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Reconstruction of $\delta^{13}C$ of chemocline CO_2 (aq) in past oceans and lakes using the $\delta^{13}C$ of fossil isorenieratene

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Abstract

High abundances of the diaromatic carotenoid isorenieratene derived from photosynthetic green sulfur bacteria (Chlorobiaceae) were found just below the chemocline in an anoxic fjord in Norway, throughout the annual cycle. The stable carbon isotope composition of this carotenoid co-varied with the $\delta^{13}C$ of CO_2 (aq) and is independent of the CO_2 and isorenieratene concentration. This constant isotopic fractionation ε_p of isorenieratene versus CO_2, $4 \pm 1‰$, was subsequently used in the reconstruction of $\delta^{13}C$ of CO_2 at the chemocline in ancient oceans and lakes. These reconstructions indicate that $\delta^{13}C$ of CO_2 at the chemocline is often influenced by isotopically light CO_2, formed by remineralization of organic matter. This process can, depending on the depth and stability of the chemocline, also effect the isotopic composition of the phytoplankton and, thus, isotopic records of sedimentary inorganic and organic carbon.

Keywords: anoxic fjord; biological carbon isotopic fractionation; fossil isorenieratene; oceanic anoxic event; photosynthetic green sulfur bacteria; $\delta^{13}C$

1. Introduction

Episodes of anoxic water column conditions were not uncommon in ancient marine environments, especially in the mid Cretaceous [1] and the Jurassic [2]. Common features of sediments deposited during these so-called oceanic anoxic events (OAEs) are their high organic carbon contents (>0.5 wt.%), distinct lamination, and impoverished benthic fauna. Globally recorded, synchronous changes in the carbon isotopic composition of organic and inorganic carbon have often been inferred to indicate substantial changes in

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Table 1
The $\delta^{13}C$ of isorenieratene derivatives found in lake and marine sediments compiled from the literature, the calculated $\delta^{13}C$–CO_2 and the respired DIC contribution at the chemocline

Location	Geological age	$\delta^{13}C$ of isorenieratene derivatives[a] (‰)	Chemocline $\delta^{13}C$–CO_2 (‰)	Palaeotemperature estimates (°C)	$\delta^{13}C$ of OC (‰)	$\delta^{13}C$ of carbonate[i] (‰)	Respired DIC contribution (%)	References
Lake Cisó, Spain	contemporary	−25.3	−21	10–14	−26.1	−0.5 to 2[j]	41–48	[15]
Lake Cadagno	contemporary	−27.0[b]	−23	8	−31.5	−0.5 to 2[j]	38–43	[35,45]
Ace Lake, sediment	contemporary	−18.1[b]	−14	5	−22.0 to −23.0	−0.5 to 2[j]	11–21	[19]
Kyllaren fjord	~1800 *AD*	−24.2[b]	**−20**	4.9 to 16	−25.4 to −25.9	−0.5 to 2[j]	34–46	[40]
Black Sea	0 to 6.2 k.y.	−14.8 to −17.4	−11 to −13	8	−23.1 to −25.0	−0.5 to 2[j]	0–19	[20,46]
East Mediterranean sapropel	Pliocene	−14.0[c]	−10	15–25	−23 to −24	0.85 ± 0.3	7–14	[47,48]
Sdom Formation, Israel	Miocene/Pliocene	−15.0[d]	−11	20–30	−26[h],−22.5[i]	0.85 ± 0.3	12–18	[49]
Gessoso solfifera Fm., Italy	Late Messinian	−10.7	−7	20–30	−22.5[i]	0.85 ± 0.3	0–3	[16,50]
	Messinian	−11.6 to −14.8	−8 to −11	20–30	−23 to −26.5	1.5 ± 0.3	2–20	[44,51]
Menilite Fm., Poland	Oligocene	−18.0	−14	10–20	−26.3	2.3 ± 0.3	23–28	[52]
Qianjiang Fm., east China	Mid–Late Eocene	−16.1 to −16.5[e]	−12 to −13	20–30	−23.9	2.05 ± 0.3	22–26	[53]
Xingouzhui Fm., east China	Palaeocene–Eocene	−17.0 to −17.3[e]	−13	20–30	−26.5 to −29.7	3.1 ± 0.3	24–30	[53]
Tarfaya, North Africa	Cenomanian–Turonian	−13.2	−9	25–35	−26.5	4.0 ± 0.3[k]	17–22	[54]
Cape Verde Basin, N. Atlantic	Cenomanian–Turonian	−13.5	−10	25–35	−22	4.0 ± 0.3[k]	21–26	[54]
Demerara Rise, N. Atlantic	Cenomanian–Turonian	−17.5	−14	25–35	−25 to −26.5	1.5 ± 0.3	25–31	[54]
Kwanza Basin, West Africa	Barremian	−14.3 to −14.5[e]	−10	25–35	−27.1 to −27.5	2.0 ± 0.3	15–20	[55,56]
Calcaires en Plaquettes, France	Late Jurassic	−19.0	−15	15–25	−27.75[i]	1.63 ± 1.3	23–33	[50]
Kimmeridge Clay, UK	Late Jurassic	−16.6	−13	15–25	−22 to −26.5	1.68 ± 1.3	15–31	[50,57]
Oxford Clay Fm., UK	Middle Callovian	−16.2 to −17.0	−12 to −13	15–25	−25.5 to −27.4	2.09 ± 1.3	14–29	[58,59]
Allgäu, Germany	Early Jurassic (Toarcian)	−19.0[a], −17.4 to −19.9[e]	−13 to −16	15–25	−29.20[i]	1.79 ± 1.3	17–34	[50,60]
Lower Rhine Basin, Germany	Late Permian	−13.4[a], −16.4[e]	−9,−12	15–25	−27.48[i]	1.69 ± 1.3	4–15, 14–25	[14]
	Permian	−12.0[c]	−8	15–25	−27.08[i]	4.05 ± 1.3	6–17	[61]
Holy Cross Mountains, Poland	Frasnian/Famennian	−13.2[e]	−9	15–25	−28.1 to −28.5	1.32 ± 1.3	2–13	[62]
	Late Frasnian	−16.5[e]	−13	15–25	−30.7	1.32 ± 1.3	12–22	[62]
Duvernay Formation, Canada	Upper Devonian	−13.2	−9	15–25	−28.5	0.89 ± 1.3	0–12	[63]
Pripyat River Basin, Belarus	Devonian	−15.0[f], −15.3[g]	−11	15–25	−28.91[i]	0.65 ± 1.3	5–17	[64]
Boas oil shale, Canada	Late Ordovician	−17.2[g]	−13	15–25	−28.73[i]	0.23 ± 1.3	12–23	[50]

The palaeotemperature estimates based on SST proxies and palaeolatitude and the average $\delta^{13}C$ of sedimentary organic carbon and of carbonate [38] required for this latter calculation are also listed. The range of the calculated respired DIC contribution includes the uncertainties in chemocline $\delta^{13}C$–CO_2, palaeotemperatures, OC, and carbonate. References are for both $\delta^{13}C_{isorenieratane}$ and $\delta^{13}C_{TOC}$. Fm. Formation.

