The authors thank Dr. Thibault de Chanvalon for his thoughtful comments on our manuscript. The reviewer's comments and suggested revisions will improve the final version of the manuscript. Our responses are in blue, below.

The manuscript investigates the change of N2O production and denitrification (DNF) in slurries after the physicochemical perturbations artificially produced. In general, the different kind of perturbations induce an increase of the N2O/DNF ratio during about one month. Based on estuarine slurries the authors also demonstrate that communities adapted to changing environment are more adapted to the changes.

The approach seems original and provides important information about the variability of N2O emissions. An important effort is made to keep the result and discussion synthetic in order to focus the reader's attention on the concepts presented, well summarized in the figure 5.

However, before publication, it seems important to lift three concerns I have concerning the experimental design:

1 – All the rates measured assume linearity of the N2O increase. From my experience, it is a transient species i.e. after a burst of NO3 it is going to increase then decrease. You wrote it increases linearly but did you measure it ? (it seems so for the pulse experiment but no time series is shown in the result) If not how can you justify your assumption?

We measured N_2O increase over time in these various experiments, typically measuring four to five timepoints during each incubation. We report the final rates calculated from the slope of the increase in N_2O over time in our manuscript. An example of our timecourse data is shown below (Fig. RC2a). We will clarify that we calculate rates using timecourse data and include this figure in the supplementary information in the revision.

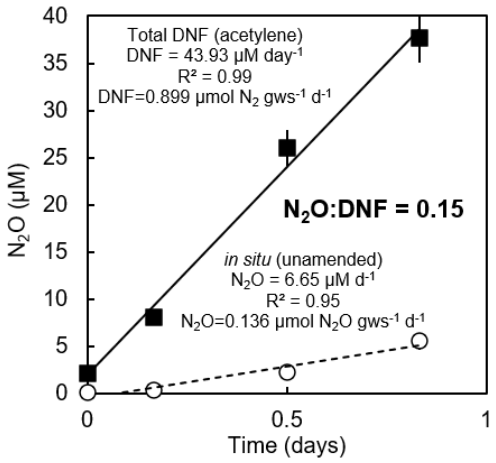

Figure RC2a. Example of timecourse concentrations of N_2O in the headspace of soil incubations for the determination of N_2O production and denitrification (DNF; amended with acetylene). These soils are from the temperature assay with agricultural soils (experiment 2).

For the increase to be linear, it is necessary to measure the initial rate, at least when less than a third of the NO3- has been consumed, however when I try to calculate mass budget of N in your experiment, it appears that almost the entire stock of nitrate have been consumed (for the higher rates reported). Can you give a maximum rate measurable for each of your experimental conditions? Did you measure the NO3 changes? Why do you not consider the importance of possible other intermediate species contributing to DNF such as NO2-?

Exp1

20g x 1 umol/g/d x 12h= 10umol

10mL x 1mmol/L = 10 umol

Exp 3

10mL (g?) of slurry diluted by 2 = ~5g x 2 umol/g/d x 12h = 5 umol

10mL x 0,4 mM = 4 umol

For experiment 1, the author is correct that there was 10 umol NO_3^- available in the slurry and that a rate of 1 umol gws-1 d-1 would consume that amount of NO_3^-. For experiment 3, there was 8 umol NO_3^-available (10 ml slurry + 10 ml amendment was brought to 0.4 mM) and a rate of about 1.5 umol gws^{-1} d^{-1} would have consumed the addition during the full incubation period. As noted above, however, we measured N_2O and DNF over time during the incubation period, typically at 0, 2, 4, 8, and 12 hours. If

rates were high enough that substrate limitation was an issue and the production of N_2O levelled off over time, we removed those timepoints from the rate calculation. The maximum rates measurable are therefore higher than any rates we report here. For instance, in experiment 1, rates would need to exceed 6 umol gws^{-1} d^{-1} to result in substrate limitation prior to determination of the linear slope of increase in N_2O. In experiment 2, the rates would need to be greater than 8 umol gws^{-1} d^{-1} to exceed our ability to calculate the linear slope of increase. Rates were consistently lower than these values. We are confident that substrate limitation did not influence the rates we report here.

