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<http://archimer.ifremer.fr>The original publication is available at <http://www.springerlink.com>**Heterogeneous energetic pathways and carbon sources on deep eastern Mediterranean cold seep communities**Carlier, A.^{1,*}, Ritt, B.², Rodrigues, C. F.³, Sarrazin, J.², Olu, K.², Grall, J.¹, Clavier, J.¹¹ LEMAR - IUEM, UMR 6539 CNRS-Université de Bretagne Occidentale, Technopôle Brest Iroise, Place Nicolas Copernic, 29280 Plouzané, France.² Laboratoire Environnement Profond, DEEP, IFREMER Brest, France.³ CESAM, University of Aveiro, Portugal.

*: Corresponding author : A. Carlier, Tel: +33-298-498838; fax: +33-298-498645, email Address:

Antoine.Carlier@univ-brest.fr**Abstract:**

Cold seep communities in the Mediterranean Sea have only been discovered two decades ago, and their trophic ecology has been the subject of very few studies. We investigated the benthic food web of two deep chemosynthesis-based ecosystems on the Napoli and Amsterdam mud volcanoes (MVs) in the eastern Mediterranean Sea (~2,000 m depth). Seeping methane has been detected at the surface of both MVs during pioneering cruises and has been hypothesised to be assimilated by benthic fauna as observed in other oceans' margins. Given the extreme oligotrophic character of the eastern Mediterranean Sea, we a priori expected that chemosynthetic food sources, especially methane-derived carbon (MDC), played a major trophic role in these deep seep communities relative to what has been observed in other seep systems worldwide. We aimed at unravelling the trophic relationships on Napoli and Amsterdam MVs through the analysis of carbon, nitrogen and sulphur isotopes both in the dominant benthic invertebrates including the small endofauna (300 μm < size < 1 cm) and in the sedimented organic matter. In particular, we assessed the fraction of MDC in the tissue of several heterotrophic and symbiotic species. Low mean $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$ values ($0.4 \pm 4.8\%$ and $-31.6 \pm 5.7\%$, respectively) obtained for mega- and macrofauna suggested that the investigated benthic food webs are virtually exclusively fuelled by carbon of chemosynthetic origin. A few grazer invertebrates ($\delta^{34}\text{S}$ up to 11‰) depart from this trend and could complement their diet with sedimented and decayed phytoplanktonic organic matter. Faunal $\delta^{13}\text{C}$ values indicated that the oxidation of sulphur is likely the predominant energetic pathway for biosynthesis on both MVs. Nevertheless, mytilid bivalves and small capitellid, ampharetid and spionid polychaetes were ^{13}C -depleted ($\delta^{13}\text{C} < -37\%$) in a way indicating they assimilated a significant portion of MDC. For these later heterotrophic species, MDC ranged between 21 and 31% (lower estimates) and 97 and 100% (upper estimates). However, our results highlighted that the origin of assimilated carbon may be complex for some symbiotic species. The vestimentiferan tubeworm *Lamellibrachia* sp., which exclusively depends on its sulphur-oxidising endosymbionts, showed a ~20‰ inter-individual $\delta^{13}\text{C}$ variability on a very small spatial scale (<1 m) at the summit of Napoli MV. This mostly reflects the variable isotopic composition of pore-water-dissolved inorganic carbon (DIC) and evidenced that tubeworms (and subsequently their endosymbionts) uptake DIC derived from multiple methane oxidation processes in varying proportions. The lower and upper MDC estimates for the vestimentum of Napoli's individuals were 11–38 and 21–73%, respectively. Finally, data on trophic ecology of Napoli and Amsterdam MVs clearly corroborate previous geophysical results evidencing the spatial heterogeneity of Mediterranean MV environmental conditions.

14 ABSTRACT

15

16 Cold-seep communities in the Mediterranean Sea have only been discovered two
17 decades ago and their trophic ecology has been the subject of very few studies. We
18 investigated the benthic food web of two deep chemosynthesis-based ecosystems on the
19 Napoli and Amsterdam mud volcanoes (MV) in the eastern Mediterranean Sea (~2000 m
20 depth). Seeping methane has been detected at the surface of both MV during pioneering
21 cruises and has been hypothesized to be assimilated by benthic fauna as observed in other
22 ocean's margins. Given the extreme oligotrophic character of the eastern Mediterranean Sea,
23 we *a priori* expected that chemosynthetic food sources, especially methane-derived carbon
24 (MDC), played a major trophic role in these deep seep communities relative to what has been
25 observed in other seep-systems worldwide. We aimed to unravel the trophic relationships on
26 Napoli and Amsterdam MVs through the analysis of carbon, nitrogen and sulfur isotopes both
27 in the dominant benthic invertebrates including the small endofauna (300µm < size <1cm)
28 and in the sedimented organic matter. In particular, we assessed the fraction of MDC in the
29 tissue of several heterotrophic and symbiotic species.

30 Low mean $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$ values ($0.4 \pm 4.8\text{‰}$ and $-31.6 \pm 5.7\text{‰}$, respectively) obtained
31 for mega- and macrofauna suggested that the investigated benthic food webs are virtually
32 exclusively fuelled by carbon of chemosynthetic origin. A few grazer invertebrates ($\delta^{34}\text{S}$ up to
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45 DIC derived from multiple methane oxidation processes in varying proportions. The lower
46 and upper MDC estimates for the vestimentum of Napoli's individuals were 11-38% and 21-

54 1. Introduction

55

56 A few years after their discovery in deep hydrothermal vents, chemosynthetic
57 communities were found on the cold seeps of the Gulf of Mexico (Paull et al. 1984). Cold
58 seeps are now known to be common habitats along active and passive continental margins
59 worldwide where cold fluids enriched in methane, hydrocarbons, sulfide and other reduced
60 compounds are emitted from the seafloor (Levin 2005). These reduced compounds are
61 exploited by free-living and symbiotic bacteria which form the basis of complex benthic food
62 webs characterised by various energetic pathways and carbon sources (Brooks et al. 1987;
63 Levin and Michener 2002). The exploration of cold seeps is still in its infancy and the trophic
64 relationships related to these particular deep ecosystems remain poorly described (Levin
65 2005). Moreover, most of the studies undertaken on cold seep trophic ecology concern the
66 benthic megafauna (size > 1-2 cm) which is often dominated by symbiotic bivalves and
67 tubeworms (Kennicutt II et al. 1992; Sibuet and Olu 1998). Only recently has more attention
68 been paid to smaller endofauna (Van Gaever et al. 2009).

69

70 Given the difficulty to reach and sample the deep-sea bottom, the measurement of
71 natural stable isotope abundance in the animal's tissues remains one of the more powerful
72 approaches to identify the energy and carbon sources of seep-related benthic fauna (see Fisher
73 1990 for review). For instance, this method was used to demonstrate for the first time that
74 marine organisms may assimilate non-photosynthetic food sources (Rau and Hedges 1979).
75 Indeed, by the use of carbon and nitrogen stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), it is possible to
76 distinguish consumers that assimilate chemosynthetically-derived carbon (more ^{13}C - and ^{15}N -
77 depleted) from those that rely on phytoplanktonic production (more ^{13}C - and ^{15}N -enriched)
78 (Paull et al. 1985; Brooks et al. 1987; Kennicutt II et al. 1992; Levin and Michener 2002). In
79 addition, bacteria that use methane, both as energy and carbon sources, are more ^{13}C -depleted
80 (usually < 40‰) than sulfur-oxidizing bacteria that fix dissolved inorganic carbon (DIC) from
81 the water column ($-35 < \delta^{13}\text{C} < -27\text{‰}$) (Paull et al. 1985). This is due to the fact that methane
82 is (1) much more ^{13}C -depleted (usually < -40‰) (Whiticar 1999; Milkov 2005) than water
83 column DIC (~0‰) and (2) assimilated by bacteria with a small carbon-isotopic fractionation
84 (Alperin et al. 1988). A large fraction of the seeping methane is consumed in the surface
85 sediment layers of cold-seeps by anaerobic oxidation of methane (AOM) coupled to sulphate
86 reduction (Boetius et al. 2000; Pancost et al. 2000). These processes occurring within archaea-

87 bacteria consortia result in the production of dissolved inorganic carbon (DIC) and sulfide and
88 generate high microbial biomass that can provide a significant supply of methane-derived
89 carbon to heterotrophic bacteria and higher-order consumers. $\delta^{13}\text{C}$ values of symbiotic and
90 heterotrophic seep-related metazoans indicate whether they preferentially rely on
91 methanotrophically-derived carbon (MDC; $< -40\text{‰}$) or thiotrophically-derived carbon
92 (Childress et al. 1986; Brooks et al. 1987) since the net $\delta^{13}\text{C}$ fractionation between a
93 consumer and its diet is small (typically $< 1\text{‰}$; Vander Zanden and Rasmussen 2001;
94 McCutchan Jr et al. 2003). However, $\delta^{13}\text{C}$ values of symbiotic species that exclusively rely on
95 sulfur-oxidising bacteria may be strongly modified when DIC uptakes originate from various
96 sources including by-products of methane oxidation (Lösekan et al. 2008). In the case of
97 cold-seep vestimentiferan tubeworms, very little is known on the processes and the organ
98 (plume and/or root) involved in the DIC uptake (Freytag et al. 2001) making the identification
99 of DIC origin challenging.

100 Sulfur stable isotopes, albeit much less used, are also very helpful in discriminating
101 between organic matter (OM) produced in the water column (by phytoplankton) and OM
102 synthesized in reduced sediments by chemosynthetic microorganisms (Brooks et al. 1987;
103 Vetter and Fry 1998). Indeed, sulfates in the water column show homogeneous $\delta^{34}\text{S}$ values
104 ($\sim +21\text{‰}$) and their fixation by phytoplankton occurs with a small negative fractionation,
105 resulting in oceanic particulate OM with similar signatures ($+17 < \delta^{34}\text{S} < +21\text{‰}$; Peterson and
106 Fry 1987). Benthic fauna that only depend on phytoplanktonic production for their food show
107 $\delta^{34}\text{S}$ values in the same range (Fry 1988; Peterson 1999) because sulfur isotopes do not
108 significantly fractionate between trophic levels (McCutchan Jr et al. 2003). In contrast,
109 dissimilatory sulphate reduction by bacteria within the sediment results in a strong
110 fractionation and much more ^{34}S -depleted (-25 to $+5\text{‰}$) sulphides. Consequently, organisms
111 that assimilate these reduced compounds (e.g. sulfur oxidizing bacteria and organisms which
112 depend on them) exhibit low $\delta^{34}\text{S}$ values as well ($< 5\text{‰}$). Therefore, in cases where the $\delta^{13}\text{C}$
113 and $\delta^{15}\text{N}$ values alone fail to unambiguously identify energetic pathways and carbon sources,
114 additional $\delta^{34}\text{S}$ analyses may allow to differentiate between input of photosynthetic *vs.*
115 chemosynthetic (or methanotrophic *vs.* thiotrophic) material for seep-related organisms
116 (Brooks et al. 1987; Kennicutt II et al. 1992; MacAvoy et al. 2005).

117

118 One of the world's major regions where hydrocarbon-enriched fluids seep from the
119 earth crust is the convergence zone, extending over 5000 km long in the Mediterranean Sea

120 and forming the contact between the African and Eurasian plates (CIESM 2006). In
121 particular, a series of mud volcanoes (MV) associated with active methane seepage have been
122 identified in the eastern part of the Mediterranean Ridge accretionary prism (Cronin et al.
123 1997). Submersible dives, undertaken during several multidisciplinary projects
124 (MEDINAUT/MEDINETH, ANAXIPROBE/TTR6, ANAXIMANDER 2003-2004),
125 unearthed specific seep-associated benthic communities which appeared to be mainly
126 chemosynthetic (Corselli and Basso 1996; Olu-Le Roy et al. 2004). The Olimpi and
127 Anaximander mud field areas (located south of Crete and south of Turkey, respectively), both
128 characterised by numerous MV and high methane emissions, host chemosynthetic
129 communities whose symbiotic megafauna is mainly composed of small sized bivalves
130 belonging to Mytilidae, Vesicomysidae, Thyasiridae and Lucinidae families (Salas and
131 Woodside 2002; Olu-Le Roy et al. 2004, Ritt et al. in prep.; Werne et al. 2004) and large
132 siboglinid vestimentiferan tubeworms recently described as a new species of *Lamellibrachia*
133 (Southward et al. submitted). The suberitid sponge *Rhizaxinella pyrifer*, the crab *Chaceon*
134 *mediterraneus* and the sea urchin *Echinus* sp. are remarkable species frequently associated
135 with these megafauna. On these deep eastern Mediterranean cold-seeps, the potentially
136 important role of thiotrophic and methanotrophic pathways in the functioning of food webs
137 have recently been revealed by (1) fluorescent *in situ* hybridisation (FISH) of endosymbiotic
138 bacteria (Duperron et al. 2007; Duperron et al. 2008; Duperron et al. 2009), (2) transmission
139 electronic microscopy (TEM) and (3) preliminary stable isotope analyses of animal tissues
140 (Olu-Le Roy et al. 2004; Werne et al. 2004) and eucaryote-specific components (Werne et al.
141 2002).

142 To date, most of the knowledge on the trophic ecology of cold-seeps originates from
143 the Atlantic (including the Gulf of Mexico) and Pacific margins (Sibuet and Olu 1998;
144 Tunnicliffe et al. 2003; Levin 2005; Cordes et al. 2009b for reviews). Isotopic data have
145 revealed that the contribution of chemosynthetic carbon to the benthos' diet depends mainly
146 on the depth and productivity of the euphotic layer (Levin and Michener 2002; Levin 2005).
147 Continental margins of the eastern Mediterranean Sea are distinguished from their
148 counterparts in other oceans by an extremely oligotrophic regime (Dugdale and Wilkerson
149 1988) and high temperatures (13°C) down to bathyal depths. These conditions are expected to
150 significantly reduce the downward flux of photosynthetically-derived organic material to the
151 deep-sea floor (Stavrakakis et al. 2000) and consequently the abundance of benthic
152 macrofauna (Kröncke et al. 2003). Such an ecological context should also *a priori* (1)
153 enhance the relative importance of chemosynthetic carbon in eastern Mediterranean cold-seep

154 communities and (2) allow a more accurate assessment of the relative contribution of the
155 different chemosynthetic food sources.

