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Biogeosciences Discussions

Interactive comment on "Heterotrophic denitrification vs. autotrophic anammox – quantifying collateral effects on the oceanic carbon cycle" *by* W. Koeve and P. Kähler

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We thank the reviewers and the editor for their thorough work and comments on our manuscript. All three reviewers seem to be generally content with our approach, but add interesting details. We will, however, not include every suggestion in our revision, the reasons being the following.

We use only four definitions of suboxic nitrogen transformations to develop our points. We know that there are numerous additional pathways (incomplete reactions, short-cuts, combinations, by-reactions) in the nitrogen cycle, which could each add interesting aspects, but would unnecessarily complicate the manuscript. Compared with the

exposition of our treatment (denitrification is heterotrophic and anammox autotrophic) we develop the question of their trophic status in as complex a system as we think is necessary to resolve the question, but not as complex as it may be in certain special, or any possible, natural situations. We confine the treatment to oceanic oxygen minimum zones.

We show in our study that the difference between denitrification and anammox in trophic status (considered to be of general interest and of importance) cannot be evaluated without considering other N transformations which, when included, change the simple picture. Adding more complexity will not necessarily improve the quality of this conclusion, but load the manuscript with lots of supplementary detail (which may though be important in other contexts). We will add a passage in our text to explain this.

Reviewer 1:

Reviewer: ... This is a well-written paper, which, beyond merely correcting an apparent mistake, provides a useful theoretical framework for understanding the stoichiometric aspects associated with the ongoing revision of our understanding of the marine nitrogen cycle with particular relevance in the light of expanding OMZs. My only general remark is that the authors should consider further stoichiometric constraints that can be deduced from the natural systems. Specifically, the ratio of nitrite accumulation to nitrate consumption which is used as master variable in the plots does not seem to reach values close to 1 in OMZs, and therefore the more extreme values of, e.g., $\Delta CO_2/\Delta N_2$, which are attained at high nitrite/nitrate ratios, are probably not realistic. I have not checked the paper but I believe that Anderson and co-workers (Deep-Sea Res. I, 29:1113-1140, 1982) concluded that the ratio never exceeds 0.7 (i.e., nitrite accumulation is always associated with some DIN deficiency, for some reason), which constrains many of the parameters to a more "boring" range. The extreme values could, e.g., be shaded in the plots.

Response: In writing the paper we recognized this issue ourselves. In our computations NO_2-accumulated and NO_3-consumed are always perfectly known, hence is their ratio. It the real ocean, however, this is only the case for NO_2-accumulated but not for NO_3-consumed and therefore the ratio is quite uncertain. At the time of submitting the paper, we where convinced that estimates of NO_3-consumed in the real ocean may be uncertain by a factor of 2 (see Devol et al., 2006, Deep-Sea Res.; comparing N_{2xs} and NO_3-deficits computed from observed NO_3, PO_4 and some N:P assumption). Earlier this year, at the Portland OS10 meeting, it became clear, that this uncertainly is perhaps much lower.

We will add a short section discussing the most likely ranges of observed NO_2-accumulated/NO_3-consumed.

Specific comments:

Reviewer: p. 1816 l. 13: DNRN, denitrification, and DNRA are not always heterotrophic processes as claimed here but corrected in section 2.3.

Response: There is a multitude of definitions and usage of the term ‘denitrification’ in the literature. This partly has historical reasons. We use denitrification in the sense of strict (heterotrophic) denitrification and as specified in the equation 2 of Table 1. It is beyond the scope of this paper to discuss in detail the variations of usage of this term in literature. The same applies to DNRN and DNRA, they are used as defined in the table. We are of course aware of processes like nitriate ammonification with H_2 or HS- as electron donors in highly reduced environment. However, to our understanding dissimilatory is clearly associated with the use (breakdown) of reduced organic carbon as energy and carbon source and is hence heterotrophic.

Reviewer: p. 1818: The stoichiometry of autotrophic CO_2 fixation by anammox bacteria (and nitrifiers) is likely not fixed due to the energy requirements of maintenance. It seems that the value of 0.07 determined under substrate replete conditions may be a maximum relative to the oligotrophic conditions found in natural waters.

Response: We agree. This will be mentioned. (See also our response to Reviewer 2 discussing the proposed stoichiometry of anammox from Strous et al., 1998)

Reviewer: p. 1825: I don’t understand the “thermodynamic” argument concerning the relative importance of nitrification and anammox. But Lam and coworkers (PNAS 2007, doi:10.1073/pnas.0611081104) observed experimentally the co-ocurrence of nitrification and anammox in the Black Sea.