It is currently not clear in the manuscript that we measured N_2O over time during the incubation periods and used the linear increase to determine the rate, which led to the concern about substrate limitation and nonlinearity in N_2O production the reviewer outlined above. We will include more specific information about the timecourse sampling for each experiment in the manuscript revision. We did not measure the change in NO_3^- or NO_2^- concentration in our experiments. The measurement of NO_3^- or NO_2^- may have supplied additional interesting information about NO_3^- uptake and the concentration of the intermediate (NO_2^-) but would not, ultimately, inform our conclusions about N_2O production and denitrification.

2 – The main goal is to understand the effect of an artificial perturbation on N2O production. To this aims the authors compare N2O production of perturbed slurry to a reference. However, the reference itself seems strongly perturbated by the experimental design. In particular, the anoxic conditions produced modify the community structures. Similarly, glucose addition would favor opportunistic species. Have you performed the experiment without these two modifications to obtain a more realistic reference rate measured? Why do you think it is mandatory to use these two perturbating conditions?

We agree that the experimental design that includes soil slurries, amendments of nitrate and organic matter, and the addition of acetylene may result in perturbation in addition to any perturbation caused by the changes in other physicochemical conditions. We include a paragraph in the discussion that outlines these concerns (lines 249-261). We fully agree that additional studies with methodology that retains undisturbed soil structure without amendments of nitrate or organic matter is required. It is possible that the perturbation response we outline here would be even higher since, as the reviewer notes, our control soils are perturbed by our design. Approaches using, for instance, isotopically labeled N to track denitrification and N_2O

production would be logical next steps to further evaluate the perturbation response we propose here.

We have explored the role of organic matter additions on the rates of denitrification and nitrous oxide production as outlined in this manuscript. For instance, we conducted a set of experiments with sediments from the Scheldt River without organic matter additions and with glucose, acetate, and lactate added at various concentrations (Fig. RC2b). We found that, while rates of denitrification increased with organic matter additions and responded to the type of organic matter (glucose>acetate>lactate), nitrous oxide production did not change appreciably and the N_2O:DNF ratio decreased with the addition of organic matter (Fig. RC2b). We felt that this data, while interesting, is not directly related to the perturbation response hypothesis we are reporting in the manuscript and so does not belong in the revision. However, these findings would suggest that the perturbation response might be even stronger than we report here given that glucose amendments were made to all three of the experiments we report here.

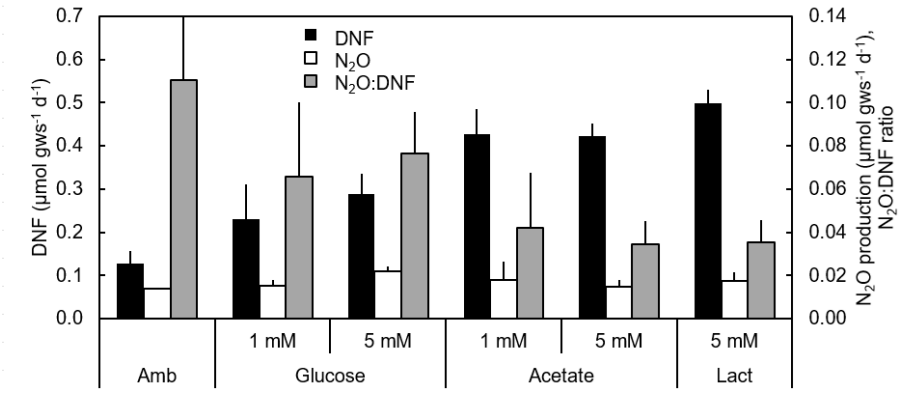

Figure RC2b. Rates of denitrification (DNF; N2+N2O), N2O production, and the N2O:DNF ratio in estuarine sediments (Scheldt River) incubated without organic matter addition (Ambient), and with 1mM or 5mM additions of glucose, acetate, or lactate.

We likewise explored the response of nitrate availability on rates of denitrification and N_2O production (Fig. RC2c). Higher N_2O production in response to greater nitrate availability is a well-known phenomenon (e.g., Firestone et al., 1980), and we observed higher N_2O production with increased nitrate availability even as rates of total denitrification reached their maximum at lower levels of nitrate availability (Fig. RC2c). We do not feel that this figure, which confirms a relatively well-known response, is needed in the revision.

