

Carbon isotope excursions during the late Miocene recorded by lipids of marine Thaumarchaeota, Piedmont Basin, Mediterranean Sea

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ABSTRACT

Group I mesophilic Thaumarchaeota fix dissolved inorganic carbon (DIC), accompanied by a biosynthetic fractionation factor of ~20‰. Accordingly, the $\delta^{13}\text{C}$ signature of their diagnostic biomarker crenarchaeol was suggested as a potential $\delta^{13}\text{C}_{\text{DIC}}$ proxy in marine basins if input from nonmarine Thaumarchaeota is negligible. Semi-enclosed basins are sensitive to carbon-cycle perturbations, because they tend to develop thermohaline stratification. Water column stratification typified the semi-enclosed basins of the Mediterranean Sea during the late Miocene (Messinian) salinity crisis (5.97–5.33 Ma). To assess how the advent of the crisis affected the carbon cycle, we studied sediments of the Piedmont Basin (northwestern Italy), the northernmost Mediterranean subbasin. A potential bias of our $\delta^{13}\text{C}_{\text{DIC}}$ reconstructions from the input of soil Thaumarchaeota is discarded, since high and increasing branched and isoprenoid tetraether (BIT) index values do not correspond to low and decreasing $\delta^{13}\text{C}$ values for thaumarchaeal lipids, which would be expected in case of high input from soil Thaumarchaeota. Before the onset of the crisis, the permanently stratified distal part of the basin hosted a water mass below the chemocline with a $\delta^{13}\text{C}_{\text{DIC}}$ value of approximately -3.5‰ , while the well-mixed proximal part had a $\delta^{13}\text{C}_{\text{DIC}}$ value of approximately -0.8‰ . The advent of the crisis was marked by ^{13}C enrichment of the DIC pool, with positive $\delta^{13}\text{C}_{\text{DIC}}$ excursions up to $+5\text{‰}$ in the upper water column. Export of ^{12}C to the seafloor after phytoplankton blooms and limited replenishment of remineralized carbon due to the stabilization of thermohaline stratification primarily caused such ^{13}C enrichment of the DIC pool.

INTRODUCTION

Marine group I (MGI) mesophilic Thaumarchaeota are among the dominant archaea in marine environments, where their occurrence can be traced by crenarchaeol, the thaumarchaeal-specific isoprenoid glycerol dialkyl glycerol tetraether (iGDGT; e.g., Besseling et al., 2020). In modern marine sediments, the $\delta^{13}\text{C}$ values of the cyclohexane ring-containing tricyclic biphytane (Bp-cren) derived from ether cleavage of crenarchaeol typically fall between -23‰ and -18‰ ($\delta^{13}\text{C}_{\text{Bp-cren}}$; Schouten et al., 2013). These values originate from autotrophic fixation of dissolved inorganic carbon (DIC) as bicarbonate, with a biosynthetic fractionation factor (ϵ) of $\sim 20\text{‰}$ (Könneke et al., 2012). Accordingly, $\delta^{13}\text{C}_{\text{Bp-cren}}$ was suggested

as a potential (paleo-) $\delta^{13}\text{C}_{\text{DIC}}$ proxy, assuming that ancient MGI Thaumarchaeota had the same metabolism as their modern heirs (Schouten et al., 2013). However, the uptake of organic carbon or the input of crenarchaeol from marine Euryarchaeota was claimed to compromise $\delta^{13}\text{C}_{\text{DIC}}$ estimates (Pearson et al., 2016). Such concerns were recently discarded (cf. Pearson et al., 2019; Besseling et al., 2020), although it has been put forward that ϵ values could depend on growth rate and carbon dioxide concentration, resulting in deviations of up to $\pm 2\text{‰}$ between the measured and the reconstructed $\delta^{13}\text{C}_{\text{DIC}}$ values of modern seawater (cf. Hurley et al., 2019). Another potential bias for $\delta^{13}\text{C}_{\text{DIC}}$ reconstructions is riverine input of crenarchaeol sourced by soil- and river-dwelling Thaumarchaeota to marine basins (Pearson et al., 2016). Elling et al. (2019) disputed the effect of such bias, even under high input

of soil-derived crenarchaeol to ancient marine sediments. Therefore, $\delta^{13}\text{C}_{\text{Bp-cren}}$ values could, indeed, be a powerful (paleo-) $\delta^{13}\text{C}_{\text{DIC}}$ proxy, as demonstrated by successful applications in tracing past carbon-cycle perturbations (Kuypers et al., 2001; Schoon et al., 2013; Elling et al., 2019).

