

## **Reply to the anonymous Referees #1 and #2**

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

We thank both reviewers for their constructive and detailed feedback on our submitted manuscript. Major issues that were addressed by both reviewers dealt with methodological issues, i.e., clarification of the methods we used, details with regards to error propagation etc.

We realized that the method section was indeed somewhat confused. Moreover, the rate calculation was in some cases based on extrapolations that were not entirely justified. Based on our explanations in the previous response letter, we double-checked the calculations and specifically looked into issues related to error propagation.

In the current manuscript, we took care to uncouple the individual rate measurements as much as possible. Gross rates of nitrification are based on nitrate addition, gross rates of ammonification are based on labelled ammonium addition, both are calculated based on isotope dilution. For the sake of clarity, net rates are now referred to as benthic fluxes, i.e., ammonium, nitrate, or N<sub>2</sub> fluxes. These are based on concentration changes in all cores from one station. We rewrote large parts of the method section. As all interpolations from the previous manuscript were removed, the number of data points is reduced in some cases, and the numbers for benthic fluxes and gross rates changed in some cases. However, the general trends and our overall line of discussion are not affected.

Below, we explain the changes and modifications following the reviewers's suggestions in more detail.

### **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 softened this statement, now referring specifically to ammonification (line 51 – 59).

**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:** For clarification, we extended the method section. We separated the denitrification measurement more clearly to avoid confusion, prepared a new Figure to demonstrate which fluxes were based on which

measurement, and included a table to the supplementary material that shall enable the reader to follow our flux calculations and gross rate measurements (line 176 – 192).

We also clarified the terminology (replacing net rates by benthic fluxes). We also address the uncertainty of measurements now explicitly in section 4.4 (line 337 – 407).

#### **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  $^{15}\text{NH}_4^+$  tracer sediment cores and the nitrification rates (net and gross) only with the  $^{15}\text{NO}_3^-$  tracer sediment cores. In the revised version, we put this more clearly (changing the method section and adding a new figure – section 4.4 – line 337 - 407). All error bars are standard deviations of measurements. Individual rate measurements and fluxes are uncoupled to minimize problems arising from error propagation, this should be well-represented in Fig. 5.

**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  $^{15}\text{NH}_4^+$  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. In the discussion section, we now also address that denitrification (measured by MIMS), measured nitrate fluxes and nitrification match in our sampled sediment cores, so that the calculated budget is closed (line 132 – 137)

**RC 1:**  $\text{NH}_4^+$  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  $\text{NH}_4^+$  from the  $^{15}\text{NH}_4^+$  tracer sediment cores. We clarified this in the revised version (see especially new Figure 2). As we stated in the initial response letter, ammonium concentrations should then 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.

In the method section, we now also point out that 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 (line 108 – 130).

**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:**

We excluded assimilation (due to high uncertainty) from Table 3 and from the manuscript.

**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:** It is correct that due to error propagation, rates are highly uncertain. In consequence, we decided to skip assimilation from the assessment. Moreover, we note that our setup as a whole was not ideal to measure assimilation, because cores were incubated in the dark (now added in the method section – line 176 – 192).

**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  $^{15}\text{N}$  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 extended the method section to clarify our calculation procedures for gross rates of nitrification, ammonification, benthic fluxes of nitrate and ammonium, and  $\text{N}_2$  production. We hope that this resolves the methodological issues mentioned here (section 2.3).

## **Referee #2**

### **Page 2 line 49**

**RC 2:** In spite of their putative relevance as an ecosystem service, very little is known about N cycling and N transformation rates in the sediment. Substantial work has been done on sedimentary nitrogen in the North Sea (e.g. see reference lists of the two papers mentioned above) and I suggest softening this statement.

**AR:** In the revised version, we softened the statement and now explicitly refer to ammonification (line 51 – 59).

**Page 4 line 109**

**RC 2:** cores. . . were incubated in a gas tight batch-incubation setup for 24 hours. The setup of this incubation is critical for the interpretation of the results and the authors need to include more detail: Were all batch incubations done on board or back in the lab? What was the time delay between coring and incubation? What was the length of the sediment cores and were all cores of equal length? What was the height of the water column above the cores, and what were the starting and end oxygen concentrations? Was the water column fully mixed in the core liners? Was stirring the same in all cores? What was the fauna in these cores and how was its activity accounted for? What were the light conditions during incubation? Were all cores incubated with the same water or with the water they came with?

**AR:** We expanded the method section and created a new figure of the core incubation setup (Fig. 2). We also added information regarding stirring and core handling etc. to the manuscript (line 108 – 130).