156

157 In the framework of the European HERMES program (Hotspot Ecosystems Research
158 on the Margins of European Seas; 2005-2009), which aimed to better understand the structure
159 and functioning of deep ecosystems on European margins, benthic communities of the Napoli
160 MV (from the Olimpi mud field) and the Amsterdam MV (from the Anaximander Mountains)
161 were revisited using an remote operated vehicle (ROV). The main objectives of this study
162 were to identify the energetic pathways and the carbon sources that fuel the dominant mega-
163 and macrofaunal species of both seep-related ecosystems through multiple stable isotopes
164 analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) of both animal tissues and sedimented organic matter (SOM).
165 We also aimed to assess the contribution of photosynthetically-derived carbon to the diet of
166 consumers, as well as the relative importance of thiotrophic vs. methanotrophic pathways
167 within the benthic food webs of both MVs. The contribution of MDC to tissues was
168 quantitatively estimated with a particular attention to the small heterotrophic endofauna.
169 Finally, we compared the isotopic signatures of the symbiotic tubeworms *Lamellibrachia* sp.
170 across different spatial scales in order to better characterise their inorganic carbon sources.

171

172 2. Material & methods

173

174 2.1. Study area

175

176 The Napoli MV is located south of Crete (Fig. 1A) and is the largest geological
177 structure of the Olimpi area, with a diameter of 4 km at its base and a vertical relief of 200 m
178 high (Fig. 1B). This circular dome lies at depth of 1950 m in a trough, and has a roughly
179 circular and almost flat summit about 1100 m in diameter (see Corselli and Basso 1996;
180 Huguen et al. 2005 for geological details). Brine lakes each covering several hundred square
181 meters and with salinity up to 83 (Charlou et al. 2003) and high methane concentrations were
182 observed on the summit plateau. Dead bivalves' accumulations and thick, dark carbonate
183 crusts with enclosed tubeworms are randomly distributed and have been interpreted as
184 evidence of important fluid seepage over long periods of time (Huguen et al. 2005).

185 Located south of Turkey and characterized by intense active tectonism, the
186 Amsterdam MV is the most prominent structure of the Anaximander Mountains (Woodside et
187 al. 1997) (Fig. 1A). At a depth of 2025 m, it is a flat-topped circular-shaped mound, about 3
188 km in diameter (Fig. 1C). It has a relief of about 20 m, except for a small actively venting
189 cone on its western side, which has a diameter of about 350 m and a height of about 90 m.
190 Methane concentrations in the water column above Amsterdam can reach 14.5 μM and
191 constitute the highest values obtained in the deep Mediterranean (Charlou et al. 2003).
192 Extended dead bivalves fields, vestimentiferan tubeworms (*Lamellibrachia* sp.) often closely
193 associated with carbonate crusts and bacterial mats were observed on Amsterdam MV (Olu-
194 Le Roy et al. 2004; Zitter et al. 2005).

195

196 2.2. Sample collection

197

198 Sampling was achieved during the MEDECO cruise (Leg1, October 2007) onboard the
199 RV "*Pourquoi Pas?*". SOM and benthic invertebrates were collected either from the surface
200 with an USNEL 0.25m²-box corer (KGS) and a multi-corer (MTB) or *in situ* with the "*Victor*
201 *6000*" ROV equipped with two manipulator arms (GBT), a suction sampler (ASPI), blade
202 corers (BC; submersible-mounted corer equipped with a guillotine-like cutter, which allows
203 efficient sampling of unconsolidated sediments) and cylinder-shaped push corer (PC). We
204 selected taxa that are dominant on both MV (Olu-Le Roy et al. 2004) and potentially display

205 different feeding behaviours, allowing us to encompass a large part of the seep benthic food
206 webs. BC and PC sampling devices collect undisturbed sediment cores. Different interval-
207 depths of each PC samples were used to analyse SOM whereas only the superficial layer (first
208 cm) of KGS and MTB samples were considered. Given that specialized benthic communities
209 are associated with different seep habitats (Levin 2005; Cordes et al. 2009a) and that intra-
210 specific isotopic variability may occur in nearby habitats (Levin 2005; Olu et al. 2009),
211 sampling with the ROV provided a unique opportunity to obtain fauna and sediments from a
212 variety of highly specific settings, including readily identifiable methane seeps, colonies of
213 vestimentiferan worms, bivalve beds and carbonate crusts.

214 On Napoli, SOM was collected from the soft sediment of the MV's summit by the
215 ROV within (PC#1, 2 and 3), 2m away (PC#30) and 5m away (PC#32) from a
216 "Lamellibrachia" microhabitat, within a "Bivalves" microhabitat (PC#4, 5 and 6) and 6m
217 (PC#26) and 22m away (PC#28) from carbonate crusts (Fig. 1B; Table 1). SOM was also
218 collected in the trough (KGS#11) and outside the MV (KGS#12). Mega- and macrofaunal
219 samples were collected with the ROV during dives #330 and #331 at 5 sites located at the
220 summit of the MV (Fig. 1B and Table 2). *Lamellibrachia* sp. were collected within an area of
221 <2m radius with 4 adjacent blade cores. This area was characterised by scattered and patchily
222 distributed colonies of tubeworms, visibly reduced sediment and white bacterial mats (Figs
223 2A and 6A). One individual of *Idas* sp. collected with BC#4 together with a single individual
224 of *Lamellibrachia* sp. was fixed on the tube of the vestimentiferan. The sponge *Rhizaxinella*
225 *pyrifera* was collected in the vicinity of brine pools, within a garden of several large and
226 clustered specimens (Fig. 2B). Four other species (*Myrtea amorpha*, *Chaceon mediterraneus*,
227 *Munidopsis acustipina*, *Echinus* sp.) were directly collected on the bottom either by the ROV
228 arm or suction sampler (Table 2; Fig. 2C).

229 On Amsterdam, surface SOM was collected on a large-scale transect going from the
230 summit (KGS#16, 22 and 25) to the eastern flank (KGS#24) and far outside of the MV
231 (MTB#5) (Fig. 1C; Table 1). SOM was also collected with the ROV within a "Bivalves"
232 microhabitat (PC#6, 8 and 10), in the close vicinity of carbonate crusts (PC#13 and 16) and
233 on a "reduced sediment" microhabitat where plumes of bubbles (assumed to be seeping
234 methane) were observed (PC#1 and 3). Mega- and macrofaunal samples were collected at 10
235 sites located at the summit of the MV with the ROV during dive #334 and also, using the
236 USNEL box corer (Fig. 1C; Table 2). The AIM camera is an autonomous camera deployed
237 during the German M70-3 cruise (on December 2006) on "reduced sediment" microhabitat
238 (Fig. 2D). The SMAC refers to a colonisation tray also deployed during M70-3 cruise and that

239 was recovered onboard the “*Pourquoi Pas?*” after a period of 11 months on the bottom (Fig.
240 2F). Four replicate BC (#5, 6, 7 and 8) were collected on the same site within a meter square
241 (Fig. 2E).

242

243 **2.3. Sample processing**

244

245 Specimens were dissected in a cold room onboard the RV and tissue samples were
246 kept at -20°C until processing at the laboratory. Exoskeletons, shells and guts (when possible)
247 were removed under a binocular microscope using fine forceps. Depending on taxa and size,
248 either whole animals (small polychaetes, bivalves) or only muscles (crabs, urchins) were kept
249 for analysis. The trophosome and vestimentum of the tubeworm *Lamellibrachia* sp. were
250 dissected and analysed separately. Tissue samples were separated in sub-samples devoted to
251 $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ analyses when the amount of material was sufficient. Sub-samples
252 devoted to $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ analyses were decarbonated by immersion in liquid HCL (10%)
253 until no further bubbling occurred (i.e. for less than 1 min), whereas sub-samples devoted to
254 $\delta^{15}\text{N}$ analysis were kept untreated. All samples were lyophilized and homogenized in a fine
255 powder using a mortar and pestle. Sub-samples devoted to $\delta^{34}\text{S}$ analyses were then re-
256 suspended in distilled water, shaken for 5 minutes, centrifuged and the supernatant was
257 discarded. This procedure was repeated twice and the samples were finally dried at 60°C and
258 re-ground.

259 Sediment samples were freeze-dried, gently ground with a mortar and pestle and
260 sieved on a 100 μm mesh to remove large detritus. A subsample (~500 mg dry weight) was
261 acidified with HCl (10%) drop by drop until the effervescence ceased and dried at 50°C under
262 a fume extractor to evaporate the acid. To prevent the loss of dissolved organic matter (Riera
263 et al. 1996), sediment samples were not rinsed but rather mixed with distilled water and dried.
264 This procedure was repeated twice. Sediment samples were then ground to a fine powder and
265 kept frozen (-20°C) until analysis of SOM.

266

267 **2.4. Stable isotope analysis**

268

269 Carbon, nitrogen and sulfur stable isotope analyses were carried out using the EA-
270 IRMS method (elemental analysis - isotope ratio mass spectrometry) with a Europa Scientific
271 20-20 IRMS coupled to a Sercon elemental analyser. The reference material used for $\delta^{13}\text{C}$ and

272 $\delta^{15}\text{N}$ analyses was IA-R001 (standard Flour, 40.2% Carbon, $\delta^{13}\text{C}_{\text{V-PDB}} = -26.43 \text{ ‰}$ and $\delta^{15}\text{N}_{\text{AIR}} = 2.55 \text{ ‰}$). Reference standards IA-R001, IA-R005 (standard Beet Sugar, $\delta^{13}\text{C}_{\text{V-PDB}} = -$
273 26.03 ‰) and IA-R006 (standard Cane Sugar, $\delta^{13}\text{C}_{\text{V-PDB}} = -11.64 \text{ ‰}$) for carbon and IA-
274 R001, IA-R045 (ammonium sulphate, $\delta^{15}\text{N}_{\text{AIR}} = -4.71 \text{ ‰}$) and IA-R046 (ammonium
275 sulphate, $\delta^{15}\text{N}_{\text{AIR}} = 22.04 \text{ ‰}$) for nitrogen were used as quality control check samples during
276 sample analysis. The reference material used for $\delta^{34}\text{S}$ analysis was IA-R036 (barium sulfate,
277 $\delta^{34}\text{S}_{\text{V-CDT}} = 20.74 \text{ ‰}$). IA-R036, IA-R025 (barium sulfate, $\delta^{34}\text{S}_{\text{V-CDT}} = +8.53 \text{ ‰}$) and IA-
278 R026 (silver sulfide, $\delta^{34}\text{S}_{\text{V-CDT}} = +3.96 \text{ ‰}$) were used for calibration and correction of the
279 ^{18}O contribution to the SO^+ ion beam. Test samples of IA-R036 and IA-R027 (whale baleen,
280 $\delta^{34}\text{S}_{\text{V-CDT}} = +16.30 \text{ ‰}$) were measured as quality control checks during sample analysis. The
281 isotopic composition of each sample was expressed as the relative difference between isotopic
282 ratios in the sample and that in conventional standards (Vienna Pee Dee Belemnite,
283 atmospheric N_2 and Canyon Diablo Triolite for carbon, nitrogen and sulfur, respectively):
284

$$285 \delta^{13}\text{C} \text{ or } \delta^{15}\text{N} \text{ or } \delta^{34}\text{S} (\text{‰}) = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000 \quad (1)$$

286 where: $R = ^{13}\text{C} / ^{12}\text{C}$, $^{15}\text{N} / ^{14}\text{N}$ or $^{34}\text{S} / ^{32}\text{S}$.

287
288 The precision for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ was $\sim 0.1 \text{ ‰}$, $\sim 0.4 \text{ ‰}$ (for samples with N content
289 $> 5\%$ dry weight) and $\sim 0.4 \text{ ‰}$, respectively. Reproducibility (expressed as standard deviation
290 of the reference standards' values) was $< 0.11 \text{ ‰}$, $< 0.24 \text{ ‰}$ and $< 0.21 \text{ ‰}$ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$,
291 respectively. C:N atomic ratios were calculated from the percentages of organic carbon and
292 nitrogen obtained for decarbonated samples.
293

294 **2.5. Contribution of CH_4 -derived carbon**

295 *2.5.1. Seeping fluids*

296
297 $\delta^{13}\text{C}$ signal of seeping methane (δ_{meth}) has recently been found to be around -50 ‰ on
298 Amsterdam MV (Pape et al. 2010) but remains poorly characterised on Napoli. Since δ_{meth}
299 varies over a large range of values worldwide (from -110 to -20 ‰) depending on gas origin
300 (biogenic or thermogenic) and location (Whiticar 1999; Milkov 2005), we used an upper and
301 a lower δ_{meth} estimates for both MV (based on the few literature data available for
302
303
304

305 Mediterranean seeps) in order to provide a range of contributions of methane-derived carbon
306 (MDC) to heterotrophic and symbiotic invertebrates. -70‰ was chosen as the lower δ_{meth}
307 since -65.6‰ has been reported for methane in the Nadir Lake which belongs to the Olimpi
308 MV province (Charlou et al. 2003). -40‰ was chosen as the upper δ_{meth} since -37.1‰ has
309 been measured on Napoli (Egorov and Ivanov 1998).

