Reply to the anonymous Referees #1

(**RC:** Referee Comment; **AR:** Author's Response)

We would like to thank the anonymous Referee #1 for the constructive feedback and thorough review of the manuscript. One main criticism is that the methods, both with regards to incubation and calculation of rates, should be explained in more detail. We agree and will address this issue further in the individual comments. We will also provide an improved description of the method, which should answer many of the reviewers' questions. Besides that, we followed all the suggestions given by the anonymous Referee #1.

Page 2 line 49-50

RC 1: "very little is known about N cycling and N transformation rates in the sediment". This is a very strong statement and has to be amply justified although I see no evidence of such being the case in the literature. At least, it should be contextually set in a much better way. The justification coming afterwards is rather confusing – are the authors suggesting nothing is known about benthic N cycling in the North Sea because previous studies failed to distinguish between mineralization and nitrification?

AR: We agree that this statement was somewhat exaggerated, even though sediment studies of course always deal with the same set of problems, i.e., that the range of sampling designs complicates intercomparisons. In a revised version, we will specify this statement, meaning indeed that ammonification / mineralization is poorly assessed.

RC 1: It is suggested that the isotope dilution method employed in this study can 'unravel several N-processes like ammonification, assimilation, nitrification, denitrification (: : :)', DNRA: : :etc within sediments. This requires demonstration, which is not clearly apparent either after this statement or in fact within the rest of the manuscript […].

AR: We agree that the isotope dilution method we applied needs to be explained in more detail. Briefly, yes, we did indeed apply the isotope dilution method using parallel enrichments with $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$ (e.g., Blackburn et al., 1979). This results in a limited number of replicates for individual rates and does indeed affect uncertainty, as the reviewer states. We will clearly state this in the revision. However, we did, precisely for this reason, attempt to assess process rates directly wherever possible, to minimize the problems arising from error propagation.

We will add a new figure as supplementary material to demonstrate which processes were deduced based on concentration changes (net ammonification for ammonium, net nitrification for nitrate concentration), and which rates were measured via tracer addition (gross nitrification and ammonification).
All isotope tracers were indeed added at a tracer level, i.e., site water was replaced by water that contained tracer, but the tracer concentration was adjusted so that it would not change the overall nutrient concentration in the water column.
In theory, it would have been possible to deduce net rates from all incubations, regardless of label additions. The net concentration changes were comparable, but we did not attempt this because gross rates were

determined by difference based on label additions, and we sought to avoid calculating net rates based on 4 cores and gross rates, by difference, based on 2 incubations. As we noted above, we will address the arising uncertainty explicitly in the results and discussion section in a revised manuscript, especially for NOAH-D, where labelled nitrate could, due to difficulties in sediment core retrieval, only be added to one incubation. We hope that by clearly addressing the number of samples and uncertainty, and by clarifying flux calculation (in form of a figure and a more detailed explanation), we have resolved the issues raised by the reviewer.

General assessment:

RC 1: Propagation of error and significance of differences encountered between different rates at different locations needs to be discussed appropriately, and concrete results presented in this regard. Rates are presented without appropriate attention to this point.

AR: As we explained above, the ammonification rates (net and gross) were calculated only with the $^{15}NH_4^+$ tracer sediment cores and the nitrification rates (net and gross) only with the $^{15}NO_3^-$ tracer sediment cores. In a revised version, we will clarify the calculation of the ammonification and nitrification rates including their significance for actual processing, given the uncertainty of measurements.

RC 1: Denitrification could be then estimated as the sum of denitrification rates measured in the two cores, and both nitrification and coupled nitrification-denitrification from the $^{15}NH_4^+$ labelled core. Indeed, it seems that was that was done (line 113-115).

AR: The denitrification rates are based on MIMS results and are not based on coupled nitrification/denitrification from label incubations. We choose this approach specifically due to the problems that arise from error propagation. If both assessments are compared, however, the rates fall within the same order of magnitude, and are roughly comparable. For the MIMS analysis, the internal precision of the samples was <0.05% for N2/Ar analyses (line153-154). We will clarify this in the revised version.

RC 1: NH_4^+ concentration in bottom water are usually very low (line 234-235) and how this would affect the accuracy of estimates of nitrification and coupled nitrification-denitrification.

AR: We measured only the net and gross ammonification rates using the concentration and isotope ratio of NH_4^+ from the $^{15}NH_4^+$ tracer sediment cores. The net and gross nitrification rates were calculated by using the concentration and isotope ratio of NO_3^- from $^{15}NO_3^-$ sediment cores. We will clarify this in the revised version. Accordingly, ammonium concentrations should not have a major effect on the measurement of nitrification rates, because these are based on concentration and isotope label changes in the nitrate pool.
As we will point out more clearly in a revised version, the addition of label (of any kind) did not change the ambient concentration, because site water was replaced with labelled water that was adjusted to in-situ concentration, so that rates should remain unaltered.

RC 1: In Table 3, in the second column, the sum of three process rates is presented with relatively low uncertainty – is this from a flux measurement and therefore this is an aggregate?

AR:
In Table 3, we indeed did not address error propagation adequately. We will revise Table 3, considering to exclude assimilation, because this process is poorly assessed in our measurement method, is associated with a relatively large uncertainty, and because rates are small, so that the sum of rates change only a little. We will of course also address uncertainty.

RC 1: In NOAH -C (Line 201) to calculate assimilation, the difference between gross and net ammonification alone if propagation of error is accounted for would already be a highly uncertain number: (8.3_2.3 - 6.8_2.3 = 1.5 _ 3.25) and we haven't yet subtracted the gross nitrification rate. The chosen mode of representing uncertainty is also not explained – how is it calculated (looking at the size of the error bars and the spread of results, I presume as the standard error of measurements, with n=2 or 1? Or n=4 or 3?), but has to be made explicit.

AR: The assimilation rates were calculated by the sum of gross ammonification minus net ammonification minus gross nitrification rates. It is correct that due to error propagation, rates are highly uncertain In consequence, we decided to skip assimilation from the assessment, because rates are (a) uncertain, and (b) relatively small (based on our measurement). Moreover, we note that our setup as a whole was not ideal to measure assimilation, because cores were incubated in the dark (details on the incubation will be provided in the revised version).

RC 1: Reference list seemingly too long compared to actual citations in text.

AR: The reference list will be shortened in the next manuscript version. We thoroughly crosschecked the reference list and found one accidental duplicate. All other references in the list are indeed represented by actual citations. However, we do see the point and will, in a revised version, restrict citations to the most relevant ones.

RC 1: Present an explicit discussion of the potential pitfalls of using the 15N dilution technique without isotope pairing (Rysgaard, Nielsen et al). In particular, the issues with underestimation of denitrification and nitrification associated with the coupling of both processes and how they could affect interpretation of the results, not mentioned on the manuscript.

AR: We will address the method (including its advantages and disadvantages) in more detail in the revision. As in any ex-situ incubation method, the results are not necessarily equal to actual natural rates in the sediment, which we will emphasize in the discussion.
Regarding nitrification and coupled nitrification/denitrification, we would like to point out that our assessment of denitrification is independent of labelling, because it is based on N2 production that was measured by membrane inlet mass spectrometry.

If denitrification removes substantial amounts of nitrate from nitrification, this should decrease the nitrate concentration (i.e., net rate), with no effect on labelling percentage. This may lead to underestimation of nitrification in our assessment, but should also result in a production of 29N2 in the MIMS measurement. We will address this in the revision where necessary.