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Heterogeneous energetic pathways and carbon sources on deep eastern Mediterranean cold seep communities

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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 $\delta^{34}S$ and $\delta^{13}C$ values (0.4 ± 4.8‰ and −31.6 ± 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 ($\delta^{34}S$ up to 11‰) depart from this trend and could complement their diet with sedimented and decayed phytoplanktonic organic matter. Faunal $\delta^{13}C$ 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 ($\delta^{13}C < -37‰$) 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 $\delta^{13}C$ 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.

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 (MV) in the eastern Mediterranean Sea (~2000 m depth). Seeping methane has been detected at the surface of both MV during pioneering cruises and has been hypothesized to be assimilated by benthic fauna as observed in other ocean's 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 to unravel the trophic relationships on Napoli and Amsterdam MVs through the analysis of carbon, nitrogen and sulfur isotopes both in the dominant benthic invertebrates including the small endofauna (300µm < size <1cm) 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 δ^{34}S and δ^{13}C values (0.4 ±4.8‰ and -31.6 ±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 (δ^{34}S up to 11‰) depart from this trend and could complement their diet with sedimented and decayed phytoplanktonic organic matter. Faunal δ^{13}C values indicated that the oxidation of sulfur 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 (δ^{13}C < -37‰) in a way indicating they assimilated a significant portion of MDC. For these later heterotrophic species, MDC ranged between 21-31% (lower estimates) and 97-100% (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 sulfur-oxidising endosymbionts, showed a ~20‰ inter-individual δ^{13}C variability on a very small spatial scale (<1m) 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-

1. Introduction

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

Given the difficulty to reach and sample the deep-sea bottom, the measurement of natural stable isotope abundance in the animal's tissues remains one of the more powerful approaches to identify the energy and carbon sources of seep-related benthic fauna (see Fisher 1990 for review). For instance, this method was used to demonstrate for the first time that marine organisms may assimilate non-photosynthetic food sources (Rau and Hedges 1979). Indeed, by the use of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), it is possible to distinguish consumers that assimilate chemosynthetically-derived carbon (more ^{13}C- and ^{15}N-depleted) from those that rely on phytoplanktonic production (more ^{13}C- and ^{15}N-enriched) (Paull et al. 1985; Brooks et al. 1987; Kennicutt II et al. 1992; Levin and Michener 2002). In addition, bacteria that use methane, both as energy and carbon sources, are more ^{13}C-depleted (usually < 40‰) than sulfur-oxidizing bacteria that fix dissolved inorganic carbon (DIC) from the water column ($-35 < \delta^{13}C < -27$‰) (Paull et al. 1985). This is due to the fact that methane is (1) much more ^{13}C-depleted (usually < -40‰) (Whiticar 1999; Milkov 2005) than water column DIC (~0‰) and (2) assimilated by bacteria with a small carbon-isotopic fractionation (Alperin et al. 1988). A large fraction of the seeping methane is consumed in the surface sediment layers of cold-seeps by anaerobic oxidation of methane (AOM) coupled to sulphate reduction (Boetius et al. 2000; Pancost et al. 2000). These processes occurring within archaea-bacteria consortia result in the production of dissolved inorganic carbon (DIC) and sulfide and generate high microbial biomass that can provide a significant supply of methane-derived carbon to heterotrophic bacteria and higher-order consumers. $\delta^{13}C$ values of symbiotic and heterotrophic seep-related metazoans indicate whether they preferentially rely on methanotrophically-derived carbon (MDC; < -40‰) or thiotrophically-derived carbon (Childress et al. 1986; Brooks et al. 1987) since the net $\delta^{13}C$ fractionation between a consumer and its diet is small (typically <1‰; Vander Zanden and Rasmussen 2001; McCutchan Jr et al. 2003). However, $\delta^{13}C$ values of symbiotic species that exclusively rely on sulfur-oxidising bacteria may be strongly modified when DIC uptakes originate from various sources including by-products of methane oxidation (Lösekann et al. 2008). In the case of cold-seep vestimentiferan tubeworms, very little is known on the processes and the organ (plume and/or root) involved in the DIC uptake (Freytag et al. 2001) making the identification of DIC origin challenging.

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

One of the world's major regions where hydrocarbon-enriched fluids seep from the earth crust is the convergence zone, extending over 5000 km long in the Mediterranean Sea and forming the contact between the African and Eurasian plates (CIESM 2006). In particular, a series of mud volcanoes (MV) associated with active methane seepage have been identified in the eastern part of the Mediterranean Ridge accretionary prism (Cronin et al. 1997). Submersible dives, undertaken during several multidisciplinary projects (MEDINAUT/MEDINETH, ANAXIPROBE/TTR6, ANAXIMANDER 2003-2004), unearthed specific seep-associated benthic communities which appeared to be mainly chemosynthetic (Corselli and Basso 1996; Olu-Le Roy et al. 2004). The Olimpi and Anaximander mud field areas (located south of Crete and south of Turkey, respectively), both characterised by numerous MV and high methane emissions, host chemosynthetic communities whose symbiotic megafauna is mainly composed of small sized bivalves belonging to Mytilidae, Vesicomyidae, Thyasiridae and Lucinidae families (Salas and Woodside 2002; Olu-Le Roy et al. 2004, Ritt et al. in prep.; Werne et al. 2004) and large siboglinid vestimentiferan tubeworms recently described as a new species of *Lamellibrachia* (Southward et al. submitted). The suberitid sponge *Rhizaxinella pyrifera*, the crab *Chaceon mediterraneus* and the sea urchin *Echinus* sp. are remarkable species frequently associated with these megafauna. On these deep eastern Mediterranean cold-seeps, the potentially important role of thiotrophic and methanotrophic pathways in the functioning of food webs have recently been revealed by (1) fluorescent *in situ* hybridisation (FISH) of endosymbiotic bacteria (Duperron et al. 2007; Duperron et al. 2008; Duperron et al. 2009), (2) transmission electronic microscopy (TEM) and (3) preliminary stable isotope analyses of animal tissues (Olu-Le Roy et al. 2004; Werne et al. 2004) and eucaryote-specific components (Werne et al. 2002).

To date, most of the knowledge on the trophic ecology of cold-seeps originates from the Atlantic (including the Gulf of Mexico) and Pacific margins (Sibuet and Olu 1998; Tunnicliffe et al. 2003; Levin 2005; Cordes et al. 2009b for reviews). Isotopic data have revealed that the contribution of chemosynthetic carbon to the benthos' diet depends mainly on the depth and productivity of the euphotic layer (Levin and Michener 2002; Levin 2005). Continental margins of the eastern Mediterranean Sea are distinguished from their counterparts in other oceans by an extremely oligotrophic regime (Dugdale and Wilkerson 1988) and high temperatures (13°C) down to bathyal depths. These conditions are expected to significantly reduce the downward flux of photosynthetically-derived organic material to the deep-sea floor (Stavrakakis et al. 2000) and consequently the abundance of benthic macrofauna (Kröncke et al. 2003). Such an ecological context should also *a priori* (1) enhance the relative importance of chemosynthetic carbon in eastern Mediterranean cold-seep communities and (2) allow a more accurate assessment of the relative contribution of the different chemosynthetic food sources.

