

The authors addressed the comments and questions as described, below. Changes were marked in yellow, green mark-ups denote modifications that were applied to previously revised parts.

Response to reviewer I

The authors thank Referee #1 for regarding this manuscript interesting. We addressed the comments and suggestions that were made, which we considered very helpful and informative. Please find our detailed point-by-point answers in the following, changes were highlighted in the text:

Specific comments:

p. 4497, line 6. “among” vs “between”?

We changed between to among.

p. 4498, lines 11-13. You switch between μM and $\mu\text{mol L}^{-1}$. Pick one.

We changed to $\mu\text{mol L}^{-1}$ throughout the manuscript.

p. 4499, lines 6-7. Something missing here. Reword. line 21. “among” vs “between”

We changed the sentence, it now reads: ‘The effects of O_2 -dependent nutrient cycling processes occurring in these relatively small regions (Codispoti, 2010) are conveyed to the rest of the ocean (see e.g. Deutsch et al. (2007)).’

We changed between to among.

p. 4501, line 29 “during daytime” or “at daybreak”? p. 4502, line 5. “during daytime”

We now wrote ‘during daytime’.

p. 4506, lines 2-6. Sounds overly dramatic. Temperature shifts will be gradual- not likely a step function which would induce massive lysogeny.

This is true. It may be worth to think about certain thresholds with regard to temperature dependent lysogeny. However, reviewer II suggested restructuring the whole paragraph on viruses in OMZs, which we did, and the respective sentence now reads:

‘If lysogeny is the prevailing mode of existence in OMZ core viruses, one would expect changing environmental conditions such as temperature shifts (Bertani and Nice, 1954; Seeley and Primrose, 1980) to induce lysis of host cells. This would consequently lead to shifts in water column nutrient budgets that cannot be accounted for in biogeochemical models by microbial processes alone.’

p. 4507, line 23. Delete “are” in this sentence.

‘Are’ has been deleted.

p. 4509, line 8. How is BATS a "less intense OMZ area"?

We rephrased this, it now reads: 'In a much less intense OMZ area (e. g. in the tropical Atlantic around the Bermuda Atlantic Time Series Station), DVM-related transport was found to account for 30% of C and 57% of N export from the euphotic zone, relative to trap particulate C and N (Steinberg et al., 2002).'

p. 4512, line 14-15. Arabian Sea- what about Ward et al. 2009 who contend denitrification is dominant in the Arabian Sea? Nature 461, 78-81 doi:10.1038/nature08276

This is true! We included the finding of Ward et al. and referenced it as follows:

Moreover, N is (i) lost by denitrification (the 4-step reduction of NO_3^- to N_2 (Devol, 2008)), which has been identified as the dominant N loss process in the Arabian Sea OMZ (Ward et al., 2009), or (ii) recycled by both DNRA (the **d**issimilatory **n**itrate **r**eduction to **a**mmonia, as hypothesized by (Lam et al., 2009)) and nitrification (the aerobic oxidation of ammonia via NO_2^- to NO_3^- under oxic to suboxic conditions (Ward, 2008)).'

lines 15-18. Could be better worded- “: : lost by denitrification or anammox or recycled by DNRA or nitrification”.

We agree and rephrased this sentence, it now reads: 'Moreover, N is (i) lost by denitrification (the 4-step reduction of NO_3^- to N_2 (Devol, 2008)), which has been identified as the dominant N loss process in the Arabian Sea OMZ (Ward et al., 2009), or (ii) recycled by both DNRA (the **d**issimilatory **n**itrate **r**eduction to **a**mmonia, as hypothesized by (Lam et al., 2009)) and nitrification (the aerobic oxidation of ammonia via NO_2^- to NO_3^- under oxic to suboxic conditions (Ward, 2008)).'

p. 4513, line 24-25. Previously you quote an upper boundary of 20 μM (p. 4501, lines 16-17). In the next paragraph on p. 4514 you use 25 μM as the upper limit.

In order to unify the borders and definitions of oxic, suboxic and anoxic and the ranges that were interpreted limiting for biogeochemical processes from our combined results, we included a table into the introduction. Based on this, we unified the definitions throughout the text.

