Author reply to reviews on manuscript by Kusch et al. (2010): Implications for chloro- and pheopigment synthesis and preservation from combined compound-specific $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$ analysis; Biogeosciences Discussions 7, 6265–6294

We thank the referees for their reviews. Overall, both reviewers evaluate the manuscript much different. For example, while referee #1 thinks *“the paper would certainly gain by a thorough work-over in terms of language and writing style”*, referee #2 considers the manuscript *“a well-written paper”*. Moreover, referee #1 states that *“the Black Sea appears to be far from ideal to tackle the problem the paper set out to explore”*, referee #2 judges *“The location of their site seems like a logical place to start with redox regime being conducive for preservation”*.
Nevertheless, both agree that the approach is novel, comparable existing data are rare (or not existing in case of ^{14}C), and that the manuscript is within the scope of BG.

Please find our comments to the reviews below (abbreviations are RC: reviewer comment; AC: author comment).

Response to the comments and suggestions of referee #1

Specific comments

RC: *“(too bad that there apparently have been no analyses of suspensions in the euphotic zone or of sediment trap materials....)”*

AC: We agree. The analysis of water column particulates would be highly interesting. Unfortunately, the amount of, e.g., filtered POC or sediment trap material needed to obtain triple compound-specific isotope analysis is extremely high and was not available for us. As an example, one of the very few studies performing compound-specific radiocarbon analysis on POC from the water column (Ingalls et al., 2006: Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon, PNAS 103, 6442–6447) required pumping of 104,000l of surface seawater (21m depth) and 208,000l of deep seawater.

RC: *“Intriguingly, some explanations immediately came to my mind that may arise from the study area with its non-standard carbon, radiocarbon and nitrogen isotope properties, and from source and product mixing in each sediment sample (authors do acknowledge, but dismiss this simple explanation in the Discussion, but not entirely convincingly so). Another explanation that came to me (and is also briefly discussed, but dismissed in the text) is whether the results reflect superpositions of sources and processes. I would expect such a mixture in a natural system that has diverse sources (land plants from a river, marine phytoplankton and presumably also cyanobacteria) and conditions of preservation (anoxic and oxic sea floors, diverse sedimentation rates etc.).*

AC: We agree that the Black Sea water body has non-standard isotope properties. In our case, however, we base the conclusions mainly on compound-specific pigment analyses. This implies that only the isotopic properties from the upper ~50m need to be considered (including all potential water mass mixing processes which, however, seems to be restricted by the pycnocline) because chlorophyll *a* and its successor products can unambiguously be assigned to photoautotrophic source organisms living in the oxic and euphotic zone. See also comment on RC10.

We also agree that source and product mixing plays a very important role. To our mind, we think we have widely addressed isotopic compositions related to mixtures of sources. For example, in section 4.1 we conclude that the ^{13}C and ^{15}N isotopic variability relates to mixtures of pigments preserved from different phytoplankton blooms. Moreover, although a general assumption in the scientific community is that pigments found in marine sediments derive from phytoplankton and survival of terrestrial pigments is normally excluded, in section 4.2 we conclude that the depleted ^{14}C concentrations of chlorophyll *a* and pheophytin *a* at stations P128 and P177 most likely derive from terrestrial intact leaves/leaf fragments stored in the Danube Delta. Also, the mixture of pigments derived from nitrate assimilation vs. those derived from N_2-fixation is discussed in section 4.1 (we conclude contribution of diazotrophic pigments at the offshore core locations).
To additionally clarify in the beginning, we have added a paragraph stating that “in natural systems isotope values always represent mixtures of a continuum of (potentially superposed) isotope values present in a certain biomarker sample. Therefore, measured pigment isotope values result from the fractional contributions of all pigment molecules present in a particular biomarker fraction,

which might be produced at different times integrated in the sediment interval investigated." at the beginning of section 4.1.

RC: *The same holds for the abstract, which indeed is a description of the contents, but gives no indication of the Ifs and Whens discussed in the body of the text.*

AC: This comment relates to RC9. In our mind, we have tried to summarize the overall conclusion of the study avoiding protracting the abstract by listing all discussed potential explanations. Referring to RC9, we have more explicitly stated our assessment of the most likely processes causing the observed isotopic variability (for $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$). These are the mechanisms summarized in the abstract, e.g., protection from decomposition by association with minerals, storage in deltaic anoxic environments, or eutrophication-induced hypoxia and light limitation for the ^{14}C isotopic variability (conclusion section 4.2). Also, seasonal variations of nutrient source, growth period, and habitat (the processes we regard most likely, conclusion 4.1) are summarized to cause the observed variability of the ^{13}C and ^{15}N isotopes.

RC: *Many sentences wind on and on, and sometimes the language is so complicated as to completely obscure the meaning.*

AC: We have addressed this comment by starting new paragraphs, and shortening and rephrasing sentences throughout the text.

Detailed comments

RC 1) *Page 6268: I don't know if the statement that chlorophyll a undergoes several diagenetic processes is correct. It is a molecule that undergoes specific chemical reactions: Cell senescence, grazing etc. may affect the cells that contain chlorophyll a, but not the molecule.....*

AC: The molecule is indeed affected by these processes (this is an essential assumption of the biomarker concept). Among many others, the references cited on page 6268, lines 7-12 (Hoyt, 1966; Owens and Falkowski, 1982; Carpenter et al., 1986; Sanger, 1988; Repeta and Simpson, 1991; Spooner et al., 1995; Gossauer and Engel, 1996; Matile et al., 1996; Chen et al., 2003; Chamberlain et al., 2006) all describe the molecular transformations caused by the described mechanisms. All described processes are associated with certain enzyme reactions causing the molecular transformations.
For another review on these processes we, e.g., may refer to Hendry et al. 1987: The degradation of chlorophyll – a biological enigma, New Phytologist 107, 255–302.

RC 2) *Page 6271: The reference for the new EA-MS method (Ogawa et al., 2010) is incomplete and not easily accessible. There should at least be a short description here. Line 15: How do you analyse the stable isotope composition of bulk sediment on an IR-MS? With an EA periphery?*

AC: We have added the missing page information to the Ogawa et al. citation. We added the missing "CE Elemental Analyser coupled with a Con-Flo II Interface".