[a] Unless stated otherwise, $\delta^{13}C$ of S-bound isorenieratane was adopted in this table, because it is possible that a kinetic isotope fractionation affected the free diaryl isoprenoids [63].
[b] Hydrogenated isorenieratene.
[c] Diagenetic products of isorenieratene (apolar fraction).
[d] Estimated $\delta^{13}C$ value due to coelution with sulfurized β-carotene.
[e] Free isorenieratane.
[f] Mixture of isorenieratane (63%) and renieratane (37%).
[g] C_{40} diaryl isoprenoid from extinct photosynthetic GSB [63].
[h] Average $\delta^{13}C$ value from algal biomarkers.
[i] From [38].
[j] $\delta^{13}C$-range for surface ocean DIC [65].
[k] Based on a 2.5 increase of atmospheric CO_2 during the OAE 2, for example [66,67].

the carbon cycle during these OAEs. Several causal links between dysoxic to anoxic water column conditions and carbon cycle perturbations have been suggested [3]. For example, the common interpretation of a positive $\delta^{13}C$ excursion in sedimentary organic and inorganic carbon during OAEs is increased organic carbon burial under anoxic conditions [4]. Negative $\delta^{13}C$ shifts in organic and inorganic carbon have been explained by release and subsequent oxidation of ^{13}C-depleted methane from gas hydrates [5]; the oxidation of the methane in the water column could have triggered anoxia. Alternatively, the process of recycling of CO_2 formed by mineralization of organic matter (i.e. respired CO_2) in long-term stratified marine basins could have played a significant role in determining $\delta^{13}C$ of organic and inorganic carbon, as was suggested for the Toarcian OAE [6,7]. To what extent this process can affect the isotopic composition of the phytoplankton and, thus, isotopic records of sedimentary inorganic and organic carbon depends on the varying contributions of respired CO_2 to the dissolved inorganic carbon (DIC) pool and this depends on the depth and stability of the chemocline. However, at this time, it is difficult to assess the importance of respired CO_2 in marine systems during OAEs, primarily due to a lack of information on $\delta^{13}C$ of CO_2 at or below the chemocline, where respired CO_2 accumulates [8–11].

We investigated the possibility of using the stable carbon isotope composition of the carotenoids derived from the photosynthetic green sulfur bacteria (GSB) (Chlorobiaceae) [12] to determine the contribution of respired CO_2. This group of microbes lives under euxinic conditions within the photic zone, requiring both light and sulfide for their metabolism. The aromatic carotenoids chlorobactene and isorenieratene and their diagenetic derivatives [13] were found in sediments of many contemporary and ancient stratified marine and lake systems (see Table 1 for an overview). The stable carbon isotopic composition of isorenieratane through time is remarkably enriched in ^{13}C compared to algal lipids [14–20]. GSB use the reductive tricarboxylic acid (TCA) cycle for CO_2 fixation and the key-enzyme, pyruvate synthase [21], has a low discrimination against ^{13}C compared to the enzyme Rubisco used by most aerobic photoautotrophs [22]. The isotopic fractionation (ε_p) between CO_2 and dry cell material is between −2.5 and −12.2‰ for several *Chlorobium* species [23,24]. For in situ brown-colored phototrophic consortia (*P. roseum*) composed for substantial part of Chlorobiaceae in Lake Dagow, Germany, an ε_p of 2 to 7‰ was measured for esterifying alcohols of BChl *e* [25], a bacteriochlorophyll synthesized exclusively by the brown strain of GSB [12]. van der Meer et al. [26] reported a 2 to 3‰ ^{13}C-enrichment for isoprenoid lipids (farnesane, chlorobactene) of *Chlorobium limicola* relative to bulk cell material, suggesting that $\delta^{13}C$ of GSB biomass can be estimated based on the $\delta^{13}C$ of their carotenoids. The relatively small fractionation between CO_2 and the characteristic carotenoids of GSB explains why these components are enriched relative to other lipids deposited in the same environment (see references in Table 1). However, the stable carbon isotopic composition of the diagenetic derivatives of isorenieratene is quite variable (i.e. between

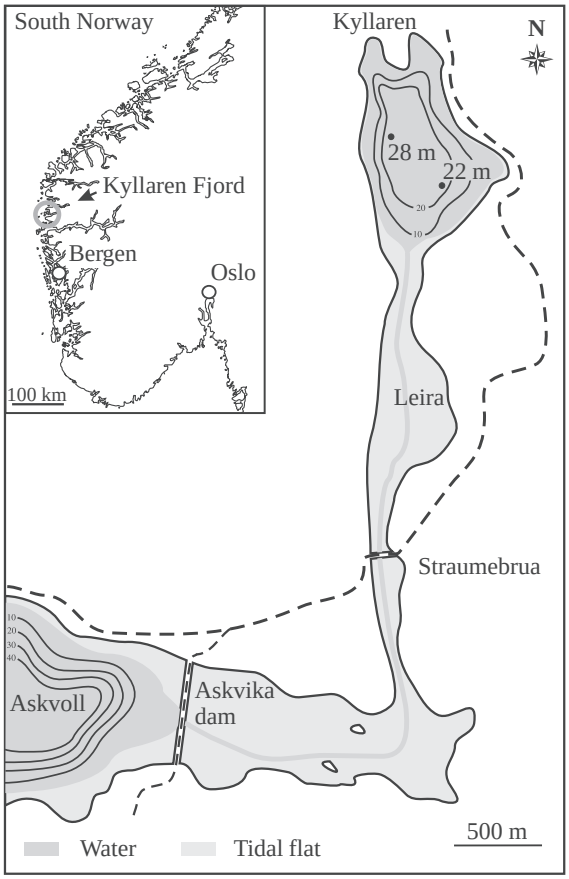

Fig. 1. Geographic setting of Kyllaren fjord, Norway. The fjord lies approximately at 61°25′N, 5°10′E [40].