Response: You refer to page 1822, we guess. Assuming that anammox is limited by the supply of ammonium in the core of the OMZ, this section discusses whether the primary ammonium maximum at the base of the euphotic zone may be a possible source of ammonium via diffusive transports. We argue that more likely aerobic nitrification will oxidize the NH_4 to NO_2 (and perhaps NO_3), which then may diffuse into the suboxic layer, where anammox may act on it. This is in good agreement with Lam et al. 2007 who show (their fig. 2d) that nitrification in their Black Sea study mainly occurs about 10m above the upper boundary of the suboxic layer, while both ammonia and NO_x consumption show a clear peak well inside this layer, about 20-25m below the nitrification maximum. It is in this layer, where they find anammox bacteria and where they measure anaerobic ammonium oxidation. In the case of the Black Sea (e.g. Lam et al. 2007) ammonium from the anoxic layer below the suboxic zone diffuses into the suboxic layer, while nitrate and nitrite, originating from nitrification enter the suboxic zone from above. Both appear to feed anammox in the suboxic layer.

Reviewer: Figures: The ratio nitrite(accum):nitrate(deficit) is referred to as the N-conversion efficiency. This is confusing when 1 indicates inefficient and 0 efficient conversion, respectively. Please find another name for this term.

Response: This is a good argument. We will change the plots (plotting 1 - NO_2-accumulated/NO_3-consumed) to reflect the term efficiency more closely.

Reviewer 2

Reviewer: ... Given that complete correct stoichiometry is one of the main goals of the paper, it seemed strange that anammox was represented without any overt carbon stoichiometry. Why not use the complete equation presented by Kartal et al (2008)? In Table 1, the authors state that the very small amount of C fixed per N oxidized allows this to be ignored, and that the 0.07 N:C ratio is included in the model. It's probably true that this small C sink makes no difference to the calculation, but it is the essence of the autotrophic argument and to my mind, omitting it undermines the argument.

Kartal et al equations:

$1NH_4$ + $1.32NO_2$ + $0.066HCO_3$ + 0.13H+ $\rightarrow$ $1.02N_2$ + $0.26NO_3$ + $0.066CH_2O_{0.5}N_{0.15}$ + $2.03H_2O$ (1)

NH_4 + NO_2 $\rightarrow$ N_2 + $2H_2O$ (2)

$0.26NO_2$ + $0.066HCO_3$ $\rightarrow$ $0.26NO_3$ + $0.066CH_2O_{0.5}N_{0.15}$ (3)

The Kartal et al. equations also include the oxidation of nitrite to nitrate, which supplies the reducing power for CO_2 assimilation in the anammox organisms. Is this additional NO_2 sink and NO_3 source included in the model? It is a rather substantial effect on the NO_3 balance, in that for 1.32 NO_2 consumed, 0.26 NO_3 is produced along with 1.02 N_2.

Response: We could not locate the paper by Kartal et al. 2008, to which the reviewer refers (i.e. which discusses this stoichiometry in more detail). However, the given equation is based on experimental work by Strous et al. 1998 (Appl. Microb. Biotechnol.) and discussed further in Kuenen, 2008 (Nature Rev. Microbiol.). We referred to the Strous et al. paper as the source of our NH_4:CO_2 ratio. Strous et al. presented results from experiments under nutrient replete conditions in a growth reactor, which yielded a contribution of anammox bacteria of 70% of the total biomass. As mentioned by reviewer 1, the CO_2 fixation per NH_4 consumed (0.066 mol:mol), likely depends on the growth conditions. With less favourable growth conditions most of the energy

gained from the reaction of NH_4 and NO_2 will be used for maintainance and will therefore not be available for CO_2 fixation. This will reduce the CO_2-fixed:NH_4-consumed ratio accordingly. This will not affect the results presented in our paper since, e.g. the plots from figs 2a and 3b (ΔCO_2:ΔN_2) will not change noticeable when CO_2-fixed:NH_4-consumed goes against zero. Unfortunately, there are no data from experiments under marine conditions, that could help to constrain this further. This uncertainty was one reason, which prevented us from using this explicit stoichiometry instead of equation 3 (Table 1, this formula is also used throughout the literature on anammox in the oceans). Changing the CO_2-fixed:NH_4-consumed ratio in the equation given above also changes the ratio of NO_2-consumed to NO_3-produced of these equations and this will affect our x-axes.

