 115 34	carbonate vein*	-53.5	+5.6
DM 115 36	carbonate vein*	-48.2	+5.9
DM 115 37	carbonate vein*	-44.2	+6.3
DM 115 38	carbonate vein*	-38.5	+6.5
DM 115 39	carbonate vein*	-37.4	+6.6
DM 115 40	carbonate vein*	-42.4	+6.4
DM142 7.1	carbonate vein	-48.7	+5.0
DM142 7.2	carbonate vein	-51.1	+4.2
DM142 7.4	carbonate vein	-47.6	+2.9
DM142 7.5	carbonate vein	-47.6	-5.4
DM142 7.6	carbonate vein	-45.7	-4.3
DM142 7.8	carbonate vein	-32.0	-1.3
DM142 7.9	carbonate vein	-27.4	-6.3

DM142 7.10	carbonate vein	-42.2	+4.8
DM111-2	clastic dikes	-14.3	+4.1
ZF103A	clastic dikes	-26.6	+6.7
DM136-2	clastic dikes	-31.7	+7.0
ZF106A	clastic dikes	+3.5	+6.7
ZF106B	clastic dikes	-32.2	+7.2
<i>Marls</i>			
DM133	Marly host-rock	-4.1	-2.1
DM134	Marly host-rock	-1.7	-3.0
DM135	Marly host-rock	-1.1	-2.8
OM1	Marly host-rock	-2.8	-1.5
OM3	Marly host-rock	-2.1	-2.0
OM9	Marly host-rock	-3.0	-2.5

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Natalicchio et al., Tab.2