310

311 2.5.2. *Heterotrophic fauna*

312

313 Estimates of the percentage of methane-derived carbon (F_m) in invertebrate' tissues
314 were generated using a two-source, single isotope mixing model. F_m was given by the
315 formula:

316

$$317 F_m = (\delta_i - \delta_{\text{wc}}) / (\delta_m - \delta_{\text{wc}}) \quad (2)$$

318

319 where δ_i , δ_{wc} and δ_m refer to the $\delta^{13}\text{C}$ signatures of the species under consideration (average of
320 all replicate samples, except for *Lamellibrachia* sp.), fauna that relies on organic carbon
321 exclusively originating from water column DIC and fauna that relies exclusively on methane,
322 respectively. No trophic shift between animal's tissue and carbon source was taken into
323 account as this is considered negligible (<1‰; Vander Zanden and Rasmussen 2001;
324 McCutchan Jr et al. 2003), even for methanotrophs (Claypool and Kaplan 1974). In order to
325 give upper and lower MDC estimates for heterotrophic fauna, we adopted the approach by
326 Levin & Mendoza (2007) adapted from Levin & Michener (2002). The lower F_m was
327 obtained with the lower δ_{meth} (-70‰) together with δ_{wc} taken as the average $\delta^{13}\text{C}$ signature of
328 thiotrophically-derived carbon (i.e. carbon synthesised from the water column DIC via
329 sulphur-oxidising energetic pathway). For both MVs we used the classical -30‰ values
330 corresponding to symbiotic fauna that exclusively depends on thiotrophic pathway (Fisher
331 1990). The upper F_m was obtained with the upper δ_{meth} (-40‰) together with δ_{wc} taken as the
332 average $\delta^{13}\text{C}$ signature of non-seep benthic invertebrates that assimilate sedimented
333 photosynthetically-derived carbon. We used for each MV the average $\delta^{13}\text{C}$ signature (-
334 18.2‰) of benthic invertebrates collected in deep (between -1425 and -1800m) western
335 Mediterranean (Polunin et al. 2001), since we were not able to analyse stable isotope ratios of
336 invertebrates recovered outside the two MVs due to insufficient abundance and biomass.
337

338 2.5.3. *Symbiotic tubeworms*

339

340 MDC was also estimated for the symbiont-bearing siboglinid *Lamellibrachia* sp. on
341 both MVs. The host assimilates carbon via fixation of ambient DIC (CO₂ and/or HCO₃.)
342 through its endosymbiotic bacteria (Felbeck 1981; Kimura et al. 2003). On cold-seeps, the
343 $\delta^{13}\text{C}$ of dissolved CO₂ surrounding the tubeworms depends on the relative abundance of
344 dissolved CO₂ derived from the normal seawater source ($\sim -7\text{‰}$), from the oxidation of
345 phytoplanktonic organic matter ($\sim -23\text{‰}$) and from oxidised methane ($< 40\text{‰}$). Given the low
346 organic matter content of sediments in the investigated area ($< 0.5\%$; Gontharet et al. 2007;
347 this study) and the low mineralization rate (between 1.7 and 2.6 $\mu\text{mol C cm}^{-2} \text{y}^{-1}$) in the
348 oligotrophic, deep eastern Mediterranean sediments (Van Santvoort et al. 2002), the
349 “phytoplanktonic organic matter” end-member was considered as negligible. Hence, we used
350 the same two end-members mixing model as above where δ_i was the $\delta^{13}\text{C}$ of the vestimentum
351 (i.e. the symbiont-free tissue) of *Lamellibrachia* sp., δ_{wc} was taken as the classical -11‰
352 values that characterises vestimentiferans (whose sulphur-oxidising endosymbiotic bacteria
353 contain the form II of Rubisco) in areas where DIC only originates from the water column
354 (Robinson et al. 2003) (implying a net -4‰ fractionation between carbon source and animal
355 tissue) and δ_m was taken as the theoretical $\delta^{13}\text{C}$ of tubeworm where DIC only originates from
356 seeping-methane oxidation (assuming no major fractionation between methane and its
357 oxidation by-products). Taking into account the above mentioned net -4‰ fractionation,
358 lower and upper δ_m were in this case -74‰ and -44‰ , respectively.

359

360 3. Results

361

362 3.1. Overall isotopic patterns

363

364 Background SOM collected outside the MV exhibited $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values that fell in
365 narrow ranges (from 4.0 to 4.4‰ and from -24.0 to -21.0‰, respectively) and that were
366 similar around Napoli and Amsterdam MV (Table 1). Overall $\delta^{15}\text{N}$ values obtained for SOM
367 at the summits of both MV showed little variation ($3.5 \pm 1.1\%$) but were slightly higher on
368 Napoli than on Amsterdam MV (Mann-Whitney U test, $p < 0.01$; Table 1). The same $\delta^{15}\text{N}$
369 trend was observed when focusing on the “Bivalves” microhabitats of Napoli and Amsterdam
370 MV (Mann-Whitney U test, $p < 0.05$). In contrast, overall $\delta^{13}\text{C}$ values of SOM collected on the
371 MV’s summits exhibited high variation ($-26.9 \pm 4.7\%$) and did not significantly differ
372 between Napoli and Amsterdam (Mann-Whitney U test, $p = 0.78$) (Table 1; Fig. 3). Mean $\delta^{34}\text{S}$
373 value obtained for SOM collected on Amsterdam (KGS 22; 0-1 cm) was $0.0 \pm 2.5\%$ (Fig. 4).
374 No $\delta^{34}\text{S}$ data were obtained on Napoli.

375

376 A total of 67 samples of benthic invertebrates belonging to 18 species of mega- and
377 macrofauna were collected at the summit of both MVs for stable isotope analyses (Table 2;
378 Fig. 5). Species known to harbour endosymbiotic bacteria, and which can be considered as
379 both producers and consumers, were consistently more ^{15}N -depleted ($-5.2\% < \text{mean } \delta^{15}\text{N} <$
380 2.5%) than species that are *a priori* heterotrophs ($2.4\% < \text{mean } \delta^{15}\text{N} < 7.8\%$). Overall, mean
381 $\delta^{34}\text{S}$ values of mega- and macrofauna varied between -6.5% and 11.3% (Table 2; Fig. 4) and
382 did not significantly differ between Napoli and Amsterdam MV (Mann-Whitney U test,
383 $p = 0.20$). In particular, the single $\delta^{34}\text{S}$ value obtained for *Lamellibrachia* sp. on Amsterdam
384 MV (-3.8%) fell within the range of those obtained on Napoli ($-4.7 \pm 3.3\%$). In general, $\delta^{34}\text{S}$
385 values of fauna were lower than 5‰, except for the skeneid gastropods (11.3‰) and one
386 individual of the trochid *Clelandella myriamae* (7.4‰). Overall, the mega- and macrofauna
387 exhibited a wide range of $\delta^{13}\text{C}$ values on both MV (Table 2; Fig. 5). On Napoli, a specimen of
388 the vestimentifera *Lamellibrachia* sp. collected at site #2 exhibited the highest $\delta^{13}\text{C}$ values ($-$
389 17.6%) whereas a specimen of the mytilid bivalve *Idas* sp. showed the lowest one (-41.6%).
390 On Amsterdam, the dorvilleid polychaete was the most ^{13}C -enriched taxon (-22.9%) whereas
391 a specimen of the polychaete Spionidae sp. 2 was the most ^{13}C -depleted (-45.4%). When only
392 considering the species for which we analysed both sulfur and carbon stable isotopes, there

393 was a significant negative correlation between $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values ($n = 40$; $r^2 = 0.172$; $p <$
394 0.001).

395 The contribution of methane-derived carbon (MDC) estimated on the basis of $\delta^{13}\text{C}$
396 values varied greatly between the different taxa (Table 3). Overall, the tubeworms
397 *Lamellibrachia* sp. collected on Napoli and Amsterdam MV derived between 11% (F_m min)
398 and 73% (F_m max) of their carbon from methane. These MDC values varied over a wide
399 range according to sampling sites (see below). Large discrepancies existed for heterotrophic
400 fauna. MDC was low (F_m max $< 56\%$) for the sponge, the skeneid gastropods, the dorvilleid
401 the echinids and decapods. Conversely, MDC was high (F_m min $> 12\%$ and F_m max $> 75\%$)
402 for the ampharetid, capitellid and spionid polychaetes. Finally, the gastropods *Taranis*
403 *moerchi* and *C. myriamae* and the glycerid polychaetes showed intermediate MDC values.

404

405 **3.2. Between-site and inter-individual isotopic variability**

406

407 SOM collected at the summits of both MVs showed a wide range of $\delta^{13}\text{C}$ values (from
408 -42.1 to -22.1‰ on Napoli MV and from -37.5 to -21.5‰ on Amsterdam MV) and was on
409 average more ^{13}C -depleted than SOM collected outside the MV, except for the “Bivalves”
410 microhabitat on Napoli and one replicate of the “reduced sediment” microhabitat on
411 Amsterdam (Table 1; Fig. 3). This heterogeneity of $\delta^{13}\text{C}$ data was due to low values obtained
412 (1) in surface sediment of the “Lamellibrachia” microhabitat and at the immediate vicinity (\sim
413 6 m away) of the carbonate crusts on Napoli MV and (2) in reduced sediments on Amsterdam
414 MV. It should be underlined that these SOM samples exhibited the lowest $\delta^{15}\text{N}$ values as well
415 (from 1.2 to 3.2‰; Table 1). $\delta^{13}\text{C}$ values obtained for the “Bivalves” microhabitats were
416 significantly higher in Napoli than in Amsterdam MV (Mann-Whitney U test, $p < 0.01$). On the
417 “Lamellibrachia” microhabitat on Napoli MV as well as on one replicate of the “reduced
418 sediment” microhabitat on Amsterdam MV, SOM was more ^{13}C -depleted at the sediment
419 surface (first cm) than deeper (9-10 cm) within the sediment (Fig. 3). However, a different
420 $\delta^{13}\text{C}$ pattern was obtained close to the carbonate crusts on Napoli where SOM was more ^{13}C -
421 depleted in depth than at surficial sediments. This trend was not observed on Amsterdam.

422 The thyasirid *Thyasira striata* showed lower $\delta^{15}\text{N}$ values than all other bivalves (Table
423 2; Fig. 5) and the difference was significant when compared with the vesicomyid *Isorropodon*
424 *perplexum* and the mytilid *Idas* sp. collected on both MVs (Kruskal-Wallis test; $p < 0.01$). *T.*
425 *striata* also showed lower $\delta^{34}\text{S}$ values than the other bivalves (Fig. 4), but the difference was

426 not significant with *I. perplexum* (Mann-Whitney U test, $p = 0.061$) and not testable with *Idas*
427 sp. due to insufficient replicate number. Overall, the lucinid, thyasirid and vesicomyid
428 bivalves exhibited more homogeneous $\delta^{13}\text{C}$ values (mean = $-30.6 \pm 2.0\%$) than the mytilid
429 *Idas* sp. (mean = $-39.4 \pm 5.1\%$; Table 2; Fig. 5). Moreover, *Idas* sp., *T. striata* and *I.*
430 *perplexum* significantly differed by their $\delta^{13}\text{C}$ values (Kruskal-Wallis test; $p < 0.01$), the
431 mytilid being significantly more ^{13}C -depleted than the two latter species (95% LSD Fisher
432 test).

433 *Lamellibrachia* sp. collected on the 4 neighbouring sites on the Napoli MV showed
434 large between-site as well as inter-individual $\delta^{13}\text{C}$ differences (from -34.8 to -17.6% ; Fig.
435 6B). Similar $\delta^{13}\text{C}$ variability (from -34.8 to -20.2%) was obtained within a single replicate
436 site (i.e. 6 individuals collected with a single blade core BC#3). $\delta^{13}\text{C}$ values significantly
437 differed between the three sites where several individuals were collected (two-way ANOVA
438 test with “sites” and “tissues” as factors, $p < 0.001$) and there was no significant interaction
439 between the two factors ($p = 0.99$). $\delta^{13}\text{C}$ values obtained in replicate site BC#2 were
440 significantly higher than those of replicate sites BC#1 and BC#3, while there was no
441 significant difference between sites BC#1 and BC#3 (95% LSD Fisher tests). As a result, the
442 contributions of methane-derived carbon (MDC) in the tissue of *Lamellibrachia* sp. collected
443 on Napoli MV varied greatly according to the replicate sites (Table 3). Minimum and
444 maximum estimates spread from low values ($F_m \text{ min} = 11\%$ and $F_m \text{ max} = 21\%$) for the most
445 ^{13}C -depleted individual collected in BC#2 to high values ($F_m \text{ min} = 38\%$ and $F_m \text{ max} = 73\%$)
446 for the most ^{13}C -enriched individual collected in BC#3. The vestimentum was significantly
447 more ^{13}C -depleted than the trophosome (paired sign test, $p < 0.05$) but the difference was
448 small ($0.8 \pm 1.3\%$) (Fig. 6B). There was no significant $\delta^{15}\text{N}$ difference between the
449 vestimentum and trophosome (paired sign test, $p = 0.48$).

450

451 4. Discussion

452

453 This study provides basic information about the energy sources that fuel the
454 Mediterranean cold-seep communities and thus pursues the ecological investigation recently
455 initiated on Napoli and Amsterdam MVs during the MEDINAUT cruise (Olu-Le Roy et al.
456 2004). The large number of species and individuals investigated in this study and the use of
457 $\delta^{34}\text{S}$ as an additional tracer of energy and assimilated carbon source bring us close to
458 understanding the trophic relationships of these two seep-related ecosystems. We also
459 analysed potential carbon sources, a prerequisite for the complete understand of the trophic
460 network of an ecosystem. The first $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data obtained for 6 megafauna species by
461 Olu-Le Roy et al. (2004) constitute a good reference for the present study, where very similar
462 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were found for the same 6 species.

463

464 **4.1. Origin of assimilated carbon on Napoli and Amsterdam MVs**

465

466 *4.1.1. Contribution of photosynthetic vs. chemosynthetic carbon*

467

468 The ecological context of the two investigated cold-seeps suggested *a priori* a very
469 low contribution of phytoplanktonic organic matter to the seep-related benthic food webs.
470 Indeed, Napoli and Amsterdam MVs are located in one of the most oligotrophic areas of the
471 world oceans, characterised by a low phytoplanktonic production ($40\text{-}250\text{ mg C m}^{-2}\text{ d}^{-1}$;
472 Turley et al. 2000). Moreover, the temperature is high ($>13^\circ\text{C}$), even at bathyal depths, in the
473 Mediterranean Sea (Charlou et al. 2003), and most of the sinking particulate organic matter is
474 likely degraded once reaching the MV's surface ($\sim 2000\text{m}$ depth). Nevertheless, recent data
475 obtained in the Cretan Sea showed relatively high fluxes of labile organic compounds and
476 bacteria attached to the settling particles in 1515 m-depth sediment traps (Danovaro et al.
477 2000), representing a potential food source for deep-sea benthic communities. So, the
478 question of the relative importance of photosynthetically-derived carbon for Napoli and
479 Amsterdam communities needed to be addressed.