In the final version of the manuscript we will discuss this issue, refer to the nitrate production process as suggested by Kuenen (2008) and the reviewer, and finally we will analyse the sensitivity of ΔCO_2:ΔN_2 to the uncertainty of these two ratios (CO_2-fixed : NH_4-consumed, NO_2-consumed : NO_3-produced .

Reviewer: One very useful purpose of this paper is to explore the theoretical possibilities, which includes evaluating the full range of ratios of denitrification to anammox and the full range of "denitrification efficiency". It would, however, be useful to the reader to point out the range of values that are actually encountered in nature. For the major OMZs, the value of the ratio NO_2 accumulated to NO_3 removed is often around 0.5. Just to present a few values, perhaps as marks on the plots, would help orient the reader and make the significance of the exercise a bit easier to comprehend.

Response: See detailed response to Reviewer 1. We will add a short section discussing the most likely ranges of observed NO_2-accumulated/NO_3-consumed.

Reviewer: I found the axes labels somewhat confusing. For example, in Figure 3, I thought at first that the slashes in the Y axis labels were functional, implying division by a denominator. Then I figured out, I think, that the text below the slash is actually a unit, an explanation

Response: We will improve the readability of the figures.

Reviewer 3

Reviewer: ... I will ask the authors to be more cautious stating the assumptions at the beginning of the paper, and not only during the Discussion section. In particular, the analysis is relevant mainly in the suboxic layer, and without considering the autotrophic photosynthetic activity in the OMZ layer (e.g. in situ organic matter production associated with the secondary peak of fluorescence and with Prochlorococcus and Synechococcus: e.g. Liu et al., 1998), and of course in the surface layer. The analysis is based on no accumulation of NH_4^+ but in accumulation of NO_2^-, which makes sense in general for the OMZs, but configurations with accumulation of NH_4^+ and/or no accumulation of NO_2^- could also occur in the OMZs. In addition, intermediate chemical forms of the nitrogen cycle, other than NO_2^- (e.g. hydrazine) could play a non-negligible role in the coupling of DNRN and anammox, for instance. What would be the consequences in terms of degree of heterotrophy and ΔCO_2:ΔN_2 ratio?

Response: We agree. To help the reader we will clarify early in the paper that our approach is to (a) start with a clear and simple case, and that that case is representative for the core of the OMZ; and (b) afterwards discuss the more complex situation at the OMZ boundaries.

Reviewer: Also, because the paper deals with a theoretical study, comments or comparisons with "real" observations will be appreciated.

Response: There are no data which allow a direct assessment of ΔCO_2: ΔN_2 production ratios which we could compare with. We already mention/discuss part of the ocean literature on anammox, as it guided the design of our experiments. As mentioned above (Rev.2) we will add a short section on realistic NO_2-accumulated:NO_3-consumed ratios to show which parts of our plots are most likely to apply in real situations.

Reviewer: *Abstract:*

- "Here, we . . .in marine oxygen minimum zones (OMZ) . . ..": mention that the study are focusing on the OMZ core (and not the oxycline where, for instance, nitrification is a very important process coupled to denitrification, known as nitrifier-denitrification).

Response: See our response above.

Reviewer: - Be more explicit with the ΔCO_2:ΔN_2 ratio: e.g. CO_2 release versus N_2 produced (here, the authors are not considering nitrogen fixation). Also with the term "nitrogen conversion": e.g. nitrite accumulated versus nitrate consumed.

Response: Ok.

Reviewer: 1. *Introduction:* - A general important comment. There is two different and distinguishable effects of the nitrogen loss on CO_2: 1) a direct effect corresponding to the topic of the paper, i.e. the autotrophic versus heterotrophic consuming and producing CO_2; 2) an indirect effect, through the nitrogen deficit, inducing less primary production (locally and/or at global scale), and then less CO_2 sequestration and carbon export. Whether the second effect could be largely more significant than the first one, is also a key-question. In your introduction, specify how the "Temporal changes of the nitrogen removal flux, .. are thought to influence the level of oceanic production and associated CO_2 fluxes", according to the authors mentioned (Altabet et al., 1995; Ganeshram et al., 1995: Codispoti, 1995). I remembered that these authors are mentioning the second indirect effect, and not the first direct effect here analysed. In addition, related to the second effect, the predominance of denitrification, and DNRA over anammox could also have an indirect effect on the local surface primary production (PP), beneficial to a NH_4-stimulated PP rather a NO_3-stimulated PP.

