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Implications for chloro- and pheopigment synthesis and preservation from combined compound-specific δ^{13}C, δ^{15}N, and Δ^{14}C analysis

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Abstract

Chloropigments and their derivative pheopigments preserved in sediments can directly be linked to photosynthesis. Their carbon and nitrogen stable isotopic compositions have been shown to be a good recorder of recent and past surface ocean environmental conditions tracing the carbon and nitrogen sources and dominant assimilation processes of the phytoplanktonic community. In this study we report results from combined compound-specific radiocarbon and stable carbon and nitrogen isotopic analysis to examine the timescales of synthesis and fate of chlorophyll-*a* and its degradation products pheophytin-*a*, pyropheophytin-*a*, and 13^2,17^3-cyclopheophorbide-*a*-enol until burial in Black Sea surface sediments. The pigments are mainly of marine phytoplanktonic origin as implied by their stable isotopic compositions. Pigment δ^{15}N values indicate nitrate as the major uptake substrate but ^{15}N-depletion towards the open marine setting indicates contribution from N$_2$-fixation. Radiocarbon concentrations translate into minimum and maximum pigment ages of approximately 40 to 1200 years. This implies that protective mechanisms against decomposition such as association with minerals or eutrophication-induced hypoxia and light limitation are much more efficient than previously thought. However, seasonal variations of nutrient source, growth period, and habitat and their associated isotopic variability are likely at least as strong as long-term trends. Combined triple isotopic analysis of sedimentary chlorophyll and its primary derivatives is a powerful tool to delineate biogeochemical and diagenetic processes in the surface water and assess their precise timescales.

1 Introduction

Primary production is the basis for sustaining life on earth. Photosynthesis by autotrophic organisms accounts for virtually all of the primary production (chemosynthesis $<1\%$) and can, thus, be called the ultimate source of metabolic energy building organic biomass (Falkowski, 2003). In both marine and terrestrial aerobic

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photoautotrophic organisms the light harvesting systems are based on the tetrapyrrole molecule chlorophyll-*a* being the major antenna pigment (Falkowski, 2003). Accordingly, chlorophyll-*a* is ubiquitously found in marine surface waters and may ultimately become buried in marine sediments. However, chlorophyll-*a* undergoes several pre- and post-depositional diagenetic processes such as (autolytic) cell senescence, microbial or viral lysis, enzymatic or hydrolysis reactions, photo-oxidation and grazing (Owens and Falkowski, 1982; Carpenter et al., 1986; Repeta and Simpson, 1991; Spooner et al., 1995; Gossauer and Engel, 1996; Chen et al., 2003) altering its original chemical structure. In particular, chlorophyll-*a* of higher plants is decomposed enzymatically during senescence of the leaves (Matile et al., 1996), by bacterial breakdown and meso- and micro-faunal grazing in soils (Hoyt, 1966; Chamberlain et al., 2006), and by oxidation and photo-degradation during transport (Sanger, 1988). Consequently, pigments found in sediments are proposed to overwhelmingly derive from marine photoautotrophs. Primary degradation products of chlorophyll-*a* include pheophytin-*a* and pyropheophytin-*a*, formed via demetallation and demethoxycarbonylation by either of the above mentioned pathways (Chen et al., 2003), and 13^2,17^3-cyclopheophorbide-*a*-enol which is considered an exclusive grazing product (Goericke et al., 2000). These chloro- and pheopigments have been shown to be extremely labile whereas their macrocycles can be preserved over very long timescales (Rho et al., 1973) allowing their stable carbon and nitrogen isotopic composition to be used to characterize past surface water environments and photoautotrophic communities (Ohkouchi et al., 2005, 2006, 2008; Kashiyama et al., 2008). Various studies have demonstrated rapid decrease of chlorophyll-*a* and pheophytin-*a* concentrations over time periods on the order of hours to days or few weeks (Owens and Falkowski, 1982; SooHoo and Kiefer, 1982; Sun et al., 1993b; Goericke et al., 2000). Carpenter et al. (1986) found very low water column half-lives of chlorophyll-*a* and pheopigments leading to photo-degradation within only 3 days. In general, degradation rates seem to be lower for sediments. Bianchi et al. (1993) report chlorophyll-*a* mean lives of up to 80 days for Hudson River sediments and Furlong and Carpenter (1988) calculate half-lives of pheopigments to be 30–50 days. This is attributed to protection of the pigments by aggregation with humic substances or protection by a sediment matrix. Overall, degradation rates were enhanced under oxic compared to anoxic conditions (Sun et al., 1993a, b) causing preferential preservation of pigments such as 13^2,17^3-cyclopheophorbide-*a*-enol in anoxic sediments (Ocampo et al., 1999). Nevertheless, the degradation rates reported to date are mainly based on relative concentration estimates prone to analytical biases and can, thus, not truly reflect natural preservation timescales. In order to precisely trace the timescales of early pigment diagenesis and its effect on the stable isotope record, we used combined δ^{13}C, δ^{15}N, and Δ^{14}C analysis of chlorophyll-*a* and its degradation products pheophytin-*a*, pyropheophytin-*a*, and 13^2,17^3-cyclopheophorbide-*a*-enol from NW Black Sea shelf sediments allowing the preservation timescales to be traced under contrasting environmental conditions including oxic, suboxic, and anoxic as well as river-influenced and open-marine sedimentary conditions.

2 Materials and methods

2.1 Sampling

Core-top sediments from stations P128, P177, P120, P169, and P167 from the NW Black Sea (Fig. 1) were taken using a multicorer on RV *Poseidon Cruise P363* in 2008. The samples were sliced into 1 cm slices and stored frozen at – 20 °C in glass jars until analysis. The uppermost 0–2 cm or 0–3 cm slices were recombined for analysis to yield enough chloropigments for radiocarbon measurements. If available, co-occurring bivalve shells were isolated from each sample and radiocarbon dated. For TOC radiocarbon analyses, subsamples of 100 to 500 mg of the freeze-dried samples were separated. Seawater samples at the respective stations were taken using a CTD rosette equipped with 30 l Niskin bottles and poisoned using $HgCl_2$ to prevent bacterial activity.