480

481 $\delta^{13}\text{C}$ values of SOM collected ~ 1 km away from the Napoli and Amsterdam MVs
482 (from -24 to -21‰) match those reported for surface sediments of the deep eastern
483 Mediterranean that receive organic matter only from phytoplanktonic production of the
euphotic layer (Van Santvoort et al. 2002). At the summit of both MVs however, SOM

484 displays $\delta^{13}\text{C}$ values that form a large continuum from the phytoplankton end-member
485 ($\sim 22\text{‰}$) to very low values ($< 40\text{‰}$) characteristic of methane-influenced sediment (Paull et
486 al. 1985; Elvert et al. 2000). The more ^{13}C -depleted SOM samples ($< -25\text{‰}$) of Napoli and
487 Amsterdam are likely due to a ^{13}C -depleted biomass fraction that assimilates products of the
488 anaerobic oxidation of methane (AOM) as reported for the Kazan MV, another active cold-
489 seep located on the Anaximander Mountains (Haese et al. 2003). On Kazan, ^{13}C -depleted
490 material has been found in anoxic sediment (12-15 cm depth; Haese et al. 2003) and ^{13}C -
491 depleted DIC pool (down to -35‰) mainly derived from methane (Werne et al. 2002).
492 However, we found a different $\delta^{13}\text{C}$ pattern within the Napoli MV sediments. Indeed, the
493 lowest $\delta^{13}\text{C}$ values were obtained for the surficial sediment (0 – 1cm) within (or very close to)
494 the “Lamellibrachia” microhabitat, where the oxygenated layer was very thin ($< 5\text{mm}$; data
495 not shown), suggesting that AOM may also occur at shallower depths in the sediments than
496 usually thought. Importantly, our results highlighted that SOM represents a very
497 heterogeneous food source for benthic consumers (in terms of carbon origin and organic C
498 content) at the summit of both MV, the organic matter mainly originating from methane at
499 some particular locations and sediment depths.

500 The $\delta^{34}\text{S}$ value of an animal not only reflects its sulfur source but also indicates, albeit
501 indirectly, the origin of the carbon it assimilates. Thus, marine invertebrates that entirely
502 depend on chemosynthetic carbon usually show $\delta^{34}\text{S}$ values lower than 5‰ (Fry et al. 1983;
503 Vetter and Fry 1998; MacAvoy et al. 2005; Becker et al. 2009), whereas benthic fauna that
504 entirely depend on phytoplanktonic carbon is much more ^{34}S -enriched ($\delta^{34}\text{S} > 18\text{‰}$) (Fry
505 1988; Becker et al. 2009). Therefore, the low $\delta^{34}\text{S}$ values we obtained for almost all
506 individuals suggest that virtually all carbon fuelling the benthic food web of Napoli and
507 Amsterdam MVs is of chemosynthetic origin. This hypothesis is supported by our $\delta^{13}\text{C}$ data
508 since most of the species were more ^{13}C -depleted ($-43.8\text{‰} < \delta^{13}\text{C} < -22.9\text{‰}$) than deep
509 benthic consumers that exclusively feed on sinking phytoplanktonic material (typically, -
510 $22\text{‰} < \delta^{13}\text{C} < -14\text{‰}$; Iken et al. 2001; Polunin et al. 2001; Carlier et al. 2009).

511 It should be underlined that in hydrothermal vent communities, free-living
512 chemoautotrophic microorganisms are a significant food source for heterotrophs and exhibit a
513 wide range of $\delta^{13}\text{C}$ values (up to -17‰) which can overlap with phytoplanktonic material
514 (Van Dover and Fry 1994). Such ^{13}C -enrichment may be explained by the use of alternative
515 CO_2 fixation pathway such as reverse tricarboxylic acid cycle (Campbell and Cary 2004).
516 Free-living bacteria have rarely been characterised isotopically on seep ecosystems (Spies and

517 DesMarais 1983; Gebruk et al. 2003) but often form conspicuous mats as observed around
518 brine pools on Napoli MV (Olu-Le Roy et al. 2004). Therefore, we cannot rule out that ^{13}C -
519 enriched local chemosynthetic production contribute to the diet of some heterotrophic
520 invertebrates in our study area. As a result, the contribution of chemosynthetic carbon may be
521 underestimated for the most ^{13}C -enriched consumers (e.g. one individual of the urchin
522 *Echinus* sp. and the suberitid sponge *Rhizaxinella pyrifer* on Napoli, and the skeneid
523 gastropods on Amsterdam). In these particular cases where $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ alone could not
524 clearly distinguish between assimilation of photosynthetic vs. chemosynthetic material, $\delta^{34}\text{S}$
525 analysis was helpful to come to a decision. The skeneids collected on the frame of the AIM
526 autonomous camera moored on the top of Amsterdam MV (Fig. 2D) showed a mean $\delta^{34}\text{S}$
527 value that suggests a mixed diet of ^{34}S -depleted chemosynthetic production and ^{34}S -enriched
528 photosynthetic production (MacAvoy et al. 2005). These gastropods are indeed grazers and
529 may have scraped the deposited film composed of both local ^{13}C -enriched free-living
530 chemosynthetic bacteria and sedimented decayed photosynthetic organic matter. Similar
531 hypothesis can be proposed for the urchin whose $\delta^{34}\text{S}$ was in the upper range of our sulfur
532 isotope data set. In the case of the sponge *R. pyrifer* (Fig. 2B), although its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
533 were consistent with a diet mainly based on sinking phytoplanktonic organic matter, its low
534 $\delta^{34}\text{S}$ rather suggests a major assimilation of chemosynthetic carbon. Since this species does
535 not bear any symbiont (Olu-Le Roy et al. 2004), it may feed by filtering suspended ^{13}C -
536 enriched free-living chemosynthetic bacteria. However, *R. pyrifer* showed no trace of
537 methane assimilation in its tissue although collected very close to brine pools suspected to be
538 methane-rich. Further analyses are then required to specify its diet, as well as to explain the
539 exceptional sizes this sponge reached on Napoli MV. The high $\delta^{13}\text{C}$ values obtained for the
540 tubeworm *Lamellibrachia* sp. also matched $\delta^{13}\text{C}$ of phytoplankton feeders but this species is
541 known to exclusively depend on its endosymbionts for carbon and this point is further
542 discussed below.

543 Overall our isotopic data set clearly indicates that Napoli's and Amsterdam's benthic
544 communities rely, to a large extent, on chemosynthetic processes (either directly via
545 symbioses or indirectly via heterotrophy). This obviously contrasts with cold-seeps located on
546 shallow continental shelf (i.e. < 50 m depth) where no significant chemosynthetic contribution
547 is reported for seep endofauna (Dando et al. 1991; Levin et al. 2000). Our results also contrast
548 with cold-seeps located at bathyal depths but whose food web is still largely fuelled by the
549 high phytoplanktonic production of the euphotic zone (Levin and Michener 2002; Sellanes et

550 al. 2008). Deep seep communities of New Zealand's continental margins also seem to depend
551 largely on photosynthetically-derived carbon, albeit in this case some heterotrophic
552 consumers may have been collected off seeps due to the use of gear operated from the surface
553 (Thurber et al. 2010). Given the food-limited character of the deep eastern Mediterranean
554 environment, the hypothesis that cold-seeps provide chemosynthetically-derived organic
555 matter to their surrounding environment is interesting. Due to insufficient recovered biomass
556 out of the active summit of both MVs, this issue could not be assessed by the stable isotope
557 approach. However, the patterns of macrofaunal abundance observed over the two large-scale
558 transects give some clues on the trophic role of MVs for the nearby benthic ecosystem.
559 Indeed, benthic macrofauna (size > 300 μ m) was abundant at the summit of Napoli (5220 ind.
560 m⁻² at site KGS#15) and Amsterdam (5152 ind. m⁻² at site KGS #16) MV. The abundances
561 sharply decreased as soon as we depart from the summit of Napoli (between 116 at KGS#13
562 and 104 ind. m⁻² at KGS#14) and Amsterdam MV (between 68 at KGS#19 and 232 ind. m⁻² at
563 KGS#18). On these surrounding areas, macrofauna reached abundance values classically
564 encountered on deep eastern Mediterranean bottoms (typically < 200 ind. m⁻²; Tselepides et
565 al. 2000; Kröncke et al. 2003). This result strongly suggests that the influence of both MVs in
566 terms of organic matter output is high but limited to the immediate vicinity (< 1 km away) of
567 the active zones. Therefore the export of seep carbon is probably highly localized as found in
568 the Gulf of Mexico (Levin 2005). This limited transfer of seep organic matter to the
569 surrounding area also agrees with the fact that, despite a high availability of methane and
570 sulphate, Mediterranean cold-seep communities exhibit lower methanotrophic microbial
571 activity (Omorieg et al. 2009) and biomass (Sibuet and Olu 1998; Levin 2005) than their
572 Atlantic and Pacific counterparts.

573

574 *4.1.2. Relative importance of thiotrophic vs. methanotrophic carbon*

575

576 Chemosynthetic symbionts probably use a wide range of different metabolic pathways
577 to gain energy from the environment and feed their hosts (Dubilier et al. 2008). The combined
578 use of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ allow the distinction of two of them, namely the thiotrophic and the
579 methanotrophic energetic pathways. To evaluate the relative contribution of thiotrophically-
580 derived and methanotrophically-derived carbon to the tissue of most Napoli and Amsterdam's
581 species, we considered a lower and an upper $\delta^{13}\text{C}$ estimate for seeping methane. The choice of
582 -40‰ for the upper δ_{meth} was supported by biological and geological clues. Firstly, most of

583 $\delta^{13}\text{C}$ values reported for the bivalve *Idas* sp., which bears methane-oxidizing symbionts
584 (Duperron et al. 2008), are lower than -40‰ on eastern Mediterranean MVs (Olu-Le Roy et
585 al. 2004; this study). As lowest $\delta^{13}\text{C}$ values of such symbiotic mytilid bivalves often closely
586 matches those of local seeping methane (Childress et al. 1986; Brooks et al. 1987; Cary et al.
587 1989; MacAvoy et al. 2002b; Olu et al. 2009), it is unlikely that methane exhibits $\delta^{13}\text{C}$ values
588 higher than -40‰ for our study area. Secondly, the lowest $\delta^{13}\text{C}$ values obtained for carbonate
589 crusts on Napoli and Amsterdam MVs were -23.2‰ and -31.0‰, respectively (Aloisi et al.
590 2000) and seeping methane is on average more ^{13}C -depleted by 16‰ than the most ^{13}C -
591 depleted carbonate crusts (calculated from 11 data sets from modern cold seeps where both
592 carbonate crusts and methane have been measured; Peckmann and Thiel 2004). Following
593 this argument, δ_{meth} would be around -39.2‰ and -47.0‰ on Napoli and Amsterdam MVs,
594 respectively (the latter value agreeing well with the -50‰ recently reported for Amsterdam;
595 Pape et al. 2010).

596 Lucinid, vesicomid and thyasirid bivalves collected on Napoli and Amsterdam MVs
597 exhibited $\delta^{13}\text{C}$ values typical of vent symbiotic bivalves that only bear sulfur-oxidising
598 bacteria and do not feed heterotrophically (Rau 1981; Kennicutt II et al. 1992; Conway et al.
599 1994). Their low $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values also support a great reliance on a thiotrophic energetic
600 pathway. These data agree with the fact that only sulfur-oxidising bacteria have been detected
601 by microscopy and genetics in Mediterranean specimens belonging to the same genus (Salas
602 and Woodside 2002; Olu-Le Roy et al. 2004; Duperron et al. 2007). Moreover, the $\delta^{13}\text{C}$
603 values of these lucinid, vesicomid and thyasirid bivalves were homogeneous (e.g. SD =
604 2.1‰ for 12 individuals of *I. perplexum* collected at 8 distinct sites), suggesting that the DIC
605 fixed by the symbionts invariably originates from the water column and that these species
606 could preferentially colonise areas weakly influenced by methane. This hypothesis is
607 reinforced by the fact that SOM of the “bivalves” microhabitat from both MVs showed
608 background $\delta^{13}\text{C}$ values that did not reflect any contribution of methane-derived organic
609 matter. Homogeneous $\delta^{13}\text{C}$ values of lucinids, vesicomids and thyasirids could alternatively
610 be explained by the fact that a high pumping rate may hide any small methane-influence by
611 diluting the methane-derived DIC. In any case, the situation observed on Napoli and
612 Amsterdam MVs contrasts with the case of the shallow (340 m depth) and active Skagerrak
613 methane seep where thyasirids showed low $\delta^{13}\text{C}$ values (<-37.4‰) clearly indicating that
614 their symbionts fix methane-derived DIC (Schmaljohann et al. 1990).

615 Low $\delta^{13}\text{C}$ data ($<-35\text{‰}$) obtained for several taxa (*Idas* sp., *C. myriamae*,
616 Ampharetidae, Capitellidae and Spionidae sp. 2) highlighted that the methanotrophic pathway
617 also contributes to the food web on Napoli and Amsterdam MVs. Accordingly, related $\delta^{34}\text{S}$
618 values were in the upper range (from 1.3 to 4.7‰) indicating a lower thiotrophic contribution
619 with respect to vesicomyid and thyasirid bivalves ($-6.0 < \delta^{34}\text{S} < -1.1\text{‰}$). As the $\delta^{13}\text{C}$ signal of
620 methane seeping from Napoli and Amsterdam MVs remains poorly characterised, we
621 provided realistic (albeit large) ranges of potential contributions of methane derived carbon
622 (MDC) in the tissues of heterotrophic and some symbiotic species, on the basis of scarce $\delta^{13}\text{C}$
623 data available for methane, methanotrophic bivalves and carbonate crusts from the eastern
624 Mediterranean (see above discussion and Material & methods for details). On Amsterdam
625 MV, small polychaetes (Spionidae sp. 2, capitellid and ampharetid) depend on methane for a
626 significant part of their assimilated carbon (at least 20-30% and possibly up to 100%). These
627 ranges of contributions are compatible with those found for the same families on the Florida
628 escarpment, one of the „ ^{13}C -lightest’ seep-macroinvertebrate assemblage, where over 50% (on
629 average) of the macrofaunal tissue carbon is derived from methane (Levin 2005; Levin and
630 Mendoza 2007). Polychaetes from the Amsterdam MV exploit MDC probably via
631 heterotrophy through surface or subsurface deposit-feeding (Fauchald and Jumars 1979;
632 Tsutsumi et al. 2001), but symbiotic relationships cannot be excluded for the undetermined
633 spionid. ^{13}C -depleted surface SOM (down to -42‰) around bushes of *Lamellibrachia* sp.
634 (Napoli) and on bubbling reduced sediments (Amsterdam) constitute plausible preferential
635 food source for such deposit-feeding polychaetes. It should be specified that the considered
636 bulk SOM food source may have contained a methane-based and ^{13}C -depleted living
637 compartment (i.e. microorganisms and meiofauna; Van Gaever et al. 2009) which is likely
638 much more nutritive for small macrofauna than the “dead” compartment (Valentine 2002).
639 For instance in Kazan MV, where high release of methane has been indentified, isotopic
640 analyses of specific compounds revealed that MDC flows through the microbial community
641 to bactivorous ciliates (Werne et al. 2002).