RC 3) *Line 20: The samples were evacuated and flame-sealed? How?*

AC: We added the reference Mollenhauer and Rethemeyer (2009), who describe the evacuation process more specifically. Flame-sealing was performed using a H_2 jet burner; we also added this.

RC 4) *Page 6272: Stuiver et al. 2010 missing in reference list*

AC: We added the Stuiver et al. (2010) citation to the reference list.

RC 5) *In the 2.4 Surface DIC model formula, I did not find the term "z" which is explained 2 lines below. The "μ" term (time constant of the first-order loss and gain term) is enigmatic to me. In general, I am not entirely sure why you need the model in this text in the first place, and why it could not go into the ancillary materials entirely. If you decide to keep it here, please devote some text to results of the model and possibly some discussion. Also, explain what the purpose is to non-specialists (not apparent to me from the following text, but maybe I missed something). On page 6279 you reference existing data, whereas the model is introduced to alleviate the lack of data.....*

AC: Equation (1) contains a typo: The denominator of the derivative on the right-hand side of the equation should read "∂z^2" instead of "∂t^2".

We apply a one-dimensional model because observations are too sparse for a more sophisticated modelling approach which would also consider upwelling/downwelling and horizontal water exchange by mixing and advection. Butzin and Roether (1994) have shown that, for a limited observational basis, these effects can be efficiently parametrized by including an additional apparent source/sink term which is proportional to the surface water concentrations with a time constant μ. Both parameters K and μ are used to fit the model results to the observations and hardly represent processes in the real ocean. To clarify, we have changed "and μ is the time constant of the first-order loss and gain term." on page 6272, lines 18-19 to "The free parameters K (an apparent vertical diffusivity) and μ (the proportionality or time constant of the apparent source (sink) term) are used to fit the model to the observations."

The model was generated to get a continuous Black Sea surface DIC bomb ^{14}C curve, which is needed to calculate the true ages of bomb-^{14}C containing pigments, i.e., all pigments despite chlorophyll *a* at station P177 and pheophytin *a* at station P128. This purpose is already described on page 6272, lines 4-6 and on page 6279, lines 12-18. To make it once clearer we have inserted the purpose again in chapter 2.4 (which we prefer to keep in the main text).

For the Black Sea very few existing data (Ostlund and Dryssen, 1986; Jones and Gagnon, 1994; Siani et al., 2000; Fontugne et al., 2009; and the results from this study) are scattered as stated on page 6273, line 13-14; in total measured Δ^{14}C values are only reported for AD1837, AD1843, AD1931, AD2004, and AD2008. Accordingly, the calculations of the "true" ages of bomb-^{14}C containing pigments would not be possible without a modelled Δ^{14}C DIC curve. In order to get the best possible results, the model was run for the time-interval AD1810 to AD2010, i.e., the range of reported values (as stated on page 6273, line 11) and, of course, it was modelled to fit these measured data.

We changed "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." (page 6272, lines 8-10) to "The history of Black Sea surface water ^{14}C concentrations after AD1950 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 in order allow calculating "true" ages of bomb-^{14}C containing pigments" as explanation to non-specialists and specified "we use model DIC ^{14}C results for AD 1810 to AD 2010 adapted to match existing data" (page 6279, lines 16-17) to "we use model DIC ^{14}C results for AD1810 to AD2010 adapted to match the few existing data available for this time interval". Additionally, we added a sentence describing the model output after the paragraph.

RC 6) *Page 6274: What is "pre-aged terrigenous organic matter" (also in other places)? Do you mean "older" or indeed "artificially aged"? Line 19: "....consistent with less bomb 14C in deeper water"?*

AC: "Pre-aged" terrestrial organic matter is a commonly used expression, describing the contributing of organic matter retained in a continental reservoir like soils before supply to the oceanic (or lacustrine) reservoir (e.g., Drenzek et al., 2007: Constraints on the origin of sedimentary organic carbon in the Beaufort Sea from coupled molecular ^{13}C and ^{14}C measurements, Marine Chemistry, 103, 146–162; Griffith et al. 2010: The radiocarbon age of organic carbon in marine surface sediments, GCA, 74, 6788-6800). We added "reworked" to clarify this.

Yes, utilization of more ^{14}C depleted DIC also implies less bomb-^{14}C in the deeper waters here. However, the expression "^{14}C-depleted DIC pool" is valid to describe the general Δ^{14}C distribution throughout the ocean controlled by the reservoir effect, i.e., the steady state balance maintained between the input of ^{14}C from the atmosphere and its removal by advection and radiodecay in the water column (see further, e.g., Bard, 1988: Correction of Accelerator mass spectrometry ^{14}C ages measured in planktonic foraminifera: paleoceanographic implications, Paleoceanography, 3, 635-645). In contrast, the description of "less bomb-^{14}C" only describes the distribution of ^{14}C in the water column, which was introduced during the last 60 years (since AD1950). We therefore prefer the expression "consistent with utilization of a more ^{14}C-depleted dissolved inorganic carbon (DIC) pool".

RC 7) *Page 6275, line 11: Different from what? This entire paragraph is one that threw me off, because each station/sample apparently is different from the others, and even repeated analyses of one sample appear to diverge significantly (or am I wrong?)? Are differences of duplicate analyses on one sample of the same magnitude as inter-sample differences?*

AC: This paragraph describes the radiocarbon results. In line 11, dual ^{14}C measurements of cyclopheophorbide *a* enol are significantly different from each other (the two numbers given in brackets are the $\Delta^{14}C$ concentrations of both cyclopheophorbide *a* enol at station P167; see also Table 1, Figure 2). In case it was not clear that station P167 was meant, we added "at this station".

Repeated (dual) analyses of one sample do only diverge significantly in case of cyclopheophorbide *a* enol at station P167. As described in Table 1 and Figure 3 error bars denote 1σ analytical uncertainties. For radiocarbon results significant differences are at the > 2σ level. We have added this to the text.