2.2 Pigment extraction and purification

The freeze-dried sediment was ground and homogenized. Dehydrated acetone was added (2× sediment volume) and samples were ultrasonicated for 15 min cooled in ice water. Afterwards samples were centrifuged at 2000 rpm for 5 min. The acetone was removed and the extraction was repeated three times. After volume reduction under a cold stream of N_2, the acetone-fraction was transferred into a hexane-MilliQ bilayer (1:3), homogenized using vortex and centrifuged at 1500 rpm for 1 min. The hexane-layer was transferred and the extraction procedure was repeated until the hexane-fraction was colorless. The hexane-fraction was then reduced in volume under cold N_2 and rinsed through a column of pre-combusted sodium sulphate. Afterwards dehydrated dimethylformamide (DMF) was added and solvents were homogenized by vortex before storing at – 30 °C overnight. For HPLC-analysis the DMF layer was separated and analysed.

The HPLC-analysis was performed using an Agilent 1200 Series HPLC/DAD System. For isolation of the chloro- and pheopigments from the extract, a semi-preparative Agilent Zorbax Eclipse XDB C18 column (9.4 × 250 mm; 5 µm) with a XDB C18 guard column (4.6 × 12.5 mm; 5 µm) were used. The chloro- and pheopigments were eluted isocratically with 75% acetonitrile/pyridine (100:0.5) and 25% ethyl acetate/pyridine (100:0.5) for 5 min, followed by a linear gradient of ethyl acetate/pyridine to 50% in 50 min. The flow rate was set to 4.2 ml/min and the oven temperature to 30 °C. Detection of the chloro- and pheopigments was achieved by photodiode array detector. To achieve high single pigment purities, a second purification step was applied using an analytical Agilent Zorbax Eclipse PAH column (4.6 × 250 mm; 5 µm) equipped with a PAH guard column (4.6 × 12.5 mm; 5 µm). Isocratic elution of the pigments was achieved using 80% acetonitrile/pyridine (100:0.5) and 20% 2-butanone/pyridine (100:0.5) for 5 min, followed by two linear gradients of 2-butanone/pyridine (100:0.5), first to 60% in 25 min and second to 100% in 10 min. The flow rate was 1 ml/min. The oven temperature was 10 °C for chlorophyll-*a* and pheophytin-*a* and 30 °C for pyropheophytin-*a* and $13^2,17^3$-cyclopheophorbide-*a*-enol.

HPLC processing blanks for each pigment methodology were determined by collection of the respective effluent volume. The solvent was dried using N_2 and the blank size (Table S1 in the Supplement) and the ^{13}C (−29.6‰), ^{15}N (5.1‰), and ^{14}C (−1000‰) isotopic composition were determined. All $\Delta^{14}C$ concentrations reported are corrected for blank carbon addition using isotope mass balance.

2.3 Isotope measurements

The stable carbon and nitrogen isotopic composition of chloro- and pheopigments were measured on a modified FlashEA1112 Automatic Elemental Analyser connected to a Thermo Finnigan Delta plus XP isotope ratio mass spectrometer (IRMS) via a ConFlow III Interface (Ogawa et al., 2010). Seawater nitrate, chemically converted to N_2O (McIlvin and Altabet, 2005), was measured for its $\delta^{15}N$ composition using an automated purge-and-trap system connected to a GV Instruments IsoPrime system. The stable carbon and nitrogen isotopic composition of bulk sediment was analysed using a Finnigan Delta plus IRMS. The isotopic compositions are expressed as conventional $\delta^{13}C$ relative to Vienna PeeDee Belemnite (VPDB) and $\delta^{15}N$ relative to atmospheric N_2.

For radiocarbon measurements, chloro- and pheopigments, and bivalve shells were converted into CO_2. Cleaned bivalves were acid-hydrolyzed. Pigment samples were transferred into pre-combusted quartz tubes and 150 µg pre-combusted copper oxide (CuO) was added as oxygen source. The samples were evacuated and flame-sealed under vacuum. Afterwards, the samples were combusted at 900 °C for 8 h. The resulting CO_2 gas was stripped of water and quantified under vacuum.

TOC was submitted as unprocessed samples and AMS measurements were performed using standard methods (McNichol et al., 1994). AMS measurements of chloropigment isolates were performed following the protocol for small samples (Pearson et al., 1998). Where sample sizes were sufficient dual measurements were obtained. Radiocarbon ages are reported as $\Delta^{14}C$ (Stuiver and Polach, 1977).

Additionally, calibrated radiocarbon ages are given for pigments for determination of their "true" age and calculation of degradation timescales. Calibration of pre-bomb samples was achieved using the calibration software CALIB 6.0 (Stuiver et al., 2010) applying a reservoir correction of 400 years (Siani et al., 2000). The "true" age of bomb-^{14}C containing pigments was determined using a modeled surface water DI^{14}C curve.

2.4 Black Sea surface water DIC model

The history of Black Sea surface water ^{14}C concentrations is poorly constrained by observations. For this reason the temporal evolution of surface water Δ^{14}C was estimated by means of a heuristic one-dimensional model. Following Butzin and Roether (2004), the input of ^{14}C via air-sea exchange and river runoff is balanced by radioactive decay, downward mixing, and by an additional first-order apparent loss or gain term which parameterizes up- or downwelling and net horizontal exchange. The temporal evolution of surface water Δ^{14}C is then given by

$$\frac{\partial\,^{14}R_{\text{wat}}}{\partial t} = K\frac{\partial^{2\,14}R_{\text{wat}}}{\partial t^{2}} - (\lambda + \mu)\,^{14}R_{\text{wat}} \qquad (1)$$

where $^{14}R_{\text{wat}}$ is the scaled surface water ^{14}C/^{12}C ratio at a given time t (following Toggweiler et al., 1989), K is an apparent vertical diffusivity, z is water depth, λ is the decay rate of ^{14}C ($= 1.2096 \times 10^{-4}\,\text{yr}^{-1}$) (Godwin, 1962) and μ is the time constant of the first-order loss and gain term. The model input are ^{14}C fluxes representing air-sea exchange ($^{14}F_{\text{air}}$) and continental river runoff ($^{14}F_{\text{riv}}$). Air-sea exchange is estimated as

$$^{14}F_{\text{air}} = \Phi pCO_2/\Sigma CO_2(^{14}R_{\text{atm}} - {}^{14}R_{\text{wat}}) \qquad (2)$$

where Φ is the average invasion rate of CO$_2$ ($= 20\,\text{mol}\,\text{m}^{-2}\,\text{yr}^{-1}/330\,\text{ppm}$) (Broecker et al., 1985), pCO_2 is the partial pressure of atmospheric CO$_2$ (Enting et al., 1994), ΣCO_2 is the average concentration of dissolved inorganic carbon in surface water

