Response to Reviewer 1

This paper reports mechanisms of fixed nitrogen loss in the sediments of Lower St. Lawrence Estuary (LSLE). Strictly speaking, N loss mechanisms are those that, regardless of the pathway, produce NO, N2O and/or N2. So far, denitrification, anammox and nitrification (producing N2O) are the best known and identified processes, all others have not been well-studied.
In particular, this research includes denitrification and anaerobic ammonium oxidation by nitrite (anammox) and by metal (Fe and Mn). The latter not well explored yet so it is a very good scientific contribution. It also have measurements of dissimilative nitrate reduction to ammonium (DNRA). The authors include the latter as a removal process, but it transforms nitrate into ammonium, a very reactive and bio-available nutrient. Therefore, strictly speaking, it is not a loss mechanism. This warrants clarification or a change in the title of this paper.

This is a well written and comprehensive manuscript, with very complete and detailed methodology involving two approaches (slurry and intact cores). Similarities and differences in rate measurements between both techniques helps to better understand the regulation of biogeochemical processes and biases of used methodologies. Both issues should, therefore, be the strength of this research.

I have two major concerns: The first is that this research only presents experiments from one station, so it may not be representative of the entire study area as the title suggests. Furthermore, the introduction is focused on the role of sediment in the N budget. I think that one station is insufficient for scaling up to large ecosystems. I believe a simple way to resolve this issue is by changing the title, and reorganizing and focusing the introduction, redirecting the MS towards a comparison of both methodologies used. My second concern is that I believe that there is an error in the interpretation of anammox rates in slurry sediment. I cannot figure out how you have obtained an anammox signal (when the addition of 15 NH4+ did not produce any results in 29N2 recovery), nor how you can compare a volumetric (from slurries) with areal rates (from intact cores). This should be clarified and emphasized, in particular the magnitude of denitrification rates and the fact that both techniques produced similar trends. In this sense, the abstract did not reflect the contents of the Ms. It started with the importance of anammox, included a rate value (only measured from intact sediment), but what about mentioning the other method and denitrification rate? Then, it mentioned the role of nitrification in oxygen utilization during the oxidation of ammonium and nitrite, but this work measured oxygen utilization rates?. Again one station is not enough to extend the conclusion to the whole of the LSLE.

Reviewer 1 raises two major concerns: 1) that a single station is not sufficient to constrain an entire ecosystem; and 2) that our finding of anammox is inconsistent with the ^{15}N ammonium labeled slurry experiments not producing $^{29}N_2$. We agree that a single station is not sufficient to scale up to a complete ecosystem. We will

state this explicitly in the manuscript and will change the title to: *Anammox, denitrification and fixed-nitrogen removal in sediments **from** the Lower St. Lawrence Estuary.* Nevertheless, as we report the first set of direct measurements for the St. Lawrence Estuary, our estimates for the importance of anammox in the entire estuary, are the best available, especially when viewed in light of other measurements made throughout the Estuary that constrain the budgets but not the pathways. We thus prefer to leave the whole estuary extensions and budgets in the MS but with statements explicitly noting their limitations. This will be reflected in the revised abstract.

The ^{15}N-ammonium labeled experiments failed to generate $^{29}N_2$ because of the absence of the ^{14}N-nitrate or ^{14}N-nitrite in the slurries, required to produce $^{29}N_2$ by anammox. Both nitrate and nitrite were entirely consumed during the pre-incubation period, and were therefore unavailable for anammox in the ^{15}N-ammonium labeled experiments. This, however, does not mean that anammox is not active in the sediments: when ^{15}N-nitrate is supplied, $^{29}N_2$ is produced (pairing of ^{15}N-nitrate with ^{14}N-ammonium) confirming anammox activity.

Reviewer 1 was uncertain how volume specific rates measured in our slurry incubations were used to estimate area specific rates. These estimates was made by assuming that the rates measured in the slurry were half the volume-specific rates of the in situ sediment (due to 50% dilution with seawater), and that these rates were representative of the upper 2cm layer of the sediment. This is discussed in lines 23,24,25 of page 15, with the additional relevant information on porosity and sediment density given on lns 20-23 of page 10. We will repeat the details of the calculation explicitly in Appendix A.