**Page 4 line 114**

**RC 2:**  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentration of the added tracer solution was the same as the bottom water concentrations (Tab. 2). This is not clear. Does this mean that you added tracer while maintaining the original nutrient concentration, i.e. removed some water and then added a mix of water and tracer that had the same nutrient concentration as the original water? If that was the case, please specify how much tracer was added and if the different tracer additions (nutrient concentrations differed up to factor 20) could have influenced the incubation experiment. In Line 116 you mention that the label addition was calculated aiming for a maximum enrichment of 5.000 ‰ in substrates and products. How was this achieved if added tracer solution concentration was the same as the bottom water concentrations, and the incubations ran with the same volume of overlaying water?

**AR:** As we outlined in the original response letter, we replaced site water with label solution. More detail regarding this addition has been added to the method section (line 109 – 130).

**Page 5 line 118**

**RC 2:** Upon sampling, incubation water was filtered with a syringe filter (material, manufacturer, 0.45  $\mu\text{m}$  pore size) Insert material and manufacturer.

**AR:** Done (line 126 – 128).

**Page 6 line 157**

**RC 2:** The surface sediment samples of the cruises HE 383 (06/07.2012) and HE 447 (06.2015) for NOAH-D were analyzed for total carbon and total nitrogen contents with an elemental analyzer (Carlo Erba NA

1500) via gas chromatography calibrated against acetanilide. Please be more specific: How deep was the sediment layer termed “surface sediment”?

**AR:** Top 1 cm – added to the manuscript (line 145).

**Page 7 line 184**

**RC 2:** Three O<sub>2</sub> profiles were measured in one sediment core of each station. Please specify the conditions: When exactly where those profiles measured, i.e. how long after retrieval of the core? What were the conditions during the measurements e.g. was there a water layer above the sediment?

**AR:** We added more detail to the method section (line 139 - 143).

**Page 7 line 195**

**RC 2:** The lowest oxygen flux was determined at the permeable sediment station NOAH-A with -10.0 mmol m<sup>-2</sup> d<sup>-1</sup>, the highest oxygen flux was measured at the impermeable sediment station NOAH-C with -53 mmol m<sup>-2</sup> d<sup>-1</sup>. The semi-permeable sediment station NOAH-D had an oxygen flux of -18.5 to -30.6 mmol m<sup>-2</sup> d<sup>-1</sup>. As pointed out by the authors in the discussion section, the fluxes in the permeable sediment vary with the flow above the sediment. For which flow setting were the fluxes reported here? The same question applies to the statement in Line 203: The lowest ammonification rates were measured in the semi-impermeable sediment at station NOAH-D.

**AR:** We amended the method section (line 176 – 192).

**Page 10 line 283**

**RC 2:** In total, though, we estimate that benthic N fluxes support between 13 % (at a water depth of 38 m) and 61 % at 10 m depth (Tab. 3) of primary production. As this is based on one time summer sampling only, I suggest softening this statement.

**AR:** We now address seasonal effects in the discussion section 4.2 (denitrification). We also rewrote the discussion, now addressing the significance of benthic fluxes in a separate section, 4.4 (line 337 – 407)

**Page 10 Line 307:**

**RC 2:** Nitrification rates are relatively independent of permeability, in contrast to ammonification. This needs further explanation. In the discussion, you mention the potential importance of the flushing of the permeable sediment, which could transport organic matter and oxygen into the sediment. This would have

direct implications for both, ammonification as well as nitrification. Why was nitrification relatively independent of permeability?

**AR:** Nitrification is controlled by the availability of oxygen and nitrate, and the substrate limitation apparently limits nitrification at the stations with higher sediment permeability. We discuss the regulation of nitrification now in the revised discussion (line 271 – 306).

**Page 11 line 311**

**RC 2:** Nitrification rates are lowest at Station NOAH-A. Here, oxygen penetration depth is highest, and the sediment has low organic matter content (Tab. 2), which obviously limits nitrification rates. This statement contradicts the statement on line 307, where you say that “nitrification rates are relatively independent of permeability”.

**AR:** As outlined above, we now discuss the regulation of nitrification in our sample set in more detail (line 271 – 306).

**Page 20 table 2**

**RC 2:** Random frictionless packing in sand produces a porosity of 0.39, and although lower numbers can sometimes be measured, a porosity of 0.29 seems unrealistic. Was the core fully water-saturated? Please check whether these numbers were reported as weight or volume ratios. Practical salinity is based on ratios and should be expressed by dimensionless number only.

**AR** As we outlined in the initial letter, this low value was an artifact, we now corrected it (new: 0.41). We also now refer to salinity as a dimensionless number. The correct porosity is 0.41 (v/v) and was measured on a fully saturated sample. We updated the manuscript accordingly, and we changed the salinity to dimensionless numbers where appropriate (Tab. 2).