−10.7 and −27‰, Table 1). The range in isotopic compositions may result from either a strong influence of environmental factors such as CO_2 concentration and growth rate or simply reflect the variable ^{13}C content of CO_2 at the chemocline as the result of varying contributions of respired CO_2 to the available DIC-pool. It is difficult to make this distinction, since no studies on the controls of the isotopic fractionation of GSB, containing isorenieratene, have been performed.

In this paper we have assessed the influence of large variations in CO_2 concentrations and $\delta^{13}C$ at the chemocline in a stratified Norwegian fjord on the $\delta^{13}C$ of carotenoids produced by a natural population of GSB. The water circulation in this 29 m deep fjord (Kyllaren) on the west coast of Norway (62°N, 5°E) is restricted by the permanent salinity stratification and the narrow, shallow connection to the Norwegian Sea (Fig. 1). The results are used to determine the $\delta^{13}C$ of CO_2 at the chemocline in ancient stratified marine basins. Implications for the extent of recycling of respired CO_2 are discussed.

2. Methods

2.1. Sampling

Water samples for the dissolved inorganic carbon concentration ([DIC]) and the stable carbon isotope ratio of DIC ($\delta^{13}C_{DIC}$) as well as the particulate organic carbon filters for carotenoid analysis were taken at a fixed sample position in Kyllaren fjord at the depocentre (28 m water depth), except for the sampling in February 2003. At this time, ice covered about 70% of the fjord and the regular sample position could not be reached. Instead, samples were taken at a position closer to the south-east coast where maximum water depth is 22.5 m (Fig. 1). Duplicate water samples were taken with a Niskin bottle from the

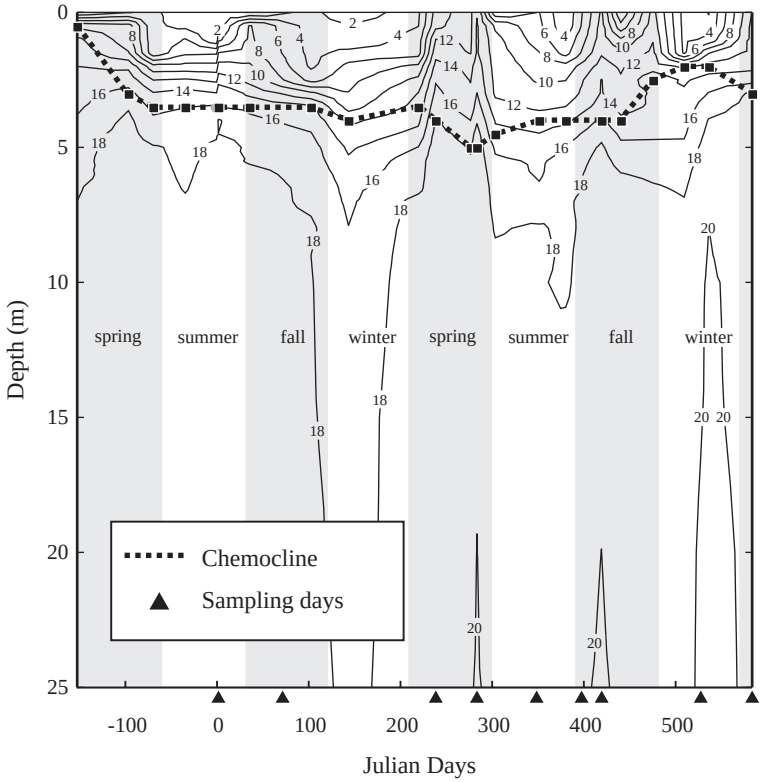

Fig. 2. Density contours (σ_t) and the O_2/HS^- interface in Kyllaren fjord through the seasons of March 2001–March 2003. Julian day 0 is set to August 20, 2001.

water column at 0 m (surface), 2, 4, 6, 8, 10, 15, 20, and 25 m depth for [DIC] and $\delta^{13}C_{DIC}$ measurements.

For determination of carotenoids, water was pumped into a 20 l pressure tank with a 12 V car-battery operated pump. 10 l of water were filtered over pre-combusted (400 °C) 0.7 μm glass fiber filters in a 150 mm in-line filter-holder. The filters were packed in aluminum foil and kept frozen in the dark.

2.2. Carotenoid analyses

The filters were freeze-dried before analysis. All subsequent work was done in a darkened room. Filters were extracted three times with cooled pico-grade acetone. The solvent was evaporated with a rotary evaporator and the extract was cleaned over a small silica column with dichloromethane as the eluent. The obtained so-called apolar carotenoid fractions were evaporated to dryness, weighed and redissolved in acetone with a concentration of 0.5 to 1 mg/ml. The apolar carotenoid fractions were analyzed on a HP 1100 series LC–MS equipped with an auto-injector, photodiode array detector, mass detector, and Chemstation chromatography manager software. Separation was achieved on a HP XDB-C18 column (2.1 × 120 mm, 5 μm particles), maintained at 30 °C, with a gradient from methanol/water (4:1, v/v) to acetone/methanol/water (19:1:1, v/v/v) in 50 min. The total run time was 60 min. Detection was achieved by in-line UV/VIS-detection and atmospheric pressure positive ion chemical ionization mass spectrometry (APCI–MS) of the eluent. Conditions for APCI–MS were as follows: nebulizer pressure was set at 60 psi, the vaporizer temperature was 325 °C, drying gas (N_2) was delivered at 7 l/min, the capillary voltage was −3 kV, and the corona needle was set at 6 μA. Compounds were identified from their retention times, UV/VIS-spectra (250–700 nm) and APCI-mass spectra (*m/z* 100–1000). Isorenieratene was quantified based on UV response by comparing the peak area at 454 nm to peak areas at 454 nm of known amounts of an authentic β-carotene standard (Aldrich).