We also rephrased the paragraph to provide a more general description of the ^{14}C results.

RC 8) *The title of part 4.1. is very ambitious (I don't believe that the data are suited to shed light on the ecology of the photoautotrophic community!) and this entire part of the discussion makes several statements that I do not agree with (below).*

AC: The use of pigment stable carbon and nitrogen isotopes in investigating the ecology of photoautotrophs has been demonstrated in various studies including Ohkouchi et al., 2005; Ohkouchi et al., 2006; Kashiyama et al., 2008a,b; Ohkouchi et al., 2008 (references given in the text). However, we have changed the 4.1 heading to "Chloro- and pheopigment origin and biogeochemical C and N cycle of the prevailing photoautotrophs" to specify.

RC 9) *Page 6275 lines 22 ff: The statements that $\delta^{13}C$ values < -26‰ "reflect a marine phytoplanktonic origin" is somewhat puzzling and unconvincing; low values such as these may occur in phytoplankton in the area (I don't know the references cited in support), but it certainly is a range of values that does not exclude a terrestrial origin. In the following lines, a series of reasons for inter-pigment differences in d13C are listed and all may be able to explain the discrepancies. But what does a long list like that of possible reasons do to help explain the differences in some samples, whereas the differences are apparently not seen in other samples?*

AC: We agree and have added that $\delta^{13}C$ values of -26‰ are also in the range previously reported for terrestrial plants (Kennicutt et al., 1992; reference added). Nevertheless, due to the large range of processes causing chlorophyll degradation on land and during transport (e.g., Hoyt, 1966; Sanger, 1988; Matile et al., 1996; Chamberlain et al., 2006) and the high amount of marine biomass production stimulated by nutrient input (Cociasu et al., 1996) a contribution of terrestrial chlorophyll *a* (and its primary degradation products investigated here) should be expected to be minor.

Generally, it has to be considered that isotope values always represent mixtures of a continuum of isotopic values present in a sample (no matter if bulk or compound-specific). Therefore, isotope values might be biased towards a seasonal signal if the fractional contribution is high compared to other seasons. If it would be assumed that pheophytin *a*, pyropheophytin *a*, and cyclopheophorbide *a* enol are produced by one homogenous chlorophyll *a* pool, their isotopic signatures should not deviate from their parent chlorophyll *a*. We have added this general assessment in line 26, page 6275. Accordingly, there have to be additional processes, i.e., the two explanations (seasonality, phytol-loss) offered, influencing the $\delta^{13}C$ values. Generally, the seasonality has an influence on all pigments, while the phytol-loss only influences cyclopheophorbide *a* enol, and may thus be considered the cause of the isotopic variability (we have also added this assessment). Moreover, this is emphasized by the $\delta^{15}N$ values, which may also be influenced by seasonality (the only explanation offered). And seasonality is again an important factor influencing the ^{14}C concentrations (4.3, page 6281ff).

RC 10) *Page 6276 line 14: I do not agree with that statement, because other observations tell another story: Water column profiles of suspended matter $\delta^{15}N$ show a slight enrichment from ca. 4‰ in the upper mixed layer to up to 8‰ at the oxycline. Below the oxycline in the Black Sea, however, $\delta^{15}N$ values shift dramatically to extremely depleted values (-8‰ at the top of the anoxic water body. A simultaneous increase in the mass of total SPON implies that 15N-depleted OM must be newly produced there. This was attributed to newly produced OM by the biomass of chemoautotrophic bacteria utilizing NH4+ as their dominant N source (Coban-Yildiz et al., 2006). Coban-Yildiz, Y., Altabet, M. A., Yilmaz, A. and Tugrul, S.: Carbon and nitrogen isotopic ratios of suspended particulate organic matter (SPOM) in the Black Sea water column, Deep-Sea Res. II, 53, 1875-1892, 2006. See also Fry, B., Jannasch, H. W., Molyneaux, S. J., Wirsen, C. O., Muramoto, J. A. and King, S.: Stable Isotope Studies of the Carbon, Nitrogen and Sulfur Cycles in the Black-Sea and the Cariaco Trench, Deep-Sea Res. I, 38, 1003-1019, 1991.*

AC: This comment refers to the statement: “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.”
We agree, bulk sedimentary $\delta^{15}N$ values show large variations throughout the shelf break Black Sea water column (the central stations do not show such strong variations) as reported by Coban-Yildiz et al. (2006) and Fuchsman et al. (2008). However, irrespective of the SPON $\delta^{15}N$ values in deeper waters, the ^{15}N isotopic composition of the investigated pigments is only determined by the ^{15}N isotopic composition of the assimilated nitrogenous nutrient source (mostly nitrate) within the upper euphotic zone in the depth range of the subsurface chlorophyll *a* maximum depth, which is reported to range from about 15m to 40m according to season (Chu et al. 2005). This is because chlorophyll *a* and its successor products can unambiguously be assigned to photoautotrophic source organisms living in the oxic and euphotic zone. We agree that the bulk value, however, is the integrated result which in this case only incidentally agrees with the surface water signal reflected by photoautotrophic pigments. Accordingly, we deleted the sentence.

RC 11) *Page 6276, line 20 ff: You are comparing nitrate $\delta^{15}N$ values (don’t say “heavy” d15N but “high” d15N) at an unknown stage of assimilation (when in the year was the cruise anyway? Overall low nitrate concentrations indicate that assimilation may be advanced and may have enriched what nitrate remained. One concentration sticks out: I guess that the upper layer near the coast may have a high $\delta^{15}N$ of nitrate due to admixtures of landderived nitrate?) with the assimilation products in sediments 0-2 or 0-3 cm.*

AC: We have changed “heavy” to “enriched”, the cruise season (March 2008) was added on page 6269, line 18 and on page 6277, line 4.

We agree. Nitrate assimilation may have been advanced during the sampling season (March 2008), as reflected in the low NO_3^- concentrations (Tab. 2), leading to enriched NO_3^- $\delta^{15}N$ values. Likewise, the enrichment may be caused by contribution of “artificial” nitrate derived from fertilizer in the Danube catchment. We have added a paragraph addressing this issue (seasonal signal of nitrate vs. integrated signal of sediments, and nitrate enrichment source) in line 23, page 6276.