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

2. Material & methods

2.1. Study area

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

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

2.2. Sample collection

Sampling was achieved during the MEDECO cruise (Leg1, October 2007) onboard the RV "*Pourquoi Pas?*". SOM and benthic invertebrates were collected either from the surface with an USNEL 0.25m^2-box corer (KGS) and a multi-corer (MTB) or *in situ* with the "*Victor 6000*" ROV equipped with two manipulator arms (GBT), a suction sampler (ASPI), blade corers (BC; submersible-mounted corer equipped with a guillotine-like cutter, which allows efficient sampling of unconsolidated sediments) and cylinder-shaped push corer (PC). We selected taxa that are dominant on both MV (Olu-Le Roy et al. 2004) and potentially display different feeding behaviours, allowing us to encompass a large part of the seep benthic food webs. BC and PC sampling devices collect undisturbed sediment cores. Different interval-depths of each PC samples were used to analyse SOM whereas only the superficial layer (first cm) of KGS and MTB samples were considered. Given that specialized benthic communities are associated with different seep habitats (Levin 2005; Cordes et al. 2009a) and that intra-specific isotopic variability may occur in nearby habitats (Levin 2005; Olu et al. 2009), sampling with the ROV provided a unique opportunity to obtain fauna and sediments from a variety of highly specific settings, including readily identifiable methane seeps, colonies of vestimentiferan worms, bivalve beds and carbonate crusts.

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

On Amsterdam, surface SOM was collected on a large-scale transect going from the summit (KGS#16, 22 and 25) to the eastern flank (KGS#24) and far outside of the MV (MTB#5) (Fig. 1C; Table 1). SOM was also collected with the ROV within a "Bivalves" microhabitat (PC#6, 8 and 10), in the close vicinity of carbonate crusts (PC#13 and 16) and on a "reduced sediment" microhabitat where plumes of bubbles (assumed to be seeping methane) were observed (PC#1 and 3). Mega- and macrofaunal samples were collected at 10 sites located at the summit of the MV with the ROV during dive #334 and also, using the USNEL box corer (Fig. 1C; Table 2). The AIM camera is an autonomous camera deployed during the German M70-3 cruise (on December 2006) on "reduced sediment" microhabitat (Fig. 2D). The SMAC refers to a colonisation tray also deployed during M70-3 cruise and that was recovered onboard the "*Pourquoi Pas?*" after a period of 11 months on the bottom (Fig. 2F). Four replicate BC (#5, 6, 7 and 8) were collected on the same site within a meter square (Fig. 2E).

2.3. Sample processing

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

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

2.4. Stable isotope analysis

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

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

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

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

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

2.5. Contribution of CH_4-derived carbon

2.5.1. *Seeping fluids*

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

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

2.5.2. *Heterotrophic fauna*

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

$$F_m = (\delta_i - \delta_{wc}) / (\delta_m - \delta_{wc}) \qquad (2)$$

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

2.5.3. *Symbiotic tubeworms*

MDC was also estimated for the symbiont-bearing siboglinid *Lamellibrachia* sp. on both MVs. The host assimilates carbon via fixation of ambient DIC (CO_2 and/or HCO_{3-}) through its endosymbiotic bacteria (Felbeck 1981; Kimura et al. 2003). On cold-seeps, the $\delta^{13}C$ of dissolved CO_2 surrounding the tubeworms depends on the relative abundance of dissolved CO_2 derived from the normal seawater source (~ -7‰), from the oxidation of phytoplanktonic organic matter (~ -23‰) and from oxidised methane (< 40‰). Given the low organic matter content of sediments in the investigated area (<0.5%; Gontharet et al. 2007; this study) and the low mineralization rate (between 1.7 and 2.6 μmol C cm^{-2} y^{-1}) in the oligotrophic, deep eastern Mediterranean sediments (Van Santvoort et al. 2002), the "phytoplanktonic organic matter" end-member was considered as negligible. Hence, we used the same two end-members mixing model as above where δ_i was the $\delta^{13}C$ of the vestimentum (i.e. the symbiont-free tissue) of *Lamellibrachia* sp., δ_{wc} was taken as the classical -11‰ values that characterises vestimentiferans (whose sulphur-oxidising endosymbiotic bacteria contain the form II of Rubisco) in areas where DIC only originates from the water column (Robinson et al. 2003) (implying a net -4‰ fractionation between carbon source and animal tissue) and δ_m was taken as the theoretical $\delta^{13}C$ of tubeworm where DIC only originates from seeping-methane oxidation (assuming no major fractionation between methane and its oxidation by-products). Taking into account the above mentioned net -4‰ fractionation, lower and upper δ_m were in this case -74‰ and -44‰, respectively.

3. Results

3.1. Overall isotopic patterns

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

A total of 67 samples of benthic invertebrates belonging to 18 species of mega- and macrofauna were collected at the summit of both MVs for stable isotope analyses (Table 2; Fig. 5). Species known to harbour endosymbiotic bacteria, and which can be considered as both producers and consumers, were consistently more ^{15}N-depleted (-5.2‰ < mean $\delta^{15}N$ < 2.5‰) than species that are *a priori* heterotrophs (2.4‰ < mean $\delta^{15}N$ < 7.8‰). Overall, mean $\delta^{34}S$ values of mega- and macrofauna varied between -6.5‰ and 11.3‰ (Table 2; Fig. 4) and did not significantly differ between Napoli and Amsterdam MV (Mann-Whitney U test, $p=0.20$). In particular, the single $\delta^{34}S$ value obtained for *Lamellibrachia* sp. on Amsterdam MV (-3.8‰) fell within the range of those obtained on Napoli (-4.7 ±3.3‰). In general, $\delta^{34}S$ values of fauna were lower than 5‰, except for the skeneid gastropods (11.3‰) and one individual of the trochid *Clelandella myriamae* (7.4‰). Overall, the mega- and macrofauna exhibited a wide range of $\delta^{13}C$ values on both MV (Table 2; Fig. 5). On Napoli, a specimen of the vestimentifera *Lamellibrachia* sp. collected at site #2 exhibited the highest $\delta^{13}C$ values (-17.6‰) whereas a specimen of the mytilid bivalve *Idas* sp. showed the lowest one (-41.6‰). On Amsterdam, the dorvilleid polychaete was the most ^{13}C-enriched taxon (-22.9‰) whereas a specimen of the polychaete Spionidae sp. 2 was the most ^{13}C-depleted (-45.4‰). When only considering the species for which we analysed both sulfur and carbon stable isotopes, there was a significant negative correlation between $\delta^{13}C$ and $\delta^{34}S$ values ($n = 40$; $r^2 = 0.172$; $p < 0.001$).