2.3. Compound specific isotope analysis

To determine the stable carbon isotopic compositions of the carotenoids, the apolar carotenoid fractions were hydrogenated with PtO_2 in ethyl acetate and acetic acid (1:1, v/v). The hydrogenated fraction was filtered over a small column of alumina oxide (activated for 2 h at 150 °C) using hexane/dichloromethane (9:1, v/v) as the eluent to obtain a fraction containing the hydrogenated carotenoids. The $\delta^{13}C$ of the hydrogenated carotenoids was determined using a Finnigan DELTA-C isotope-ratio-monitoring GC–MS (irmGC–MS) system. A fused silica capillary column (25 m × 0.32 mm) coated with CP-Sil 5 (film thickness 0.12 μm) was used with helium as carrier gas. The samples were dissolved in hexane and injected at 70 °C. Subsequently, the oven was programmed to 130 °C at 20 °C min^{-1} and then at 4 °C min^{-1} to 320 °C at which it was maintained for 20 min. The $\delta^{13}C$ values for individual compounds are reported in the standard delta notation relative to VPDB standard and are the means of duplicate runs with differences between runs varying from 0.2 to 1.1‰.

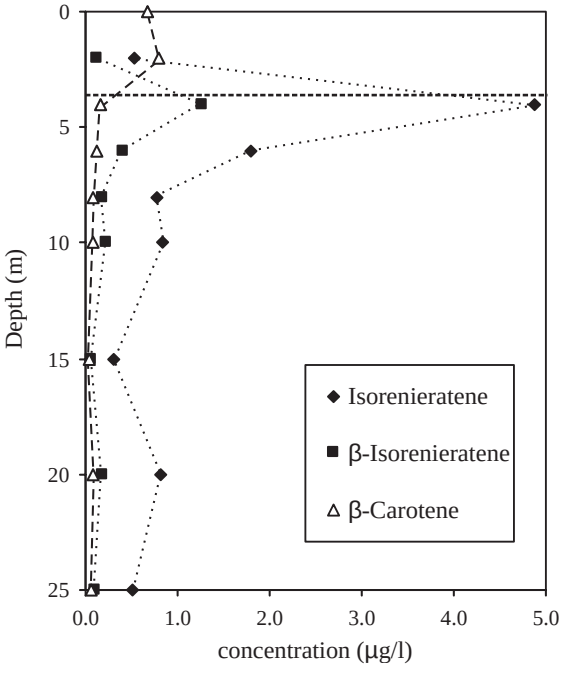

Fig. 3. Concentration profile of isorenieratene, β-isorenieratene, and β-carotene in Kyllaren fjord, Norway, in August of 2001. The horizontal dashed line indicates the position of the chemocline at that time.

3. Results

The position of the chemocline in Kyllaren fjord is at ca. 3.5 m depth in the water column during the largest part of the year (Fig. 2). In early summer 2002 the chemocline moved below 4 m depth and in winter 2002–2003 the chemocline shoaled to less than 2 m depth (Fig. 2).

The apolar carotenoids detected in the water column of Kyllaren fjord throughout the year are dominated by isorenieratene, β-isorenieratene, and β-carotene, with concentrations up to 9.2, 3.0, and 4.5 μg/l, respectively. The concentrations of isorenieratene and β-isorenieratene generally peak at 4 m depth, just below the chemocline (Fig. 3). However, this is not the case in June and August of 2002, when the β-carotene concentration peaked at this depth, and in February and April of 2003 (Fig. 4). Interestingly, no regular seasonal variability is discernable in the studied period. A high concentration of β-carotene (0.8 μg/l) is found at 0 and 2 m depth in August of 2001 and at 4 m depth isorenieratene is the dominant carotenoid with a concentration of 4.9 μg/l (Fig. 4). In August of 2002 at 4 m depth β-carotene is the dominant carotenoid and the isorenieratene concentration was reduced to 1.2 μg/l. In April of 2003 the isorenieratene concentration at 4 m depth is still low (0.2 μg/l) in contrast to April of 2002 when the concentration is as high as 4.8 μg/l. At 8 m or deeper the isorenieratene concentrations are constant at ca. 0.6 μg/l throughout the year (data not shown).

The $\delta^{13}C$ of the carotenoids was determined for particulate organic matter (POM) samples at 4 m depth with relatively high carotenoid concentrations (Table 2). Isorenieratene and β-isorenieratene exhibit strikingly low $\delta^{13}C$ values in August and November of 2001 and in April of 2002 (−29 to −32‰). In August, September and October of 2002, the $\delta^{13}C$ of isorenieratene and β-isorenieratene increased by ~5‰ (Table 2 and Fig. 5). The ^{13}C content of β-carotene is similar to isorenieratene in the months August and November of 2001, but in August of 2002 the $\delta^{13}C$ of β-carotene is depleted in ^{13}C by ca.

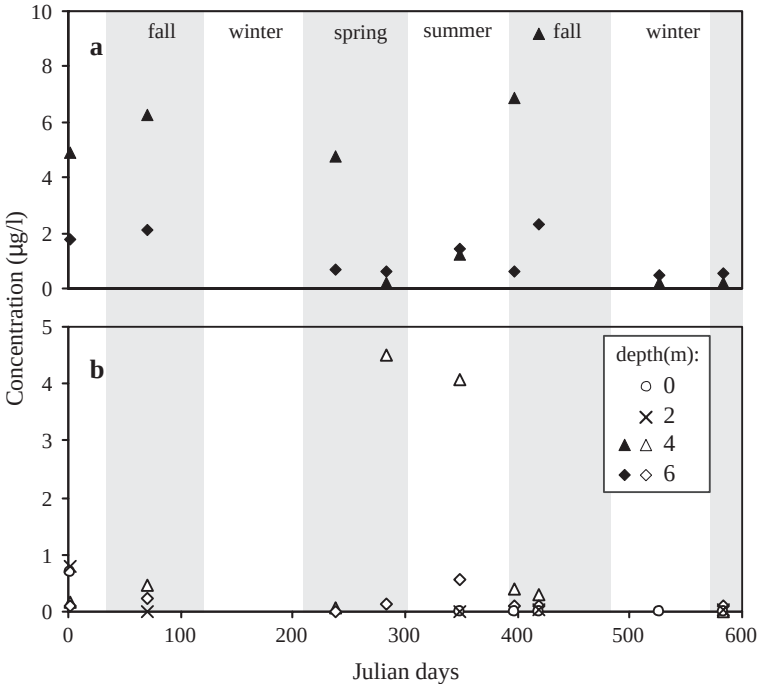

Fig. 4. Concentrations of a) isorenieratene (closed symbols) and b) β-carotene (open symbols) in Kyllaren fjord at 0, 2, 4, and 6 m depth in the period August 2001 to April 2003. Isorenieratene concentrations at 0 and 2 m depth were below the detection limit.