Response: We will clarify the indirect effect on PP in adding a more detailed sentence on glacial-interglacial variatons of denitrification and possible impact on global N-inventories and PP. We regard the aspect of NH_4-stimulated vs.NO_3-stimulated PP of little significance to our paper. We could only speculate here, but generally think that if phytoplankton is N-limited its primary production will not depend on the N-species, except perhaps under very low light conditions. This is outside the focus of our current paper.

- Line 6, correct the typo “intoN_2” into“into N_2”.

Response: Ok.

Reviewer: *2.1. Background and definitions*:

- Denitrification and DNRA are not always heterotrophic. Also maybe here, or in the conclusion, you could mention that recent studies suggest that anammox bacteria could reduce itself nitrate into nitrite from organic acids (e.g. Den Campf et al., 2006), i.e. could be heterotrophic. In that case, DNRN will be still performed heterotrophically, but by anammox bacteria and with different stoichiometry. In addition, some denitrifying bacteria could have anammoxosomes (e.g. Hu et al., 2006) and could use ammonium and nitrite, i.e. a scenario similar to scenario II (DNRN+A+DNRA). This is not affecting the conclusion of the paper.

Response: For us anammox, denitrification, DNRN, DNRA are processes. When combining for example DNRA and anammox in our computations, it does not matter whether this takes place inside one organism or in different ones, as long as it takes place in relative proximity, i.e. in the same water. If some of the archaea performing anammox use certain organics quantitatively (and if these organics are available in the ocean and not only in the experiments) these anammox bacteria are clearly heterotrophic. As the reviewer states, this is not affecting the conclusion of the paper.

Reviewer: - About the historical presentation of the anammox, you can add that Hamm and Thompson (1941) are the first to write the anammox chemical equation.

Response: We try to get a copy of that paper (no digital version is available unfortunately), check it, and cite if appropriate.

(HAMM, R . E., AND T. G. THOMPSON, 1941. Dissolved nitrogen in the sea water of the Northeast Pacific with notes on the total carbon dioxide and the dissolved oxygen. J. Marine Res.. 4 : 11-27.)

Reviewer: - Lines 14-18, pages 1817: here, the hypothesis and its statement are very strong, even if this hypothesis is discussed latter in the Discussion section (Cf my general comments). For instance, Lam et al. (2009) estimate that 33% of the nitrite is produced by nitrification, and a large part of the ammonium by micro-aerobic respiration. This hypothesis is correct, if you specify that this analysis is focused on the suboxic OMZ core layer.

Response: As we stated above, we will add an explanation of the structure/approach/range of our paper.

Reviewer: *2.2. Stoichiometric constraints:* - R1: why not a more simple equation with HNO_3 and CO_2, as in Table 1, and since you are not commenting any carbonate effect.

Response: Equations in table 1 are generic ones, i.e. can be used for any composition of organic matter. That is very convenient for our computations which provide the plots presented in the paper. However, to provide an easy start into the computations and a first order estimate of ΔCO_2: ΔN_2, we use the explicit equation R1, in which the reader can recognize the ratio immediately. Our choice is one from the literature which uses the Anderson organic matter composition. This is in the form given and it would not be good citation style to change this equation to make it look more similar to those used in Table 1. Therefore we will keep the current form.

Reviewer:- Lines 23-24, pages 1818: here, the hypothesis and its statement are again very strong, even if this hypothesis is discussed latter in the Discussion section (Cf my general comments).

Response: As stated above, we will explain the approach and range of our paper more thoroughly in the revision. We where surprised to see that NH_4 is not always reported in detail in studies from oxygen minimum zones in general, or of anammox. However, the data presented e.g. in Kuypers et al., 2005, PNAS; Kuypers et al., 2003, Nature; Thamdrup et al., 2006, Limnol. Oceanogr. from the Benguela upwelling, the Black Sea and off Chile, respectively, support our statement that NH_4 does not accumulate in suboxic waters. This is in particular the case when regarding these waters to be those to which nitrogen loss processes are confined (p1816, line 19-20, Devol, 2008.)

Reviewer: - Line 14-15, page 1819: instead of "indistinguishable", "not significantly different" is

maybe more correct. - Line 25, page 1819: "inefficiency" seems more correct than "efficiency".

Response: We will re-plot with x-axes showing (1 - NO_2-accumulated:NO_3-consumed), making "efficiency" a correct term.