642 MDC contribution was much more difficult to estimate for the mytilid *Idas* sp.
643 because this bivalve is known to harbour sulfur- and methane-oxidizing (among others)
644 endosymbiotic bacteria (Duperron et al. 2008) and could be mixotroph (i.e. assimilates carbon
645 by filter-feeding as well) as evidenced for other symbiotic mytilids (Page et al. 1990).
646 Nevertheless most of the individuals were more ^{13}C -depleted ($<40\text{‰}$) and more ^{34}S -enriched
647 (4.5‰) than thiotrophic bivalves (from -6.0 to -1.1‰) and $\delta^{34}\text{S}$ values of thiotrophic species

648 are usually lower than those of methanotrophic ones (Brooks et al. 1987; Cary et al. 1989;
649 Paull et al. 1992; Vetter and Fry 1998). Taking into account the very low input of
650 phytoplanktonic material to the deep eastern Mediterranean seafloor, *Idas* sp. from Napoli and
651 Amsterdam MVs likely derives a significant part of its carbon from methane. On Amsterdam
652 for instance, assuming no assimilation of phytoplanktonic organic matter (which give a F_m
653 min) and a δ_{meth} of -50‰ (Pape et al. 2010), *Idas* sp. could derive up to 69% of its carbon
654 from methane.

655 Although the consumers of higher trophic levels (e.g. the predator gastropod *Taranis*
656 *moerchi*, the decapods *Munidopsis acustipina* and *Chaceon mediterraneus*, and the glycerid
657 polychaete) exhibited $\delta^{13}\text{C}$ values that mostly fall in the classical “-30‰ group”, it remains
658 difficult to assess the energetic pathway they predominantly depend on. As reported in other
659 cold-seeps (MacAvoy et al. 2002a; MacAvoy et al. 2008), the thiotrophic energetic pathway
660 likely plays an important role for the benthic predators of Napoli and Amsterdam MVs.
661 However, it should be kept in mind that the organic matter transferred to the top of benthic
662 food webs may originate from a wide variety of energetic and carbon end-members (e.g.
663 methanotrophs; thiotrophs using water column- and/or methane-derived DIC, and containing
664 various forms of carbon-fixing enzymes).

665 Our results showed that an important proportion of carbon assimilated by macro- and
666 megafauna at the summit of the MVs is derived from seeping methane (even when using a
667 lower estimate of δ_{meth}) as concluded for the microbial and ciliate communities and the
668 ciliates of the Kazan MV (Werne et al. 2002). Overall, the multiple isotopic patterns we
669 obtained (albeit qualitative) suggest that seeping methane plays a more important trophic role
670 on the Amsterdam than on the Napoli MV. This would be in agreement with the fact that seep
671 areas of the Anaximander Mountains are more active in terms of gas seepage and show higher
672 methane concentrations close to the seafloor than MVs of the Olimpi province (Charlou et al.
673 2003; Zitter et al. 2005). In the same line of evidences, video spatial analyses of these seep-
674 related communities (number of living specimens, species diversity) have suggested a higher
675 chemosynthetic activity on the Anaximander Mountains than in the Olimpi field (Olu-Le Roy
676 et al. 2004). Nevertheless, more accurate measurement of the isotopic signal of methane and
677 of its spatial variability on our study area would be necessary to better refine the possible
678 MDC contributions in animal's tissues and then the trophic importance of methane in the
679 related seep-communities.

680

681 **4.2. Small-scale spatial heterogeneity of nutrients sources**

682

683 Seeping methane seems to be an important primary source of carbon for the
684 vestimentiferan *Lamellibrachia* sp. both on Napoli and Amsterdam MVs. The MDC
685 contributions were highly variable between individuals as suggested by the large range of
686 $\delta^{13}\text{C}$ obtained for the tubeworms' tissue. The case of this species is discussed here in the light
687 of the heterogeneous character of cold seeps. It seems now well established that
688 vestimentiferan tubeworms (including the genus *Lamellibrachia*) depend exclusively on the
689 activity of their sulfur-oxidizing endosymbiotic bacteria for their nutrition (Cary et al. 1989;
690 Dubilier et al. 2008). A large carbon flow from the endosymbionts to the host's tissues is
691 evidenced by similar isotopic compositions in the bacteria-containing trophosome and the
692 bacteria-free vestimentum of *Lamellibrachia* sp. (Thurber et al. 2010; this study) and
693 *Escarpia laminata* (Cary et al. 1989).

694 Vestimentiferan symbionts contain Rubisco form II and there is to date no evidence of
695 the presence of form I (Naganuma et al. 2005; Duperron et al. 2009). As the form II enzyme
696 discriminates less against ^{13}C during the carbon fixation than the form I, the host's tissues
697 exhibit $\delta^{13}\text{C}$ values around -11‰ (-16 to -9‰), provided that the fixed DIC only originates
698 from the water column (Robinson et al. 2003). Such high $\delta^{13}\text{C}$ values are usually observed on
699 hydrothermal vents (Rau 1981; Desbruyères et al. 1983; Fisher et al. 1990), but not on cold-
700 seeps where vestimentiferans (including *Lamellibrachia*) are much more ^{13}C -depleted (from -
701 58 to -20‰; Brooks et al. 1987; Kennicutt II et al. 1992; MacAvoy et al. 2005; Sellanes et al.
702 2008; Thurber et al. 2010). Low $\delta^{13}\text{C}$ values obtained for *Lamellibrachia* sp. on Napoli and
703 Amsterdam MVs fell within the latter range but more importantly, individuals from Napoli
704 exhibited unusual 20‰ $\delta^{13}\text{C}$ variability over a very small distance (~1m). Significant $\delta^{13}\text{C}$
705 differences have already been observed for macrofauna inhabiting nearby seep microhabitats
706 showing different seepage activities (Levin 2005; Levin and Mendoza 2007). However intra-
707 specific $\delta^{13}\text{C}$ differences comparable to 15‰-variation obtained within a single blade-core
708 sample (representing a surface of 0.02 m²) have never been reported. Several factors may
709 account for the large inter-individual $\delta^{13}\text{C}$ variability observed for *Lamellibrachia* sp. of
710 Napoli MV.

711 Between-sex isotopic discrepancies potentially exist for vestimentiferan tubeworms
712 since, for instance, females bear eggs that are rich in ^{13}C -depleted lipids (Jarnegren et al.
713 2005). Eggs were clearly visible in of some of the tubeworms collected on Napoli MV, but

714 only in their trunk. However, since similar isotopic patterns were obtained for both the
715 vestimentum and the trophosome parts, we do not think that presence or absence of eggs
716 contributed to inter-individual $\delta^{13}\text{C}$ differences of *Lamellibrachia* sp.

717 A more plausible explanation could be that the limitation effect of DIC during its
718 transfer from the environment to the endosymbionts (via its uptake by the host) and the
719 subsequent isotopic fractionation depends on the growth rate and, therefore, on the size of
720 each individual. Indeed it has been shown for two different vestimentiferan species that
721 discrimination against ^{13}C was more pronounced in the smaller than in the larger individuals,
722 resulting in 8‰ inter-individual $\delta^{13}\text{C}$ variation (Fisher et al. 1990). In our case, it was not
723 possible to measure the lengths of each individual because the use of blade corers does not
724 allow recovery of the entire animal's roots. Without testing such a relationship for our study
725 area, we cannot rule out the hypothesis that variable ^{13}C -fractionation occurring during DIC
726 fixation by endosymbionts contributes to the inter-individual $\delta^{13}\text{C}$ differences. However it is
727 improbable that this factor explains the entire 20‰ $\delta^{13}\text{C}$ variability obtained on Napoli MV.

728 More likely, most of the observed inter-individual $\delta^{13}\text{C}$ variations resulted from the
729 assimilation of DIC of diverse origins and in variable proportions. The origin of pore water
730 DIC is more complex in cold-seeps than in vent environments (Brooks et al. 1984; Suess and
731 Whiticar 1989) and its $\delta^{13}\text{C}$ depends on the relative abundance of dissolved carbon derived
732 from i) the water column source ($\sim 1.0\text{‰}$), ii) mineralisation of phytoplanktonic organic
733 matter ($\sim -23\text{‰}$) and iii) methane oxidation ($\sim -110\text{‰}$ to $\sim -30\text{‰}$). Considering a negligible
734 “marine organic matter” end-member (see Material & methods) together with the lower δ_{meth}
735 estimate (-70‰), minimum MDC in the host's tissue of *Lamellibrachia* sp. varied between
736 10.8 and 38.2% over the Napoli's investigated area. With δ_{meth} approaching -40‰ , the MDC
737 values could be much more important ($>50\%$), as found for the vestimentifera *Escarpia* in the
738 Florida Escarpment (Paull et al. 1989). These calculated methane contributions should be
739 regarded with care since they assume DIC source as a major factor governing the $\delta^{13}\text{C}$
740 variations of vestimentiferans. Anyway, the $\delta^{13}\text{C}$ variability we found for *Lamellibrachia* sp.
741 on a small spatial-scale strongly supports the hypothesis of Dattagupta et al. (2006) that these
742 seep-vestimentifera uptake DIC mainly through the thin tube at their posterior (buried) end in
743 the same manner as sulphide (Scott and Fisher 1995; Freytag et al. 2001), and to a lesser
744 extent by their gill plume. Indeed, at small spatial scale (e.g. a blade-core surface area) the
745 DIC pool is likely much more heterogeneous within the sediment pore water than in the
746 overlying water column. Following this hypothesis, the relative proportion of MDC that is

747 fixed by the endosymbionts of *Lamellibrachia* could be linked to the length of the
748 tubeworm's root, i.e. the sediment depth its posterior end can reach. But as we could not
749 measure the length of sampled individuals, we were not able to test this hypothesis.

750 Overall, our data suggest that a significant proportion of DIC fixed by the
751 endosymbionts originated from seeping methane. This stresses the fact that, although
752 vestimentiferan siboglinids likely exclusively depend on the thiotrophic energetic pathway for
753 the synthesis of their organic compounds, their sources of inorganic carbon are complex and a
754 significant part of fixed CO₂ is a by-product of (aerobic and/or anaerobic) methane oxidation.
755 Data we acquired on the trophic ecology of Napoli and Amsterdam MVs clearly corroborates
756 the spatial heterogeneity of Mediterranean mud volcano environments revealed by previous
757 geophysical studies (Werne et al. 2004). Our results also underline the importance to
758 characterise the $\delta^{13}\text{C}$ of DIC when attempting to identify the energetic pathway and the
759 carbon source of an animal on the base of its tissue's stable isotope signature.

760

761 The $\delta^{15}\text{N}$ discrepancy between the vesicomyid *I. perplexum*, the lucinid *M. amorpha*
762 and the thyasirid *T. striata* tackle interesting questions on the relative availability of
763 nitrogenous compounds for these co-occurring bivalves that all depend on their thiotrophic
764 endosymbionts. The inter-specific $\delta^{15}\text{N}$ variations may be due to species-specific types of
765 symbionts characterised by different fractionation factor occurring during the assimilation of
766 dissolved inorganic nitrogen (DIN) and/or due to the location of the symbionts (extracellular
767 for thyasirid vs. intracellular for vesicomyid and lucinids). Another hypothesis could be the
768 relative availability of reduced compounds for these co-occurring bivalves depending on the
769 depth at which they live in the sediment. The thyasirid are known to live deeper in the
770 sediment than the two other species and, thanks to their extensible foot, they are able to reach
771 dissolved compounds (e.g. sulfur) at even greater depths (Dufour and Felbeck 2003). As a
772 result, the low $\delta^{15}\text{N}$ values of *T. striata* could be explained by the fact that its endosymbionts
773 fractionate less against ^{15}N than those of the two other species, possibly due to higher
774 concentrations of nitrogenous compounds deep in the sediment. Additional data on the
775 concentrations of nitrogenous compound over a sediment depth profile would help verify this
776 hypothesis.

777

778 **4.3. Conclusions**

779

780 Overall, our multiple isotope approach clearly indicated that Napoli's and
781 Amsterdam's benthic communities rely to a large extent on chemosynthetic processes (either
782 directly via symbioses or indirectly via heterotrophy). Our results agreed with the *a priori*
783 prediction that the oligotrophic eastern Mediterranean euphotic zone supplies virtually no
784 phytoplanktonic food source to its deep benthic fauna. The food webs of both MVs appeared
785 mainly based on the thiotrophic energetic pathway and the production of free-living and
786 symbiotic sulfur-oxidising bacteria is then transferred up the food chains to active benthic
787 predators. Our data also showed that seeping methane fuels several food web's components
788 (1) as both energy and carbon source (e.g. for the mytilid *Idas* sp. and its symbionts) or (2) as
789 carbon source only for capitellid, ampharetid and some spionid polychaetes (via fixation of
790 methane-derived DIC by thiotrophic free-living or symbiotic bacteria). On Amsterdam MV,
791 methane is thus transferred to macro- and megafaunal benthic species which reinforces
792 preliminary isotopic data obtained on the neighbouring Kazan MV for bulk organic matter,
793 authigenic carbonates, specific biomarker compounds, and macrofaunal tissues.

794 More importantly, variable isotopic signatures obtained for SOM and symbiotic
795 megafauna (especially *Lamellibrachia* sp.) on very small spatial scales (<1m) highlighted that
796 chemosynthetic communities depend on complex sources of DIC. They reflected the highly
797 heterogeneous character of the MV biotopes, a feature currently evidenced for abiotic (nature
798 of substrate, intensity of seeping fluxes, oxic/anoxic interface depth in the sediment) and
799 biotic parameters (microbial and macrobenthos abundances) during multidisciplinary projects.