Lines 18-19. You said this on the last page (4512).

The statement on N turnover in the ETNA has been removed, here.

p. 4514, line 14-16. How can “ratios” be a source of a nutrient?

We agree, this of course can't be, we changed it from 'N:P ratios' to 'excess phosphorous'.

p. 4515, lines 2-3. Please note: Dekaezemacker, J. et al. (2013); Bonnet, S., et al. (2013) and Turk-Kubo, K. A., et al. (2013).

Thanks for this input- we added all three references to the text.

Line 3. Whose unpublished data?

We added the missing information: Joshi and Löscher, unpublished.

Lines 15-21. Also should mention the recent kerfuffle regarding contaminated N₂ stocks Dabundo, R., et al. (2014).

Yes, this is true and an important topic when talking about diazotrophy in terms of rates. Overall, all of us that have used contaminated or potentially contaminated gas stocks may have misinterpreted their data. Thus we added the following explanation:

‘A very recent study however demonstrated, that N₂ fixation rates may have largely been misinterpreted as the applied gas stocks were to different degrees contaminated with other ¹⁵N compounds, such as nitrate or ammonia (Dabundo et al., 2014). This study raised concern about all previously generated N₂ fixation rates.’

p. 4516, lines 27-28. Not sure I fully get this. For both anammox and nitrification through regeneration of NH₄⁺?

This may indeed happen at certain oxygen ranges; Kalvelage et al., 2011, showed a strong overlap of both processes at O₂ ranges between ~5 and 20 μmol kg⁻¹. The presence of organic matter in particulate form has very recently been shown, by Ganesh et, 2015 in ISMEj, to promote both processes, and it has been suggested that ammonia availability is the reason for this. We added this reference to the text:

‘Additionally, the strong correlation between nitrification and anammox activity to the modeled export production rates (Kalvelage et al., 2011) indicates an impact of organic matter supply also for autotrophic N-cycling processes, which has been suggested to result from ammonia availability (Ganesh et al., 2015).’

p. 4517, line 16. “Oxygenic” means O₂ producing. Perhaps use “oxic” or “aerobic”?

We changed this to ‘aerobic’.

lines 24-26 and then some on the next page. Seems a redundant passage to earlier discussions.

We shortened the repetitive statements.

p. 4518, lines 7-10. Needs to be explained more fully.

We now introduced the feedback effects as proposed by Landolfi and by Canfield and discuss positive vs. negative feedbacks in this section:

‘Model studies, however, show that denitrification of N₂ fixation-derived organic matter may lead to a net N loss that further stimulates N₂ fixation, because 120 moles of nitrate per mole of phosphorus are used to remineralize Redfield organic matter via denitrification (Landolfi et al., 2013). In contrast, N₂

fixation fixes only 16 moles N (per mole P). Because of those stoichiometric constraints, denitrification of newly fixed N would lead to a net loss of N, which would then enhance the N deficit, promoting further N₂ fixation, a cycle that ultimately leads to a runaway N loss (Landolfi et al., 2013). Only by decoupling N₂ fixation and N loss, e.g. by iron limitation or dissolved organic matter cycling, the N inventory may stabilize, otherwise the OMZ would become completely void of fixed inorganic N and the OMZ sulfidic conditions would potentially evolve.'

Lines 18-20. Should clarify – from an “N” cycle perspective, or N:P ratios, N₂ fixation should be a negative (stabilizing) feedback compensating for N losses as proposed by Deutsch et al. Indeed, the N₂ fixation may be a positive feedback within the OMZ with respect to O₂.

This is a very interesting topic to me; I included some additional discussion on feedback effects (see also comments above) and discussed this topic a bit more detailed from an N cycle perspective.

p. 4520, lines 6-9. See also Dekaezemacker et al. (above) for their 10oS transect to 100oW

We included this reference and their results on Fe and inorganic carbon stimulation of N₂ fixation.