Table 2
$\delta^{13}C$ (in ‰ vs. VPDB) of carotenoids at 4 m depth in Kyllaren fjord and the calculated fractionation factor ε_p vs. CO_2(aq)

Sampling date	Juliann	CO_2(aq)	Isorenieratene		β-isorenieratene		β-carotene	
	Day	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	ε_p (‰)	$\delta^{13}C$ (‰)	ε_p (‰)	$\delta^{13}C$ (‰)	ε_p (‰)
21-08-2001	1	−27.4	−30.2 ± 1.1	2.9	−28.7 ± 0.7	1.4	−31.1 ± 0.8	3.8
01-11-2001	71	−26.0	−30.3 ± 1.0	4.5	−29.5 ± 0.9	3.6	−30.8 ± 1.0	5.0
18-04-2002	238	−26.6	−31.8 ± 0.2	5.4	−31.2 ± 0.1	4.8	n.a.	
03-06-2002	283	−13.8	n.a.		n.a.		n.a.	
08-08-2002	348	−21.4	−25.4 ± 0.8	4.1	−24.9 ± 0.5	3.6	−35.3 ± 0.2	14.4
27-09-2002	397	−21.5	−24.6 ± 0.6	3.2	−24.8 ± 1.0	3.4	−27.5 ± 0.5	6.2
19-10-2002	419	−19.7	−26.0 ± 1.0	6.5	−25.8 ± 0.8	6.2	n.a.	
07-02-2003	527	−26.9	n.a.		n.a.		n.a.	
03-04-2003	583	−27.4	n.a.		n.a.		n.a.	

n.a.: not analyzed.

10‰ relative to isorenieratene and β-isorenieratene (Fig. 5).

4. Discussion

4.1. Origin of the carotenoids in Kyllaren fjord

The high abundances of isorenieratene and β-isorenieratene are an indication that brown strains of GSB thrive at 4 m depth in Kyllaren fjord [27]. The brown strains of GSB produce typically isorenieratene and β-isorenieratene, whereas the green-colored strains produce predominantly chlorobactene [28]. A substantial shift in the microbial community at 4 m depth occurred in June of 2002 as indicated by the shift in carotenoid distribution from predominantly isorenieratene to predominantly β-carotene (Fig. 4). At this time the chemocline, and consequently the ecological niche for the GSB, moved below 4 m depth (Fig. 2). Microscopic observations indicate a high density of a *Euglena* species. The *Euglena* bloom at 4 m depth in June of 2002 is, therefore, a likely source for the β-carotene, which fits with its substantially depleted $\delta^{13}C$ value (−35.3‰) consistent with the larger fractionation associated with the enzymatic fixation by Rubisco. Heterotrophic growth of the *Euglena* sp. seems not likely as we would expect a $\delta^{13}C$ value similar or slightly ^{13}C-enriched relative to their diet [29], and we found no potential food-source with a similar $\delta^{13}C$ value as the *Euglena*. In August and November of 2001, the $\delta^{13}C$ of β-carotene is similar to that of isorenieratene and β-isorenieratene, suggesting that

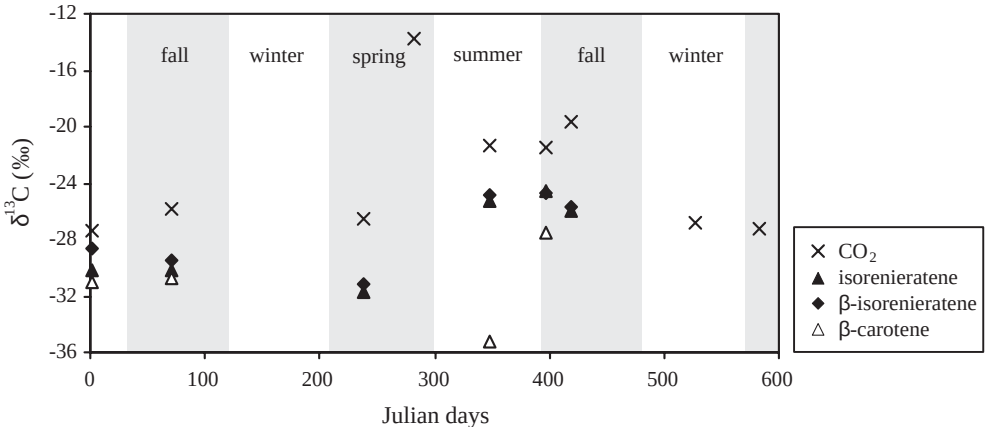

Fig. 5. $\delta^{13}C$ of CO_2 (aq) [9] and of isorenieratene, β-isorenieratene and β-carotene at 4 m depth in Kyllaren fjord in the period August 2001 to April 2003.

all apolar carotenoids are derived from GSB. Indeed, GSB can produce β-carotene [28].

4.2. Carbon isotopic fractionation by GSB

The $\delta^{13}C$ of isorenieratene will be primarily determined by the $\delta^{13}C$ of CO_2aq. The $\delta^{13}C$ of CO_2 in Kyllaren fjord at 4 m depth varied between −14 and −27‰ [9]. When the $\delta^{13}C$ of isorenieratene is plotted against the $\delta^{13}C$ of CO_2 a strong correlation becomes apparent (Fig. 6). The biological isotopic fractionation (ε_p) for both isorenieratene and β-isorenieratene versus CO_2 at the chemocline was similar and varied for isorenieratene from 2.9 to 6.5‰ (Table 2). This small fractionation is consistent with the use of the reductive TCA cycle as the predominant metabolic pathway of the GSB in Kyllaren fjord. These values are in accordance with the small ε_p values (2.5 to 4‰) determined for several *Chlorobium* species grown in laboratory cultures [23] (Fig. 6), assuming that $\delta^{13}C$ of the carotenoids is similar to the $\delta^{13}C$ of whole cell material [26]. With these data an average ε_p of 4 ± 1‰ ($n = 12$) was calculated. The $\delta^{13}C$ data from two field studies, in the Black Sea and in Lake Dagow, agree well with these data (Fig. 6). An ε_p of 3.5‰ was calculated from the $\delta^{13}C$ of isorenieratene derivatives present in the surface sediment of the Black Sea (−16.1‰) [20] and the $\delta^{13}C$ of CO_2 in the Black Sea at 85 m depth (−12.7‰) [8,30], where the isorenieratene concentration peaked in the water column in 1988 [31,32]. The $\delta^{13}C$ of esterifying alcohols of BChl *e* from in situ brown-colored phototrophic consortia in Lake Dagow, Germany [25] and the corresponding in situ $\delta^{13}C$ of CO_2 also fit this relationship (Fig. 6). This is to be expected since van der Meer et al. [26] reported similar ^{13}C contents for a carotenoid (i.e. chlorobactene) and the esterifying alcohol of BChl *e* (i.e. farnesol) of *Chlorobium limicola*.