Carbon-cycle perturbations are common for modern semi-enclosed basins, which tend to develop thermohaline stratification, altering the $\delta^{13}\text{C}_{\text{DIC}}$ of the water column (e.g., Fry et al., 1991). Water masses below the chemocline tend to become ^{13}C depleted due to pronounced organic matter recycling, resulting in $\delta^{13}\text{C}_{\text{DIC}}$ values as low as -19‰ (e.g., van Breugel et al., 2005). Conversely, seasonal eutrophication and preferential $^{12}\text{CO}_2$ loss via enhanced degassing in surface waters can produce ^{13}C enrichment, with $\delta^{13}\text{C}_{\text{DIC}}$ values as high as $+16.5\text{‰}$ in lacustrine environments (e.g., Stiller et al., 1985; Oren et al., 1995). Akin to modern basins, thermohaline stratification may have caused the alteration of $\delta^{13}\text{C}_{\text{DIC}}$ pools also in ancient semi-enclosed basins (e.g., Schoon et al., 2013).

In the late Miocene, the Mediterranean Basin developed thermohaline stratification (García-Veigas et al., 2018) due to its close-to-complete tectonic isolation from the global ocean during the Messinian salinity crisis (MSC; 5.97–5.33 Ma). Although the global carbon cycle was likely affected by this event (e.g., Capella et al., 2019), the effect of the advent of the MSC on the $\delta^{13}\text{C}_{\text{DIC}}$ pool of the late Miocene Mediterranean water column remains unassessed. To fill this gap, we conducted a case study on the Piedmont Basin (northwestern Italy), the northernmost subbasin of the late Miocene Mediterranean Sea (Dela Pierre et al., 2011). There, MGI Thaumarchaeota represented the main group of planktonic archaea at the onset of the MSC (Natalicchio et al., 2017; Sabino et al., 2021).

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After assessing potential biases in the applicability of $\delta^{13}\text{C}_{\text{Bp-cren}}$ values as paleo- $\delta^{13}\text{C}_{\text{DIC}}$ proxies, we reconstructed the $\delta^{13}\text{C}_{\text{DIC}}$ of the stratified water body in proximal (Pollenzo section) and distal (Govone section) sectors of the margin of the Piedmont Basin. The approach used in this study shows great promise to decipher ancient carbon-cycle perturbations.

MATERIALS AND METHODS

We analyzed five lithologic cycles (shale-marl couplets) deposited across the MSC onset (5.97 Ma) at a water depth >200 m in a more proximal (Pollenzo; 43 samples) and a distal (Govone; 14 samples) position along the southern margin of the Piedmont Basin (Figs. 1 and 2; for details, see the Supplemental Material¹). The cycles reflect astronomically driven moister (shales) and drier (marls) climate oscillations (cf. Natalicchio et al., 2019; Sabino et al., 2020). In the studied sections, the MSC onset was placed at the base of marls of cycles Pm5 (Pollenzo; Natalicchio et al., 2019) and Gm30 (Govone; Gennari et al., 2020; Fig. 2). An aliquot of the total lipid extract of the 57 samples was used to obtain data on *i*GDGTs and branched GDGTs (*b*GDGTs), analyzed through high-performance liquid chromatography–mass spectrometry. We calculated the branched and isoprenoid tetraether (BIT) index and the *i*GDGT-2/*i*GDGT-3 ratio ([2]/[3] ratio) to assess input from soil Thaumarchaea and the relative contributions of upper-water-column versus deeper-water-column thaumarchaeal communities, respectively (Hopmans et al., 2004; Kim et al., 2015). Ether-bound isoprenoids were released from *i*GDGTs by treating another aliquot of the extracts with HI/LiAlH_4 , while the asphaltene fraction of 21 samples (Pollenzo: 7 samples;

Govone: 14 samples) was desulfurized to release sulfur-bound compounds from macromolecules. The hydrocarbon fractions obtained from ether cleavage and desulfurization were analyzed using gas chromatography–mass spectrometry. The $\delta^{13}\text{C}$ analyses of ether-cleaved biphytanes (Bp) and desulfurized phytane and C_{27} to C_{29} steranes obtained after column chromatography were performed on 41 samples (Pollenzo: 27 samples; Govone: 14 samples). The $\delta^{13}\text{C}$ values are reported in per mil (‰) versus the Vienna Pee Dee belemnite (V-PDB) standard, and the average analytical standard deviation was 0.4‰ (see the Supplemental Material for details).