Lines 16-19. Perhaps something else is constraining diazotrophic cyanobacteria here (e.g. Fe). See also Turk-Kubo et al. and Bonnet et al. (above)

For sure, but comparing our bioassay experiments to the ones of Julien Dekaezemacker, we found large variability. It could be speculated that it is a combination of factors, such as Fe availability along with organic matter and/or P. We included the references and their results on Fe-dependent N₂ fixation.

p. 4521, lines 8-9. This point here is unclear to me. Clarify.

We rephrased this sentence, it now reads:

'However, one of the predictions of the optimality-based model of N₂ fixation by Pahlow et al. (2013), which is based on the assumption that natural selection should tend to produce organisms optimally adapted to their environment, is that the competitive advantage of diazotrophs is most pronounced under conditions of low DIN and increased DIP availability (Houlton et al., 2008). The ability to compete for DIP is less important at high DIP; based on this, high phosphate concentrations above the ETSP OMZ might actually reduce the selective advantage of diazotrophs compared to ordinary phytoplankton.'

p. 4522, lines 14-15. Perhaps cite the early Dugdale et al. 1977 observation here?

We included this reference as follows, again thanks for this hint!

'An early observation from the Peruvian OMZ brought the development of H₂S into context with full denitrification (Dugdale et al., 1977).'

Line 23. “Diffusive” vs “diffuse”?

We changed ‘diffuse’ to ‘diffusive’.

p. 4525, line 16. To “increase” rather than to “be increasing”?

This has been changed, accordingly.

line 18. Do you mean “>” rather than “<” here? How is this level of O₂ “classical”? A reference might help. Line 23. “: remains to be proven”. Is there a suggestion that anammox and/ or DNRA produces N₂O? References. Or change to “assessed” or “demonstrated”.

Thanks for this hint, we modified the sentence and added the following references:

‘The production of N₂O by archaea (and bacteria) depends on dissolved O₂ concentrations and is increasing with decreasing O₂ concentrations (Frame and Casciotti, 2010; Löscher et al., 2012). Denitrifying bacteria do not produce N₂O in the presence of O₂ (> 10 μmol L⁻¹); however, when O₂ concentrations are approaching 0 μmol L⁻¹, N₂O is consumed during denitrification. There is no N₂O production under anoxic conditions. The significance of N₂O production during anammox (Kartal et al., 2007) and DNRA (Giblin et al., 2013) in OMZ (see section 5) remains to be proven.’

p. 4526. This observation goes pretty far back- Firestone’s work in soil. Also see Cohen, Y. (1978), Consumption of dissolved nitrous oxide in an anoxic basis, Saanich Inlet, British Columbia., Nature, 272, 235-237

We are aware of Firestones work but we considered the Cohen reference on the Saanich Inlet more adequate and included into the text - thanks for this hint.

p. 4529. Line 17. “Classical” is an odd word to use with Anammox.

Probably true, we removed ‘classical’.

Response to reviewer II

As several points were criticized concerning the structure and readability of this manuscript, we restructured it. Major changes were made by merging the section on remineralization with the N cycle section; in addition, the manuscript was shortened considerably. Of course, this is a product of different authors, however, we now tried to integrate the different sections better, in order to make it more concise and connect the different parts better. It has further been remarked that a certain bias may be present in this study, as it synthesizes the results of a large scale project from GEOMAR and Kiel University. We now tried to compare our studies to other ones more obviously, to reduce the impression of biased opinions.

A table of content, two additional figures and one table have been added.

P4500: I.14-15 Can Fe and Mn and SO₄ reduction really be distinguished based on O₂ levels?

No, a certain overlap has e.g. been described in Canfield and Thamdrup, 2009. We removed the sentence on O₂ levels as it was confusing.

4501, I.10 connect sentence.

The sentences were connected

4501, I.24-25: Explain how? In a review such a sentence sounds odd and offers nothing.

We decided to remove the sentence as we agree that there is no additional insight provided by it.

4502: I.2 leave out visual.

'Visual' was removed.

4502, I.7 leave out brackets.

The brackets were removed.