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

3.2. Between-site and inter-individual isotopic variability

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

The thyasirid *Thyasira striata* showed lower $\delta^{15}N$ values than all other bivalves (Table 2; Fig. 5) and the difference was significant when compared with the vesicomyid *Isorropodon perplexum* and the mytilid *Idas* sp. collected on both MVs (Kruskal-Wallis test; $p < 0.01$). *T. striata* also showed lower $\delta^{34}S$ values than the other bivalves (Fig. 4), but the difference was not significant with *I. perplexum* (Mann-Whitney U test, $p = 0.061$) and not testable with *Idas* sp. due to insufficient replicate number. Overall, the lucinid, thyasirid and vesicomyid bivalves exhibited more homogeneous $\delta^{13}C$ values (mean = -30.6 ±2.0‰) than the mytilid *Idas* sp. (mean = -39.4 ±5.1‰; Table 2; Fig. 5). Moreover, *Idas* sp., *T. striata* and *I. perplexum* significantly differed by their $\delta^{13}C$ values (Kruskal-Wallis test; $p < 0.01$), the mytilid being significantly more ^{13}C-depleted than the two latter species (95% LSD Fisher test).

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

4. Discussion

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

4.1. Origin of assimilated carbon on Napoli and Amsterdam MVs

4.1.1. Contribution of photosynthetic vs. *chemosynthetic carbon*

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

$\delta^{13}C$ values of SOM collected ~1 km away from the Napoli and Amsterdam MVs (from -24 to -21‰) match those reported for surface sediments of the deep eastern 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 displays $\delta^{13}C$ values that form a large continuum from the phytoplankton end-member (~22‰) to very low values (<40‰) characteristic of methane-influenced sediment (Paull et al. 1985; Elvert et al. 2000). The more ^{13}C-depleted SOM samples (< -25‰) of Napoli and Amsterdam are likely due to a ^{13}C-depleted biomass fraction that assimilates products of the anaerobic oxidation of methane (AOM) as reported for the Kazan MV, another active cold-seep located on the Anaximander Mountains (Haese et al. 2003). On Kazan, ^{13}C-depleted material has been found in anoxic sediment (12-15 cm depth; Haese et al. 2003) and ^{13}C-depleted DIC pool (down to -35‰) mainly derived from methane (Werne et al. 2002). However, we found a different $\delta^{13}C$ pattern within the Napoli MV sediments. Indeed, the lowest $\delta^{13}C$ values were obtained for the surficial sediment (0 – 1cm) within (or very close to) the "Lamellibrachia" microhabitat, where the oxygenated layer was very thin (<5mm; data not shown), suggesting that AOM may also occur at shallower depths in the sediments than usually thought. Importantly, our results highlighted that SOM represents a very heterogeneous food source for benthic consumers (in terms of carbon origin and organic C content) at the summit of both MV, the organic matter mainly originating from methane at some particular locations and sediment depths.

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

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

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

Overall our isotopic data set clearly indicates that Napoli's and Amsterdam's benthic communities rely, to a large extent, on chemosynthetic processes (either directly via symbioses or indirectly via heterotrophy). This obviously contrasts with cold-seeps located on shallow continental shelf (i.e. < 50 m depth) where no significant chemosynthetic contribution is reported for seep endofauna (Dando et al. 1991; Levin et al. 2000). Our results also contrast with cold-seeps located at bathyal depths but whose food web is still largely fuelled by the high phytoplanktonic production of the euphotic zone (Levin and Michener 2002; Sellanes et al. 2008). Deep seep communities of New Zealand's continental margins also seem to depend largely on photosynthetically-derived carbon, albeit in this case some heterotrophic consumers may have been collected off seeps due to the use of gear operated from the surface (Thurber et al. 2010). Given the food-limited character of the deep eastern Mediterranean environment, the hypothesis that cold-seeps provide chemosynthetically-derived organic matter to their surrounding environment is interesting. Due to insufficient recovered biomass out of the active summit of both MVs, this issue could not be assessed by the stable isotope approach. However, the patterns of macrofaunal abundance observed over the two large-scale transects give some clues on the trophic role of MVs for the nearby benthic ecosystem. Indeed, benthic macrofauna (size > 300μm) was abundant at the summit of Napoli (5220 ind. m^{-2} at site KGS#15) and Amsterdam (5152 ind. m^{-2} at site KGS #16) MV. The abundances sharply decreased as soon as we depart from the summit of Napoli (between 116 at KGS#13 and 104 ind. m^{-2} at KGS#14) and Amsterdam MV (between 68 at KGS#19 and 232 ind. m^{-2} at KGS#18). On these surrounding areas, macrofauna reached abundance values classically encountered on deep eastern Mediterranean bottoms (typically < 200 ind. m^{-2}; Tselepides et al. 2000; Kröncke et al. 2003). This result strongly suggests that the influence of both MVs in terms of organic matter output is high but limited to the immediate vicinity (< 1 km away) of the active zones. Therefore the export of seep carbon is probably highly localized as found in the Gulf of Mexico (Levin 2005). This limited transfer of seep organic matter to the surrounding area also agrees with the fact that, despite a high availability of methane and sulphate, Mediterranean cold-seep communities exhibit lower methanotrophic microbial activity (Omoregie et al. 2009) and biomass (Sibuet and Olu 1998; Levin 2005) than their Atlantic and Pacific counterparts.

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

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

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

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

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

MDC contribution was much more difficult to estimate for the mytilid *Idas* sp. because this bivalve is known to harbour sulfur- and methane-oxidizing (among others) endosymbiotic bacteria (Duperron et al. 2008) and could be mixotroph (i.e. assimilates carbon by filter-feeding as well) as evidenced for other symbiotic mytilids (Page et al. 1990). Nevertheless most of the individuals were more ^{13}C-depleted (<40‰) and more ^{34}S-enriched (4.5‰) than thiotrophic bivalves (from -6.0 to -1.1‰) and $\delta^{34}S$ values of thiotrophic species are usually lower than those of methanotrophic ones (Brooks et al. 1987; Cary et al. 1989; Paull et al. 1992; Vetter and Fry 1998). Taking into account the very low input of phytoplanktonic material to the deep eastern Mediterranean seafloor, *Idas* sp. from Napoli and Amsterdam MVs likely derives a significant part of its carbon from methane. On Amsterdam for instance, assuming no assimilation of phytoplanktonic organic matter (which give a F_m min) and a δ_{meth} of -50‰ (Pape et al. 2010), *Idas* sp. could derive up to 69% of its carbon from methane.

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

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

4.2. Small-scale spatial heterogeneity of nutrients sources

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

Vestimentiferan symbionts contain Rubisco form II and there is to date no evidence of the presence of form I (Naganuma et al. 2005; Duperron et al. 2009). As the form II enzyme discriminates less against ^{13}C during the carbon fixation than the form I, the host's tissues exhibit $\delta^{13}C$ values around -11‰ (-16 to -9‰), provided that the fixed DIC only originates from the water column (Robinson et al. 2003). Such high $\delta^{13}C$ values are usually observed on hydrothermal vents (Rau 1981; Desbruyères et al. 1983; Fisher et al. 1990), but not on cold-seeps where vestimentiferans (including *Lamellibrachia*) are much more ^{13}C-depleted (from -58 to -20‰; Brooks et al. 1987; Kennicutt II et al. 1992; MacAvoy et al. 2005; Sellanes et al. 2008; Thurber et al. 2010). Low $\delta^{13}C$ values obtained for *Lamellibrachia* sp. on Napoli and Amsterdam MVs fell within the latter range but more importantly, individuals from Napoli exhibited unusual 20‰ $\delta^{13}C$ variability over a very small distance (~1m). Significant $\delta^{13}C$ differences have already been observed for macrofauna inhabiting nearby seep microhabitats showing different seepage activities (Levin 2005; Levin and Mendoza 2007). However intra-specific $\delta^{13}C$ differences comparable to 15‰-variation obtained within a single blade-core sample (representing a surface of 0.02 m^2) have never been reported. Several factors may account for the large inter-individual $\delta^{13}C$ variability observed for *Lamellibrachia* sp. of Napoli MV.