800

801

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803

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811

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1102

1103 Figure captions:

1104

1105 Fig. 1: (A) Location of the Napoli and Amsterdam MVs. Study areas and sampling sites on
1106 Napoli (B) and Amsterdam (C) MVs. SOM and fauna were collected either from the surface
1107 with an USNEL box corer (KGS#) and a multicore sampler (MTB5), or *in situ* at the summit
1108 of both MV (enlarged boxes). *In situ* sampling was carried out by the ROV with manipulator
1109 arms (GBT), a suction sampler (ASPI), blade corers (BC) and push corer (PC); AIM:
1110 autonomous camera; SMAC: colonisation tray.

1111

1112 Fig. 2: Sampling the mega- and macrofauna on the Napoli (A-C) and Amsterdam (D-F) mud
1113 volcanoes using Victor 6000 ROV. (A) Sampling the “Lamellibrachia” microhabitat with a
1114 blade core, (B) a field of large *Rhizaxinella pyrifer*, (C) sampling an *Echinus* sp. with the
1115 ROV arm, (D) AIM camera deployment site, where ascending bubbles were visible, (E)
1116 sampling the “bivalves” microhabitat with a blade core and (F) two colonization devices
1117 (SMAC and RACK) deployed on the MV. A few animals used for isotopic analyses were
1118 sampled on the SMAC (rear device).

1119

1120 Fig. 3: $\delta^{13}\text{C}$ values of SOM collected on (A) Napoli and (B) Amsterdam MVs at different
1121 sites and at different depth below the seafloor. Left dark grey area indicates $\delta^{13}\text{C}$ of methane-
1122 derived SOM (considering the upper δ_{meth} estimate); right soft grey area represents the range
1123 of $\delta^{13}\text{C}$ values obtained for surface SOM collected outside the two MVs ($n=4$). Mean $\delta^{13}\text{C} \pm$
1124 SD ($n=3$) are shown for the “Lamellibrachia” and the two “Bivalves” microhabitats only. All
1125 other points refer to a single analysis.

1126

1127 Fig. 4: Mean $\delta^{34}\text{S}$ values of consumers and SOM collected on Napoli (black symbols) and
1128 Amsterdam (open symbols) mud volcanoes (No $\delta^{34}\text{S}$ values were available for SOM from
1129 Napoli). Left grey area indicates typical $\delta^{34}\text{S}$ values of animals that assimilate
1130 chemosynthetically-derived carbon (Vetter and Fry 1998); right shadow area indicates typical
1131 $\delta^{34}\text{S}$ values of animals that exclusively depend on phytoplanktonic production (Becker et al.
1132 2009).

1133

1134 Fig. 5: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD when $n \geq 3$) of invertebrates collected on the top of
1135 Napoli and Amsterdam MVs (See Table 2 for species codes). Left dark grey area and “CH₄”
1136 indicate the assimilation of methanotrophically-derived carbon (considering the upper δ_{m}
1137 estimate; see text for details). Right soft grey area indicates the range of $\delta^{13}\text{C}$ values obtained
1138 for benthic consumers that exclusively depend on phytoplanktonic carbon. Dotted lines show
1139 the upper $\delta^{15}\text{N}$ limit (3‰) for species known to bear endosymbiotic bacteria. Dashed boxes
1140 represent the ranges of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained for SOM on the summit of each MV.

1141

1142 Fig. 6: (A) Locations of the 4 adjacent replicate blade core (BC) sampling within the
1143 “Lamellibrachia” microhabitat on Napoli MV. The 3 push-cores visible on the picture
1144 correspond to SOM sampling (PC#1, 2 and 3). (B) Ranges of $\delta^{13}\text{C}$ values (vertical bar within
1145 boxes = median) obtained for *Lamellibrachia* sp. collected at these 4 replicate sampling.
1146 Vestimentum and trophosome were analysed separately. The number of analysed individuals
1147 is specified in bracket for each BC sampling.

1148

Figure 1
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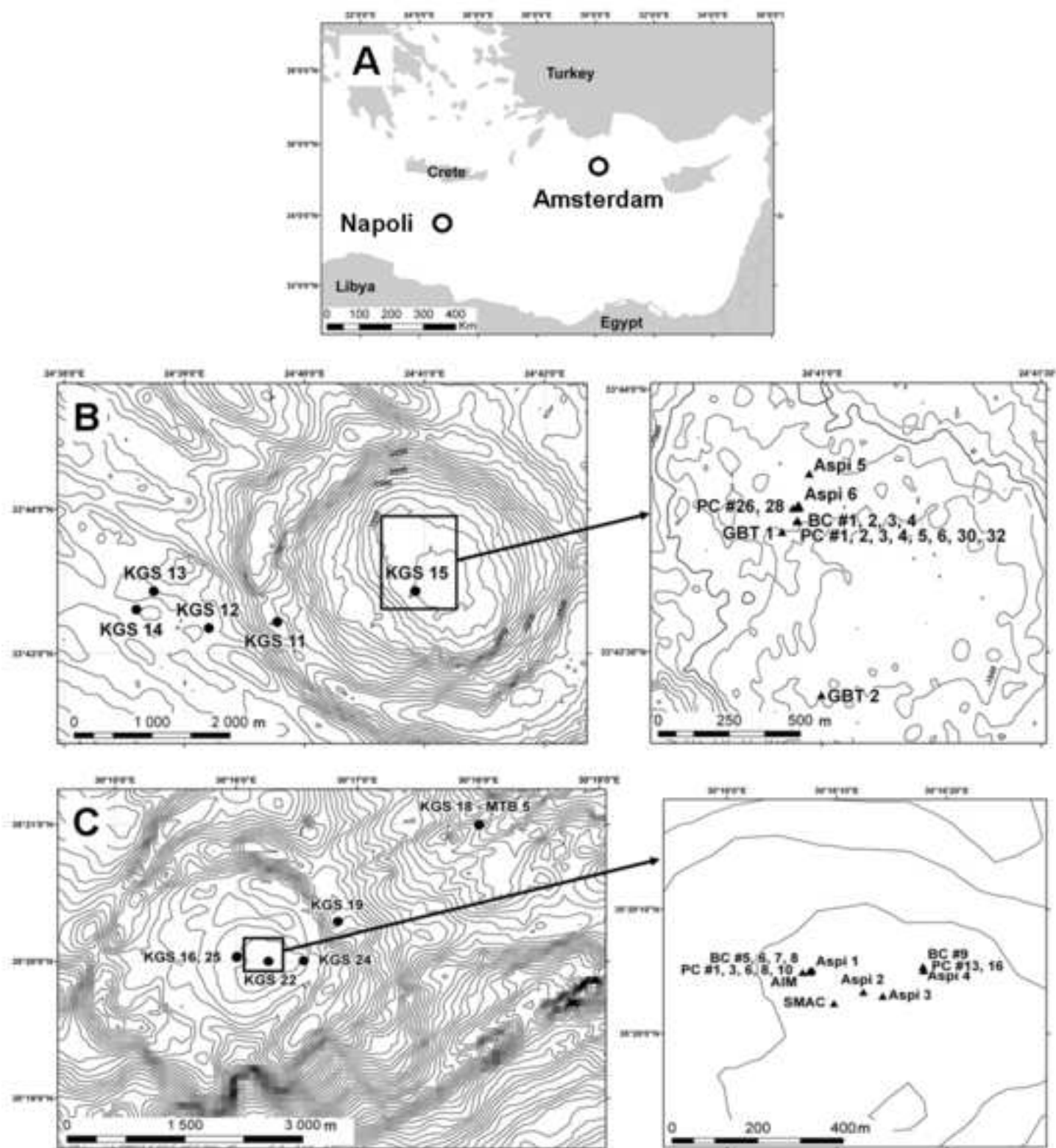


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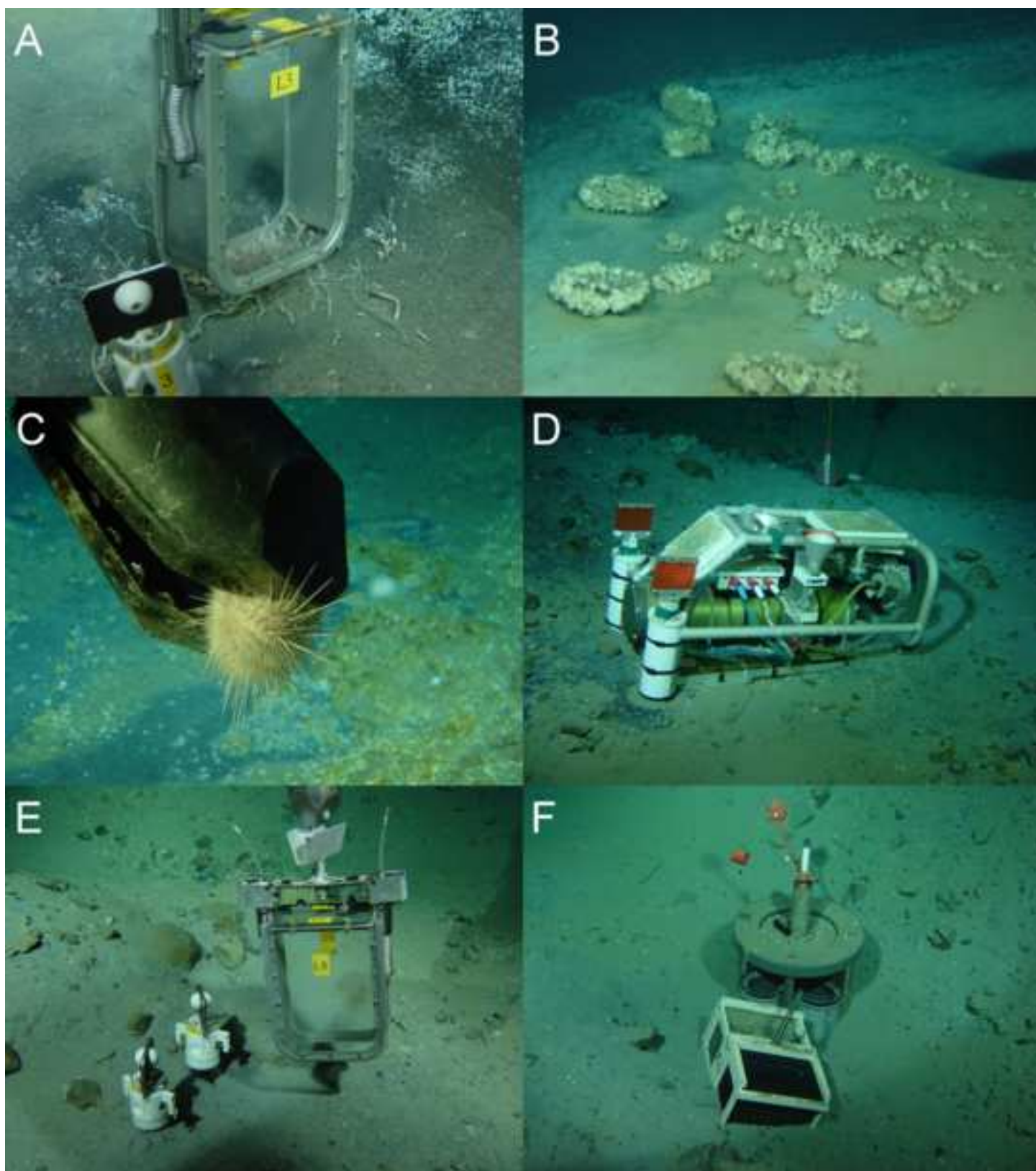


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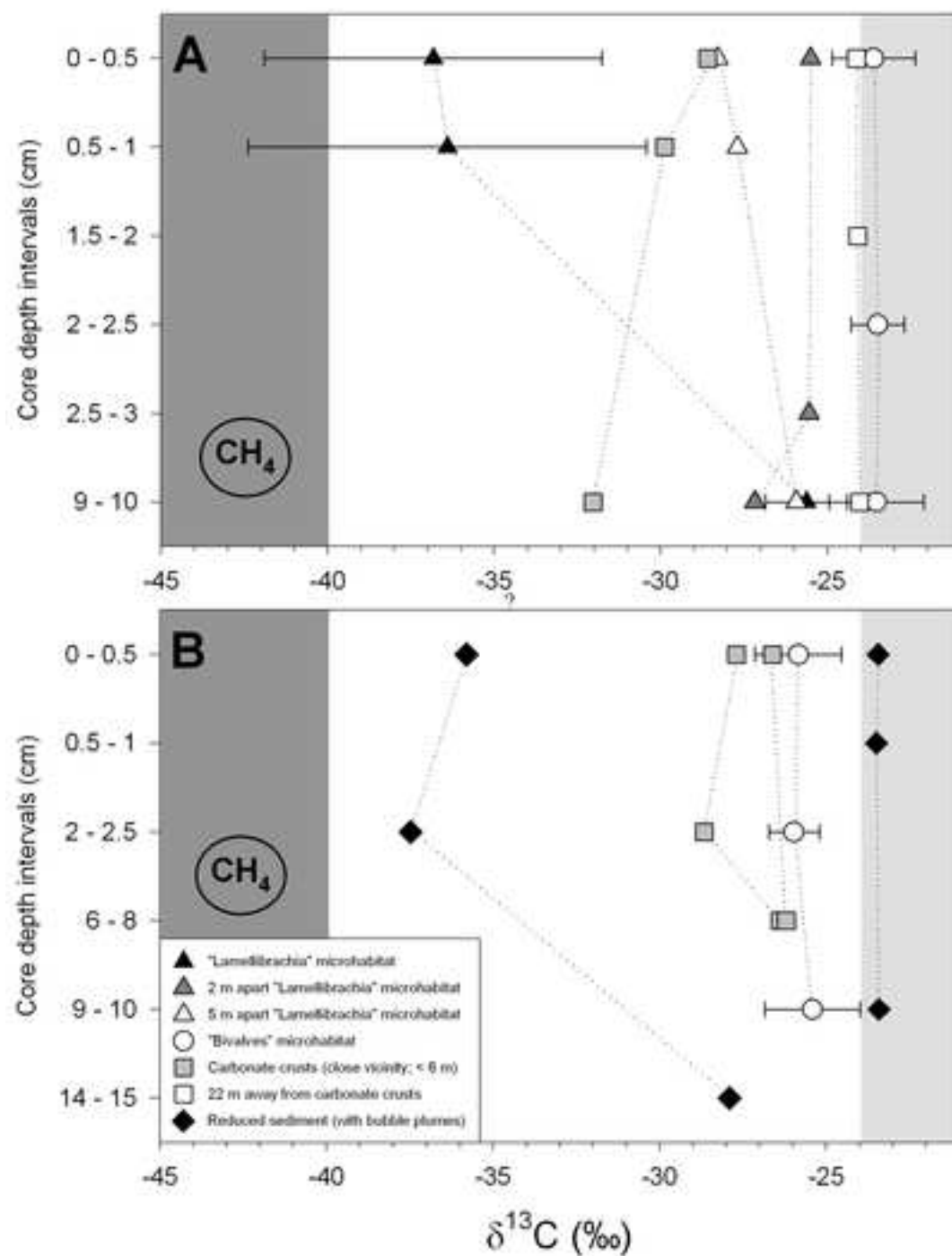