RC 12) *Page 6276, Line 15 ff: The sediments and pigment/chlorophyll extracts certainly integrate over at least one entire seasonal cycle or several years, so that arguments about late-season origin of chlorophyll are in my opinion meaningless. Also, the inference of increasing diazotrophic N2-fixation with distance from the river mouth is based on conjecture only and me need to be phrased more cautiously. To my knowledge there has been no experimental or observational evidence for diazotrophic N2 fixation in the Black Sea. Does synechococcus (see citation Uysal, 2000) produce the pigments analysed here? That N2 fixation is indeed a significant input at least to the nitrate pool is unlikely in my opinion considering the nitrate d15N values: The average d15N of nitrate in the upper layer above the chemocline (no nitrate below, of course) appears to be 7-8 permil. This is significantly higher than open ocean deep-water nitrate (of course no connection with the Black Sea) and nitrate in the intermediate water of the Mediterranean Sea. But it is known that rivers draining fertilized catchments bring nitrate enriched in d15N (>8 permil), which may set the level of nitrate d15N in the Black Sea surface layer.*

AC: As mentioned above (see reply to RC9), isotope values always represent admixtures of a continuum of isotopic values present in a sample and these for a particular sample may be biased towards one season if the fractional contribution is higher in this sample than the fractional contribution from the same season in another sample.
We agree that there is no experimental/observational evidence of N_2-fixation in the Black Sea. However, various studies (e.g., McCarthy et al., 2007; Fuchsman et al., 2008; added to references) have proposed N_2-fixation as an important component to balance the N cycle in the Black Sea, which has high N-losses (e.g., Murray et al., 2005 report twice as much flux of N out as N_2, than flux of N in as NO_3^- and NH_4^+) by denitrification and anammox (see also McCarthy et al., 2007).
The cyanobacterium Synechococcus, which has been identified to have nitrogenase genes (e.g., Huang et al., 1988; Ikemoto and Mitsui, 1994; added to reference list), has been observed throughout Black Sea waters (Uysal, 2006; added to references). We consider these data in support of our pigment $\delta^{15}N$ data, which suggest an additional relatively N-depleted source at the offshore core location (added to the text). Isotopically, such ^{15}N-depleted pigments can unlikely derive from assimilation of nitrate from deeper, suboxic water depths, which are highly ^{15}N-enriched (e.g., Murray et al., 2005; Fuchsman et al., 2008). Such enrichment would also be reflected in enriched pigment $\delta^{15}N$ values if such suboxic nitrate was assimilated by the photoautotrophs, but the opposite (^{15}N depletion) is obvious.

Yes, Synechococcus has chlorophyll *a*, thus, all other pigments investigated in this study may derive from degradation of this chlorophyll *a*. We have added a paragraph stating that Synechococcus is a likely source of diazotrophic pigments and that N_2-fixation was not yet determined in situ but was proposed in several publications to balance the Black Sea N cycle after line 10 on page 6277.

As stated in reply to RC11, we have added a paragraph addressing the nitrate $\delta^{15}N$ value in line 23, page 6276. The average nitrate $\delta^{15}N$ value of 7-8‰ (note that the P128 $\delta^{15}N$ value in 15m water depth is 4.9‰) is slightly higher compared to open ocean deep-water nitrate, where reported values range from 5-7‰ (e.g., Liu and Kaplan, 1989; Brandes et al., 1998; Sigman et al., 2000, 2005; added to references). This must either be caused by nitrate depletion by blooming phytoplankton (sampling in March 2008) supported by generally low nitrate concentrations (Tab. 2) or by input of enriched agricultural nitrate from the Danube River as implied by the high nitrate concentrations at the mouth of the Danube River (P128 and P177, Tab. 2). Unfortunately, Danube River $\delta^{15}N$ data are not available. However, if seawater nitrate is enriched in ^{15}N by source depletion or agricultural fertilizer input it may mask the N_2-input to the nitrate pool.

RC 13) *Page 6277, line 5 ff: I don't understand: river input with N:P>80 dominating and across pycnocline transport of phosphate (no nitrate in deeper waters) inhibited favors N2-fixation?*

AC: We have rephrased this paragraph to clarify. We propose (6277, line 2-10) that the very high N:P ratios at the river mouth do not limit nitrate assimilation since N is in excess of P. Thus primary production is theoretically only limited by phosphate. In contrast, offshore N:P ratios are below the Redfield ratio (16:1), thus, P is in excess over N and primary production will be limited by nitrate. Moreover, due to the stratification of the Black Sea, no suboxic nitrate seems to be upwelled/admixed since assimilation of this pool would be reflected in significantly enriched pigment $\delta^{15}N$ values, since suboxic nitrate has been shown to be enriched by ~7-10‰ compared to oxic water nitrate (e.g. Fuchsman et al., 2008). Because the opposite is obvious (depletion), this suboxic nitrate seems to not be effectively admixed and assimilated. Accordingly, due to the overall nitrate limitation in the central basin, additional N may be supplied by diazotrophs (N_2-fixation).

RC 14) *Page 6277 ff (4.2): I found the entire discussion on "timescales of chloro- and pheopigment synthesis" (who synthesises pheopigments anyway?) very difficult to follow....and the text does nothing to assess the controlling parameters and instead again offers a lot of assumptions and possible explanations.....I hope that other reviews may provide a more informed assessments of the contents. What does the last sentence before the Conclusions want to say?*

AC: "Pheopigments" is a general term for those chlorophyll derivatives that lack the central Mg-ion like pheophytin (see textbooks like "Photosynthesis: A Comprehensive Treatise" edited by A. S. Raghavendra, 1998 or "Primary processes of photosynthesis: principles and apparatus", vol. 1, edited by G. Renger, 2008). Pheophytins are originally present in photosystem II where they serve as electron acceptors (we mention this on page 6279, lines 3-5) but most pheopigments found in sediments are degradation products as described on page 6268, lines 4-18.