Between-sex isotopic discrepancies potentially exist for vestimentiferan tubeworms since, for instance, females bear eggs that are rich in ^{13}C-depleted lipids (Jarnegren et al. 2005). Eggs were clearly visible in of some of the tubeworms collected on Napoli MV, but only in their trunk. However, since similar isotopic patterns were obtained for both the vestimentum and the trophosome parts, we do not think that presence or absence of eggs contributed to inter-individual $\delta^{13}C$ differences of *Lamellibrachia* sp.

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

More likely, most of the observed inter-individual $\delta^{13}C$ variations resulted from the assimilation of DIC of diverse origins and in variable proportions. The origin of pore water DIC is more complex in cold-seeps than in vent environments (Brooks et al. 1984; Suess and Whiticar 1989) and its $\delta^{13}C$ depends on the relative abundance of dissolved carbon derived from i) the water column source (~1.0‰), ii) mineralisation of phytoplanktonic organic matter (~-23‰) and iii) methane oxidation (~-110‰ to ~-30‰). Considering a negligible "marine organic matter" end-member (see Material & methods) together with the lower δ_{meth} estimate (-70‰), minimum MDC in the host's tissue of *Lamellibrachia* sp. varied between 10.8 and 38.2% over the Napoli's investigated area. With δ_{meth} approaching -40‰, the MDC values could be much more important (>50%), as found for the vestimentifera *Escarpia* in the Florida Escarpment (Paull et al. 1989). These calculated methane contributions should be regarded with care since they assume DIC source as a major factor governing the $\delta^{13}C$ variations of vestimentiferans. Anyway, the $\delta^{13}C$ variability we found for *Lamellibrachia* sp. on a small spatial-scale strongly supports the hypothesis of Dattagupta et al. (2006) that these seep-vestimentifera uptake DIC mainly through the thin tube at their posterior (buried) end in the same manner as sulphide (Scott and Fisher 1995; Freytag et al. 2001), and to a lesser extent by their gill plume. Indeed, at small spatial scale (e.g. a blade-core surface area) the DIC pool is likely much more heterogeneous within the sediment pore water than in the overlying water column. Following this hypothesis, the relative proportion of MDC that is fixed by the endosymbionts of *Lamellibrachia* could be linked to the length of the tubeworm's root, i.e. the sediment depth its posterior end can reach. But as we could not measure the length of sampled individuals, we were not able to test this hypothesis.

Overall, our data suggest that a significant proportion of DIC fixed by the endosymbionts originated from seeping methane. This stresses the fact that, although vestimentiferan siboglinids likely exclusively depend on the thiotrophic energetic pathway for the synthesis of their organic compounds, their sources of inorganic carbon are complex and a significant part of fixed CO_2 is a by-product of (aerobic and/or anaerobic) methane oxidation. Data we acquired on the trophic ecology of Napoli and Amsterdam MVs clearly corroborates the spatial heterogeneity of Mediterranean mud volcano environments revealed by previous geophysical studies (Werne et al. 2004). Our results also underline the importance to characterise the $\delta^{13}C$ of DIC when attempting to identify the energetic pathway and the carbon source of an animal on the base of its tissue's stable isotope signature.

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

4.3. Conclusions

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

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

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Figure captions:

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

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

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

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

Fig. 5: δ^{13}C and δ^{15}N values (mean ±SD when $n \geq 3$) of invertebrates collected on the top of Napoli and Amsterdam MVs (See Table 2 for species codes). Left dark grey area and "CH_4" indicate the assimilation of methanotrophically-derived carbon (considering the upper δ_m estimate; see text for details). Right soft grey area indicates the range of δ^{13}C values obtained for benthic consumers that exclusively depend on phytoplanktonic carbon. Dotted lines show the upper δ^{15}N limit (3‰) for species known to bear endosymbiotic bacteria. Dashed boxes represent the ranges of δ^{13}C and δ^{15}N values obtained for SOM on the summit of each MV.

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

Figure 1

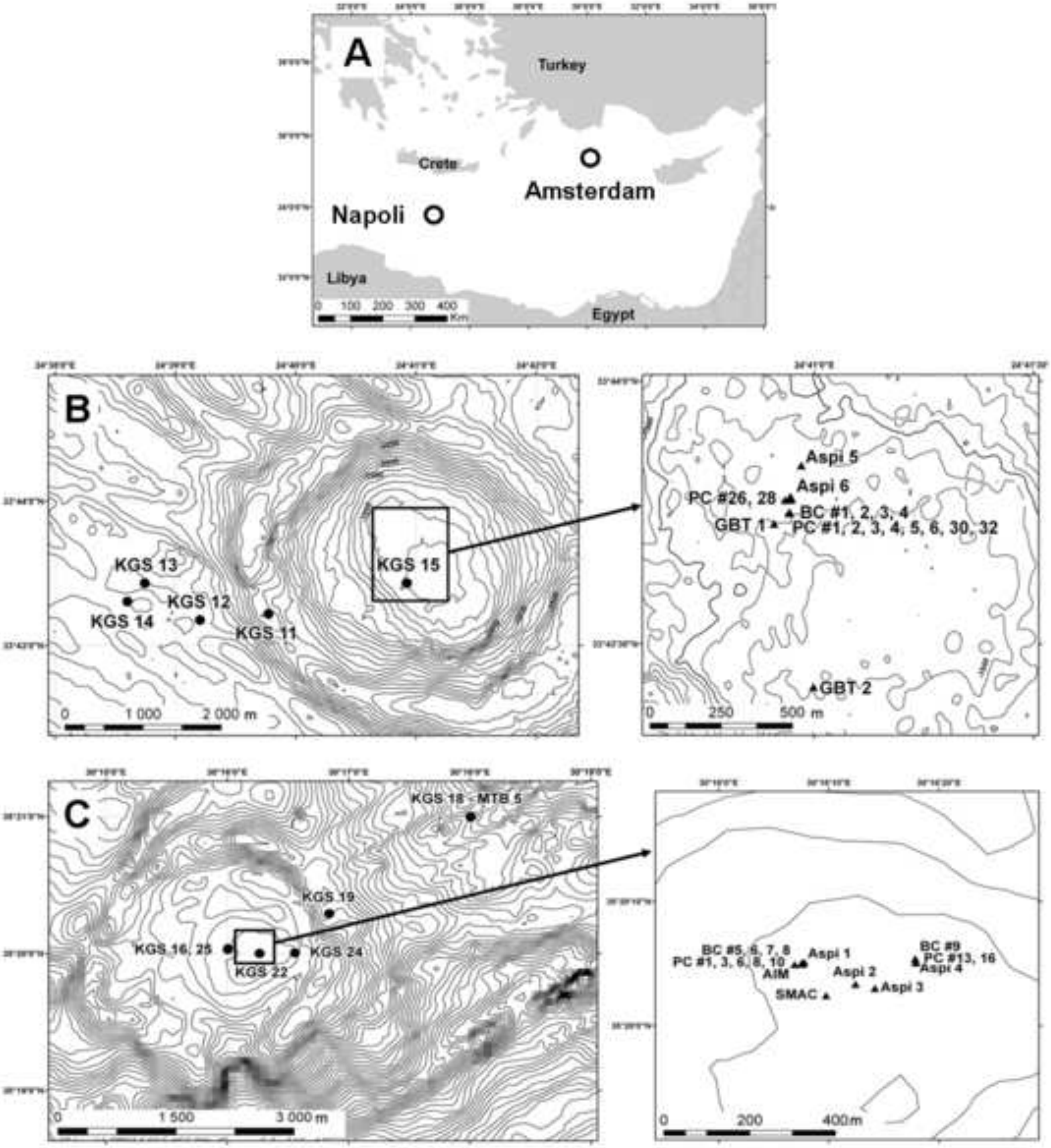
A
Turkey
Crete
Amsterdam
Napoli
Libya
Egypt
0 100 200 300 400 Km
B
KGS 13
KGS 14
KGS 12
KGS 11
KGS 15
0 1 000 2 000 m
Aspi 5
Aspi 6
PC #26, 28
BC #1, 2, 3, 4
GBT 1
PC #1, 2, 3, 4, 5, 6, 30, 32
GBT 2
0 250 500 m
C
KGS 18 - MTB 5
KGS 19
KGS 16, 25
KGS 22
KGS 24
0 1 500 3 000 m
BC #5, 6, 7, 8
PC #1, 3, 6, 8, 10
AIM
Aspi 1
Aspi 2
Aspi 3
SMAC
BC #9
PC #13, 16
Aspi 4
0 200 400m