(= 2 mol m^{-3}), and $^{14}R_{\mathrm{atm}}$ is the atmospheric ^{14}C/^{12}C ratio in the Northern Hemisphere (Enting et al., 1994; Levin et al., 2008). The ^{14}C delivery due to river runoff is crudely estimated as

$$^{14}F_{\mathrm{riv}} = V_{\mathrm{run}}/A\,^{14}R_{\mathrm{riv}} \quad (3)$$

where V_{run} is the runoff to the Black Sea (= 350 km^3 yr^{-1}) (e.g. Tolmazin, 1985), A is the surface area of the Black Sea (= 420 000 km^2), and $^{14}R_{\mathrm{riv}}$ is the fluvial ^{14}C/^{12}C ratio. As observations of $^{14}R_{\mathrm{riv}}$ were not available, $^{14}R_{\mathrm{riv}} = {}^{14}R_{\mathrm{atm}}$ was assumed, i.e., atmospheric ^{14}C deposited in catchment areas enters the Black Sea instantaneously and without dilution. While this is clearly an overestimation, the contribution of $^{14}F_{\mathrm{riv}}$ amounts only about 1‰ of the atmospheric input flux. Equation (1) is numerically integrated from year 1810 to 2010 and evaluated at 20 m depth since highest chlorophyll-*a* concentrations have been found between 10 m and 30 m water depth (Krupatkina et al., 1991; Repeta and Simpson, 1991; Chu et al., 2005). The solution is fitted to scattered observations (Ostlund and Dryssen, 1986; Jones and Gagnon, 1994; Siani et al., 2000; Fontugne et al., 2009; this study) using K and μ as fit coefficients. Values of K and μ are about 7 × 10^3 m^2 yr^{-1} (∼ 2 cm^2 s^{-1}) and ∼ 1 × 10^{-3} yr^{-1}, respectively.

3 Results

3.1 Stable carbon and nitrogen isotopic composition

Bulk sedimentary δ^{13}C values increase from – 26.1 ± 0.1‰ (P128) to – 23.2 ± 0.1‰ (P167) with distance seawards, which is in agreement with an increasing contribution of marine production at the offshore locations (Table 1, Fig. 2). In contrast, δ^{13}C values of chloro- and pheopigments range from – 24.5 ± 0.1‰ to – 26.7 ± 0.1‰ without a distinct spatial pattern. Pigment isotopic values within samples are heterogeneous covering a ∼ 2‰ range except for P128, where all pigments have virtually identical δ^{13}C values.

The δ^{13}C values are in the range previously reported for marine phytoplankton (Sachs et al., 1999). The pigment δ^{15}N-values range from 4.0 ± 0.4‰ to – 2.8 ± 0.4‰. Generally, chlorophyll-*a* is the most ^{15}N-enriched whereas pyropheophytin-*a* is the most ^{15}N-depleted pigment within each sample. Along-transect data reveal a gradual isotopic depletion (~ 4‰) with increasing distance from the river mouth. Only chlorophyll-*a* δ^{15}N values at stations P120 and P169 deviate from this trend. The general trend is, moreover, consistent with a steady decrease of bulk sedimentary δ^{15}N values (ranging from 8.5 ± 0.5‰ to 4.2 ± 0.5‰) with distance offshore, which is in agreement with previously reported data (Reschke et al., 2002). Seawater nitrate (NO_3^-) δ^{15}N values from 0 m to 92 m water depth range from 4.9 ± 0.2‰ to 8.4 ± 0.2‰ (Table 2).

3.2 Radiocarbon composition

TOC radiocarbon concentrations (Table 1, Fig. 3) are lowest at stations P128 (– 166.6 ± 2.1‰) and P177 (– 161.0 ± 3.4‰) in front of the Danube river mouth. At these stations contribution of pre-aged terrigenous organic matter is likely (Kusch et al., 2010). With further distance offshore Δ^{14}C values increase to – 64.2 ± 3.2‰ (P120) and – 51.2 ± 3.2‰ (P169) and show bomb-^{14}C contribution (44.5 ± 2.5‰) at anoxic core location P167. All bivalve shell Δ^{14}C values are bomb-influenced and range from 56.1 ± 3.3‰ at the shallowest nearshore station (P128, *Scarpharca* sp.) to 9.9 ± 3.8‰ further offshore (P169, *Modiolus* sp.), which is consistent with utilization of more ^{14}C-depleted dissolved inorganic carbon (DIC) with increasing water depth.

Chloro- and pheopigment Δ^{14}C values (Table 1, Fig. 3) vary over a range of ~ 290‰ with lowest values for pheophytin-*a* at station P128 (– 127.9 ± 11.9‰ and – 109.3 ± 11.6‰) and chlorophyll-*a* at station P177 (– 183.2 ± 15.3‰) in front of the Danube River. However, in both samples the other pigments are significantly less depleted in ^{14}C. Pyropheophytin-*a* from station P128 contains bomb-^{14}C (32.9 ± 12.8). Likewise, Δ^{14}C values of pheophytin-*a* (-6.6±18.1‰) and pyropheophytin-*a* (– 55.8 ± 12.9‰) from station P177 are notably increased relative to chlorophyll-*a*. At intermediate station P120 pheophytin-*a* (– 50.6 ± 7.6‰) and chlorophyll-*a* (– 36.7 ± 15.5‰) agree within 1σ-analytical uncertainty. Pyropheophytin-*a* from the same sample, however, shows bomb-^{14}C contribution (32.9 ± 12.8‰). Chlorophyll-*a* at station P169, in similar water depth as P120, shows the most enriched Δ^{14}C value of the data set (181.1 ± 17.1‰). Co-occurring pyropheophytin-*a* (21.7 ± 14.3‰) is significantly less enriched in ^{14}C. At anoxic station P167 dual measurements of pheophytin-*a* (13.6 ± 12.2‰ and 1.7 ± 9.2‰) are within 1σ-analytical uncertainty. Chlorophyll-*a* from this station yields Δ^{14}C values of 19.9 ± 13.2‰ and – 34.1 ± 16.5‰, agreeing within 2σ-analytical uncertainty. In contrast, Δ^{14}C values of 13^2,17^3-cyclopheophorbide-*a*-enol (48.3 ± 12.2‰ and – 7.1 ± 8.3‰) are significantly different (> 2σ-uncertainty), while pyropheophytin-*a* shows the highest bomb-^{14}C incorporation (105.5 ± 18.0‰).