We disagree with the comment that the abstract does not accurately reflect the contents of the manuscript, but we will add values for the other rates in addition to the anammox rates. O_2 utilization rates during nitrification were computed from the O_2 microprofile and the nitrification rates.

Minor comments:

In this section the location of station 23 should be mentioned (upper or middle part of the estuary?). Also, the hydrographic setting should be provided: what kind of estuary is LSLE? Is there any temporal variation along an annual cycle?

Co-ordinates for Stn. 23 will be added to the methods section. (The core used for the incubations was recovered at: 48°42.032'N/68°39.171'W; 345m. Overlying water oxygen, nitrate and phosphate concentrations were 63 µM, 34µM and 2.7µM, respectively)

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A more complete description of the St. Lawrence Estuary will be added. We don't feel that the hydrographic data would add sufficient value to the MS to warrant an additional figure.

Some text will be added to the introduction:

The 300 Km long, 50 Km wide, and 0.3 Km deep Lower St. Lawrence Estuary (LSLE) occupies the landward portion of the Laurentian Trough, a glacial bathymetric feature that extends 1200 Km landward from the edge of the continental shelf. Because of its great depth, the water column in the LSLE is permanently stratified with net seaward flow in the suface layer and net landward flow in the bottom layer.

And some text will be added to the a new section entitled 'site description' which will include a map and the following text:

The dominant bathymetric feature in the LSLE is a 300-350 m deep glacially-modified submarine trough, known as the Laurentian Channel (or Trough), that extends from Tadoussac all the way to the eastern Canadian continental shelf break (Fig. 1). Sediments in the channel are composed of fine-grained particulates (pelites) with, on average, 60 % clay, 35 % silt and 5 % sand (Nota and Loring 1964). The sediments are dark yellowish-brown in the first 1-3 cm below the sediment-water interface, reflecting the presence of detrital and authigenic ferric iron [Fe(III)] and manganic [Mn(IV)] minerals (Loring and Nota 1968; Lyle 1983; König et al. 1997). Below this oxidized layer, the sediments are dark greenish-grey (Loring and Nota 1968).

Regarding the slurry, sediment parameters should be included (apparent density and porosity), in order to ensure reproducible measured rates. In order to do so, it is necessary to estimate real rates, taking into consideration the proportion of water (porewater and added water) vs. used sediment (solid matrix).

Density and Porosity values were reported (lns 20-23 of page 10), but will be repeated in Appendix A

Table 1 is not well explained and expected results for each treatment should be included. Also, the role of ATU (a metabolic inhibitor of aerobic ammonium oxidation)should be clarified.

We will improve the explanation of Table 1 and add columns with the outcomes we predict. We will add a sentence explaining that ATU is a specific inhibitor of nitrification, blocking the oxidation of ammonium to nitrite.

Another point, did you expect coupling between nitrification and denitrification. If possible,you could distinguish between 29N2 and 30N2.

We have partitioned the coupling of nitrification to denitrification and presented the results in table 13. The coupling is discussed extensively in the original manuscript. (lns 25-29 pg. 17 and lns 1-3 of pg 18).

They must be put into a scientific context. In terms of style, do not use slurry incubation, extractions, etc as sub-titles. Replace these terms by the processes being quantified.

We disagree and feel that organizing the results according to the respective experiments/datasets is the correct way to do it.

The first paragraph is very general and must be moved to the introduction.

We agree with the reviewer and will move the first paragraph of the discussion to the introduction

Regarding the vertical pore-water profiles, explaining whether ammonia profiles reflect the observed ammonium consuming processes (e.g. aerobic and anaerobic ammonium oxidation vs. organic matter regeneration) would be a scientific contribution.

We do not agree that this would enhance the scientific value of the manuscript. Based on the oxygen microprofiles, ammonium produced below about 1 cm depth is anaerobic. Though interesting, partitioning the pathways of organic matter degradation between the different electron acceptors is beyond the scope of this study.