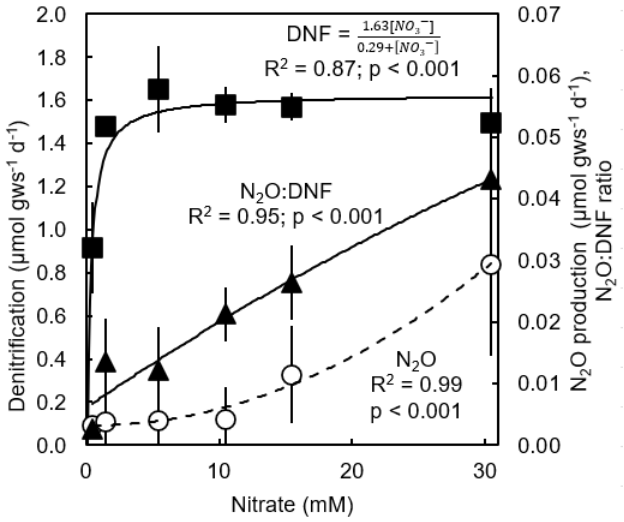

Figure RC2c. Rates of denitrification (DNF; N2+N2O), N2O production, and the N2O:DNF ratio in estuarine sediments (Scheldt River) at various nitrate concentrations.

We elected to use concentrations of nitrate low (<2 mM) relative to the tens of mM that elicit high N_2O production irrespective of perturbation effects in our experimental design. The sediments we used for the perturbation experiments were reducing wetland sediments that has very low ambient nitrate, and the acetylene method (anoxic conditions and the presence of acetylene) does not allow for nitrification. The addition of nitrate is meant to alleviate substrate limitation and produce a "potential" rate of denitrification. As stated above and in the manuscript, building on this investigation of the role of perturbation on nitrous oxide dynamics with methods that do not require amendments or manipulation of soils/sediments is needed. We do feel, however, that the current manuscript outlines a relatively novel way of better understanding N_2O production dynamics that, even with some methodological challenges, warrants publication.

3 – In the first experiment, the reference incubation (for ΔT= ΔZinc = Δmoisture = ΔSalinity = ΔpH =0) for the soil treatments present in Figure 1 should correspond to the same slurry. However, very different production rates are reported (from 0 to 0.8 for N2O production and from 0.2 to 2.8 for DNF) which cast serious doubt about the reproducibility of the experiments, and the validity of the experimental design.

The moisture treatment entailed air-drying soil prior to amending soil with water to achieve various soil moisture treatments. The drying of the soil prior to the rate measurements likely resulted in the lower rates measured in this treatment compared to the other treatments. The reference condition does not differ as much for the other treatments, though we agree there is some variation. These differences may stem from the timing of the incubations and the condition of the soil. Given the large number of

treatments (a total of 360 incubations were performed with more than 1400 N_2O samples analyzed on the gas chromatograph in the first experiment), each of the five perturbation incubations were performed at different times. The incubations were all performed within a 2 month period, but changes in the environmental conditions of the source soil may have influenced the reference rates of DNF and N_2O production. We do not make any comparisons of rates across treatments, but rather we compare rates of DNF and N_2O production within each incubation to elucidate the role of perturbation relative to the reference. The differences between the rates in the reference conditions in each treatment, therefore, are not of concern. We will clarify this in the revision.

Additionally, the methods need much better description of the methodology used and the associated limitation (see below). It could be done in a supplementary file.

I would also appreciate the author to propose a hypothesis about the underline mechanism responsible for such common type of answer: does N2O producer adapt faster than N2 producer? Is it due to thermodynamic barrier?

We outline the underlying mechanism(s) that we argue are likely responsible for the observed perturbation response in the final paragraph of the discussion (lines 313-331). The nosZ and nirS expression results point to inhibition in the expression of nosZ and not of nirS following salinity perturbation. This is followed by a recovery in nosZ expression together with a reduction in additional perturbation response, indicating that initial perturbation confers resilience on the microbial community. Whether these responses are due to changes in enzyme expression within the existing microbial community or changes to the overall microbial community (or some combination of the two) is beyond the scope of our study.

Details remarks:

Sites characterization:

Why selected the 0-2 cm depth layer? did you check the absence of oxygen and the decrease of NO3 to identify the layer with the most active denitrifying community?

We did not verify absence of oxygen or nitrate update rates when selecting the soil/sediment depth, but surface soils are typically the most biogeochemically active layer within the soil/sediment column.

Do you have other ancillary parameters that could give some chemical context:

Do you know the natural NO3 concentration, does it vary between your samples, could that play a role?