RESULTS AND DISCUSSION

$\delta^{13}\text{C}_{\text{Bp-cren}}$ Values as a Robust $\delta^{13}\text{C}_{\text{DIC}}$ Proxy

The BIT index suggests high (0.4–1.0, proximal sector, Pollenzo) to moderate (0.3–0.7, distal sector, Govone) soil-derived organic matter input into the Piedmont Basin at the advent of the MSC (Fig. 2A). A high BIT may imply high input of crenarchaeol sourced from group I soil Thaumarchaeota to marine sediments (Weijers et al., 2006), hampering the applicability of crenarchaeol and its derivative Bp-cren as a $\delta^{13}\text{C}_{\text{DIC}}$ proxy in this case. The $\delta^{13}\text{C}_{\text{Bp-cren}}$ values from MGI Thaumarchaeota range from -23‰ to -18‰ in modern marine sediments (Schouten et al., 2013, and references therein). In contrast, the $\delta^{13}\text{C}_{\text{Bp-cren}}$ values of soil Thaumarchaeota are approximately -30‰ in soils dominated by C_3 plants and -23‰ in soils where C_4 plants prevail (Weijers et al., 2010). Because C_3 plants dominated the Piedmont Basin hinterland in the late Messinian (Bertini and Martinetto, 2011), a bias from soil archaea should result in high and increasing BIT values corresponding to low and decreasing $\delta^{13}\text{C}_{\text{Bp-cren}}$ values. However, the reverse trend was found for the studied samples, with lower $\delta^{13}\text{C}_{\text{Bp-cren}}$ values occurring when BIT values decreased, and vice versa (Figs. 2B and 3). The input of soil thaumarchaeal lipids was therefore apparently negligible. The *b*GDGTs were likely rather sourced from bacteria dwelling in rivers, coastal marine sedi-

ments, and in oxygen-deficient waters (e.g., Liu et al., 2014; Crampton-Flood et al., 2021). This agrees with increasing BIT values coinciding with higher river discharge and an expansion of oxygen-deficient waters toward the margin of the Piedmont Basin after the MSC onset (Natalicchio et al., 2019; Sabino et al., 2020, 2021). Because Thaumarchaeota have also been reported to dwell in rivers (e.g., Kim et al., 2007; Yang et al., 2013), river-derived crenarchaeol with increasing fluvial discharge can also affect $\delta^{13}\text{C}_{\text{DIC}}$ reconstructions of seawater. Today's rivers draining the former catchment area of the Piedmont Basin are typified by a mean $\delta^{13}\text{C}_{\text{DIC}}$ value of -8‰ (Marchina et al., 2016). Assuming a similar value for the late Miocene and an $\varepsilon = 20\text{‰}$ (Könneke et al., 2012) for fluvial Thaumarchaeota, a trend toward ^{13}C depletion of Bp-cren with increasing river discharge is likely. Although river discharge increased in the Piedmont Basin after the MSC onset (cf. Natalicchio et al., 2019; Sabino et al., 2020), Bp-cren became increasingly ^{13}C enriched (Fig. 2B). Hence, we infer negligible input also from fluvial Thaumarchaeota to the sedimentary pool and conclude that the measured $\delta^{13}\text{C}_{\text{Bp-cren}}$ values reflect the signature of MGI Thaumarchaeota. These values can consequently be used as $\delta^{13}\text{C}_{\text{DIC}}$ proxy, despite the moderate to high BIT index (see the Supplemental Material for details on the acyclic and cyclic biphytanes).