4 Discussion

4.1 Chloro- and pheopigment origin and ecology of the photoautotrophic community

The stable carbon and nitrogen isotopic compositions of primary pigments reflect those of the substrates, the assimilation pathway, and the growth conditions of the photoautotrophic community (Sachs et al., 1999; Ohkouchi et al., 2005, 2006, 2008; Kashiyama et al., 2008). Empirically it has been shown that chlorophyll-*a* is enriched in ^{13}C and depleted in ^{15}N relative to total cellular carbon and nitrogen by 1.8 ± 0.8‰ and 4.8 ± 1.4‰, respectively (Sachs et al., 1999; Ohkouchi et al., 2006, 2008). Therefore, after accounting for fractionation, our data imply that mean $\delta^{13}C_{cell}$ values of the phytoplanktonic community (– 26.3 ± 0.8‰ to – 28.5 ± 0.8‰) are in agreement with previous estimates of surface water particulate organic matter from the NW Black Sea shelf area (Banaru et al., 2007) and reflect a marine phytoplanktonic origin of the chloro- and pheopigments (Sachs et al., 1999). The observed ~2‰ scatter among the pigment

$\delta^{13}C$ values might be due to growth season effects (Sachs et al., 1999) coupled with seasonally varying particle fluxes and spatially and temporarily varying pigment fluxes (King, 1995). Seasonal variations in $\delta^{13}C_{DIC}$ could, e.g., be driven by decreasing carbon isotopic fractionation during times of high productivity and low surface water $CO_{2(aq)}$ concentrations (Gruber et al., 2002) or admixture with seasonally varying riverine $\delta^{13}C_{DIC}$ (Kanduč et al., 2007). In case of $13^2,17^3$-cyclopheophorbide-*a*-enol an additional potential small isotopic effect could be the cleavage of the phytol side chain, which is normally more ^{13}C-depleted than the tetrapyrrole macro cycle (Sachs et al., 1999; Ohkouchi et al., 2008).

Fractionation-corrected $\delta^{15}N_{cell}$ values range from approximately 8.8 ± 1.5‰ to 2.0 ± 1.5‰ in very good agreement with bulk sedimentary $\delta^{15}N$ (8.5 ± 0.5‰ to 4.2 ± 0.5‰). Such good agreement between the cellular and bulk sedimentary $\delta^{15}N$ values indicates that bulk sedimentary $\delta^{15}N$ values reliably mirror the surface water phytoplankton nitrogen isotopic composition at the study sites. Nitrogen isotopic variations within each sample and especially the noticeable ^{15}N-enrichment of chlorophyll-*a* at stations P120 and P169 could be caused by seasonal variations of the nitrogenous nutrient source. Chlorophyll-*a* produced at the end of a phytoplanktonic bloom limited by nitrogen would be reflected in heavy $\delta^{15}N$ values resulting from ^{15}N enrichment of the substrate when the source becomes depleted (Altabet and Francois, 1994). Since chlorophyll-*a* $\delta^{15}N_{cell}$ values (8.8 ± 1.5‰ to 4.7 ± 1.5‰) at all stations show good agreement with the respective seawater nitrate $\delta^{15}N$ values (Table 2), nitrate must be the major substrate assimilated by the phytoplanktonic community (presuming a high NO_3^- uptake/availability ratio). The good spatial agreement of seawater nitrate $\delta^{15}N$ values of river mouth and offshore study sites indicates an isotopically homogenous nitrate pool. Therefore, an overall trend of pigment ^{15}N-depletion with distance offshore indicates that distinct groups of phytoplankton using different nitrogen assimilation processes occur in the surface waters of the study sites. Heavier $\delta^{15}N$ values in front of the Danube river mouth imply predominant NO_3^- assimilation, whereas lighter values towards the central basin may result from contribution of pigments derived from diazotrophs with typical stable nitrogen isotopic composition in the range of – 3‰ to 0‰ (Minagawa and Wada, 1986; Montoya et al., 2002). Such contribution from N_2-fixing biomass is consistent with the nutrient availability observed during the sampling campaign P363. In front of the Danube delta observed nitrogenous nutrient supply was high enough (N:P > 80) to maintain nitrate assimilation; here phosphorus is the limiting nutrient (Cauwet et al., 2002). In contrast, further offshore towards station P167 nitrogen became limiting over phosphorus (N:P < 10). Since the Black Sea is strongly stratified, the permanent pycnocline prevents upward mixing of nutrients from deeper waters. Thus, N_2-fixation by cyanobacteria (Uysal, 2000) might account for a significant fraction of total nitrogen assimilation (McCarthy et al., 2007).