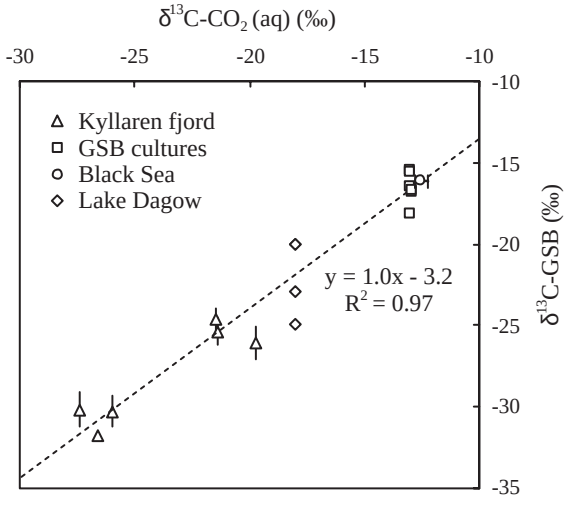

Fig. 6. The stable carbon isotopic compositions of isorenieratene from in situ GSB (Kyllaren fjord) and $\delta^{13}C$ of cell-material from cultures of GSB [23] display a 1 to 1 covariance with the $\delta^{13}C$ of CO_2 (aq). The $\delta^{13}C$ for isorenieratene in the Black Sea surface sediment [20] and of esterifying alcohols of BChl *e* from in situ brown-colored phototrophic consortia in Lake Dagow, Germany [25] fit this relationship.

CO_2 concentrations in Kyllaren fjord varied widely, from 140 (April of 2002) to 880 μM, but these variations did not influence ε_p for GSB. This is in agreement with our expectation that [CO_2] is not a limiting factor for growth below the chemocline where respired CO_2 has accumulated [8]. A correlation between the isorenieratene concentration and ϵ_p is also not evident, suggesting that growth rate is not a determining factor, as is the case for marine algae [33]. It should be noted, however, that ε_p could not be determined in waters with low concentrations of isorenieratene in February and April of 2003, when it is likely that growth rates were at their lowest. However, our results combined with culture [23] and other field studies [20,25] suggest that the dominant factor determining the $\delta^{13}C$ of isorenieratene is the $\delta^{13}C$ of CO_2. The ^{13}C-fractionation of the enzyme pyruvate synthase used by GSB seems insensitive to external environmental factors, or no limiting external factors persist in the chemocline environment. This indicates that the $\delta^{13}C$ of isorenieratene can be used to calculate the $\delta^{13}C$ of CO_2 at the chemocline in past environments, using the ε_p value of 4‰ established by our study.

4.3. Reconstruction of past $\delta^{13}C$–CO_2 (aq) at the chemocline of stratified marine basins and lakes

Isorenieratene derivatives have been detected in euxinic sediments throughout the geological record up to the Late Ordovician, and their $\delta^{13}C$ values have often been measured to confirm their origin from GSB. Because aryl isoprenoids, diagenetic products formed by C–C cleavage [34], might be partly

derived from β-carotene [17], the only confident confirmation of the occurrence of GSB at the time of deposition is the presence of (possibly aromatized) C_{40} diaryl isoprenoids, enriched in ^{13}C relatively to other lipids. These cases, compiled in Table 1, show a $\delta^{13}C$ range for isorenieratene derivatives of −11 to −27‰ over the last 450 Ma. From these data, the $\delta^{13}C$ of CO_2 at the chemocline can be estimated using our determined ε_p value (Table 1). The estimated $\delta^{13}C$ of chemocline CO_2 in lakes and in Kyllaren fjord is low (−14 to −23‰) compared to that in marine palaeoenvironments (−7 to −16‰), suggesting a substantial contribution of respired CO_2. The accumulation of respired CO_2 in relatively small basins of lakes and fjords is well known [10,35–37], and is ascribed to the restricted water circulation.

The range of the estimated $\delta^{13}C$ values of chemocline CO_2 in marine palaeoenvironments does not immediately suggest a contribution of respired CO_2. To assess if respired CO_2 accumulated and contributed to the CO_2 at the chemocline in these palaeoenvironments, the $\delta^{13}C$–$CO_{2\ \text{chemocline}}$ needs to be compared to the $\delta^{13}C$–CO_2 of surface waters in equilibrium with atmospheric CO_2 (atm). The $\delta^{13}C_{DIC}$ of surface waters can be estimated using the average $\delta^{13}C$ values of carbonate minerals [38], by correcting for the average isotopic depletion of carbonate minerals relative to DIC (~1.2‰, Fig. 7). The $\delta^{13}C$–CO_2 can then be calculated from $\delta^{13}C_{DIC}$ by calculating the ε_p for the equilibrium between CO_2 and HCO_3^- [39]

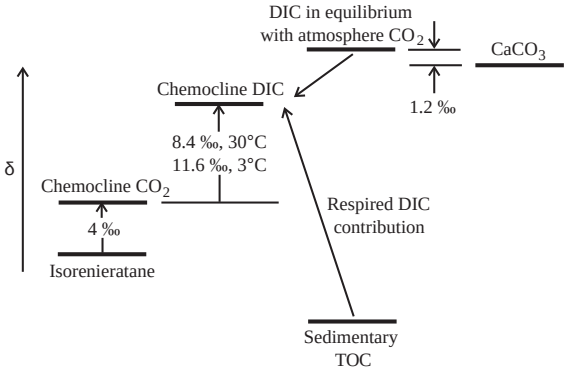

Fig. 7. Schematic overview of carbon isotopic relationships between (fossilized) isorenieratene, dissolved CO_2, dissolved inorganic carbon (DIC), both at the surface and at the chemocline, sedimentary organic carbon (TOC) and carbonate minerals. Modified after Hayes et al. [38].