Overall comments to section 2.2: This section lacks a distinct thought structure. The reader is exposed to a lot of information that does not seem to have a particular logic or goal. In addition, this section does not arrive at a conclusion that is used at the detailed level that is provided here. Please reduce to the essential that is needed to support your major line of argument.

We restructured this section and merged it with section 4.2 in order to clarify the importance of diel vertical migrating species for the OMZ biogeochemistry.

4503, I.25 "to move forward.." The conclusion comes at an odd place and is phrased oddly. Move this together with your other conclusions to the end and synthesize better with the rest of the text.

We removed this sentence, as well as the other future objectives in the text, and included them into the summary (former section 10).

Section 3: This section is very interesting, but as for the other section, it is poorly integrated. The information density is high, but what is critical for the reader to know? A hierarchy is needed. Better to be guided by a particular argument one wants to put forward than to present everything that is known even it is very interesting. What is known about the temperature dependence of viral activity in the ocean?

We restructured this paragraph and set the focus on the meaning of viruses in OMZ waters- which are to date a rather understudied topic.

4504, I.25: none-particle bound community: odd term.

As the chapter was restructured, this sentence changed, too. Thus this term got removed.

4506. I. 27: Do you imply that microbes are protected in aggregates? Is the more direct evidence for this claim?

Yes, this has been demonstrated, before, we added the respective references to the sentence: By attachment to particles such as marine snow and fecal pellets, microbes get direct access to the nutrients stored therein. Moreover, they can create protective microenvironments via biofilm formation (Hall-Stoodley et al., 2004 and references therein).

4507, I.1: Again the term " move forward " is used in the middle of the summary. Such a sentence is not needed in this summary.

We removed this sentence and included it into the summary (section 10).

4507, I.1 'lead' instead of 'led':

As the chapter was restructured, this sentence changed, too. Thus this term got removed.

Section 4:

4507, I.23 leave out 'are'

We removed that.

4508, I.2-5. Again, recommendations for further research are made. If you find this so important to include, why don't you move your recommendations to a separate section rather than repeatedly distracting from the flow of the text with these inserts? It would be more useful if the sections are tied together better.

We removed the recommendations and included them into the summary (section 10).

4508, I.11, who is 'they'?

This refers to pelagic species conducting DVM, we added this information to the sentence.

4508: I.20 Is it necessary to use present the 5kPa partial pressure unit instead of one commonly used unit. This adds just unnecessary extra info.

It is probably unnecessary and confusing, as well. Thus, we removed it.

4508, I.28: Move up verb in this sentence. Very hard to understand meaning.

We rephrased the sentence:

'The expansion and intensification of OMZs may thus reduce zooplankton and nekton mediated fluxes by decreasing DVM.'

4508: Section 4.2 fits better with section 2.

We included section 4.2 to section 2 and re-structured section 2, accordingly.

4509, I.2: replace 'to the' with 'in'

This has been replaced.

4510, I.1 'pronounced'. Do you mean 'higher'?

Yes, this has been changed.

4510, I.3-5. I don't understand this sentence very well. Can vertical velocities drive vertical tracer fluxes, or aren't vertical velocities derived from vertical tracer fluxes?

Yes, vertical velocities indeed drive vertical fluxes of dissolved tracers such as DOC or oxygen. We recommend in this case the review paper of Levy et al. (2012) 'Bringing physics to life at the submesoscale' in GRL, where a comprehensive overview of the role of submesoscale processes for marine life is given. The mathematical definition of a turbulent tracer flux is $F = \text{mean}(w'T')$, where w is the vertical velocity fluctuation and T' the tracer fluctuation. However, it is also true that vertical tracer fluxes can be used to quantify vertical velocities, as it is very difficult to measure the vertical velocities directly. However in this case we talk about the general role of vertical velocities and not about observational approaches how to measure them. We guess that the word 'tracer' might have caused confusion in this case as and we now use the word 'solutes'.

Section 5: Needs some restructuring. Again, the level of detail needs to be funneled such that the reader does not get completely confused by the sheer amount of information.