Reviewer: - Line 29, page 1819: not directly clear on Fig. 2a. Clearer on Fig. 5.

Response: Will be left as is, the reference to the figure is only to the last part of the sentence, not to 'goes along with', this is obvious when comparing Fig. 2a and 1a, as in fact shown in Fig. 5, presented later in the text.

Reviewer: -Line 2, page 1820: after ". . . N_2-production", add maybe Fig. 2a (or Fig. 5).

Response: Ok.

Reviewer: - Line 3, page 1820: instead of Fig. 2b, would be clearer on a figure "N_2 fraction from anammox versus N_2fraction from N_{org}".

Response: We don't think that an additional figure is necessary here.

Reviewer: - Line14, page 1820: "assuming" is perhaps better than "using".

Response: Perhaps.

Reviewer: 2.3. Allochthonous substrate sources: - Line 25, page 1822: kinetic grounds are also important.

Response: We may try to explore this issue in more detail in the final version of the paper. However, there is the problem that most of the ^{15}N-isotope experiments on nitrification as well as on anammox likely give only potential rates (Hamersley et al. (2007, Limnol. Oceanogr.), hence little can be said about kinetics here, apart from speculations.

Reviewer: - Lines 28-29, page 1822: anammox and nitrification often are co-existing in the OMZs (e.g. Lam et al., 2008).

Response: Lam et al. (2008, Geochim Cosmochim Acta, 72, 2268) is on microbial ammonia oxidation in a hydrothermal plume. We guess the reviewer refers to Lam et al. (2009, PNAS). Lam et al. (2009) refer to Hamersley et al. (2007) for experimental details. These authors state (p. 931) that their experiments 'where made under conditions of excess substrate availability, and we did not attempt to derive in situ anammox rates from them'. We conclude that the rates given in Lam et al. 2009 are potential rates as well. From this it is very difficult to quantify whether anammox and nitrification really co-exist (in a given liter of seawater) in the real ocean. Experimental work with tracer additions that do not violate fundamentals of tracer work (i.e. not to change the ambient substrate concentration significantly) are very much in need here.

Reviewer: *3. Discussion*: - The discussion, focused in the aphotic zone, is very interesting. Maybe add at the end (Lines18-22, page 1825) "in the aphotic zone".

Response: Ok.

Reviewer: *References*: - Bange et al. (1996) and Silva et al. (2009) are not cited in the text.

Response: Ok.

Reviewer: *Tables and Figures*: - Table 1: why S in the bulk organic matter, without comment about S in the text? In addition, because the paper is focused on the effects on CO_2, it will be better to include CO_2 in equation (3) of the anammox, even if the effect is negligible and not visible on Figures 2a, 3b and 5.

Response: There are other applications of the generic equations given in Tab. 1 where S matters. These will be published elsewhere, but we plan to refer to this paper for reference. Including CO_2 and organic matter in equation (3) explicitly requires an electron donor. As we have explained in detail in response to reviewer 2 we will discuss a generic version of the complete stoichiometry proposed by Strous et al., 1998 in the final version of the paper.

Reviewer: - Table 2: in the caption, add after “scenario I”, “with DNRN, denitrification and anammox”. For the 2nd column, why are you not using the notation “DNRN:den” instead of “den: DNRN”?

Response: We will clarify both points in the final version of the paper.

Reviewer: For the 4th column, write “N_2-anammox:Total N_2-production” instead of “ Anammox:N2-production”.

Response: We agree.

Reviewer: In footnote a, use the same notation than for X-axis of the figures (cf remark for the figures): I suggest “NO_2- produced (DNRN) to mol NO_2- CONSUMED (denitrification)” instead of “NO_2- produced (DNRN) to mol NO_2- USED (denitrification)”.

Response: We agree.

Reviewer: - For the figures, give information, if it is possible, about where the “real system” is. And also be more explicit with the axis title, using the same notations than in the text. E.g., use “NO_2 accumulated / NO_3 consumed”, instead of “NO_2 (accum) / NO_3 (deficit), since NO_3 deficit classically deals with a deficit involving directly the phosphate concentration. Here, I understood that it deals with the moles of NO_3- consumed for 1 mole of phosphate released.

Response: It is obviously a good idea to distinguish better between our computed term and the use of NO_3-deficit in the open ocean literature. As suggested, we will use ‘NO_3-consumed’, accordingly. All reviewers requested that we mention where the real system is and we will discuss this in the text.

Interactive comment on Biogeosciences Discuss., 7, 1813, 2010.