Impact of Water-Column Stratification on the $\delta^{13}\text{C}_{\text{DIC}}$ Pool

The Piedmont Basin developed water-column stratification approaching the MSC onset (Dela Pierre et al., 2011), following the geodynamic and oceanographic evolution of the Mediterranean Basin (Roveri et al., 2014). Before the MSC onset, the $\delta^{13}\text{C}_{\text{Bp-cren}}$ values from the distal sector reflected ^{13}C depletion relative to the proximal sector (mean $\Delta_{\text{dist-prox}} = -2.7\text{‰}$; Figs. 2B and 3), close to the lowest $\delta^{13}\text{C}$ values reported for crenarchaeol and Bp-cren for modern and ancient marine sediments ($\sim -24\text{‰} \pm 1\text{‰}$; Figs. 2B and 3; Schouten et al., 2013, and references therein; Schoon et al., 2013; Elling et al., 2019). Smittenberg et al. (2005) explained ^{13}C depletion of this magnitude in thaumarchaeal lipids as the result of the uptake of ^{13}C -depleted DIC when the chemocline rises to the base of the photic zone. We propose that the $\delta^{13}\text{C}_{\text{Bp-cren}}$ values from Govone reflect a similar mechanism. Considering the mean $\delta^{13}\text{C}_{\text{Bp-cren}}$ value of -23.5‰ from samples indicating photic zone euxinia (presence of isorenieratane; Fig. 2; Sabino et al., 2021), and applying an $\varepsilon = 20\text{‰}$ (Könneke et al., 2012), we estimate a $\delta^{13}\text{C}_{\text{DIC}}$ of -3.5‰ and a possible $\pm 2\text{‰}$ deviation (maximum value; cf. Hurley et al., 2019) for the DIC pool below the chemocline in the Piedmont Basin. In the proximal Pollenzo sector, the higher $\delta^{13}\text{C}_{\text{Bp-cren}}$ values

¹Supplemental Material. Geological setting, methodology, and lipid abundance, carbon stable isotopes, and sources (Tables S1–S4; Figures S1–S3). Please visit <https://doi.org/10.1130/GEOL.S.15832095> to access the supplemental material, and contact editing@geosociety.org with any questions.

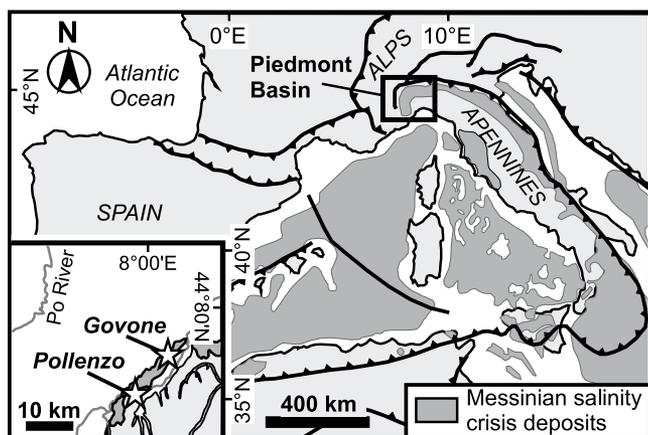


Figure 1. Distribution of Messinian salinity crisis deposits in the Mediterranean Basin. Inset: Location of proximal Pollenzo (44°41'08\"/>