We restructured this section in order to avoid repetitions, major changes made to this section are that we merged it with parts of the previous section 6 and that we structured it in 4 subsections:

1. O₂ - a major control N cycle processes in two contrasting OMZ regions
2. The role of nutrient stoichiometry for primary production and N turnover in OMZ waters
3. N₂ fixation- an underestimated term of the N budget in OMZs
4. Feedback controls of the N cycle in OMZ regions
- 5.

4512, I.21. Complicated sentence. Shouldn't this sentence be moved up? The key new paradigm is the spatial connection of N₂ fixation and N₂ loss. Make this more obvious.

Has been moved to the beginning of the paragraph.

4513, I.3. replace 'give'. The word give implies a causal relationship, whereby the N regime is a cause of the O₂ regime. There is, however, a mutual effect.

Due to the restructuring of this section, this sentence was removed.

4513, I.17: Nitrification cannot be only N turnover process. In I.11 you say that dissimilar processes occur.

The statement on dissimilar N cycle processes seems to be confusing, here, we changed it to :
'This difference between the ETNA and ETSP OMZs is mirrored by a diverging $\delta^{15}\text{N}$ -nitrate signal, which is strongly positive in the ETSP but has negative values in nitrate depleted surface waters of the ETNA (Ryabenko et al., 2012), indicating different N turnover processes characteristic for these two regions.'

4513 I.27 - 4514: I.10 The extrapolation of the occurrence and significance of a process merely based on O₂ tolerance levels is very tentative and should be treated as such.

It is very tentative, thus the statement of the increased N loss area has been removed.

4514, I.12 leave out 'we could identify'. In essence N limitation was implied from experiments.

Changed as suggested:

'Despite the fundamental differences between the OMZs of the ETNA and ETSP with regard to N loss, the results of short-term mesocosm experiments implied N limitation of surface plankton communities in both areas (Franz et al., 2012a; Franz et al., 2012b).'

4514, I.11 to I.21. This is a very important section and strangely not given very much space or depth altogether compared to other detailed sections in the text. DOP discussion:

Different paradigms or current views should be juxtaposed more clearly. What about Poly-P storage? Is the assumption of a Redfield stoichiometry justified? There is altogether a need for a separate P section or a section on CNPO stoichiometry in this paper.

By restructuring this section, (we now included a subsection 'The role of nutrient stoichiometry in OMZ waters') we added a discussion of the role of P including DOP.

'Part of the excess phosphorus was consumed by non-Redfield production, predominantly by diatoms. Release of dissolved organic phosphorus (DOP) by phytoplankton further diminished excess P. N:P of the accumulated biomass generally exceeded the supply ratio (Franz et al., 2012b). These results demonstrate that low nutrient N:P conditions in upwelling areas overlying O₂-deficient waters represent a net source for DOP. Whether accumulated DOP stimulates growth of diazotrophic phytoplankton is presently unknown. However, a very recent model suggested an important role of DOP for stimulating growth of N₂ fixing organisms based on large-scale surface data sets of global DON and Atlantic Ocean DOP (Somes and Oschlies, 2015). This model indicated that the marine N- budget seems to be sensitive to DOP and that access to the relatively labile DOP pool expands the ecological niche for diazotrophs. First direct evidence for a stimulating effect of DOP on N₂ fixation was obtained from a mesocosm experiment, the authors of the study noted that a general stimulating effect of DOP on N₂ fixation has been observed (Meyer et al., 2015). :

4515, l.11. I saw a paper in ISME on N cycling on cyanobacterial aggregates by Klawonn et al. (2015). This might be a very useful reference to demonstrate co-existing N cycle processes in a cyanobacterial aggregate.

Thanks for this hint; we included the information on co-occurring N turnover processes in Baltic Sea cyanobacterial aggregates and the Klawonn reference to the text.

4516, l.1-2. Last sentence provides nothing.

We removed this sentence.

Section 5.4. The title promises more than the text provides. This paragraph is more about the connection between Anammox and export fluxes than nutrient regeneration and primary production. Consider changing the title or do better justice to the title by including relevant information on nutrient regeneration and primary production.

The complete section has been restructured; the content has been integrated into the subsection on Feedback controls of the N cycle in OMZ waters, see also comments to reviewer I.