4.2 Timescales of chloro- and pheopigment synthesis and assessment of controlling parameters

Chlorophyll-*a* is generally expected to rapidly decompose after cell death. A sediment trap study in the Black Sea showed that the flux of chlorophyll-*a* to a water depth of 100 m is only 0.2% of the production in 0–55 m water depth (Repeta and Simpson, 1991). Due to this instability of primary pigments, the $\Delta^{14}C$ concentrations of pigments deposited in surface sediments would be expected to mirror recent surface water DIC ^{14}C concentrations. Instead, ^{14}C concentrations of chlorophyll-*a* (– 183.2 ± 15.3‰) and pheophytin-*a* (– 127.9 ± 11.9‰ and – 109.3 ± 11.6‰) at stations P177 and P128, respectively, are much lower than DIC $\Delta^{14}C$ values represented by co-occurring bivalves from 17 m (56.1 ± 3.3‰) to 96 m water depth (9.9 ± 3.8‰) and previously reported values (Jones and Gagnon, 1994; Fontugne et al., 2009), translating into ages of 570 to 1260 cal yrs BP (Table 1). Potential explanations for the low $\Delta^{14}C$ concentrations include utilization of different DIC pools, protective mechanisms associated with sedimentary matrices or faecal pellets, and contribution of pre-aged terrestrial pigments. Although the Danube River catchment is geologically characterized by large areas of carbonaceous rocks, the old pigment ages at stations P177 and P128 are unlikely an artefact of riverine hardwater DIC utilization by marine phytoplankton since corresponding bivalve shells from shallow waters at both stations show bomb-^{14}C concentrations, thus, unambiguously reflecting a modern DIC pool. Moreover, the contribution of pigments from riverine freshwater organisms, which might indeed be influenced by Danube hardwater DIC, should be minor considering that the riverine nutrient input favours large marine phytoplanktonic blooms in the near-shore area producing high amounts of marine biomass (Cociasu et al., 1996). Aggregation with humic substances or protection by sediment matrices in general has previously been proposed to improve the preservation potential of pigments (Bianchi et al., 1993). Adsorption might occur both as surface monolayer and inside mineral pores large enough for pigments protecting them from photo-oxidation and hydrolytic enzymes (Mayer, 1994). Adsorption appears likely because of very high organic carbon/surface area ratios found for sediment water interface samples of the Black Sea (Mayer, 1994). The sediment discharge from the Danube River (Jaoshvili, 2002) should provide minerals appropriate for this process to act effectively either during water column transit or immediately after initial sedimentation. A similar protective mechanism might be the packaging of pigments into dense and rapidly sinking fecal pellets after grazing by herbivores (Taguchi et al., 1993), which are quickly exported from the photic zone protecting pigments from photo-oxidation. Such a process, however, would only account for the preservation of digestive pigments like pheophytin-*a*, pyropheophytin-*a*, and 13^2,17^3-cyclopheophorbide-*a*-enol but not for the original chlorophyll-*a*. Chloro- and pheopigments protected by either of the above ways may afterwards be carried into younger sediments through bioturbation. However, such process should not discriminate against pheophytin-*a*, which is significantly younger at both stations. Contribution of terrestrial biomass as indicated by low TOC and leaf-wax lipid (Kusch et al., 2010) radiocarbon concentrations, could account for older chloro- and pheopigments, if intact leaf fragments have been sequestered on land before delivery to the Black Sea. Such sequestration might occur in the Danube Delta, which is a periodically flooded wetland area characterized by interconnected lakes and ponds. A flood event or dredging processes may release plant leaves previously preserved in anoxic lake sediments preventing degradation. Contribution from pre-aged intact leaves could also explain why only chlorophyll-*a* and pheophytin-*a* are noticeably depleted in ^{14}C whereas pyropheophytin-*a* is enriched in most samples, and why only those stations immediately in front of the river mouth are affected. Both chlorophyll-*a* and pheophytin-*a* are primary pigments in the photosystem II and, thus, would be the only pigments in preserved intact leaves or leaf fragments. Furthermore, the southward deflection of Danube River sediments inhibits large scale dispersal of terrigenous organic matter across the NW Black Sea shelf (Panin and Jipa, 2002) and restricts its deposition to the river mouth area.

Except for chlorophyll-*a* and pheophytin-*a* at stations P177 and P128, respectively, all investigated pigments contain bomb-^{14}C, i.e., they are enriched relative to the pre-bomb surface water DIC ^{14}C concentration of $-54.8‰$ (Jones and Gagnon, 1994). Thus, they were synthesized after AD 1950 (Stuiver and Polach, 1977). Accordingly, pigment ages of bomb-^{14}C containing pigments can only be estimated by comparison with the surface water DIC ^{14}C record from AD 1950 until the sampling year AD 2008. Since measured bomb surface water DIC ^{14}C values are only available for AD 1988 (Jones and Gagnon, 1994), we use model DIC ^{14}C results for AD 1810 to AD 2010 (Fig. 4; Table S2 in the Supplement) adapted to match existing data (Ostlund and Dryssen, 1986; Jones and Gagnon, 1994; Siani et al., 2000; this study). Estimated average calendar years of pigment synthesis derived from comparison with the DIC ^{14}C record range from AD 1956 to AD 1969 (Table 1) because post-bomb surface DIC ^{14}C concentrations have not equilibrated to values $<30‰$ (Fig. 4; Table S2 in the Supplement) until AD 2008. Only for pyropheophytin-*a* at station P128 (AD 1963 or AD 2009), and pyropheophytin-*a* (AD 1969 or AD 1981) and $13^2,17^3$-cyclopheophorbide-*a*-enol at station P167 (AD 1964 or AD 2002) two possible average synthesis years can be derived. However, in all cases the earlier possible date is regarded more likely because of its similarity for estimated years of pigment synthesis at the other stations (AD 1956 to AD 1964) and, in the case of $13^2,17^3$-cyclopheophorbide-*a*-enol, with the date estimated for a second aliquot (AD 1962) analysed separately. The modelled surface DIC ^{14}C concentrations do not reach values high enough to correspond to that of the most ^{14}C-enriched pigment, chlorophyll-*a* at station P169 (181.12 ± 17.1‰). This might be caused by either uncertainties of the model related to the scarcity of input data, since the chlorophyll-*a* Δ^{14}C value is in the range previously reported for Northern Hemisphere oceanic surface water DIC (e.g. Druffel, 1987), or contribution of terrestrial chlorophyll-*a* such as from a recent leaf fragment, reflecting atmospheric bomb-^{14}C concentrations. Overall, also bomb-^{14}C containing pigments are older than expected. Considering that our estimate of blank carbon introduced during HPLC processing is associated with an uncertain error and that we cannot accurately determine the blank introduction during wet chemical sample preparation, the reported blank-corrected Δ^{14}C concentrations may be slightly erroneous. An additional contribution of roughly 1–11 µg ^{14}C-free C for samples sizes of approximately 30 µg to 230 µg C would change the ^{14}C concentrations to values between 100 and 110‰. Such values equal surface water DIC ^{14}C concentrations integrated from AD 1970 to AD 1990. This time period of highest surface DI^{14}C concentrations is coincident with an up to tenfold increase of the phytoplankton photosynthetic activity on the NW Black Sea shelf caused by anthropogenic eutrophication (e.g. Aubrey et al., 1996; Oguz, 2005). Besides higher biomass quantity, pigment preservation should have been enhanced through reduced light penetration in the uppermost water column (Aubrey et al., 1996; Oguz, 2005), which likely limited photo-oxidation of pigments during that time. Moreover, the eutrophication-triggered recurrent hypoxia of the shallow shelf bottom waters (Aubrey et al., 1996) should have further enhanced pigment preservation by decreasing both oxidation and grazing. Therefore, the preservation efficiency should have been considerably enhanced during this time period obscuring more recent pigment ^{14}C concentrations.

