GEOMAR
Helmholtz-Zentrum für Ozeanforschung Kiel

GEOMAR | Düsternbrooker Weg 20 | 24105 Kiel | Germany

D. L. Arévalo-Martínez
Dr.
Tel +49 431 600-4207
Fax +49 431 600-4202
darevalo@geomar.de

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Dear Dr. Ward

I hereby submit the revised version of our manuscript: *"Influence of mesoscale eddies on the distribution of nitrous oxide in the eastern tropical South Pacific",* in order to be considered for publication in Biogeosciences.

We thank both referees for their comments and in general we agree with them in that some of our results could be presented more precisely, also acknowledging and discussing the advantages and potential caveats of our approach.

The modifications done to our manuscript are highlighted in the main text, and detailed replies to the referees' comments and suggestions are included in the point-by-point responses which you will find at the end of this letter. Along with the main manuscript I'm also attaching a supplementary material file which we considered necessary in order to account for the referees' comments.

We appreciate your attention and look forward to your reply.

Kind regards

Damian L. Arévalo-Martínez

GEOMAR
Helmholtz-Zentrum für
Ozeanforschung Kiel

Düsternbrooker Weg 20
24105 Kiel | Germany

Tel +49 431 600-0
Fax +49 431 600-2805
www.geomar.de

Deutsche Bank AG Kiel
BLZ 210 700 24
Kto. 144 8000

SWIFT/BIC DEUTDEDB210
IBAN DE 69210700240144800000

Steuernummer 1929401912
VAT DE281295378

Author's response to referee's comments

On the following I list the changes done in response to the comments and suggestions by Referee #1 (Prof. Dr. Nicolas Gruber):

Major points:

Comment by Prof. Gruber:

"Overinterpretation of the data: While I admire the authors for their very thorough and deep discussion of the data, sometimes I had the feeling that they went too far and started to pick up simply "noise", resulting from the fact that they investigate a rather dynamic environment. For example, some of the differences in "aging" could simply be just within eddy variations, stemming from differences in formation, transport, initial nutrient levels, etc. I suggest that the authors acknowledge this alternative interpretation more strongly and adjust their wording accordingly."

Reply by authors:

We acknowledge that due to the station density and time scales of our survey, it is difficult to assess at what extent the changes in N_2O concentrations between coastal and open ocean features were due to biogeochemical cycling during their lifetime, as opposed to variability induced by their properties at the time of formation. Furthermore, analysis of our full data set showed that, for example, stations within the center of the same eddy might have different anomalies (difference between center and outside) despite of sharing the same distribution in the water column. This clearly points out to an unaccounted variability within the eddies which might be indeed reflected in these N_2O concentration differences. Since with our methods we cannot directly evaluate this effect, and we are not aware other studies dealing with N_2O and mesoscale eddies in the ETSP, our discussion in centered in our "best guess" of how the N_2O-cycling proceeds within mesoscale eddies during their transport offshore. In the revised manuscript we included anomaly plots in Fig. 3 (p. 31 of main text file) and supplementary Fig. S1 (p. 1 of supplement file) in order to better depict the differences in N_2O concentrations between stations in the center of the eddy and stations outside. For this, we also used additional stations located between the center and the edges of the sampled eddies so as to illustrate the spatial variability in N_2O and O_2 concentrations (Fig. S3). We also discuss now alternative explanations for our observations given the potential limitations of our sampling methods (p. 8 – 9, l. 236 – 259; p. 10, l. 276 – 278, p. 14, l. 397 – 407).

Comment by Prof. Gruber:

"Stronger synthetic view: The paper would greatly benefit from the authors taking a more synthetic view of their results. As it stands, the authors emphasize differences and much less the common aspects. Thus, the reader comes away with the impression that every eddy is different, preventing them from formulating more general principles. I thus strong encourage the authors to add a synthesis section where they develop a diagrammatic view of how N_2O is formed, consumed and transported in such a dynamic environment such as the ETSP."

Reply by authors:

We agree with this suggestion and therefore we included a new section in the revised manuscript (4.4 Synthesis, p. 18 – 19, l. 513 – 540).

Comment by Prof. Gruber:

"Molecular genetic methods. I applaud the authors' combination of the (bio)geochemical measurements with those using molecular genetic methods. But in the text, the integration is not as strong as it could be, as the genetic information is used in a rather qualitative manner. In particular, one wonders whether the bacterial biomass present would suffice to produce/consume the amount of N_2O needed in order to produce the environmental concentrations and gradients in N_2O."