The "assumptions and possible explanations" describe the processes (contribution of pre-aged intact leaf fragments, eutrophication-induced light limitation and hypoxia) most likely controlling the observed radiocarbon isotopic composition (i.e. age structure). To stronger emphasize this, we changed "parameters" in the 4.2 heading to "processes". Additionally, we more specifically added our evaluation of the likelihood of the described processes within the paragraph, since this appears not to have been entirely clear before.

The last sentence before the conclusion summarizes and concludes chapter 4.3. As pointed out on page 6282, lines 4-12 the stable isotopic compositions of those pigments that show the largest age range (stations P128 and P177) do not differ significantly as might have been expected over long-term timescales if there were temporal gradients in nutrient supply (e.g., by anthopogenic influence on the river nitrate load). In contrast, the stable isotopic compositions of those pigments that show minimum age offsets (stations P120 and P169) differ significantly likely resulting from seasonal changes and/or different nutrient assimilation pathways as pointed out in lines 14-16. The final sentence therefore draws the conclusion that the stable isotopic variability during the last millennium is not stronger (in fact potentially weaker) than the variability associated with differences of the nutrient assimilation pathway and seasonal (isotopic) nutrient source variations. We have rephrased the sentence.

RC 15) *Page 6278, line 8 ff: Pigments as monolayers? Mayer (1994) does not deal with pigments.*

AC: We agree that Mayer (1994) does not explicitly investigate pigments. But he investigates organic carbon (OC) in general and found OC concentrations tightly coupled to the surface area of soil and sediment minerals (monolayer-equivalent) leading inter alia to his hypothesis that the OC is protected by its location inside the mineral pores too small to allow functioning of the hydrolytic enzymes necessary for organic matter decay. Since pigments are part of the organic carbon, we offer this process as a potential explanation for enhanced pigment preservation.

Response to the comments and suggestions of referee #2

Unfortunately, the page and line numbering provided by the referee does not agree with the BGD manuscript page and line numbering as available at http://www.biogeosciences-discuss.net/7/6265/2010/bgd-7-6265-2010.pdf (this information was forwarded to the editorial office on 29 October 2010 but no help could be provided). In the following we assumed that the page numbering is as following:
Page 1 = BGD page 6267,
Page 2 = BGD page 6268,
Page 3 = BGD page 6269, etc.
Our assumptions of the BGD page and line numbering are highlighted in red.

Specific Comments:

RC 1) *The introduction does not really spell-out the overall goals of this work. On line 12, page 3* (BGD 6269, line 8ff)*, there is an attempt to do this but I would suggest a new paragraph with a more explicit statement of goals to end the introduction section.*

AC: We extended the paragraph to more explicitly state the overall goals.

RC 2a) *On page 4* (BGD 6270) *the method section on pigment extraction and purification makes no reference to any prior literature (e.g., Sachs et al., 1999), does this mean that all of these techniques are original? If so, I would like to see a chromatogram.*

AC: The extraction techniques were developed at JAMSTEC, and we were not able to cite references when we originally submitted this manuscript. As of 23 September 2010 a new paper was published by Tyler et al. (Tracking aquatic change using chlorin - specific carbon and nitrogen isotopes: The last glacial-interglacial transition at Lake Suigetsu, Japan, Geochem. Geophys. Geosyst., 11, Q09010, doi:10.1029/2010GC003186) describing the technique established at JAMSTEC (the datasets were produced simultaneously) and providing chromatograms. We now refer to this paper and added the respective citation.

RC 2b) *Also, on page 5, line 10* (BGD 6271, line 23)*, please elaborate on what is meant by "unprocessed", does this mean they were not decarbonated?*

AC: The samples were submitted unprocessed and not analysed unprocessed. At NOSAMS the ^{14}C measurements were performed using a standard method including acidification, combustion, and graphitization as described in McNichol et al. (1994) and cited on page 6271, line 24. To emphasize this, we have added "including acidification, combustion, and graphitization".

RC 3a) *On page 6* (BGD 6273, line 7)*, the refer to a large assumption they make which involves radiocarbon input from river and atmospheric sources - they are assume they are equal. Does this affect some of issues that arise in section 4.2 where they refer to 3 possible explanations on why the chlorophyll-a and pheophytin C-14 are much lower than the DIC-14? If not, they still need to better justify this assumption.*

AC: We do not assume that the inputs from riverine and atmospheric sources are equal, but we assume that (at a given time) the ^{14}C concentrations in river water and in the atmosphere are equal. The time-integrated input from river discharge $^{14}F_{\mathrm{riv}}$ amounts only about 1‰ of the total atmospheric deposition flux $^{14}F_{\mathrm{air}}$. Therefore, it is unlikely that this assumption affects the issues discussed in Section 4.2.

RC 3b) *Now, back to explanation on why the pigment and DIC differ in radiocarbon. I am not convinced that by comparing the bivalve number, which agree with the DIC C-14, that this eliminates the possibility of a different aged DIC source being used by phytoplankton. It is always assumed that* *calcareous bivalves use similar sources of DIC as phytoplankton in a region? If so, please provide references.*

> **AC:** We agree, in general, it cannot be assumed that bivalves use similar DIC sources as phytoplankton. With respect to ^{14}C DIC sources differ by the water depths phytoplankton and bivalves reside in and the DIC species (whether CO_2 (aq), HCO_3^-, or CO_3^{2-}) taken up by each group. In this particular case, the isotopic fractionation between the DIC species is negligible (see, e.g., Zeebe & Wolf-Gladrow, 2001: CO_2 in seawater: Equilibrium, kinetics, isotopes, Elsevier Oceanography Series, 65, 1-346) compared to the differences in ^{14}C concentrations between the bivalves and the most depleted pigments at the river mouth (>150‰). Accordingly, the water depth may explain such larger differences, however, the water depth the bivalves grew in (P128, 17mbsl and P177, 22mbsl) is coincident with the subsurface chlorophyll *a* maximum depth (Chu et al. 2005) suggesting the DI^{14}C source utilized by both groups was identical.
> Another argument against utilization of different DIC pools is the variability of pigment ^{14}C concentrations at both stations P128 and P177. Phytoplanktonic utilization of hardwater DIC would result in depleted ^{14}C concentrations of all analysed pigments at these two stations, but only chlorophyll *a* (P177) and pheophytin *a* (P128) are depleted while pheophytin *a* and pyropheophytin *a* at P177 and pyropheophytin *a* at P128 reflect post-bomb ^{14}C concentrations, i.e., are not influenced by and hardwater. We additionally added this argument.