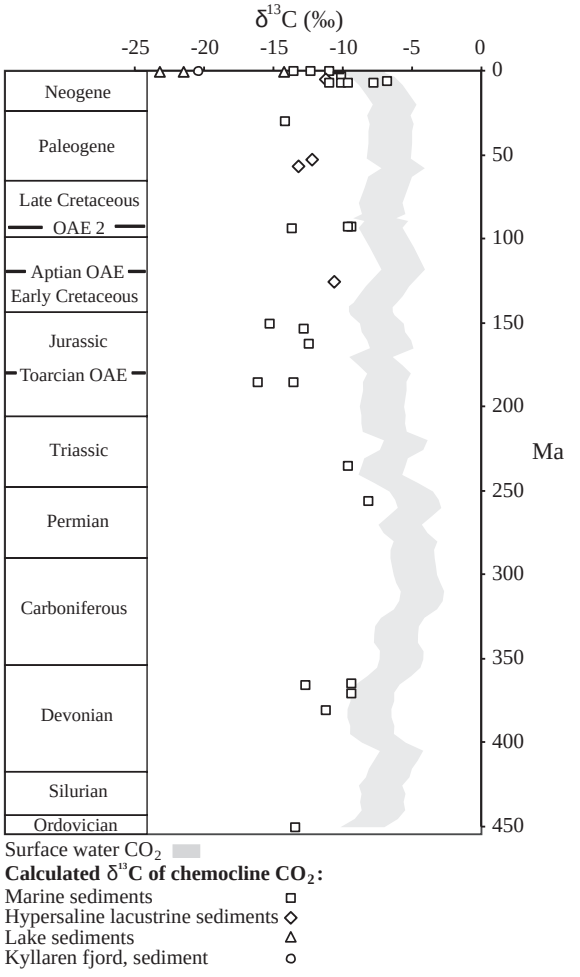

Fig. 8. The reconstructed $\delta^{13}C$ of CO_2 at the chemocline of stratified marine and lacustrine basins, calculated from fossilized isorenieratene (Table 1), plotted versus time (up to the Late Ordovician). The $\delta^{13}C$ of surface water CO_2 (grey shaded area), reconstructed from carbonate data compiled by Hayes et al. [38,68] and assuming a temperature range from 3 to 30 °C, is plotted for comparison. With exception of few cases, the $\delta^{13}C$ of chemocline CO_2 is depleted relative to the $\delta^{13}C$–CO_2 (atm) because of the contribution of respired CO_2 in these environments.

and with the assumption that $\delta^{13}C_{DIC}$~$\delta^{13}C$–HCO_3^-. As this fractionation is temperature dependent, we use surface temperature boundary conditions between 3 and 30 °C (so that ε_p is 11.6 to 8.4‰). The $\delta^{13}C$–CO_2 (atm) ranges from −2.7 to −11.7‰ and is indicated in Fig. 8 by the grey shaded area. The CO_2 at the chemocline is, with few exceptions (Messinian Gessoso solfifera Fm., Permian Holy Cross Mountains,

and Duvernay Fm.), always ^{13}C-depleted compared to CO_2 (atm) (Fig. 8), indicating a contribution of respired CO_2 at the chemocline in these marine palaeoenvironments.

The contribution of remineralized organic carbon to the dissolved inorganic carbon (DIC) pool at the chemocline can be estimated with the use of the following isotopic and concentration mass-balance equations (Eqs. (1) and (2)),

$$\delta^{13}C_{chem} \times [DIC_{chem}] = \delta^{13}C_{atm} \times [DIC_{atm}] + \delta^{13}C_{resp} \times [DIC_{resp}] \quad (1)$$

$$[DIC_{chem}] = [DIC_{atm}] + [DIC_{resp}] \quad (2)$$

with DIC_{chem}=chemocline DIC, DIC_{atm}=DIC in equilibrium with atmospheric CO_2 and DIC_{resp}=DIC formed by remineralization of organic carbon ('respired' DIC). Eqs. (1) and (2) can be rearranged to yield the following equation (Eq. (3)) to calculate the respired DIC contribution.

$$\text{Respired DIC contribution: } \frac{DIC_{resp}}{DIC_{chem}} \times 100\% = \frac{\delta^{13}C_{chem} - \delta^{13}C_{atm}}{-\delta^{13}C_{atm} + \delta^{13}C_{resp}} \times 100\% \quad (3)$$

We used the stable carbon isotopic composition of sedimentary organic carbon ($\delta^{13}C_{TOC}$) as an estimate for the isotopic composition of respired DIC (Fig. 7). A range of $\delta^{13}C_{TOC}$ values was used when parallel measurements of $\delta^{13}C_{TOC}$ and the $\delta^{13}C_{isorenieratane}$ were not available (Table 1). When no $\delta^{13}C_{TOC}$ values were available at all, average $\delta^{13}C$ values of marine organic carbon for that time period, as compiled by Hayes et al. [38], were used. To convert the $\delta^{13}C$–$CO_{2\ chemocline}$ (Table 1) into $\delta^{13}C$–$DIC_{chemocline}$ [39], palaeotemperatures were estimated (Table 1) based on SST proxies and palaeolatitude, with a ± 5 °C uncertainty range, and the assumption was made that $\delta^{13}C$–DIC ~ $\delta^{13}C$–HCO_3^-. Calculated in this way, respired DIC contributions at the chemocline are ca. 40% in Kyllaren fjord and in all lakes, except Ace Lake. In the marine palaeoenvironments, respired DIC contributions at the chemocline varied from 0 to a maximum of ca. 30%.