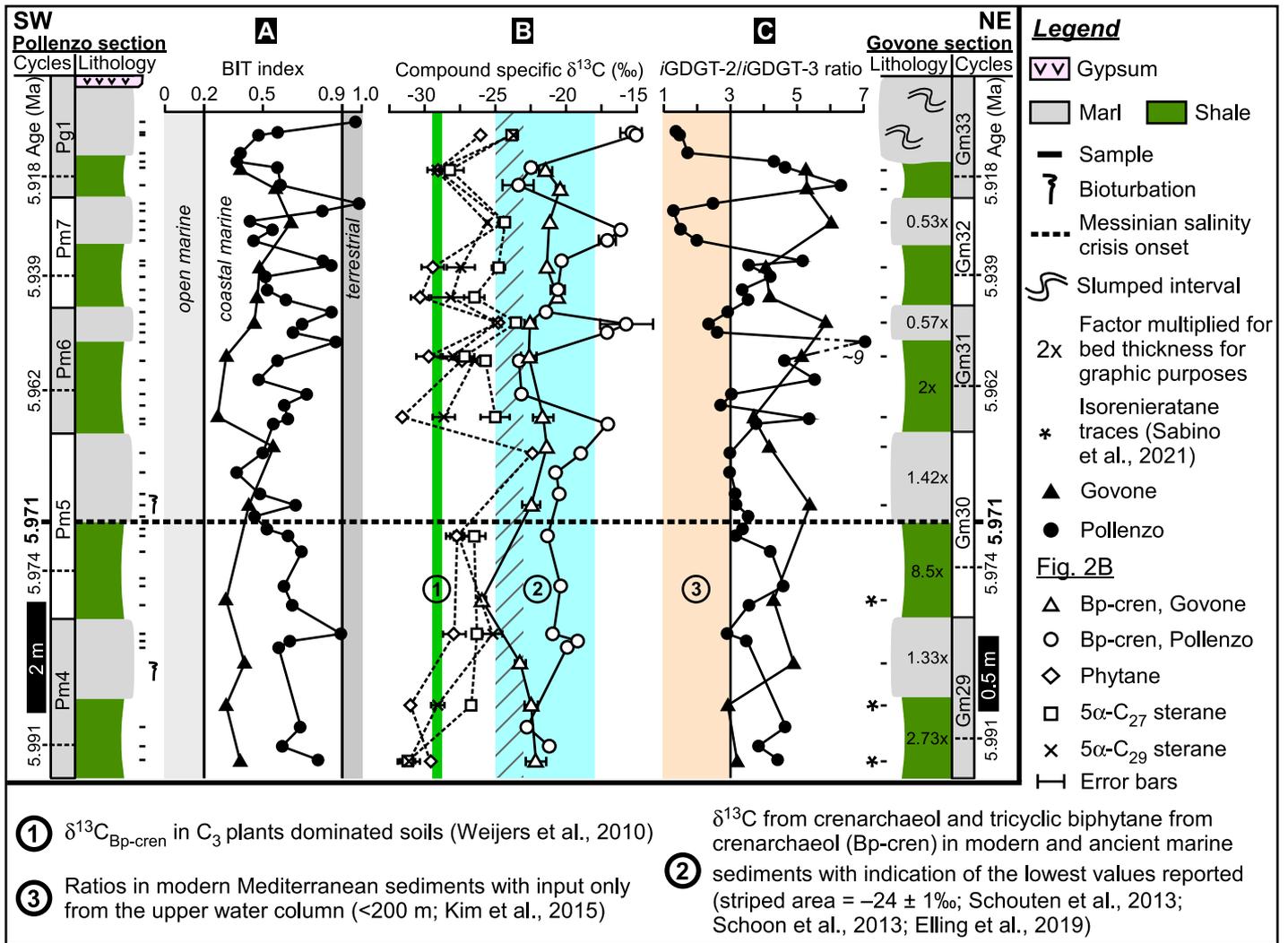


Figure 2. (A) Branched and isoprenoid tetraether (BIT) index, (B) compound-specific carbon ($\delta^{13}\text{C}$) stable isotopes, and (C) $i\text{GDGT-2}/i\text{GDGT-3}$ ratio in Pollenzo section (after Natalicchio et al., 2019) and Govone section (after Sabino et al., 2021) in the Piedmont Basin. $i\text{GDGT}$ —isoprenoid glycerol dialkyl glycerol tetraether; Bp-cren—tricyclic biphytane from crenarchaeol.

(-20.8‰ on average; Figs. 2B and 3) probably reflect mixing (Natalicchio et al., 2017), redistributing the remineralized carbon and maintaining a $\delta^{13}\text{C}_{\text{DIC}} = -0.8\text{‰} \pm 2\text{‰}$, close to that of modern seawater ($-1\text{‰} < \delta^{13}\text{C}_{\text{DIC}} < +2\text{‰}$; Schmittner et al., 2013).