RC 3c) *Why was Chlorophyll b and its degradation products not measured, this is a better source for higher plants and something that could be quite useful when they make reference to the terrestrially-derived sources in a paper that we cannot at this is in review in GCA? This again is highly relevant to explanation on possible sources of pigments from vascular plants that are older than the DIC.*

> **AC:** The intention of analysing chlorophyll *a* in this study was not to get a measure of higher plant-derived chlorophyll *a* but phytoplanktonic chlorophyll *a*. We didn't expect to find significant amounts of terrestrial chlorophyll *a* because of the multitude of degradation processes affecting terrestrial chlorophyll *a* (see page 6268 lines 9-14) and the high abundance of marine phytoplankton. This was confirmed by minor amounts of chlorophyll *b* in the samples. Such chlorophyll *b* may, however, not only derive from terrestrial plant but also from phytoplankton (e.g., Vernet and Lorenzen, 1987: The presence of chlorophyll *b* and the estimation of phaeopigments in marine phytoplankton Journal of Plankton Research, 9, 255-265; and reference herein). Irrespective of its origin, the low amount of chlorophyll *b* was not sufficient to perform any compound-specific isotope analysis. Overall, we purified all pigments which were sufficiently abundant in the samples to perform isotope analysis.
> In the GCA paper we refer to on page 6278, line 24 (Kusch et al. 2010) radiocarbon concentrations of leaf wax lipids (long-chain *n*-alkanes and *n*-fatty acids) are presented from core-top sediments around the Black Sea, which were found to be significantly depleted in ^{14}C (up to 7300 ^{14}C yrs BP at the Danube river mouth). Such depletion can be attributed to retention on the continent before final supply to the Black Sea. We use this citation to emphasize that the shelf area in front of the Danube River does indeed receive pre-aged higher plant biomarkers. In comparison to chloro- and pheopigments, however, the leaf wax lipids are highly refractory and survive millennial-scale storage on land as is usually not expected for primary pigments, which instead are converted into a multitude of chlorins (and potentially porphyrins) by loss of functional groups.
> The GCA paper was formally accepted for publication one week after submission of this BG manuscript and was available online since 9 September 2010. Unfortunately the citation guidelines of BG didn't allow to make this fact any clearer than stating the manuscript status as "in review". It is now published in GCA, 74, 7031-7047; doi: 10.1016/j.gca.2010.09.005.

RC 4) *On page 10, line 28* (BGD 6278, line 28)*, they make references to the role of flooding in releasing plant leaves, any data for flooding events in this region to cite?*

> **AC:** Yes, there are flooding data to cite. We have added the citations Jugara Tiron et al. (2009), who mention 89 flooding events on a data set for 160 years (1840–2000) and the 2006 extreme flood, and Bakker (2007), who investigated transboundary floods for a data set of 20 years (1985-2005).

RC 5) *On page 11* (BGD 6280, line 7-10)*, the statement about possible blank carbon, which they "cannot accurately determine" hits the reader like "a ton of bricks." This just comes out of nowhere and adds considerable skepticism about the validity of the data. Now, they do make an adjustment of roughly 1 to 11 µg 14C-free for samples 30 to 230 µg. Where does this number come from? Once they apply, the numbers seems to come in range with the DIC14C, but without any source or justification for this correction factor the reader is left with no basis for accepting this correction factor.*

AC: We have revised the entire paragraph in order to better clarify our interpretation. We regard the Black Sea eutrophication phase the most likely time interval of bomb-^{14}C pigment production. However, the estimated values of the pigment synthesis years (AD1956 to AD1969) slightly deviate from the time period of eutrophication (AD1970 to AD1990). We consider the possibility that this deviation could be caused either by the uncertainties of the ^{14}C measurement and the surface water DIC ^{14}C model (scarcity of input data during the initial incorporation of bomb-14C into the surface water DIC pool) or a potential minor error in the blank C determination. The additional blank carbon amount required to equal the pigment ^{14}C concentrations with the surface water DIC ^{14}C concentrations integrated from AD1970 to AD1990 can be calculated using isotope mass balance defined as

$$^{14}C_{eu} = f_m\, ^{14}C_m + f_{bl}\, ^{14}C_{bl} \qquad (4)$$

where $^{14}C_{eu}$ is the surface water DIC ^{14}C concentration during the eutrophication phase, $^{14}C_m$ is the measured and blank corrected pigment ^{14}C concentration, $^{14}C_{bl}$ is the additional undetermined blank C ^{14}C concentration, and f_m and f_{bl} are the respective fractional contributions with $f_m + f_{bl} = 0$.

This mass balance results in roughly 1 to 11 µg C for samples sizes of approximately 30 µg to 230 µg C required to change the pigment ^{14}C concentrations to values between 100 and 110‰ equalling the surface water DIC ^{14}C concentrations for the period AD1970 to AD1990. However, less blank C would be required to change the pigment ^{14}C concentrations to values equalling the surface water DIC ^{14}C concentrations to very recent values. Nevertheless, this requires heterogeneous blanks. While pyropheophytin *a* would require some ^{14}C-free blank C, all other pigments require modern blank C. The blank C should, however, be the same for all samples since they were purified with the same method. We therefore consider a correction towards very recent ^{14}C concentrations rather unlikely.

In order to avoid any further scepticism potentially arriving from such blank error mass balance, the deleted the calculation and only added a paragraph describing the processes themselves which might lead to potential temporal deviations between the estimated values of the pigment synthesis years and the time period of eutrophication.