Figure 2

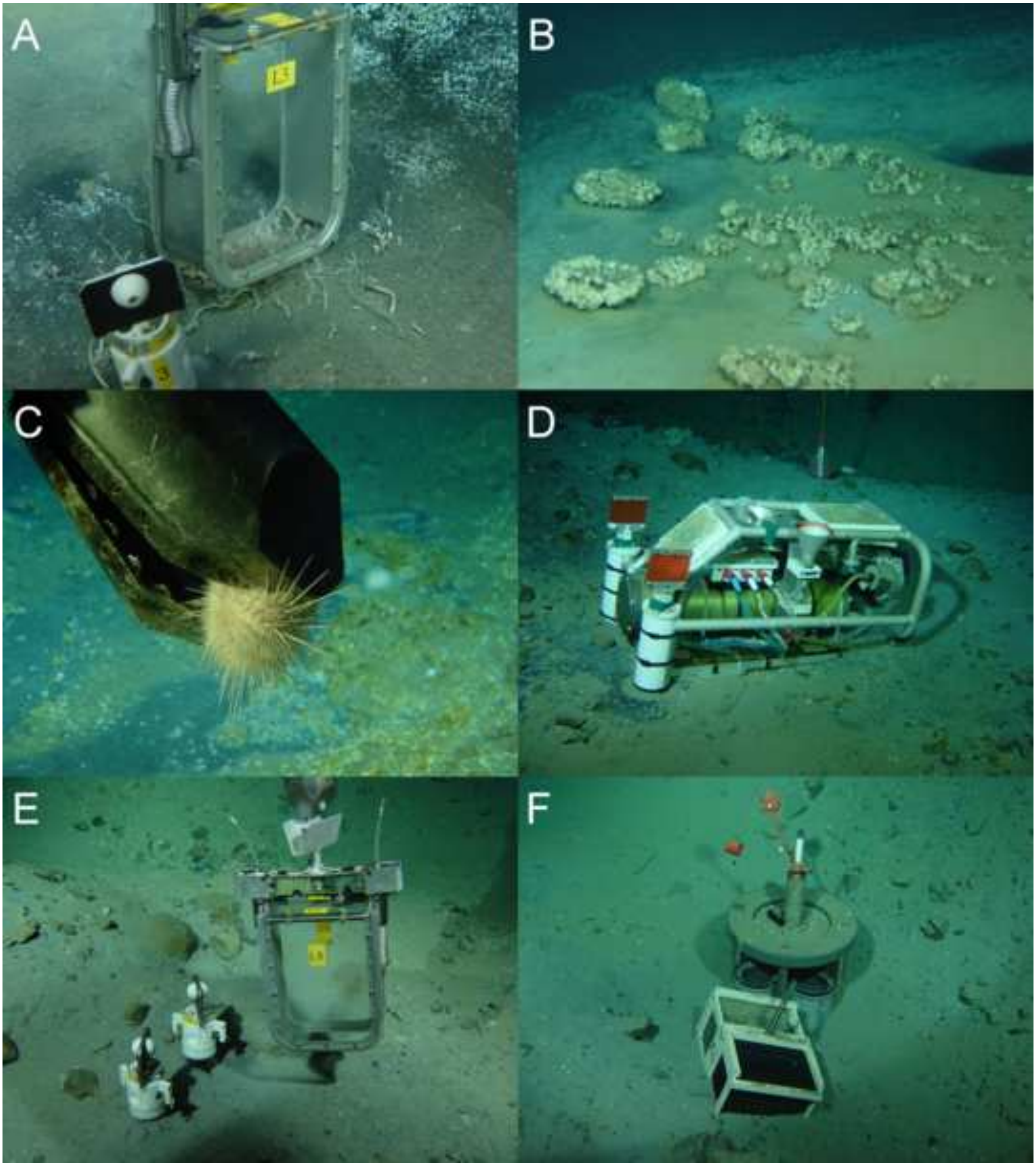

Figure 3

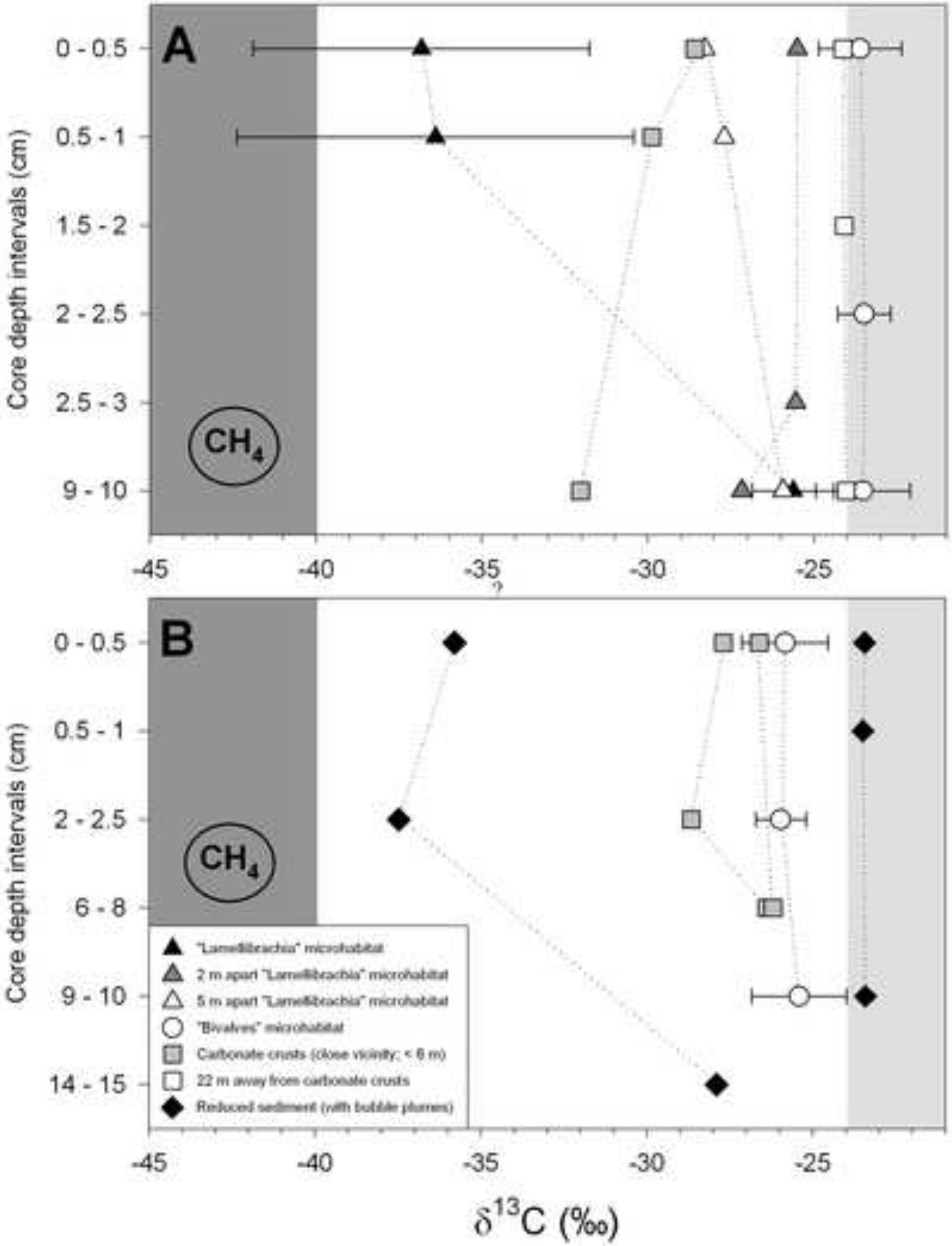
A
B
CH$_4$
CH$_4$
Core depth intervals (cm)
0 - 0.5
0.5 - 1
1.5 - 2
2 - 2.5
2.5 - 3
9 - 10
6 - 8
14 - 15
-45
-40
-35
-30
-25
δ^{13}C (‰)
"Lamellibrachia" microhabitat
2 m apart "Lamellibrachia" microhabitat
5 m apart "Lamellibrachia" microhabitat
"Bivalves" microhabitat
Carbonate crusts (close vicinity; < 6 m)
22 m away from carbonate crusts
Reduced sediment (with bubble plumes)