Why estimate diffusional flux if most of the used nitrate for the dissimilative process comes from nitrification?

Diffusional fluxes are calculated to estimate the assimilative pathways.

Part of the discussion should be focused on why anammox rates are rather different depending on the methodology used. Please discuss about what others process couldbe responsible for unaccounted N sink?

The differences between slurry and whole-core incubations are discussed in the original manuscript (page 16 lns 1-19).

Mass balance based on a single station can be imprecise and, as noted by the reviewer, is therefore speculative.

We propose this as one possible explanation for the apparent missing N-sink (lns 12-15 pg 18).

Table 3 seems skewed: values do not correspond to column titles.

Table 3 is viewed correctly in our version of the MS. See above comment.

Response to Reviewer 2

General comments: The nitrogen cycle seems to get more complex every time I read another paper. And it's no wonder given all the thermodynamically-favorable reactions that can potentially occur in nature and the remarkable ability of microbes to evolve new ways of making a better living through chemistry. But, just because a biochemical reaction is possible does not mean it actually occurs in nature or plays a significant role in the nitrogen cycle. Crowe et al. address the important question of which reactions really matter in the sedimentary nitrogen cycle of the St. Lawrence Estuary. They approach this problem by inoculating sediments with stable isotopes of N in both slurry and whole-core incubation experiments. More specifically, the authors examined the relative importance of nitrification, denitrification, anammox, dissimilatory nitrate reduction, and ammonium oxidation by Mn(III, IV) and Fe (III), and organically-complexed Mn(III) in sediments from one station in the St Lawrence Estuary. The authors found relatively modest rates of denitrification, that most of the nitrate fueling denitrification was produced by nitrification, that two-thirds of the N2 was produced by the "classical" denitrification mechanism whereas the remainder was produced by anammox, and that the other processes they examined were relatively unimportant. Overall, the paper was well written and I appreciated the detailed description of the methodology. The results mostly confirm what other researchers have found, that coupled nitrification denitrification is important and that anammox contributes significantly to N2 gas production. The more novel N-pathways examined appeared to be of minor importance, which is an important finding. I have just two comments that I would like the authors to consider.

Reviewer 2 had two comments to address: 1) that our results need not apply to the entire estuary; and 2) that sulfide may have accumulated to inhibitory concentrations during our experiments.

First, it is a stretch to draw conclusions about the N-cycle of the St. Lawrence Estuary using data from one station. Thus, although these results are important for improving understanding of the N cycle, I think the authors should restrict the scope of interpretation to this one particular location.

We addressed this first comment in our response to reviewer 1. We will tone down the extensions to the entire estuary in the revised manuscript.

Second, the slurry experiment showed little NH_4^+ oxidation by Mn and Fe (hydr)oxides. However, it is not clear whether this result is due to experimental conditions or if this type of anaerobic ammonium oxidation is in fact not occurring at the station. It is well known that even a brief exposure to sulfides in slurry

experiments inhibits ammonium oxidation to NOx. Although this phenomenon is usually interpreted as inhibition of nitrification (aerobic ammonium oxidation), it is unknown whether sulfide also inhibits ammonium oxidation by Mn and Fe (hydr)oxides. Thus, I interpret these findings with caution. Sulfide concentrations in the slurry experiments were not reported, but would have likely accumulated during the 12h pre-equilibration period and would be consistent with the lack of nitrification observed. I would not be surprised if microbes can carry out these thermodynamically-favorable reactions under the right conditions. At this point, we still don't know.

In response to this comment, we noted in the manuscript that anaerobic respiration during the course of our slurry incubations was unlikely to have exhausted the pools of reactive Fe and Mn species.. Given that these are thermodynamically more favorable electron acceptors than sulfate and,therefore inhibit sulfate reduction and the ensuing sulfide production, it unlikely that much sulfide was produced over the course of our incubations. If, in fact, sulfate reduction were active, at the respiration rates described in the manuscript, this sulfide would have been rapidly consumed by re-oxidation with the oxidized forms of Fe and Mn present. It is unlikely that sulfide would have accumulated to levels inhibitory to putative anaerobic nitrificiation over the course of our incubations.