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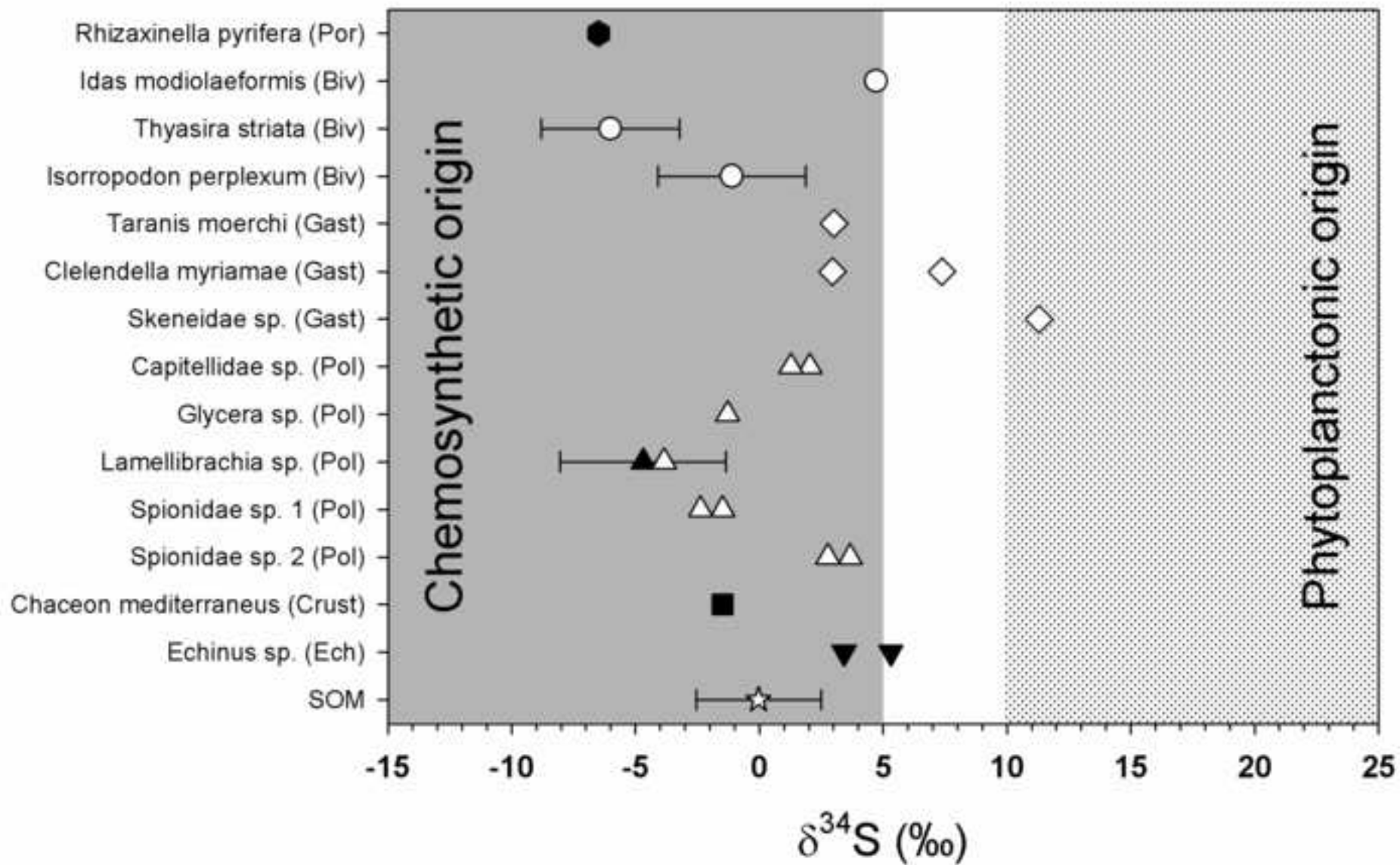


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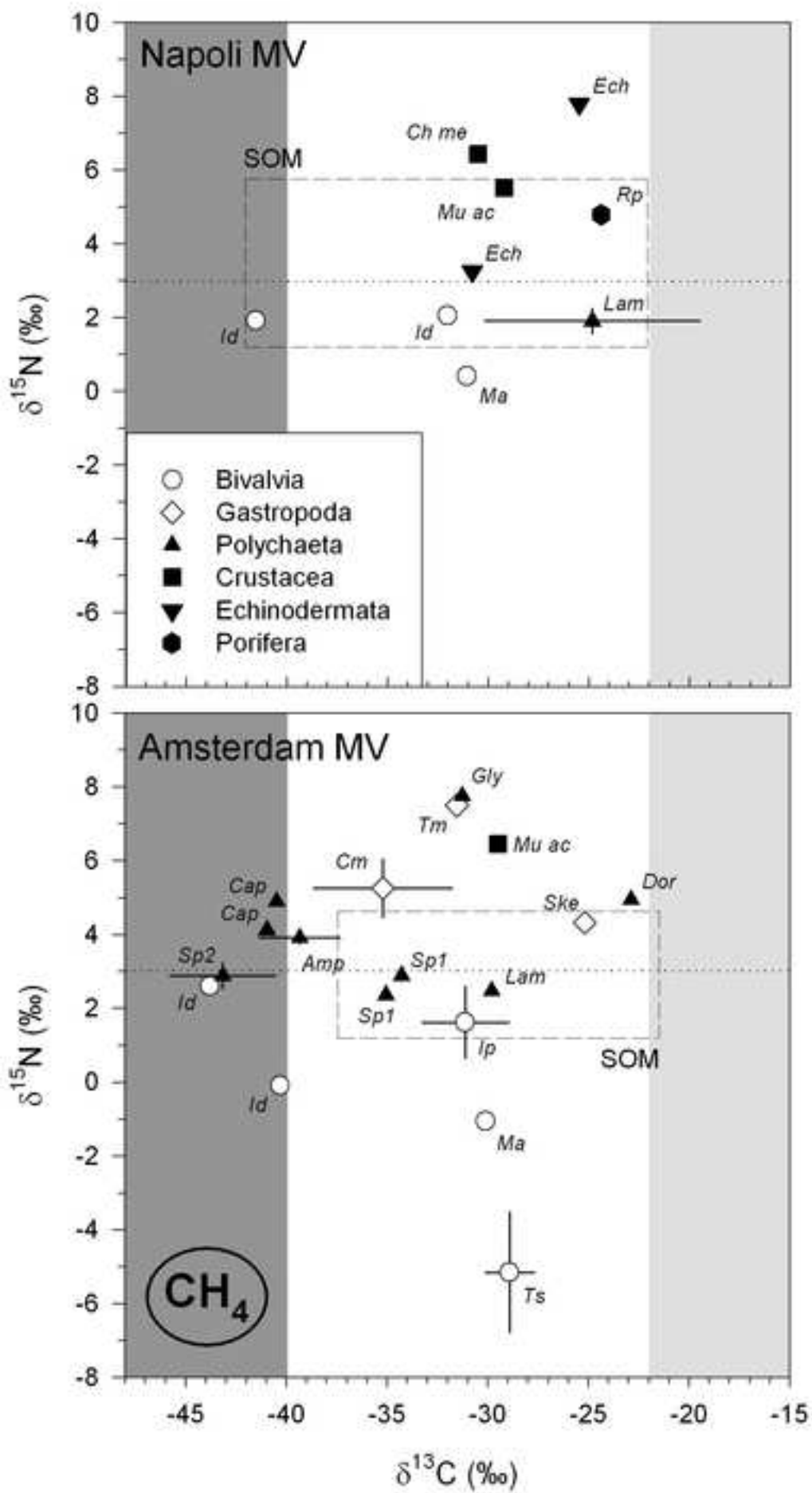


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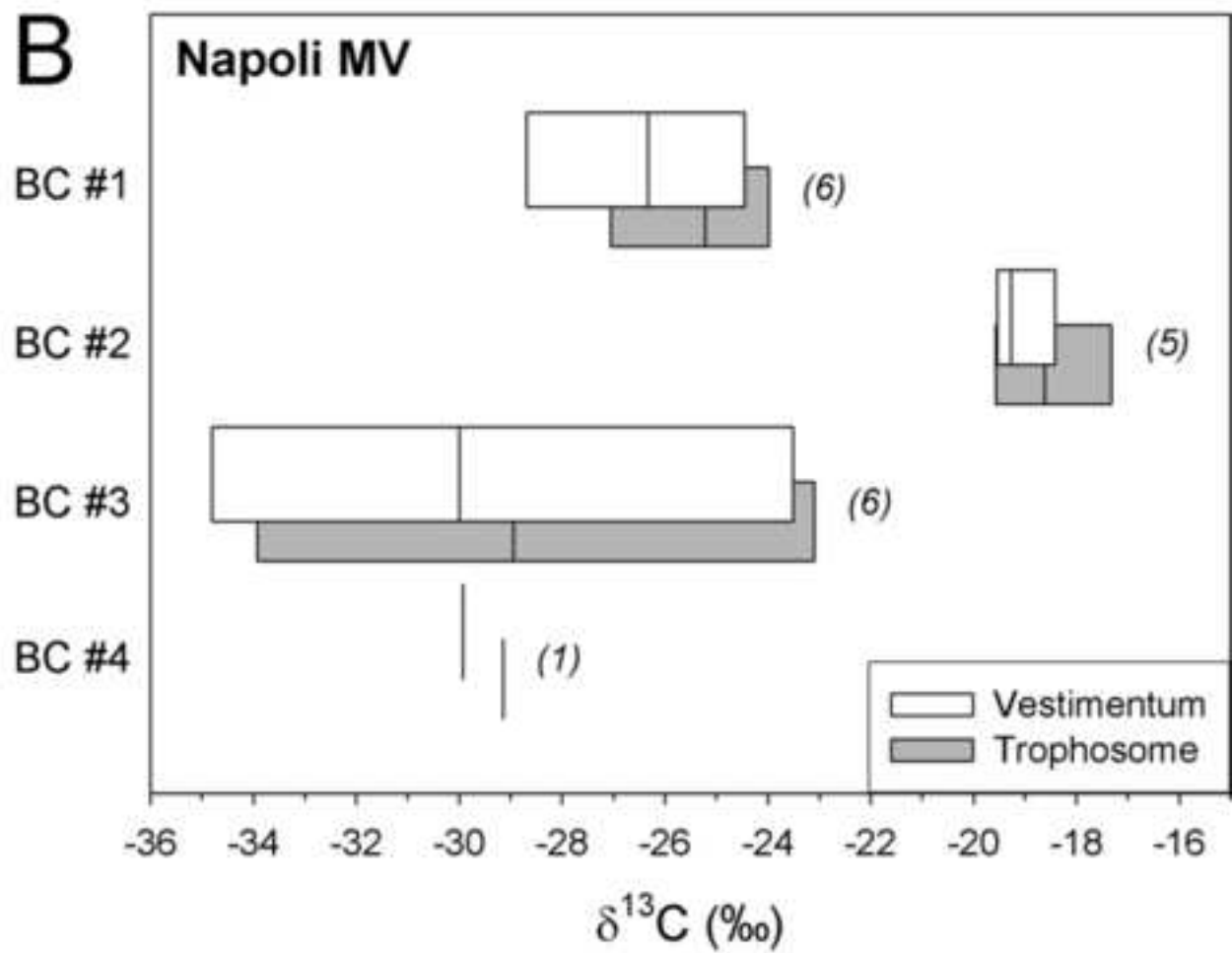
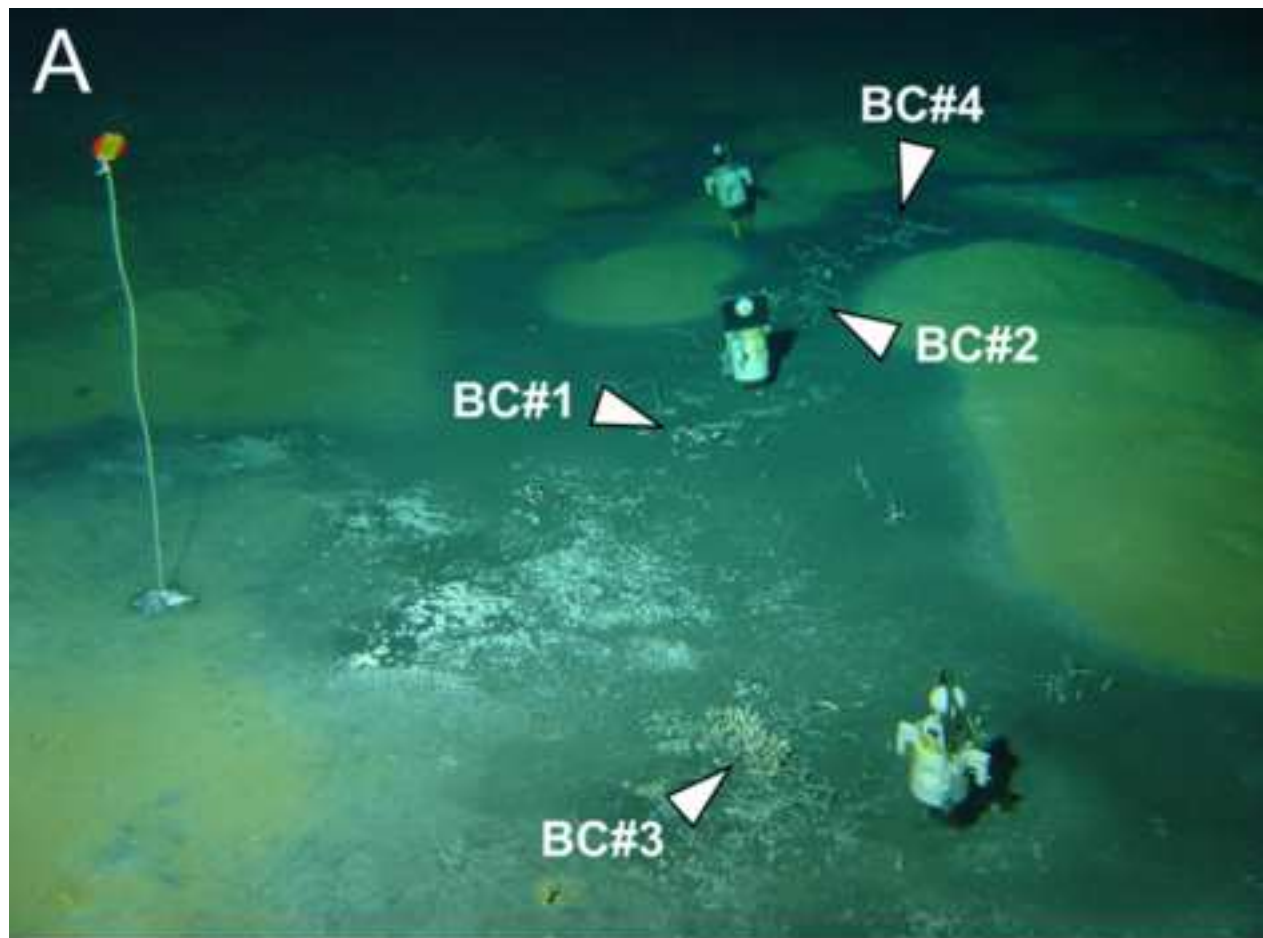


Table 1: Nitrogen and organic carbon content, C:N value as well as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of SOM collected on various microhabitats on the summit of Napoli and Amsterdam MVs and in the surrounding areas (shaded lines) of both MVs. nd = not determined.