Reply by authors:

We thank Prof. Gruber for the positive evaluation of our approach. The overall problem with gene abundance data is that they give an idea of what could possibly be found in an environment under certain conditions rather than providing indication of the activity or importance of a process. Therefore, we used the molecular data only in a qualitative way to show that the potential for nitrification and denitrification (or also for anammox) is present. Our data mirror the high abundance of archaeal ammonia oxidizers as it is typically found in coastal waters of that region, and a comparably lower abundance of denitrifiers. Since *amoA* (as key gene for nitrification) dominates the upper part of the water column, we consider nitrification to be a quantitatively more important process here. Nevertheless, both genes (*nirS*, *amoA*) were present where the deeper maximum in N_2O could be found, and therefore both processes may contribute to the N_2O budget. We modified the respective part of the manuscript (p. 11 – 12, l. 319 – 339) in order to better integrate the molecular data. From our point of view it is however difficult to determine what part of the biomass is sufficient to shape the observed gradients, particularly because the potential for N_2O production/consumption does not only depend on the overall abundance of microbes or their relative importance regarding the biomass but rather on the efficiency of the process and the environmental conditions, such as substrate availability and O_2. (For example, N_2-fixers are considered to account for only 2% in ocean surface metagenomes, and yet they contribute up to 80% of the new N to these waters). We are therefore critical towards a comparison with the overall biomass.

<u>Minor comments:</u>

Comment by Prof. Gruber:

"Introduction: I suppose the authors want to refer also to their Nature Geoscience paper."

Reply by authors:

This reference has been included as suggested.

Comment by Prof. Gruber:

"page 9215, line 22: "This result can be explained by lower water column O_2 concentrations in eddy A than in eddy B (36.4 and 42.9 molm^{-2})" This is an example of a possible "overinterpretation" of the results. This is a rather small mean difference, which I doubt is big enough to really explain the difference."

Reply by authors:

This paragraph has been reorganized for more clarity and the interpretation of this result is now presented in a way that our observational constraints are clearly stated (p. 8 – 9, l. 236 – 259). In particular for O_2 we computed integrated water column concentrations from additional stations (even though there was no N_2O sampling) in order to provide a measure of the uncertainty of our estimates (p. 9, l. 247 – 248).

Comment by Prof. Gruber:

"page 9252, line 19 "Therefore it is likely that the decaying primary production of eddy C during its transit away from the shelf led to a diminished supply of organic matter which could fuel N-loss within the OMZ's core, explaining the relatively high N_2O concentrations observed in comparison to eddies A and B." This speculation is reasonable, but again, the difference is not as marked as the authors portray it to be. Hence, I would be more cautious in the interpretation of these differences."

Reply by authors:

The text has been changed accordingly (p. 10, l. 271 – 278) and it is supported by anomaly plots (Fig. 3) which better depict the actual extent of the observed differences between the center and the edges of this eddy as well as its differences with respect to mode water eddies.

Comment by Prof. Gruber:

"page 9254, line 13ff "[..] show that denitrifiers produce increasing N_2O:N_2 ratios as the O_2 concentrations increase..." It would be very interesting if the authors were able to be more quantitative here. Shouldn't it be possible to estimate this ratio by combining an estimate of the N-loss with the increase in N_2O?"

Reply by authors:

In the revised version of the manuscript we used our N_2O concentration values as well as N^* (as an indicator for N-loss) in order to obtain a rough N_2O:N_2 ratio for eddy A. This was used to support our arguments in p. 12 (l. 340 – 351), giving an indication of the sensitivity of N_2O to the oxygen gradient through the water column within the eddy A.

Comment by Prof. Gruber:

"page 9257, line 5ff "After integrating ΔN over the depth range of the OMZ, we obtained values of 8.9 and 0.02 molm^{-2} for eddy A during M90 and M91, respectively". I don't understand this result. A nitrogen deficit that is once created cannot be easily alleviated. One of the few options is to have N_2-fixation to kick in, providing a lot of newly fixed N to compensate for the lost N_2. Thus, it is puzzling to me how this change in ΔN can happen."