Figure 4

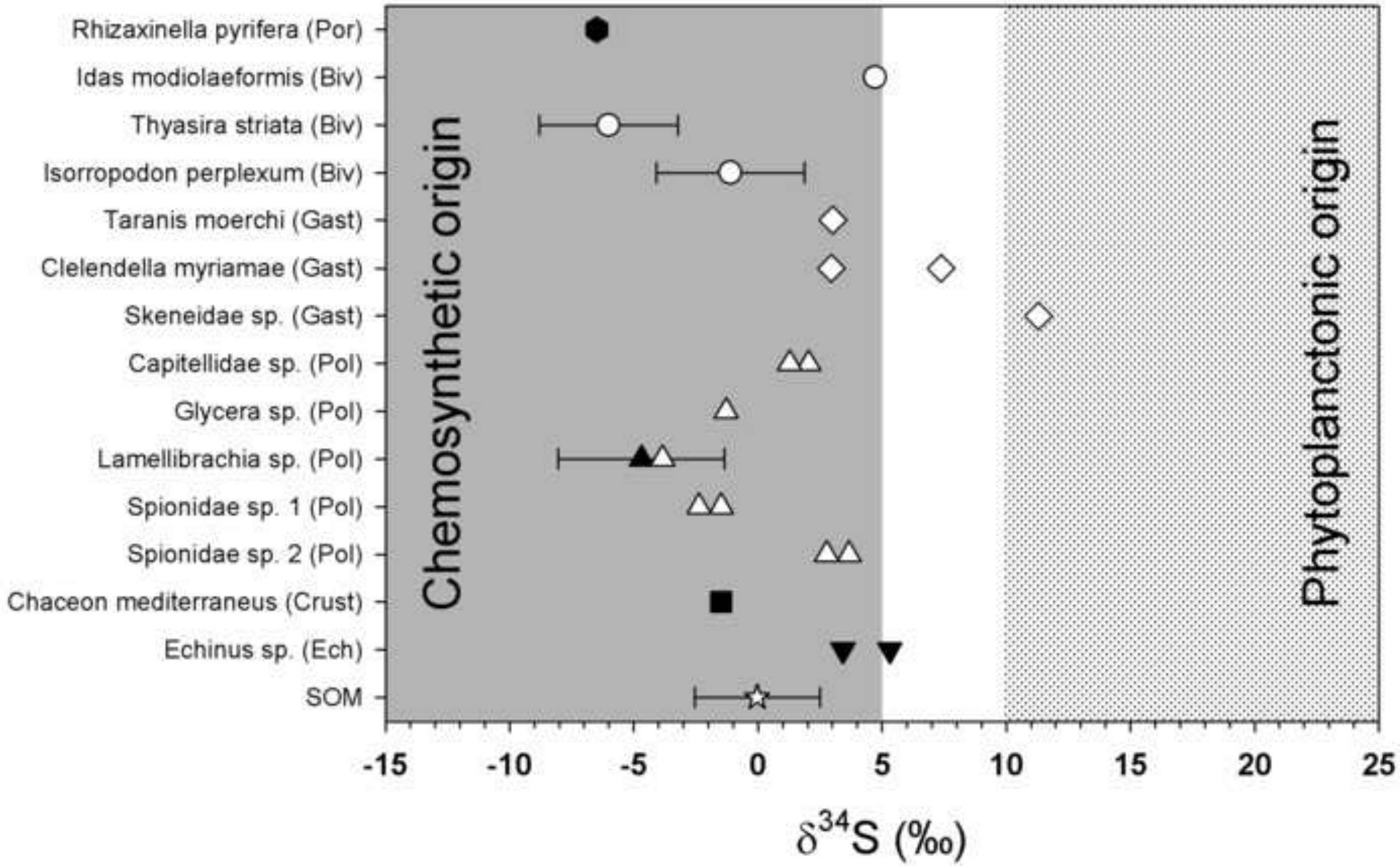
Rhizaxinella pyrifera (Por)
Idas modiolaeformis (Biv)
Thyasira striata (Biv)
Isorropodon perplexum (Biv)
Taranis moerchi (Gast)
Clelendella myriamae (Gast)
Skeneidae sp. (Gast)
Capitellidae sp. (Pol)
Glycera sp. (Pol)
Lamellibrachia sp. (Pol)
Spionidae sp. 1 (Pol)
Spionidae sp. 2 (Pol)
Chaceon mediterraneus (Crust)
Echinus sp. (Ech)
SOM
Chemosynthetic origin
Phytoplanctonic origin
-15
-10
-5
0
5
10
15
20
25
δ34S (‰)