The MSC onset coincided with intensification of water-column stratification in the Piedmont and other Mediterranean subbasins (Natalicchio et al., 2017; García-Veigas et al., 2018). This change is mirrored in the studied samples by ^{13}C enrichment in thaumarchaeal lipids and algal steranes and phytane (see also the Supplemental Material; Fig. 2B). Excursions toward $\delta^{13}\text{C}_{\text{Bp-cren}}$ values as high as -15‰ in the proximal Pollenzo sector coincide with [2]/[3] ratios lower than 3 (Figs. 2B and 2C). Remarkably, such low ratios are also found in modern sediments of the Mediterranean Sea for which thaumarchaeal lipids are derived dominantly from the upper water column (<200 m; cf. Kim et al., 2015). Accordingly, the pattern in the

proximal sector is likely due to lipids sourced from Thaumarchaeota dwelling in the upper water column and fixing carbon from a DIC pool reaching $\delta^{13}\text{C}$ values as high as $+5\text{‰} \pm 2\text{‰}$ during the earliest MSC phase. The drier climate in the Mediterranean during the MSC (Roveri et al., 2014) makes preferred $^{12}\text{CO}_2$ degassing via enhanced seawater evaporation a possible explanation for such ^{13}C enrichment (e.g., Horton et al., 2016). However, in the northern Mediterranean, humid conditions persisted (Bertini and Martinetto, 2011), and the climate was only relatively dry during some excursions (cf. Natalicchio et al., 2019; Sabino et al., 2020). Therefore, $^{12}\text{CO}_2$ degassing is unlikely to have been the sole mechanism responsible for the positive $\delta^{13}\text{C}$ excursions. Interestingly, similar positive excursions have been reported for the DIC pool of the superficial water of the modern Dead Sea (above the pycnocline; $+2.5\text{‰} \leq \delta^{13}\text{C}_{\text{DIC}} \leq +5\text{‰}$; cf. Oren et al., 1995). Such $\delta^{13}\text{C}$ excursions are caused by phytoplankton blooms preferen-

tially taking up ^{12}C after episodes of enhanced runoff, which supplied additional nutrients to the basin (Oren et al., 1995). Similarly, periodic increases of riverine runoff and nutrient input were shown to have led to phytoplankton blooms in the Piedmont Basin (Natalicchio et al., 2019). We therefore suggest that massive export of ^{12}C to the seafloor following productivity pulses resulted in the observed positive excursions in the DIC pool of the upper water column (Fig. 4). Concurrently, stabilization of thermohaline stratification limited the replenishment of remineralized carbon to the upper water column, agreeing with the coinciding ^{13}C enrichment of algal lipids (Fig. 2B).

In contrast, the MSC sediments of the distal Govone section lack abnormally high $\delta^{13}\text{C}_{\text{Bp-cren}}$ values and show [2]/[3] ratios constantly higher than 3 (Fig. 2C). These ratios hint at input of lipids from MGI Thaumarchaeota dwelling in deeper waters (Kim et al., 2015), probably fixing at least partially carbon from the DIC pool

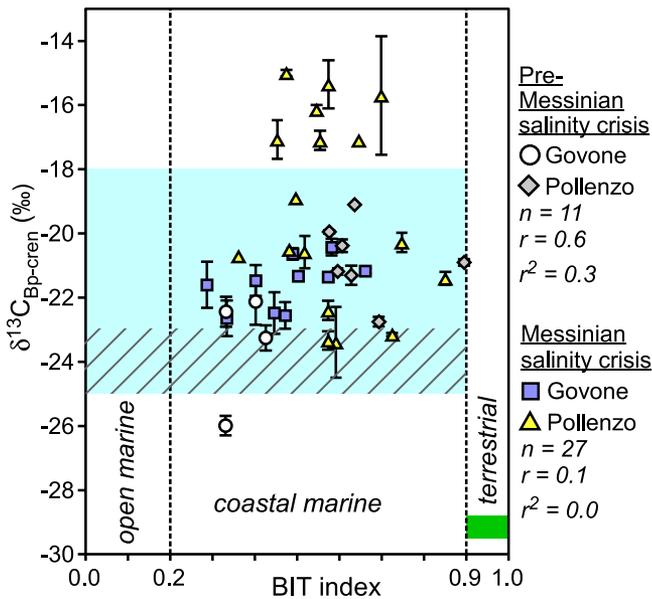


Figure 3. Branched and isoprenoid tetraether (BIT) index versus carbon stable isotope composition of tricyclic biphytane from crenarchaeol ($\delta^{13}\text{C}_{\text{Bp-cren}}$).