Reply by authors:

During the second survey of the coastal eddy A (M91, December) we observed an overall reduction of N_2O concentrations in its center, despite the fact that right at the boundaries of the OMZ concentrations were high. Considering the evidence of N-loss during M91, it would be reasonable to assume the N-deficit (and also the N_2O consumption at the OMZ core) to be higher during that cruise. However, our computed N-deficit suggests that what we measure during M91 is most likely a remaining signal of enhanced N-loss activities during a period in which the eddy A stayed (and strengthened) on the shelf. As for other comparisons between M90 and M91 within our manuscript, however, it should be considered that the station density for N_2O during our study does not fully guarantee that we sampled exactly the same waters, even if the criteria for defining the center of the eddy was the same (i.e. the sea surface anomaly height data). On the other hand, recently Löscher et al. [2015a] presented N_2-fixation data for the same cruises, showing enhanced N_2-fixation rates in the center of mode water eddies. Moreover, they also observed co-occurrence of N_2-fixation and N-loss within these eddies, suggesting a spatial link between both processes in the ETSP. However, a comparison of the eddy A during M90 and M91 showed that in general N_2-fixation rates tended to be lower during M91, most likely indicating a decline of biological production during "aging" of the eddy. Hence, although N_2-fixation rates were lower within the mode water eddy A during M91, its occurrence, together with the fact that during M91 we most likely sampled the eddy after a period of intense N-cycling might help to explain the alleviation on the N-deficit, as pointed out by Prof. Gruber. In the revised version of the manuscript, we present a more robust calculation of the N-deficits. We also corrected our N^* values from the M91 cruise since there was a mistake in the original calculation, as pointed out by Referee #2 (see Fig. 4). We opted for not showing property plots of nutrients and N^* comparing both cruises since this has been already presented in the work of Bourbonnais et al. (2015) (this paper was published after our original submission). Likewise, we adjusted the discussion paragraph accordingly and include the reference to Löscher et al. [2015a] since that work was not published at the time of submission of our manuscript (see p. 11 – 12, l. 319 – 339).

Author's response to referee's comments

On the following I list the changes done in response to the comments and suggestions by Referee #2:

Major points:

"There are several issues that need to be resolved, the first being data quality assurance. While I acknowledge that the primary research group involved is internationally recognized for its N_2O work, I am concerned about the N_2O concentration data in Fig 3b. Here the center profile is lower than outside throughout the water column with the difference greatest at 1000 m where eddy influence should be minimal and I have similar concerns for Fig. C. I suggest double checking these data and if they stand up explicitly address this point in the text."

Reply by authors:

In attention to the suggestion by the Referee #2, we thoroughly re-checked the N_2O depth profiles depicted in Fig. 3. After revision we found that the anomalies in N_2O concentrations do persist through the water column and that indeed the size of the anomalies in these stations can also be observed for temperature (T), salinity (S) and oxygen (see Fig. 3 in the revised manuscript and Fig. S1 in the supplement file). Likewise, error bars have been added to the plots showing depth profiles of N_2O concentrations in order to depict the uncertainties of the measurements. The findings of Stramma et al. [2013] suggest that although the strongest effect of the eddies could be found in the upper 600 m of the water column, the associated zonal and meridional velocities could also be detected at greater depths and therefore N_2O anomalies could also be expected below this depth. Nevertheless, we do acknowledge that the spatial resolution of the N_2O sampling as well as the actual location of the stations might complicate the interpretation: in this manuscript we presented data from selected stations across the three eddies in order to illustrate the conditions outside and inside of the corresponding eddy (the center being the location with the largest sea surface height anomaly (SSHA); see Stramma et al. [2013]). However, if we include stations that we consider intermediate between, for example, the center and the edge of a given eddy, this would change the magnitude of the anomaly despite having a similar N_2O distribution. Thus, although we agree with Referee #2, in that observational constraints make it difficult to judge in absolute terms the anomalies in the vertical distribution of N_2O due to mesoscale eddies, our results show that, as for other biogeochemical properties, the physical changes within the eddy can also affect N_2O in the water column, and in particular within the OMZ (see Fig. 3 in the revised manuscript as well as Figs. S1 and S2 in the supplement file).