Collection site	Sampling device (and number)	Core depth interval (cm)	Nitrogen (%)	Elemental carbon (%)	Atomic C:N	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Napoli MV							
Outside of MV	KGS#11	0 - 1	0.1	0.4	7.1	4.4	-23.5
Far outside of MV	KGS#12	0 - 1	0.0	0.7	17.8	4.3	-21.2
"Lamellibrachia" microhabitat	PC#1	0 - 0.5	nd	1.7	nd	nd	-42.1
		0.5 - 1	0.3	1.2	4.7	1.7	-37.3
		9 - 10	0.1	0.4	6.3	5.1	-24.3
	PC#2	0 - 0.5	0.3	1.3	4.7	1.2	-32.0
		0.5 - 1	0.1	0.8	5.1	2.2	-30.0
		9 - 10	0.1	0.4	6.6	4.8	-25.9
	PC#3	0 - 0.5	0.2	0.7	4.7	3.2	-36.4
		0.5 - 1	0.2	0.9	4.4	3.1	-41.9
		9 - 10	0.1	0.5	6.6	4.2	-26.7
Close periphery of "Lamellibrachia" microhabitat	PC#30	0 - 0.5	0.1	0.4	6.4	4.0	-25.5
		2.5 - 3	0.1	0.4	3.6	3.7	-25.6
		9 - 10	0.1	0.4	5.9	4.1	-27.2
	PC#32	0 - 0.5	0.2	1.0	5.8	4.3	-28.3
		0.5 - 1	0.2	1.0	6.2	3.7	-27.7
		9 - 10	0.1	0.8	6.5	2.4	-25.9
"Bivalve" microhabitat	PC#4	0 - 0.5	0.0	1.0	17.2	4.6	-22.2
		2 - 2.5	0.0	0.8	17.6	4.8	-22.7
		9 - 10	0.0	0.4	5.0	5.8	-22.1
	PC#5	0 - 0.5	0.1	0.4	6.4	4.9	-24.7
		2 - 2.5	0.1	0.4	6.3	5.2	-24.3
		9 - 10	0.1	0.4	6.5	5.2	-24.9
	PC#6	0 - 0.5	0.1	0.5	6.8	4.4	-24.0
		2 - 2.5	0.1	0.5	7.1	4.0	-23.5
		7 - 8	0.2	1.9	9.3	2.0	-23.6
Close periphery of carbonates crusts	PC#26	0 - 0.5	0.1	0.6	6.1	3.5	-28.6
		0.5 - 1	0.1	0.5	6.2	2.9	-29.9
		9 - 10	0.0	0.2	4.1	3.7	-32.0
	PC#28	0 - 0.5	0.1	0.4	6.4	4.9	-24.1
		1.5 - 2	0.1	0.4	6.9	4.7	-24.1
		9 - 10	0.1	0.3	5.6	4.4	-24.0
Amsterdam MV							
Outside of MV	KGS#24	0 - 0.5	0.0	0.7	21.7	4.0	-24.0
Far outside of MV	MTB#5	0 - 1	0.1	0.5	6.8	4.3	-21.0
Summit of MV	KGS#16	0 - 0.5	0.1	0.9	8.8	4.5	-21.5
		0 - 0.5	0.1	0.7	18.5	3.4	-25.4
		0 - 0.5	0.0	0.6	20.7	4.1	-24.4
"Bivalve" microhabitat	PC#6	0 - 0.5	0.1	0.8	10.1	2.4	-26.4
		2 - 2.5	0.1	0.6	11.7	2.8	-26.7
		9 - 10	0.0	0.6	19.4	4.6	-25.5
	PC#8	0 - 0.5	0.0	0.8	14.0	3.7	-24.3
		2 - 2.5	0.1	0.6	12.7	2.7	-25.2
		9 - 10	0.0	0.5	18.2	4.2	-23.9
	PC#10	0 - 0.5	0.1	0.8	11.0	2.9	-26.8
		2 - 2.5	0.0	0.6	13.9	3.5	-26.0
		9 - 10	0.0	0.6	21.6	3.4	-26.8
Close periphery of carbonates crusts	PC#13	0 - 0.5	0.1	0.8	13.6	2.8	-27.7
		2 - 2.5	0.1	0.7	15.8	2.7	-28.7
		7 - 8	0.0	0.6	10.3	3.0	-26.4
	PC#16	0 - 2	0.1	0.7	16.3	2.5	-26.6
		6 - 8	0.0	0.6	19.4	3.2	-26.2
		0 - 0.5	0.1	0.4	5.2	2.9	-23.4
"Reduced sediment" microhabitat	PC#1	0 - 0.5	0.1	0.4	5.2	2.9	-23.4
		0.5 - 1	0.1	0.3	5.2	3.0	-23.5
		9 - 10	0.1	0.3	5.2	2.9	-23.4
	PC#3	0 - 1	0.1	1.0	9.1	1.3	-35.8
		2 - 3	0.1	0.9	10.1	1.2	-37.5
		14 - 15	0.0	0.6	18.9	3.4	-27.9

Table 2

Table 2: Carbon, nitrogen and sulfur stable isotope abundance (range of values or mean \pm SD when number of replicates >2) in the tissue of mega- and macrofauna collected on Napoli and Amsterdam MV. The number of replicates is specified in brackets. Taxa: Por. = Porifera; Biv. = Bivalvia; Pol. = Polychaeta; Crust. = Crustacea; Ech. = Echinodermata; Gast. = Gastropoda. The meanings of sampling device's acronyms are detailed in Material & methods section. For *Lamellibrachia* sp., each replicate represents the average of vestimentum's and trophosome's values.

Species	Taxa	Code	Dive #	Sampling device / number	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{34}\text{S}$ (‰)
Napoli MV							
<i>Rhizaxinella pyrifer</i>	Por.	<i>Rp</i>	330	GBT2	4.8	(1) -24.4	(1) -6.5
<i>Idas</i> sp.	Biv.	<i>Id</i>	330	BC#4; GBT1	1.9 ; 2.1	(2) -41.6 ; -32.0	(2)
<i>Myrtea amorpha</i>	Biv.	<i>Ma</i>	331	ASPI#6	0.4	(1) -31.1	(1)
<i>Lamellibrachia</i> sp.	Pol.	<i>Lam</i>	330	BC#1, 2, 3, 4	1.9 \pm 0.4	(19) -24.8 \pm 5.4	(19) -4.7 \pm 3.3
<i>Chaceon mediterraneus</i>	Crust.	<i>Ch me</i>	330	GBT2	6.4	(1) -30.5	(1) -1.5
<i>Munidopsis acustipina</i>	Crust.	<i>Mu ac</i>	331	ASPI#5	5.5	(1) -29.2	(1)
<i>Echinus</i> sp.	Ech.	<i>Ech</i>	330	GBT1	3.3 ; 7.8	(2) -30.8 ; -25.5	(2) 3.4 ; 5.3
Amsterdam MV							
<i>Idas</i> sp.	Biv.	<i>Id</i>	334	ASPI#3; AIM camera	-0.1 ; 2.6	(2) -43.8 ; -40.3	(2) 4.7
<i>Myrtea amorpha</i>	Biv.	<i>Ma</i>		KGS#25	-1.0	(1) -30.1	(1)
<i>Isorropodon perplexum</i>	Biv.	<i>Ip</i>	334	BC#5, 6, 7, 8; ASPI#3; AIM camera; SMAC; KGS#22	1.6 \pm 1.0	(12) -31.1 \pm 2.1	(12) -1.1 \pm 3.0
<i>Thyasira striata</i>	Biv.	<i>Ts</i>	334	SMAC; KGS#16, 22, 25	-5.2 \pm 1.6	(4) -28.9 \pm 1.2	(4) -6.0 \pm 2.8
<i>Taranis moerchi</i>	Gast.	<i>Tm</i>		KGS#22	7.5	(1) -31.5	(1) 3.0
<i>Clelendella myriamae</i>	Gast.	<i>Cm</i>	334	ASPI#1, 3, 4; SMAC; KGS#22	5.3 \pm 0.8	(5) -35.2 \pm 3.5	(5) 3.0 ; 7.4*
Skeneidae sp.	Gast.	<i>Ske</i>	334	on AIM camera	4.3	(1**) -25.2	(1**) 11.3
Ampharetidae sp.	Pol.	<i>Amp</i>	334	BC#8; AIM camera; SMAC	3.9 \pm 0.2	(3) -39.4 \pm 2.0	(3)
Capitellidae sp.	Pol.	<i>Cap</i>	334	SMAC	4.1 ; 4.9	(2) -41.0 ; -40.5	(2) 1.3 ; 2.1
Dorvilleidae sp.	Pol.	<i>Dor</i>	334	BC#8	5.0	(1) -22.9	(1)
Glyceridae sp.	Pol.	<i>Gly</i>		KGS#22	7.8	(1) -31.3	(1) -1.3
<i>Lamellibrachia</i> sp.	Pol.	<i>Lam</i>	334	BC#9	2.5	(1) -29.8	(1) -3.8
Spionidae sp. 1	Pol.	<i>Sp1</i>		KGS#16, 22	2.4 ; 2.9	(2) -35.0 ; -34.3	(2) -1.9 \pm 0.6
Spionidae sp. 2	Pol.	<i>Sp2</i>	334	BC#8; ASPI#3; AIM camera; SMAC	2.9 \pm 0.3	(3) -43.2 \pm 2.6	(3) 3.2 \pm 0.6
<i>Munidopsis acustipina</i>	Crust.	<i>Mu ac</i>	334	ASPI#2	6.5	(1) -29.5	(1)

* this value refers to a single individual collected on the AIM camera and not analysed for d13C and d15N

** represents a single analysis of about 50 pooled individuals

Table 3: Minimum (F_m MIN) and maximum (F_m MAX) estimates of methane-derived carbon (MDC) contribution (%) to the tissues of symbiotic and heterotrophic species collected on Napoli and Amsterdam MVs. For *Lamellibrachia* sp. collected on Napoli, estimates are given for the most and the less ^{13}C -enriched individuals (indicated by BC#2 and BC#3, respectively). Values in *italic* refer to literature data; TS = this study.

Species	Taxa		F_m MIN	F_m MAX	References
Symbiotic					
<i>Lamellibrachia</i> sp.	Pol.	Napoli MV, BC#2	11	21	TS
		Napoli MV, BC#3	38	73	TS
		Amsterdam MV	30	57	TS
Vestimentifera	Pol.	Florida escarpment	42*	64*	Paul et al., 1989
Heterotroph					
Seep heterotrophic fauna	mixed**	New Zealand	21	73	Thurber et al., 2009
<i>Rhizaxinella pyrifer</i>	Por.	Napoli MV	0	28	TS
<i>Taranis moerchi</i>	Gast.	Amsterdam MV	4	61	TS
<i>Clelendella myriamae</i>	Gast.	Amsterdam MV	13	78	TS
Skeneidae sp.	Gast.	Amsterdam MV	0	32	TS
Gastropods	Gast.	Unimak, AK	23		Mendoza & Levin, 2007
		Kodiak, AK	46		Mendoza & Levin, 2007
Ampharetidae sp.	Pol.	Amsterdam MV	23	97	TS
<i>Amphisamytha</i> sp. (ampharetid)	Pol.	Florida escarpment	25	60	Mendoza & Levin, 2007
Capitellidae sp.	Pol.	Amsterdam MV	27	100	TS
		Florida escarpment	5	24	Mendoza & Levin, 2007
Dorvilleidae sp.	Pol.	Amsterdam MV	0	21	TS
		Unimak, AK	28		Mendoza & Levin, 2007
Glyceridae sp.	Pol.	Amsterdam MV	3	60	TS
		Unimak, AK	2		Mendoza & Levin, 2007
Spionidae sp. 1	Pol.	Amsterdam MV	12	75	TS
Spionidae sp. 2	Pol.	Amsterdam MV	33	100	TS
Spionidae sp.	Pol.	Unimak, AK	11		Mendoza & Levin, 2007
<i>Munidopsis acustipina</i>	Crust.	Napoli MV	0	50	TS
		Amsterdam MV	0	52	TS
Galatheid crab	Crust.	Kodiak, AK	53	66	Mendoza & Levin, 2007
<i>Chaceon mediterraneus</i>	Crust.	Napoli MV	1	56	TS
<i>Echinus</i> sp.	Ech.	Napoli MV	0	46	TS

* based on $\delta^{13}\text{C}$ and ^{14}C content measurements

** sipunculids, arthropods, molluscs and annelids showing $\delta^{13}\text{C}$ values $\leq -40\text{‰}$