4.3 Chloro-and pheopigment preservation timescales and implications for the stable carbon and nitrogen isotopic composition

If the decomposition of chlorophyll-*a* to its successor pigments in the sediment occurred on timescales of several years to decades, the ^{14}C concentrations of the latter within the same sedimentary layer as the precursor should decrease with increasing degradative loss of functional groups, i.e., a decrease in ^{14}C concentrations would be expected from chlorophyll-*a* to pheophytin-*a* and further to pyropheophytin-*a* due to increasing time needed for the decompositional process. An evaluation of the timescales of pre- and post-depositional degradation processes is only possible for station P167, the only core location situated well below the oxycline where bioturbation can be excluded to cause pigment age differences. Likewise, no age artefact from low sediment accumulation is expected since the sedimentation rate at this station is ~ 0.9 mm/yr years (extrapolated from Gulin et al., 2002). Except for pyropheophytin-*a* all pigment ^{14}C concentrations agree within 2σ-analytical uncertainty, thus, their decompositional conversion occurs within a few years. Although the ^{14}C concentrations of pyropheophytin-*a* are significantly different from those of the other pigments at station P167, the calendar age difference is also only a few years (Table 1) caused by the steep gradient of bomb-^{14}C incorporation into surface water DIC during the 1960s. However, other than expected pyropheophytin-*a* is younger than chlorophyll-*a* and pheophytin-*a*. This pattern is also evident at stations P128, P177, and P120. These younger ages are coupled with a ^{15}N-depletion of pyropheophytin-*a* compared to chlorophyll-*a* and pheophytin-*a* at the respective stations, which might be caused by seasonal nutrient source variations. Since the spring bloom is associated with a pyropheophytin-*a* mass flux maximum and the autumn bloom is coupled to a pheophytin-*a* flux maximum (King, 1995), the ^{14}C concentration differences between pyropheophytin-*a* and the other pigments might also be driven by seasonality at the more offshore locations (P120 and P167). A difference in the average dwelling depths of diatoms (spring bloom) and coccolithophorides (autumn bloom) or seasonally varying DIC ^{14}C concentrations (Broecker and Peng, 1980) could explain the pigment Δ^{14}C differences. For the Black Sea seasonal variations in subsurface chlorophyll-*a* depths have been reported (Chu et al., 2005) but seasonally integrated DIC ^{14}C data are not available.

The stable carbon and nitrogen isotopic compositions of the individual pigments reflect the conditions at the time of synthesis. At station P128 the oldest pigment (pheophytin-*a*) is approximately 700 years older than the youngest pigment (pyropheophytin-*a*). Our data suggest that during this time period the pigment nitrogen isotopic composition and, thus, the nutrient assimilation pathway and source nitrate isotopic composition has not changed substantially if pigments are derived from phytoplankton. Likewise, no major changes of the nutrient source and assimilation pathway are obvious at station P177 where the oldest (chlorophyll-*a*) and the youngest (pheophytin-*a*) measured pigments are even ~1200 years apart. If chlorophyll-*a* and pheophytin-*a* at these two river mouth stations are derived from terrestrial plants, such temporal assessment is invalid. All pigments derive from phytoplankton at stations P120 and P169 where chlorophyll-*a* δ^{15}N values differ considerably from the δ^{15}N values of the other pigments while their ^{14}C concentration differences are insignificant. Apparently, for the NW Black Sea shelf spatial differences of the nutrient assimilation pathway and seasonal variations of the isotopic composition of a nutrient source seem to have a stronger imprint on the stable carbon and nitrogen isotopic composition than potential temporal changes during the last millennium.

5 Conclusions

This study reports the first results from combined compound-specific radiocarbon and stable carbon and nitrogen isotopic analysis of chloro- and pheopigments. The combined δ^{13}C and δ^{15}N isotope analysis of chlorophyll-*a* and its degradation products pheophytin-*a*, pyropheophytin-*a*, and 13^2,17^3-cyclopheophorbide-*a*-enol from NW Black Sea surface sediments provides evidence for their origin from a mainly nitrate assimilating phytoplanktonic community with additional contribution from diazotrophs

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towards the central ocean basin. The chloro- and pheopigment ^{14}C concentrations are much lower than expected translating into minimum and maximum pigment ages of approximately 40 to 1200 years. This is most likely the result of protection against degradation by processes such as association with minerals or eutrophication-induced hypoxia and light limitation. Nevertheless, the isotopic heterogeneity is also caused by seasonally varying isotopic compositions of the nutrient source, habitat, and the growth period which appear to be at least as strong as the long-term trends.

Overall, the combined triple isotopic analysis of sedimentary chlorophyll and its primary degradation products can delineate biogeochemical and diagenetic processes in surface waters and simultaneously assess the timescales of these processes.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/7/6265/2010/ bgd-7-6265-2010-supplement.pdf.

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Table 1. Blank-corrected radiocarbon (Δ^{14}C) and stable isotopic composition (δ^{13}C and δ^{15}N) of purified chloro- and pheopigments, bivalve shells, total organic carbon (TOC), and bulk sediment. Where sample sizes were sufficient, samples were split prior to ^{14}C analysis. Errors are given as 1σ-analytical uncertainties. n.d., Not determined.