Comment by Referee #2:

"Since much is made of the temporal evolution of N_2O in Eddy A, profile plots for comparing M90 and M91 data should be included. I have similar concerns about the gene abundance data as much it appears noisy and there is not visual comparison between M90 and M91 results. The text needs to include and evaluation of the reproducibility of these data."

Reply by authors:

The revised version of the manuscript includes a new figure (Fig. 6) in which the N_2O and O_2 distributions in eddies A and B from the cruise M90 and in eddy A during M91 are depicted. We opted for not showing section plots of T and S since they have been shown already in the paper by Bourbonnais et al. (2015). Nevertheless T-S diagrams of all the stations we used for our survey (with and without N_2O measurements) are now presented in the Fig. S3 of the supplement, in order to support the discussion in p. 14 (l. 397 – 407). On the other hand, we consider that section plots of M90 vs. M91 for N_2O are no longer necessary since we added the Fig. 6. A visual comparison of the vertical distribution of the molecular markers *amoA*, *nirS* and *hzo* between M90 and M91 was not included in the manuscript since the data from M91 was already presented within the context of a discussion on sources and sinks of N_2O in the coastal eddy A (Fig. 5, section 4.2, p. 11 – 12, l. 319 – 357). Thus, we considered redundant to use these data again in the subsequent section. Furthermore, unlike M91, for the M90 cruise we only have molecular data from stations located at the center of the eddy. This is the reason why we decided to focus the discussion in those stations and present the results as integrated values in the water column. As pointed out by Referee #2, there might be some caveats with the use of that approach and therefore this point is now addressed in the revised version of the manuscript (e.g., p. 9, l. 249 – 259).

Comment by Referee #2:

"The profile comparisons all use a depth scale. Eddies are characterized by raising or lowering of isopycnal surfaces and it would more accurate to make comparisons of properties between eddy interior and exterior in sigma-theta space"

Reply by authors:

We agree with Referee #2 in that comparisons of properties across the eddies could be better described in terms of density surfaces. Therefore in the new version of the manuscript we use both density surfaces and depths to refer to the vertical distribution of N_2O and other physical and biogeochemical properties (p. 8, l. 4, 10). Moreover, we added density contour lines to the velocity plots in Fig. 2 so as to visually support the comparison. However, we kept water depth as vertical axis in all plots since we consider that this is more intuitive for the reader.

Comment by Referee #2:

"(...) Having said this, a more general issue is that the station density for which N_2O data are available are too sparse to well characterize distributions. The authors need to satisfy themselves with just establishing whether N_2O concentration is significantly different inside eddies and admit that discussion of any mechanisms are speculative. In this regard, more statistical rigor is needed in terms of establishing an average background N_2O profile for comparison and the authors have substantial data of their own to draw upon (e.g. Ryabenko et al., 2012)."

Reply by authors:

As pointed out before, in this study we presented a selection of profiles which we identified to be located within the center, edge and outside of the corresponding eddy. The inclusion of anomalies for each eddy in Fig. 3 provides a more clear view of the extent

of the observed differences between N_2O concentrations inside and outside of the eddies. In order to establish a background concentration of N_2O for comparison purposes we used the data from Kock et al. (2015) since it includes data collected during the same cruises in which we performed the field work of this study. The calculation procedure for the background profile has been included in the methods section (p. 6, l. 152 – 157) and the corresponding interpretation can be found in section 4.1 (p. 10, l. 279 – 285). Visual aid for this point is given in supporting figure S2. The study by Kock et al. (2015) has been included in the reference list.

Comment by Referee #2:

"Because the distributions of N_2O within the eddies are not well characterized, I don't see how there can be any certainty in the integrated values in Table 1. Clearly they cannot be taken as representative of the entire eddy. Even if representative of eddy center, it is unknown if the center represents the point of maximum difference (especially given the transect data in Fig. 4) regardless of whether the center was actually sampled. These problems also lead to difficulties in making comparison between M90 and M91 observations of Eddy A since differences are just as likely to be the result of sampling different portions of the eddy."