We tested this proxy on two modern-day settings. In the Black Sea, the $\delta^{13}C$ of fossil isorenieratane of three time-intervals were measured (0–1000, 1000–1200, and 5200–6200 yBP) [20]. The respired DIC contribution at the chemocline (3 to 13 %), calculated with the $\delta^{13}C$ of isorenieratane in the most recent sediments, is consistent with the present-day respired DIC contribution (12%), as calculated with the isotopic and concentration mass-balance equations (Eqs. (1) and (2)) and the in situ DIC data of a cruise in 1988 [8,30]. Earlier in the Black Sea history (i.e. 1200 yBP and 6200 yBP), respired DIC contributions at the chemocline were 0–8% and 9–19%, respectively. These calculations suggest that our proxy gives reasonable estimates for the contribution of respired DIC at the chemocline in ancient Black Sea-like environments. Another test can be made with a smaller anoxic basin, i.e. Kyllaren fjord. The contribution of respired DIC at the chemocline, calculated as described above with isorenieratene in sediments dated at ~1800 AD [40], was 38–43%. This is less than the average 74% calculated from isotopic DIC data of the chemocline during the period 2001–2003 [9]. A possible explanation for this discrepancy is that the isotopic composition of the sedimentary organic carbon (ca. −26‰) was not similar to that of the respired CO_2 as is assumed in our approach, but is relatively depleted in ^{13}C in Kyllaren fjord [9]. The high terrestrial (refractory) organic carbon contribution to the sediment [40] is probably responsible for the lower $\delta^{13}C$ values in the sediment relative to $\delta^{13}C$ of aquatic organic matter. When −20‰ is employed for respired carbon (DIC_{resp}), the calculated contribution of DIC_{resp} at the chemocline is 44–57%, still not as high as we found during 2001–2003. The shoaling of the chemocline position and increasing stabilization of the stratification in the fjord since the building of dams in the connecting channel of Kyllaren fjord with the open sea in 1954 and 1988 [40], is likely to have caused the increase in respired DIC contribution at the chemocline since 1800 AD.

Apart from the Mediterranean, the Black Sea and the two North Atlantic OAE 2 sites, all locations where fossilized isorenieratene was found were lacustrine, lagoonal or shallow marine, so that a terrestrial contribution to $\delta^{13}C_{TOC}$ can be expected. In these cases, the respired DIC contributions at the chemocline might be underestimated, as marine derived organic matter is generally depleted in ^{13}C relative to DIC by 25‰ [41] and terrigenous organic

matter typically exhibits $\delta^{13}C$ values of −28 to −32‰ [42,43]. The estimates for the contribution of respired CO_2 in Table 1 should thus be considered as conservative.

4.4. Temporal and spatial variations in respired DIC contributions in palaeoenvironments

Variations in the respired DIC contributions with time seem to have occurred in most palaeoenvironments for which data are available, as in the Messinian Vena del Gesso Basin (Gessoso solfifera fm.), in the Early Jurassic basin along the Tethyan continental margin (Allgäu), in the Permian Lower Rhine Basin and in the Frasnian/Famennian carbonate shelf sea (Holy Cross Mountains), and could signify either a change in the depth of the chemocline position or a change in the stability of the stratification in the basin resulting from changes in palaeoproductivity or

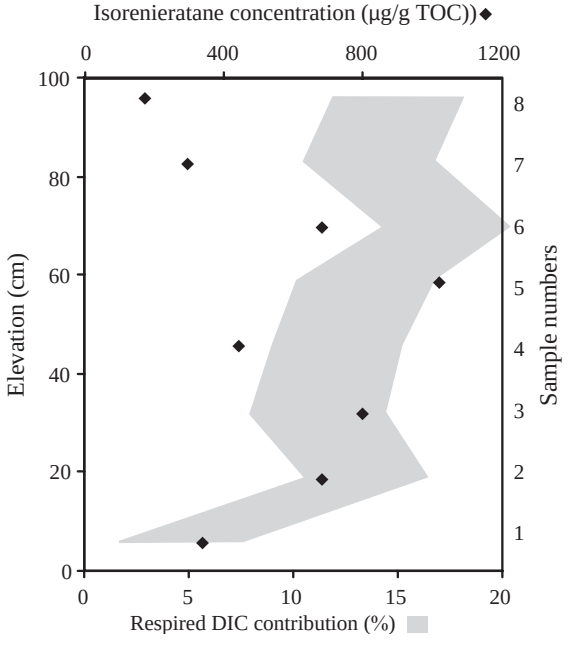

Fig. 9. Respired DIC contribution at the chemocline calculated from S-bound isorenieratane in 8 samples from a marl bed of one evaporitic cycle (IV) of the Gessoso solfifera Formation, Italy. The range of the calculated respired DIC contribution (grey shaded area) is determined by the range in palaeotemperature estimates (20 to 30 °C) and the average standard deviation of $\delta^{13}C$ of inorganic carbon for this time period [38]. Diamonds represent the concentration of isorenieratane, corrected for the extent of sulfurization [44].

(palaeo)circulation in the euxinic environments. For example, a detailed examination of the nature of change at the chemocline with time can be made with a previously published $\delta^{13}C_{isorenieratane}$ record of the Messinian Gessoso solfifera Formation [44]. We calculated a general increase in the respired DIC contribution at the chemocline with the progressing evaporitic conditions (Fig. 9). This would be in agreement with the idea that due to evaporation the water levels lowered in this shallow lagoonal system and thus respired CO_2 would have an increasing impact on the carbonate system.

We also find a contribution of respired DIC to the chemocline in the proto North Atlantic, calculated from the $\delta^{13}C$ of isorenieratane deposited during or prior to OAE 2. Because of the widespread occurrence of ocean anoxia, respired DIC contributions at different localities can be compared. Our calculations show that the relative contribution was similar at the different localities, even in the abyssal Cape Verde Basin (Table 1), and also similar to that in the present-day Black Sea. Thus, our results suggest that during OAE 2 oceanographic conditions in large parts of the proto North Atlantic were comparable to those of the present-day Black Sea.

5. Summary and conclusion

The biological carbon isotope fractionation, ε_p, of isorenieratene and β-isorenieratene versus CO_2, by photosynthetic GSB displays an annual average of 4‰ (±1.5‰) in the anoxic Kyllaren fjord, independent of CO_2 concentration and growth rate, and is similar to that in cultures of GSB. We applied this ε_p to calculate $\delta^{13}C$ of chemocline CO_2 (aq) in ancient stratified marine and lacustrine environments from previously determined $\delta^{13}C$ of fossilized isorenieratene. The reconstructed $\delta^{13}C$ of chemocline CO_2 ranges from −7 to −16‰, in the different marine palaeoenvironments where isorenieratene derivatives were found, and even down to −23‰ in lake settings. The contribution of respired DIC to DIC at the chemocline was estimated, using the $\delta^{13}C$ of sedimentary organic carbon as an estimate for respired DIC and the carbonate $\delta^{13}C$ as a proxy for surface water DIC in equilibrium with atmospheric CO_2. We found maximum respired DIC contributions (~40%) in (semi)

enclosed environments such as the lakes and Kyllaren fjord. In the ancient marine basins, respired DIC contributions varied from 0 to 30%, with substantial temporal and spatial variations.

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