Sample	δ^{13}C [‰][a]	δ^{15}N [‰][a]	Δ^{14}C [‰][a]	cal year AD[b]	ID No.[c]
P128 (45°04.69′ N, 29°46.64′ E; 17 m b.s.l.) 0–2 cm					
chlorophyll-*a*	−25.8 ± 0.1	2.1 ± 0.4	n.d.		
pheophytin-*a*	−26.1 ± 0.1	2.5 ± 0.4	−127.9 ± 11.9	1230 ± 105	70 736
pheophytin-*a*	−26.1 ± 0.1	2.5 ± 0.4	−109.3 ± 11.6	1380 ± 70	70 737
pyropheophytin-*a*	−25.8 ± 0.1	1.5 ± 0.4	32.9 ± 12.8	1963 ± 1/2009 ± 6	70 738
Scarpharca sp.	−1.1 ± 0.1		56.1 ± 3.3		70 752
TOC 0–1 cm[d]	−25.7 ± 0.1		−166.6 ± 2.1		66 558
bulk sediment	−26.1 ± 0.1	8.5 ± 0.5			
P177 (44°35.76′ N, 29°11.43′ E; 22 m b.s.l.) 0–3 cm					
chlorophyll-*a*	−26.3 ± 0.1	2.0 ± 0.4	−183.2 ± 15.3	690 ± 160	71 683
pheophytin-*a*	−24.6 ± 0.1	2.6 ± 0.4	−6.6 ± 18.1	1962 ± 2	70 746
pyropheophytin-*a*	−24.8 ± 0.1	1.9 ± 0.4	−55.8 ± 12.9	1950 ± 1	70 747
Abra fragilis	−0.6 ± 0.1		54.0 ± 3.3		70 754
TOC 0–1 cm[d]	−25.2 ± 0.1		−161.0 ± 3.4		66 564
TOC 2–3 cm	−24.7 ± 0.1		−103.4 ± 4.2		71 682
bulk sediment	−25.7 ± 0.1	8.4 ± 0.5			
P120 (44°30.66′ N, 30°40.93′ E; 91 m b.s.l.) 0–3 cm					
chlorophyll-*a*	−26.0 ± 0.1	3.1 ± 0.4	−36.7 ± 15.5	1959 ± 2	71 685
pheophytin-*a*	−25.3 ± 0.1	−0.5 ± 0.4	−50.6 ± 7.6	1956 ± 2	70 748
pyropheophytin-*a*	−24.5 ± 0.1	−0.2 ± 0.4	6.2 ± 12.4	1962 ± 1	70 749
TOC 0–1 cm[d]	−23.7 ± 0.1		−64.2 ± 3.2		70 734
TOC 2–3 cm	−23.6 ± 0.1		−209.7 ± 3.2		71 681
bulk sediment	−24.0 ± 0.1	6.7 ± 0.5			
P169 (44°15.48′ N, 30°29.19′ E, 96 m b.s.l.) 0–3 cm					
chlorophyll-*a*	−26.4 ± 0.1	4.0 ± 0.4	181.1 ± 17.1	n.d.	71 684
pheophytin-*a*	−24.7 ± 0.1	−0.9 ± 0.4	21.7 ± 14.3	1963 ± 1	70 750
pyropheophytin-*a*	−25.0 ± 0.1	−0.9 ± 0.4	n.d.		
Modiolus sp.	0.9 ± 0.1		9.9 ± 3.8		70 753
TOC 0–1 cm[d]	−23.4 ± 0.1		−51.2 ± 3.2		70 735
bulk sediment	−25.4 ± 0.1	6.7 ± 0.5			
P167 (43°58.88′ N, 31°30.83′ E; 1336 m b.s.l.) 0–2 cm					
chlorophyll-*a*	−26.7 ± 0.1	−0.1 ± 0.4	−34.1 ± 16.5	1959 ± 3	70 743
chlorophyll-*a*	−26.7 ± 0.1	−0.1 ± 0.4	19.9 ± 13.2	1963 ± 1	70 751
pheophytin-*a*	−26.3 ± 0.1	−1.2 ± 0.4	13.6 ± 12.2	1963 ± 1	70 740
pheophytin-*a*	−26.3 ± 0.1	−1.2 ± 0.4	1.7 ± 9.2	1962 ± 1	70 741
pyropheophytin-*a*	−24.7 ± 0.1	−2.8 ± 0.4	105.5 ± 18.0	1969 ± 3/1981 ± 6	70 742
$13^2,17^3$-cyclopheophorbide-*a* enol	−25.5 ± 0.1	−0.7 ± 0.4	48.3 ± 12.2	1964 ± 1/2002 ± 6	70 744
$13^2,17^3$-cyclopheophorbide-*a* enol	−25.5 ± 0.1	−0.7 ± 0.4	−7.1 ± 8.3	1962 ± 1	70 745
TOC 0–1 cm[d]	−24.6 ± 0.1		44.5 ± 2.5		66 557
bulk sediment	−23.2 ± 0.1	4.2 ± 0.5			

[a] Blank-corrected.

[b] Calendar year AD calibrated using CALIB 6.0 for years <AD 1950 and modelled surface DI^{14}C values for years ≥AD 1950.

[c] Data from Kusch et al. (2010).

Table 2. Seawater chemical properties and nitrate nitrogen isotopic composition. n.d. Not determined.

Sample	Water depth [m]	Temperature [°C]	Salinity [psu]	O_2 concentration [µmol/l]	NO_3^- concentration [µM]	$\delta^{15}N$ NO_3^- [‰]
P128	1.5	7.5	15.3	368	8.4	8.2 ± 0.2
						7.2 ± 0.2
	15	6.3	18.0	307	0.3	4.9 ± 0.2
P177	6	6.9	16.8	359	2.3	8.1 ± 0.2
	12	6.8	16.8	360	2.3	8.0 ± 0.2
	22	6.2	17.1	330	3.0	8.4 ± 0.2
						7.0 ± 0.2
P120	5	8.1	18.0	324	0.2	n.d.
	40	7.9	18.0	320	0.3	n.d.
	68	7.3	18.2	303	0.5	n.d.
	88	7.4	18.3	292	0.5	n.d.
P169	5	8.1	17.7	333	0.2	n.d.
	75	7.5	18.3	281	1.1	4.9 ± 0.2
	92	8.1	19.1	142	1.9	5.0 ± 0.2
P167	2	8.3	17.2	341	0.2	n.d.
	25	8.1	18.1	322	0.0[a]	7.2 ± 0.2
	52	7.2	18.5	321	0.2	n.d.
	70	8.1	19.3	113	1.1	7.7 ± 0.2
	150	8.5	21.1	4	0.0	n.d.
	500	8.9	22.0	0	0.0	n.d.

[a] This measurement is considered prone to an analytical artifact.

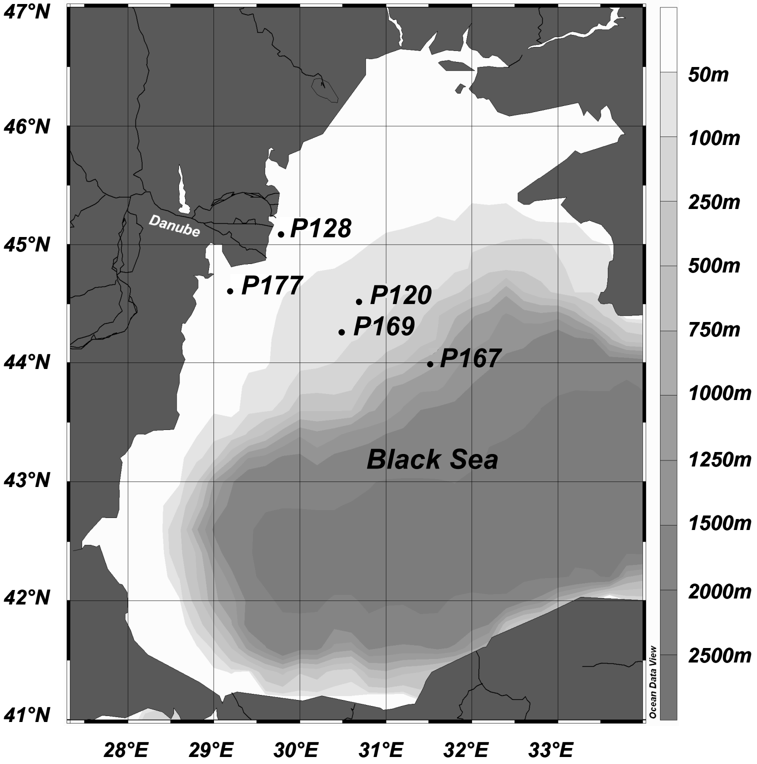

Fig. 1. Locations of sampling stations.