Reply by authors:

We acknowledge that estimating integrated concentrations in the water column based on single profiles could be ambiguous due to the station density we had, and therefore in the revised version of the manuscript we wrote part of the interpretation in a more cautious manner (p. 8 – 9, l. 236 – 259; p. 10, l. 276 – 278, p. 14, l. 397 – 407). However, even under these observational constrains, and as suggested by Stramma et al. [2013], the mesoscale eddies which we tracked and sampled during M90 and M91 were stable structures whose center could be clearly identified based on the SSHA data. Hence, we think choosing the center of the eddies based on SSHA data (as we do in this study) is a save assumption which allows us to provide a fairly good description of the N_2O distribution across these features. Although we don't have a station density that fully represents the N_2O vertical structure of the eddies during M90 and M91 (which would be optimal), our estimates represent a good approximation to the distribution changes that can be observed under the influence of recently formed (coastal) and aged (open ocean) eddies, in particular since we now provide a more robust analysis of background concentrations of N_2O (p. 6, l. 152 – 157, p. 10, l. 279 – 285, and Fig. S2), as well as anomaly plots (Fig. 3) in addition to the concentration values. As for the comparison between M90 and M91, we acknowledge that different portions of the eddy could have been sampled despite the fact that the definition of center of the eddy was consistent. However, analysis of T and S profiles during M90 and M91 suggests that the water masses within the eddy were the same at the time of sampling. Although this has been shown before by Bourbonnais et al. [2015], we included T-S diagrams in the revised version of the manuscript (Fig. S3) in order to further support this argument.

Comment by Referee #2:

"(...)This can explain why the NO_3^- deficit appeared to decrease between the two time points (see next)."

Reply by authors:

Please see our reply to the following comments. Part of the interpretation in this section has been modified accordingly (Section 4.2 and also 4.3, p. 11 – 13 and 13 – 15, respectively).

Comment by Referee #2:

"I found the whole last section (pg 9256 line 20) of the Discussion, which assessed changes in integrated N-loss over time in Eddy A, rather confusing. First, after having shown N^* data, a switch is made to "NO" to assess N deficits. N^* relies of deviation from Redfield N:P and is the current standard so the switch to "NO" (which assumes a relationship with O2) is unclear."

Reply by authors:

We used "NO", a quasi-conservative water mass tracer in order to independently estimate denitrification based on the available data for eddy A. Since this geochemical approach has been used for previous studies in order to provide quantitative estimates of N-loss and N_2O production during denitrification (see corresponding references in section 4.3 of the manuscript), we considered appropriate to use the same methodology in order to compare our results. In order to avoid further confusion we will explain this in the new version of the manuscript and still use the N* approach in section 4.2 which is focused in N_2O-cycling during M91. This section is now included under: "4.3.1 Changes in N-cycling" (p. 15 – 18, l. 427 – 512).

Comment by Referee #2:

"(….)Perhaps it is because the N^* scale in figure 3 is well beyond the bounds typically observed, but these calculations need to be rechecked as reasonable N^* data for these cruises has been published."

Reply by authors:

Many thanks for pointing this out. Indeed, after re-checking the numbers we found a slight mistake in the N^* computation which in turn shifted all our values out of the normal range. Thus, although the features of the water column distribution of N^* remained the same, the absolute values had to be corrected. This issue was addressed by presenting the corrected values in Fig. 4 of the revised manuscript.

Comment by Referee #2:

"(…) If the authors used N deficit data only from the stations with N_2O data (not clear), then they still have the same issues here regarding insufficient sampling and characterization of the eddy. There are also logic gaps here as a reduction in N deficit could only come about by mixing with water with little or no N deficit. This parameter represents an integration of N-loss rate over time, but the authors interpret the apparent result as a change in rate. The apparent decrease in N deficit is probably due to 1) having sampled different regions of the eddy at each time point or 2) problems with using "NO" instead of N^* as erroneously including any region with O_2 present in the integration will reduce the deficit."

Reply by authors:

This section of the manuscript has been rewritten considering the replacement of "NO" for N*, and the necessity of correcting out N* values from the M91 cruise. Likewise we strengthened the discussion about N-loss by including and discussing the findings of new publications such as Bourbonnais et al. (2015) and Löscher et al. (2015a) (Fig. 4 and p. 11 – 12, l. 317 - 339). Nevertheless, we do agree with Referee #2 in that a potential caveat of the interpretation of this data is the fact that we couldn't sample exactly the same part of the eddy center during M90 and M91 (see above).

Comment by Referee #2:

"(...) Finally, this section has a lot of speculation about the processes producing N2O and corresponding yield that is not substantiated."