Figure 5

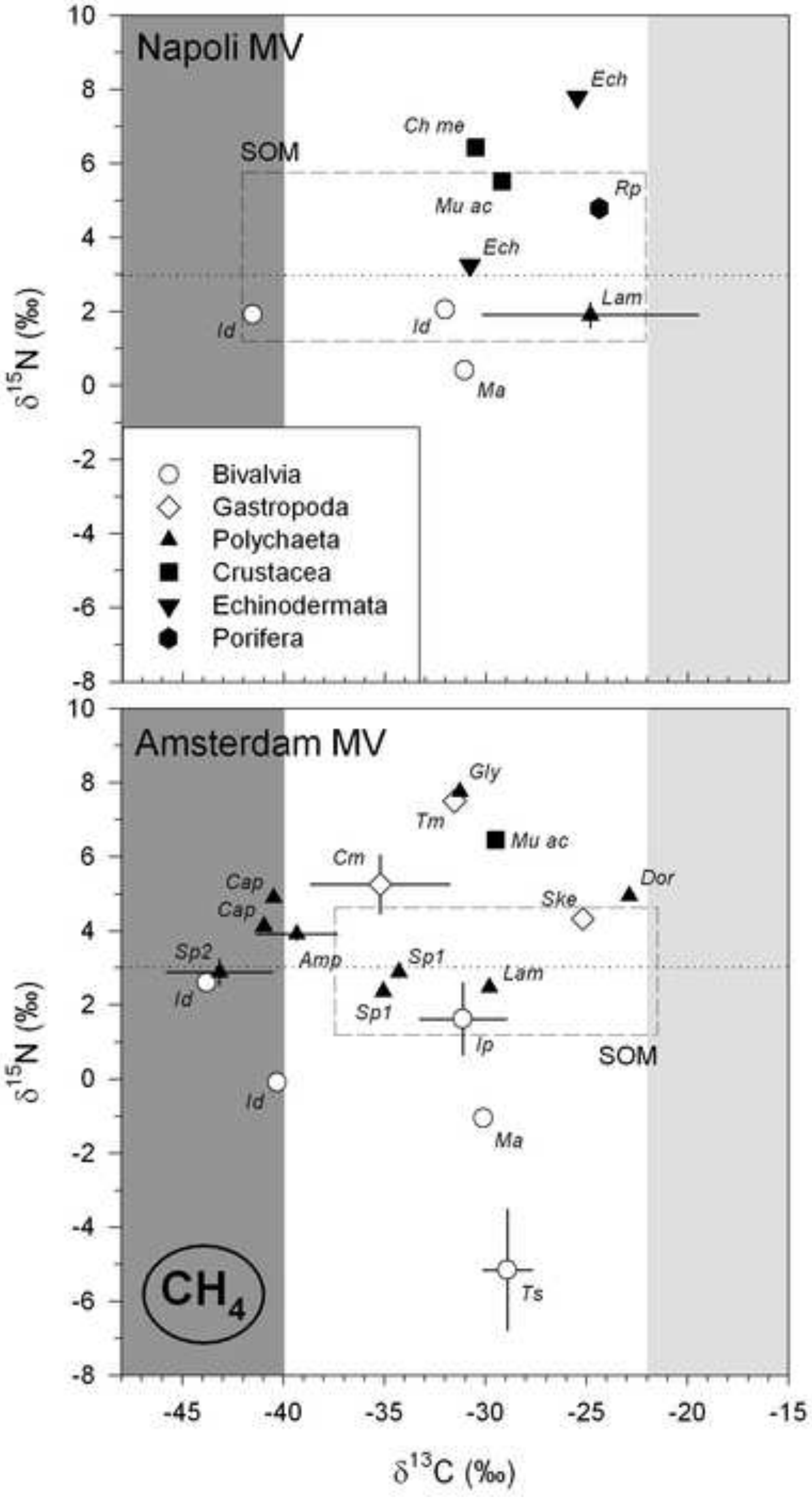
Napoli MV
Ech
Ch me
SOM
Mu ac
Rp
Ech
Lam
Id
Id
Ma
○ Bivalvia
◇ Gastropoda
▲ Polychaeta
■ Crustacea
▼ Echinodermata
⬢ Porifera
Amsterdam MV
Gly
Tm
Mu ac
Cm
Cap
Cap
Dor
Ske
Amp
Sp2
Sp1
Lam
Id
Sp1
Ip
SOM
Id
Ma
Ts
CH_4
$\delta^{15}N$ (‰)
$\delta^{15}N$ (‰)
$\delta^{13}C$ (‰)
10
8
6
4
2
0
-2
-4
-6
-8
-45
-40
-35
-30
-25
-20
-15

Figure 6

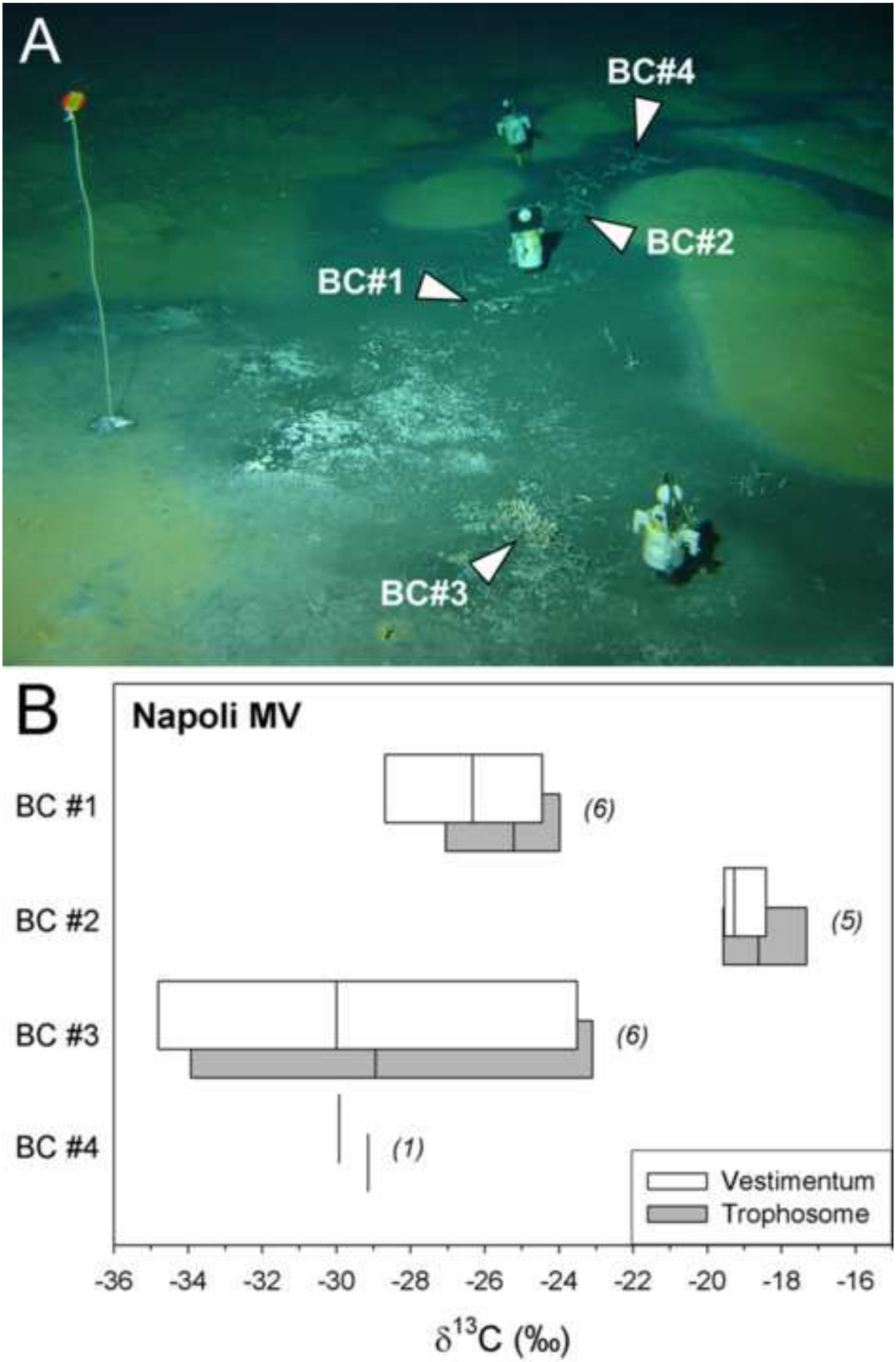
A
BC#4
BC#2
BC#1
BC#3
B
Napoli MV
BC #1
BC #2
BC #3
BC #4
(6)
(5)
(6)
(1)
Vestimentum
Trophosome
-36
-34
-32
-30
-28
-26
-24
-22
-20
-18
-16
$\delta^{13}C$ (‰)