RC 6) *Section 4.3 is very confusing and the arguments are not convincing. For example, on page 12 from line 27* (BGD 6281, line 23) *to page 13, line 5* (BGD 6282, line 3)*, there is this confusing argument about the role of seasonality that may be affecting the availability of old versus young DIC, also thrown into this is the location of where phytoplankton might spend time in the water column. First of all, I have no idea when these samples were even taken so I do not know if it is spring or fall. Secondly, the domination of one pheopigment in a particular season (e.g., pryo. mass flux with autumn bloom) should not decouple with its parent chlorophyll, and how does the assumption about 14Riv? Moreover, the discussion continues with speculation about how C14 DIC can vary seasonally in the ocean but that there is no seasonal information for the Black Sea, I am not sure how constructive this discussion is? Also, if the pycnocline is such a barrier how much sediment flux to water affects this seasonality of DIC? This seems to be inferred here, at least to me; or is there something else occurring over different seasonal timescales with DIC?*

AC: We have added the sampling season (March 2008) in paragraphs 2.1 and 4.1 (in case of the latter we address the issue of the sampling season on the seawater δ^{15}N values and sedimentary pigment isotope values. However, since the sediments investigated integrate the mass flux over at least one annual cycle (depending on the sedimentation rates) the sediment sampling season itself is not relevant, since pigments derived from all seasons are present and not only those from the sampling season.

We have reworded the paragraph to clarify the potential role of seasonality on the isotope record. Seasonally varying isotopic compositions of nutrients (δ^{15}N) and DIC (δ^{13}C and Δ^{14}C) could potentially result in differences of the isotopic compositions of the individual pigments extracted from the sediments, if each of them is predominantly derived from a different phytoplankton bloom occurring at different seasons. This implies that the parent chlorophyll *a* molecules are also different for the specific blooms and may isotopically vary. Accordingly, the respective derivatives would vary as well. For the Black Sea, evidence for different pigment fluxes associated with certain phytoplankton blooms is reported in King (1995).

The ^{14}C concentration in seawater is depth-dependent. Therefore, the ^{14}C concentration of DIC utilized by certain plankton groups and, as a result, preserved in their respective pigments, might vary with season caused by seasonal variations of the average dwelling depths of blooming phytoplankton groups. Evidence for such differenced in dwelling depth/subsurface chlorophyll *a* maximum depths have been reported for the Black Sea by Chu et al. (2005). Unfortunately, this assumption cannot be further tested since seasonally resolved DIC ^{14}C data are not available (in fact there hardly are DIC ^{14}C data necessitating the model we obtained).

For ^{14}Riv there is no influence (see comment on RC3a).

The pycnocline should not be a major barrier for pigments (and other biomarkers) since these are most likely exported from the euphotic zone by means of ballast material. Such ballast material includes, e.g., lithogenic mineral grains or faecal pellets packaging the organic matter into larger, dense particles.

RC 7) *Table one is essentially not discussed in the paper and more importantly, as I mentioned earlier it does not provide the month samples were taken.*

AC: We cannot clearly interpret this comment. Both, Table 1 and Table S1 from the Supplement (if that was meant?) are discussed in the paper. Table 1 reports the isotope data and is cited on page 6273, line 21, on page 6274, lines 12 and 21, on page 6277, line 23, on page 6279, line 20, and on page 6281, line 17. Table S1 reports the blank sizes and is cited on page 6271, line 4.

Corrections:

RC 1) *Page 2, line 10* (BGD 6267, line 23?), *should state "much of the sustaining life" when considering the deep vent communities, just as one notable example.*

AC: We address this in the following sentence (page 6267, line 23-26) stating "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).", i.e., explaining that primary production is based on both photo- and chemosynthesis.

RC 2) *Page 2* (BGD 6268), *Eliminate "the" on line 22*

AC: We cannot assign this comment, line 22 on BGD page 6268 does not contain a "the".

RC 3) *Page 2* (BGD 6268, line 13?) *Add "be" before overwhelmingly*

AC: We added "be" and changed "derive" to "derived".

RC 4) *Page 2* (BGD 6268, line 14?) *New paragraph should start with "primary degradation….", this is a problem throughout the text, there are huge paragraphs that go on too long*

AC: We started a new paragraph.

RC 5) *Page 3, line 5* (BGD 6269, line 1?), *insert "to" between 30-50.*

AC: We inserted "to" and deleted the hyphen.

RC 6) *Page 3, line 8* (BGD 6269, line 4?), *should be Sun et al., 1993a,b*

AC: We deleted the space.

RC 7) *Page 3, start new paragraph around line 10* (BGD 6269, line 8?) *to include goals as stated earlier.*

AC: We started a new paragraph.

RC 8) *Page 3, line 22* (BGD 6269, line 18?), *multicore has a hyphen, and what season in 2008, this is really important!*

AC: We cannot assign this comment, in the BGD manuscript is no hyphen. We inserted the sampling month.

RC 9) *Page 3, line 24* (BGD 6269, line 20?), *in "to" between both number ranges*

AC: We inserted "to" and deleted the hyphen.

RC 10) *Page 3, line 28* (BGD 6269, line 25?), *use upper-case for liters (L)*

AC: We changed "l" to "L".

RC 11) *Page 3, line 33* (BGD 6270? line 3?), *it is important to report the type of sonicator and frequency used, the time means nothing when considering the variability in the power of these devices.*

AC: We added that an ultrasonication bath was used.

RC 12) *Page 4* (BGD 6270), *eliminate "The" on line 8*

AC: We cannot assign which "the" in sentence "The hexane-layer was transferred and the extraction procedure was repeated until the hexane-fraction was colorless." is meant?

RC 13) *Page 4* (BGD 6270), *inset spaces between "x" and numbers for dimensions of the columns*

AC: There already are spaces between the "x" and the numbers in the BGD manuscript.

RC 14) *Page 4, Insert new paragraph starting on line 15* (BGD 6270, line 22?) *with "To achieve…"*

AC: We started a new paragraph.

RC 15) *Page 4* (BGD 6270), *Use superscripts to describe flow to be consistent with units throughout manuscript*

AC: We changed the units of flow rates to superscripts.