Section 6

Section 6.1 can be tied with the previous section 5.4. The section on stoichiometry comes too late. It would tie the different arguments together.

See comments above; we merged this section with section 5 and moved the section on nutrient stoichiometry up.

Section 6.2: The information here implies a major paradigm shift. I doubt many readers will understand this. Broaden this section, bring out its true significance and explain better. Section 6.2 is a

very critical section, but unfortunately not explained well at all. It also ends very abruptly. Consider rewriting the whole paragraph. It is based on the argument put forward in Landolfi et al. (2013). Emphasize that this is a paradigm, but that the paradigm doesn't work too well for different reasons. The problem is that the section is quite confusing for a reader to figure out. I have difficulties understanding the meaning of the sentence 'Denitrification partly reverses the role of remineralisation in the nitrogen cycle of OMZ and acts to transform them into net sinks of fixed N, because.....' It's a huge sentence, very convoluted in style and very dense in information. The flow of the argument is interrupted by section 6.3 and then picks up in section 6.4. Restructuring is needed.

See comments above; we merged this section with section 5, re-structured it and put more emphasize on the different modes of feedback regulation as discussed by Landolfi et al..

4517, I.16 Oxygenic respiration is the wrong term. Use Oxygen respiration. Biochemically the first sentence is misleading. A respiratory process does not regenerate an inorganic form of nitrogen.

We used the term 'aerobic' instead.

4517, I.24 '... OMZs harbor diverse bacteria of the N cycle'.

According to the comments of reviewer I, this sentence has been restructured, thus this expression has been removed.

4517, I.26, there are no nitrates, use singular.

This has been changed.

Section 6.3 conveys a confusing message. Back to zooplankton, but very briefly. Is this section necessary or can this information be included elsewhere?

The information has now been included into section 2.

Section 6.4. This is a very informative section, but again, consider which key arguments you intend to put forward and focus on these. Introduce the optimality-based model. The term is not introduced prior.

This section has been moved to section 5 and has been re-written, we added an explanation of the optimality-based model to it:

'However, one of the predictions of the optimality-based model of N₂ fixation by Pahlow et al. (2013), which is based on the assumption that natural selection should tend to produce organisms optimally adapted to their environment, is that the competitive advantage of diazotrophs is most pronounced under conditions of low DIN and increased DIP availability (Houlton et al., 2008). The ability to compete for DIP is less important at high DIP; based on this, high phosphate concentrations above the ETSP OMZ might actually reduce the selective advantage of diazotrophs compared to ordinary phytoplankton.'

Section 7: Section 7 should be tied with the previous section.

We do not agree on that point, the particular importance of sulfidic events with regard to climate change and future development of OMZ waters requires an own section. We however tried to better connect it to the previous and the following sections.

Section 8:

Retain style by providing an introduction paragraph

We included an introduction to this chapter.

4525, l.25, remove 'indeed'

Has been removed.

4526, l.25: What is exactly the recent hypothesis by Canfield et al. (2010)? This is written as if every reader should know this.

An explanation has been added to the sentence:

'This is in line with the recent findings from the anoxic event off Peru by Schunck et al. (2013) and similar to the recent suggestion of a cryptic sulfur cycle where sulfate reduction is coupled to rapid H₂S oxidation by NO₃⁻ proposed for the OMZ off Chile by Canfield et al. (2010).'

4527, l.21. 'implies'

This has been changed.

Section 9:

4529, l.13: core community: State how you define a core community and what this means for OMZ.

We changed the sentence by adding information on what is special about the microbes in the OMZ- namely that most of them are there, wherever you are :

'In accordance with several previous studies (Stevens and Ulloa, 2008; Stewart et al., 2012) a large part of the microbial community has been identified to be phylogenetically similar throughout the OMZ.'

Section 10: Cut out all your open questions from the text and paste them into this section.

We included all open questions in the text into section 10.

4533, l.5. 'In detail, we aim....' . Who is 'we'? Shouldn't this be a general recommendation for further work?

This has been rephrased:

'In detail, future studies may address the following key questions'