Table 1

Table 1: Nitrogen and organic carbon content, C:N value as well as δ^{15}N and δ^{13}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	δ^{15}N (‰)	δ^{13}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
	KGS#25	0 - 0.5	0.1	0.7	18.5	3.4	-25.4
	KGS#22	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
"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 ± 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	δ^{15}N (‰)		δ^{13}C (‰)		δ^{34}S (‰)	
Napoli MV										
Rhizaxinella pyrifera	Por.	*Rp*	330	GBT2	4.8	*(1)*	-24.4	*(1)*	-6.5	*(1)*
Idas sp.	Biv.	*Id*	330	BC#4; GBT1	1.9 ; 2.1	*(2)*	-41.6 ; -32.0	*(2)*		
Myrtea amorpha	Biv.	*Ma*	331	ASPI#6	0.4	*(1)*	-31.1	*(1)*		
Lamellibrachia sp.	Pol.	*Lam*	330	BC#1, 2, 3, 4	1.9 ± 0.4	*(19)*	-24.8 ± 5.4	*(19)*	-4.7 ± 3.3	*(6)*
Chaceon mediterraneus	Crust.	*Ch me*	330	GBT2	6.4	*(1)*	-30.5	*(1)*	-1.5	*(1)*
Munidopsis acustipina	Crust.	*Mu ac*	331	ASPI#5	5.5	*(1)*	-29.2	*(1)*		
Echinus sp.	Ech.	*Ech*	330	GBT1	3.3 ; 7.8	*(2)*	-30.8 ; -25.5	*(2)*	3.4 ; 5.3	*(2)*
Amsterdam MV										
Idas sp.	Biv.	*Id*	334	ASPI#3; AIM camera	-0.1 ; 2.6	*(2)*	-43.8 ; -40.3	*(2)*	4.7	*(1)*
Myrtea amorpha	Biv.	*Ma*		KGS#25	-1.0	*(1)*	-30.1	*(1)*		
Isorropodon perplexum	Biv.	*Ip*	334	BC#5, 6, 7, 8; ASPI#3; AIM camera; SMAC; KGS#22	1.6 ± 1.0	*(12)*	-31.1 ± 2.1	*(12)*	-1.1 ± 3.0	*(4)*
Thyasira striata	Biv.	*Ts*	334	SMAC; KGS#16, 22, 25	-5.2 ± 1.6	*(4)*	-28.9 ± 1.2	*(4)*	-6.0 ± 2.8	*(4)*
Taranis moerchi	Gast.	*Tm*		KGS#22	7.5	*(1)*	-31.5	*(1)*	3.0	*(1)*
Clelendella myriamae	Gast.	*Cm*	334	ASPI#1, 3, 4; SMAC; KGS#22	5.3 ± 0.8	*(5)*	-35.2 ± 3.5	*(5)*	3.0 ; 7.4*	*(2)*
Skeneidae sp.	Gast.	*Ske*	334	on AIM camera	4.3	*(1**)*	-25.2	*(1**)*	11.3	*(1**)*
Ampharetidae sp.	Pol.	*Amp*	334	BC#8; AIM camera; SMAC	3.9 ± 0.2	*(3)*	-39.4 ± 2.0	*(3)*		
Capitellidae sp.	Pol.	*Cap*	334	SMAC	4.1 ; 4.9	*(2)*	-41.0 ; -40.5	*(2)*	1.3 ; 2.1	*(2)*
Dorvilleidae sp.	Pol.	*Dor*	334	BC#8	5.0	*(1)*	-22.9	*(1)*		
Glyceridae sp.	Pol.	*Gly*		KGS#22	7.8	*(1)*	-31.3	*(1)*	-1.3	*(1)*
Lamellibrachia sp.	Pol.	*Lam*	334	BC#9	2.5	*(1)*	-29.8	*(1)*	-3.8	*(1)*
Spionidae sp. 1	Pol.	*Sp1*		KGS#16, 22	2.4 ; 2.9	*(2)*	-35.0 ; -34.3	*(2)*	-1.9 ± 0.6	*(2)*
Spionidae sp. 2	Pol.	*Sp2*	334	BC#8; ASPI#3; AIM camera; SMAC	2.9 ± 0.3	*(3)*	-43.2 ± 2.6	*(3)*	3.2 ± 0.6	*(2)*
Munidopsis acustipina	Crust.	*Mu ac*	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

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		Fm MIN	Fm MAX	References
Symbiotic					
Lamellibrachia 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*	Paull et al., 1989
Heterotroph					
Seep heterotrophic fauna	mixed**	New Zealand	21	73	Thurber et al., 2009
Rhizaxinella pyrifera	Por.	Napoli MV	0	28	TS
Taranis moerchi	Gast.	Amsterdam MV	4	61	TS
Clelendella myriamae	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
Amphisamytha 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
Munidopsis acustipina	Crust.	Napoli MV	0	50	TS
		Amsterdam MV	0	52	TS
Galatheid crab	Crust.	Kodiak, AK	53	66	Mendoza & Levin, 2007
Chaceon mediterraneus	Crust.	Napoli MV	1	56	TS
Echinus sp.	Ech.	Napoli MV	0	46	TS

* based on δ^{13}C and 14C content measurements

** sipunculids, arthropods, molluscs and annelids showing δ^{13}C values ≤ -40‰