$\delta^{13}\text{C}$ from crenarchaeol and tricyclic biphytane from crenarchaeol (Bp-cren) in modern and ancient marine sediments with indication of the lowest values reported (striped area = $-24 \pm 1\text{‰}$; Schouten et al., 2013; Schoon et al., 2013; Elling et al., 2019). Typical $\delta^{13}\text{C}_{\text{Bp-cren}}$ values in C_3 plants-dominated soils (Weijers et al., 2010).

below the chemocline. This scenario explains the lower $\delta^{13}\text{C}_{\text{Bp-cren}}$ values ($\sim -21.5\text{‰}$ on average), the lack of $\delta^{13}\text{C}$ excursions, and the partial mismatch with the trends observed for algal lipids (Fig. 2B). Periodic input of ^{13}C -depleted DIC into the upper water column is, however, testified by the lowering of the $\delta^{13}\text{C}$ values of algal lipids (Fig. 2B). Given that mixing was suppressed by the stable thermohaline stratifi-

cation, fluvial discharge was the most probable source of ^{13}C -depleted DIC ($\delta^{13}\text{C}_{\text{DIC}} = -8\text{‰}$, see above; Fig. 4) at these times. In conclusion, permanent stratification of the water column after the onset of the MSC promoted the development of strong vertical chemical gradients, establishing a ^{13}C -enriched upper water column overlying a ^{13}C -depleted water mass in the Piedmont Basin.

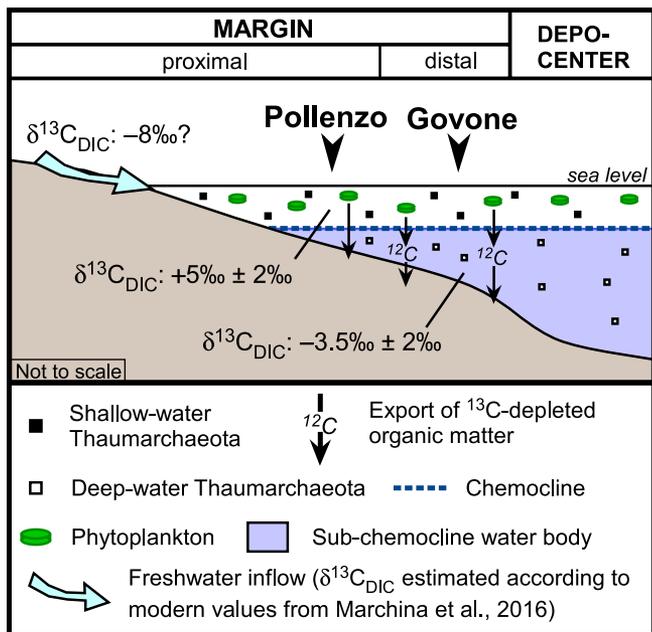


Figure 4. $\delta^{13}\text{C}_{\text{DIC}}$ values in stratified water mass of the Piedmont Basin at the advent of the Messinian salinity crisis. DIC—dissolved inorganic carbon.

SUMMARY AND IMPLICATIONS

The carbon stable isotope signature of crenarchaeol-derived tricyclic biphytane ($\delta^{13}\text{C}_{\text{Bp-cren}}$) from marine Thaumarchaeota allows us to trace the composition of the pool of DIC in the Piedmont Basin—the northernmost late Miocene Mediterranean subbasin— ~ 6 m.y. ago at the advent of the Messinian salinity crisis. The water column was typified by a stagnant, deeper body with a $\delta^{13}\text{C}_{\text{DIC}}$ of approximately -3.5‰ during the basin depocenter before and during the advent of the crisis. The onset of the event was marked by significant ^{13}C enrichment of the DIC pool in the upper water column ($\delta^{13}\text{C}_{\text{DIC}}$ as high as $\sim +5\text{‰}$) due to the basinwide stabilization of thermohaline stratification. The ^{13}C enrichment is interpreted to primarily reflect preferential export of ^{12}C to the seafloor after phytoplankton blooms and limited replenishment of remineralized DIC.

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