RC 16) *Page 4, line 30* (BGD 6270, line 3-5?) *eliminate "The" and add "s" to composition*

AC: We infer page 6271, line 3-5 are meant and deleted "the" and added "s" to composition.

RC 17) *Page 5* (BGD 6271), *line 3, no new paragraph…*

AC: We cannot find a new paragraph on page 6271, line 3 in the BGD manuscript.

RC 18) *Page 5, line 13* (BGD 6271, line 26?), *insert "and" after sufficient…*

AC: We added a comma after "sufficient".

RC 19) *Page 5, line 17* (BGD 6272? line 3?), *Stuiver et al., 2010 not listed in refs.*

AC: We added the reference.

RC 20) *Page 5, equation on line 30* (BGD 6272? line 15?) *needs to be numbered "1"*

AC: Equation 1 already is numbered "1" in the BGD manuscript.

RC 21) *Page 5, line 34* (BGD 6272? line 18?), *insert comma after (Godwin, 1962)*

AC: We added a comma.

RC 22) *Page 6, line 14* (BGD 6272, line 16 or 22?), *insert comma after "where"*

AC: We consider adding a comma grammatically incorrect.

RC 23) *Page 6* (BGD 6272), *line 30, delete "an"*

AC: We cannot assign this comment; there is no line 30 on page 6271 in the BGD manuscript.

RC 24) *Page 7, reword line 12* (BGD 6274? line 13?) *to be "at the mouth of the… "in front of" is not appropriate*

AC: We reworded this.

RC 25) *Page 7, line 22* (BGD 6274? line 23?), *Same as on line 12*

AC: We reworded this.

RC 26) *Page 8, line 22* (BGD 6274, line?), *insert "the" after In*

AC: We cannot assign this comment; there is no "in" on pages 6274/6275/6276, line 22 in the BGD manuscript.

RC 27) *Page 8, line 23* (BGD 6276? line 7?), *omit "potential small"*

AC: We deleted “potential small”.

RC 28) *Page 8, line 32* (BGD 6276? line 16-17?), *eliminate “the” before nitrogenous and make source plural*

AC: We changed “source” to “sources”.

RC 29) *Page 8, line 33* (BGD 6276? line 17-19?), *eliminate “be”, change reflected to reflect and add an “and” after values, change resulting to result and in line 34, change from to in*

AC: We have reorganized this sentence in accordance to RC11 of reviewer #1.

RC 30) *Page 9, line 7* (BGD 6276? line 27-28?), *change to mouth not in front of*

AC: We reworded this.

RC 31) *Page 9, line 20* (BGD 6277? line 11?) *insert hyphen into timescales*

AC: We inserted a hyphen here and, thus, throughout the manuscript.

RC 32) *Page 9, line 35* (BGD 6277? line 27?), *any idea about the age of these carbonaceous rocks, and delete “the”*

AC: These rocks will have an infinite ^{14}C age, i.e., do not contain any measurable radiocarbon. Thus, a contribution of carbonate-derived DIC would bias the ^{14}C concentration of DIC towards “old” ages (“hardwater effect”). As long as the carbonates are older than ~50,000 years, which is a save assumption for most consolidated carbonate rocks outcropping in mountain ranges, the precise age is irrelevant for the radiocarbon hardwater effect.
We deleted “the”.

RC 33) *Page 10, line 1* (BGD 6277? line 28?), *insert comma after phytoplankton*

AC: We added a comma.

RC 34) *Page 10, line 6* (BGD 6278? line 5?), *omit “area”*

AC: We changed “…blooms in the near-shore area…” to “…blooms at the river mouth…”.

RC 35) *Page 10, line 7* (BGD 6278? line 6?), *start new paragraph with Aggregation…*

AC: We started a new paragraph.

RC 36) *Page 11, line 2* (BGD 6279? line 8?), *omit “area”*

AC: We deleted “area”.

RC 37) *Page 11, line 13* (BGD 6279? line 20?), *insert comma after (Tab 1)*

AC: We added a comma.

RC 38) *Page 11, line 14* (BGD 6279? line 21?), *replace have with “are’*

AC: We replaced “have” by “were”.

RC 39) *Page 11, line 21* (BGD 6279? line 28?), *Insert new paragraph starting with “the modeled…”*

AC: We started a new paragraph.

RC 40) *Page 11, line 27* (BGD 6280? line 6?), *delete “also”*

AC: We deleted “also”.

RC 41) *Page 11, line 31* (BGD 6280? line 11?), *insert “to” between number range*

AC: We inserted “to” and deleted the hyphen.

RC 42) *Page 12, line 2* (BGD 6280, line 17?), *insert “and” after quantity*

AC: Adding “and” would obscure the meaning of the sentence.

RC 43) *Page 12, line 5* (BGD 6280? line 20?)*, delete "the" both times*

AC: We deleted "the".

RC 44) *Page 12, line 28* (BGD 6281? line 18?)*, reword sentence that begins with "However, …*

AC: "However, other than expected pyropheophytin *a* is younger than chlorophyll *a* and pheophytin *a*" was changed to "In contrast to what would be expected, pyropheophytin *a* is younger than chlorophyll *a* and pheophytin *a*."

RC 45) *Page 13, line 4* (BGD 6282? line 2-3?)*, insert 4 after (Chu et al., 2005)*

AC: We cannot interpret this comment. The citation is Chu et al. 2005, not Chu et al. 2004.

RC 46) *Page 13, line 6* (BGD 6282? line 4?)*, insert "in the Danube delta" after "pigments"*

AC: The stable carbon and nitrogen isotopic compositions of the individual pigments reflect the conditions at the time of synthesis not only in the Danube delta but for all stations. We therefore refrain from adding "in the Danube delta".

RC 47) *Page 13, line 10* (BGD 6282? line 8?)*, insert comma after "pathway"*

AC: We added a comma.

RC 48) *Page 13, line 18* (BGD 6282? line 17?)*, delete "apparently" and insert comma after Sea*

AC: We rephrased the entire sentence in accordance to RC 15 of reviewer #1.

RC 49) *Page 13, line 34* (BGD 6283? line 6?)*, delete "the"*

AC: We deleted "the".