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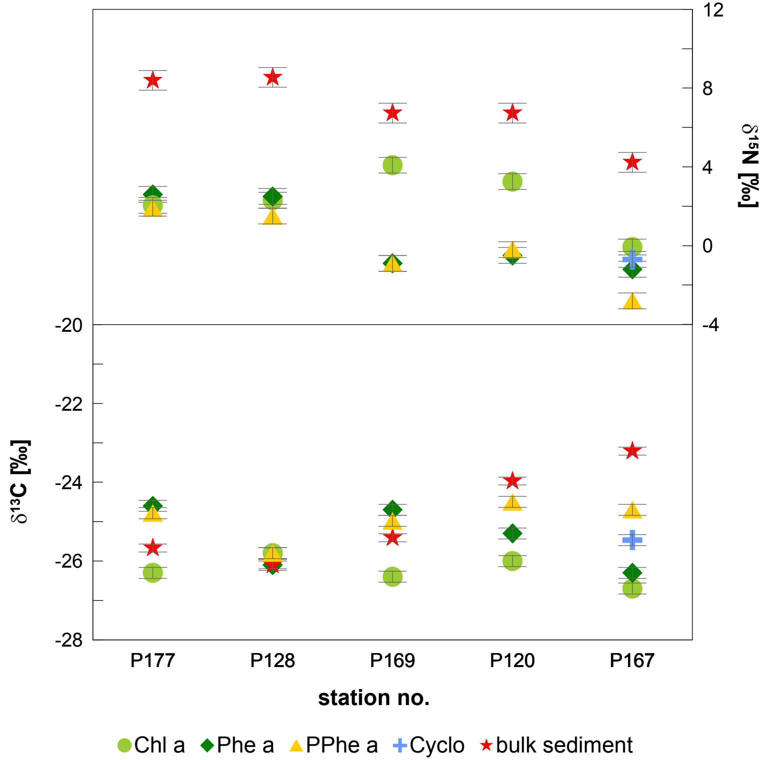

Fig. 2. Stable nitrogen and carbon isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of purified chloro- and pheopigments Error bars give 1σ-analytical uncertainty. Chl *a*: chlorophyll-*a*, Phe *a*: pheophytin-*a*, PPhe *a*: pyropheophytin-*a*, Cyclo: $13^2,17^3$-cyclopheophorbide-*a*-enol.

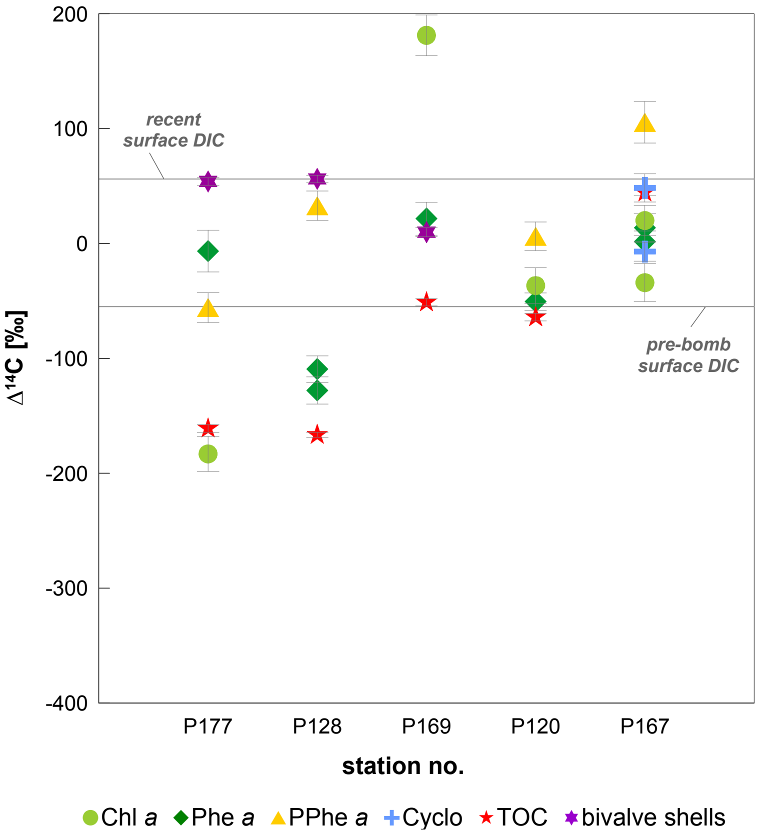

Fig. 3. Radiocarbon concentrations ($\Delta^{14}C$) of purified chloro- and pheopigments. Error bars denote 1σ analytical uncertainties. Chl *a*: chlorophyll-*a*, Phe *a*: pheophytin-*a*, PPhe *a*: pyropheophytin-*a*, Cyclo: $13^2,17^3$-cyclopheophorbide-*a*-enol.

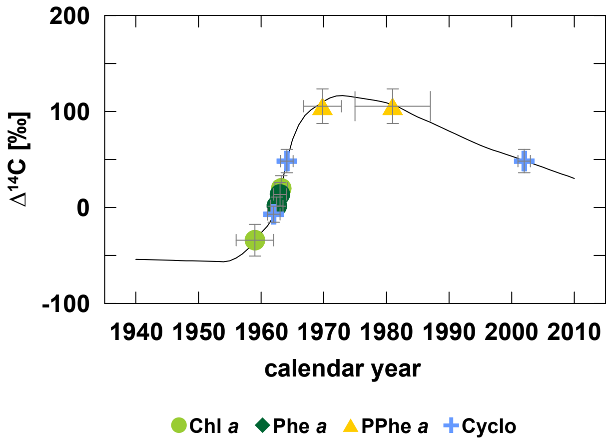

Fig. 4. Modelled Black Sea surface water DIC Δ^{14}C concentrations. As example, potential calendar years of pigment synthesis derived from correlation with Δ^{14}C concentrations are given for station P167. Error bars denote 1σ analytical uncertainties. Chl *a*: chlorophyll-*a*, Phe *a*: pheophytin-*a*, PPhe *a*: pyropheophytin-*a*, Cyclo: 13^2,17^3-cyclopheophorbide-*a*-enol.