Reply by authors:

We agree with Referee #2 in that some of the arguments provided cannot be supported with our data. As it is written however, our discussion warns the reader about it and furthermore highlights the need for multidisciplinary, highly resolved surveys in order to better understand the net impact of mesoscale eddies in the distribution of N_2O, in particular when longer time scales (seasonal to interannual) are considered (see e.g. p. 9, l. 249 – 259). Part of the discussion has been reorganized, however, in order to show more clearly our arguments (p. 16, l. 449 – 473).

Other points

1) *"In many locations citations can be improved to include a broader selection of relevant literature (e.g. Frame and Casciotti, 2010; papers from Bess Ward's group) as well newer highly relevant literature that one or more of the authors are also co-authors of (e.g. Ryabenko et al., 2012.) In particular, Bourbonnais et al., 2015 (GBC) needs to be referenced as they examine N deficit distributions in Eddy A during M90 and M90."*

Reply by authors:

Following the recommendation from Referee #2, the following references have been added: Bourbonnais et al. (2015), Ji et al. (2015), Ryabenko et al. (2012), Kalvelage et al. (2011) and Babbin et al. (2014) (see reference list starting in p. 21)

2) *"pg 9251 line 7-8, the claim about higher N_2O in the center as opposed to other, within eddy locations is not well substantiated."*

Reply by authors:

This sentence and the whole paragraph have been re-organized for more clarity (p. 8 – 9, l. 236 – 259).

3) *"pg. 9251 line 27, need to be careful not to confuse substantiated findings with hypotheses/speculation in prior papers."*

Reply by authors:

This sentence has been rewritten for more clarity (p. 9, l. 253 – 259).

4) *"pg. 9253 line 5-10, not clear what is the basis of the assertion of lack of eddy impact on surface layer, as this depends on vertical velocity and exchange rates. Satellite Chl a often shows impact from eddy circulation."*

Reply by authors:

Given that enhanced concentrations of N_2O can be found within the upper oxycline of the ETSP (e.g. Fig. 3), shoaling of the thermocline within mode water eddies would mean higher N_2O concentrations for a given depth as compared to a background profile. Since coastal upwelling waters off Peru are a known source of extremely high N_2O concentrations to the surface, one could get the impression that this eddy-driven shoaling of waters with relatively high N_2O could also contribute to that effect. However, our observations show that the anomalies caused by these eddies seemed to be far from the reach of surface waters and thus did not contribute to the N_2O fluxes out of the ocean at this location. Independent verification of our bottle data for the surface comes from underway measurements performed during the same cruises in the ETSP (see Arévalo-Martínez et al. (2015)). From these data we can say that there wasn't any appreciable variation of N_2O concentrations in the surface during the several cross-eddy sections carried out in the M90 and M91 cruises. This explanation is now included in the revised version of the manuscript (p. 10 – 11, l. 290 – 305)

5) *"pg 9253 line14, not clear what is meant by "O_2 minima" as the whole region as effectively zero O2 at depth."*

Reply by authors:

In this context O_2 minima means the core of the OMZ (i.e. O_2 concentrations < 5 µmol L^{-1}). This has been now explicitly stated in the revised version of the manuscript (p. 11, l. 310).

6) *"pg 9255, last line, appears to be confusion between 'concentration' and 'content', this may be behind the problem in #5. Content derives from depth or volume integrated parameters but local concentration is one factor determining rates of processes. The biogeochemical significance of depth integrated parameters can also be distorted by vortex stretching."*

Reply by authors:

The word "concentration" has been replaced as suggested because in this context we meant to discuss the N_2O, O_2 and nutrient content in the whole water column (p. 14, l. 412). Likewise we agree with Referee #2 in that the apparent content of a given biogeochemical property can change due to the shoaling/deepening of isoclines. However, we assume this effect to be marginal for the time scales considered in this study.

Other changes:

We decided to remove the panel (a) from Fig. 1 in which the satellite-derived chlorophyll concentration for the ETSP at the time of sampling was presented. Since a similar image has been presented by Stramma et al. (2013) and it is not essential for the purposes of this study, we considered appropriate to only present the SSHA data.

We included density line contours in the velocity plots of Fig. 2 in order to visually support the description of the results, in particular in section 4.1.

We would like to point out that the reference Kock et al. (2015) has been already accepted for publication in Biogeosciences and at the moment is in press. Therefore before final submission we will need to change that reference to the most updated one.