Did you characterize by any way the natural heterotrophic activity? (organic matter lability? Oxygen consumption -DBO5 of your slurries? Enzymatic activities?)

Did you measure other elements that could interfere in the N cycle such as redox element (Fe, H2S, ...) ? or other N species that could better describe the natural N cycle of your site such as NO2- ?

We did not measure these soil/sediment characteristics uniformly across the sites/experiments. We argue that these parameters would be important in comparing rates of denitrification and/or N_2O production across sites, they are less important in understanding the perturbation response in a laboratory setting as reported here.

Incubation Design:

For experiment 3: “the jars were amended to a final concentration of 0.4 mM NO 3- and 0.8 mM glucose weekly” it is not clear if the chemicals were added to increase the slurries concentration ***by*** 0.4 and 0.8 mM or ***to*** 0.4 and 0.8 mM (which implicate you measured the NO3 and glucose concentration)

The slurries were amended by 0.4 NO_3^- and 0.8 mM glucose weekly. We did not measure the glucose levels. This will be corrected in the revision.

For experiment 1 and 2, 10mL of water is mixed with 2g of soil or sediment, where does the water come from?

In experiment 1, the water mixed with soils was deionized water amended with nitrate and glucose. In experiment 2, the water was site water collected from the sites and diluted with water from the Apples site (freshwater) or seawater to achieve the target salinity. This will be detailed in the manuscript.

Is the use of N2 purge to take off O2 could change your community behaviour ? Why do not use Ar instead ? why did you use He for the second set of experiment?

He was used in the 2nd experiment versus N_2 in the 1st and 3rd experiments simply because of the various resources available to us in the laboratories in which we were conducting our research (Villanova University in Pennsylvania, USA for experiment 1 and 3, and the Netherlands Institute of Ecology and the University of Georgia, USA for experiment 2). N_2 is ~80% of the atmosphere that these soils/sediments are exposed

to, and we expect that purging oxygen with 100% N_2 would not change the microbial community behavior relative to argon or helium.

Analytical strategy :

How did you took your aliquots from your jars for N2O analyses? is there any risk of O2 contaminations during this sampling, in particular for pulse experiment where a time serie was performed with He in the headspace (a very volatile gas)? What the introduction of O2 could produce? Did you check the absence of oxygen ?

For experiments 1 and 3, headspace samples for N_2O analysis were removed using 10 ml syringes (with valve) and injected into gas chromatograph within hours of collection. The system we use is gas tight, though there is always risk of oxygen contamination. The introduction of a small amount of oxygen is unlikely to alter results in a significant way. Denitrifiers are typically not as sensitive to oxygen as microbes performing terminal respiration with more reduced electron acceptors (such as sulfate reduction and methanogenesis), and oxygen would be quickly consumed in the incubation jars. We carefully reviewed the production of N_2O in each incubation jar (i.e., Fig. RC2a) that would indicate nonlinearity in N_2O production in response to any major disruption (in addition to substrate limitation). We do not feel that the introduction of oxygen in these experiments was likely. We will clarify the details of the sampling in the revised manuscript.

How long did you store your gas aliquots before measurement ? In which vessel ? what is the detection limit of your instrument ? are you always far above the detection limit?

The samples in experiment 1 and 3 were analyzed within hours of collection (they were sampled into 10 ml syringes with a valve). The samples in experiment 2 were sampled into gas-tight, evacuated vials with a septa closure. These samples were stored for several weeks prior to analysis. These details will be clarified in the revision. The electron capture detector gas chromatography approach has very low detection limits. While our initial samples during the timecourse measurements (i.e., Fig. RC2a) are sometimes below detection, the production of N_2O was easily detectable in subsequent measurements.

How did you estimate the mass of sediment? Is it the mass of solid calculated from porosity or the mass of the bulk sediment (ie mainly water)?

We report values as rates per gram fresh/wet sediment/soil. This will be clarified in the text of the manuscript.

Writing:

In figure 2, I recommend to put two black lines in the oligohaline and mesohaline sites to delimitated the daily changes of salinity.

This is a good suggestion. However, we do not have data sufficient to bracket salinity at these sites on a daily basis. Salinity changes significantly over seasonal cycles as well due to changes in discharge. In the text, we will include additional information about the salinity range at these sites. From van Damme et al. (2005; 10.1007/s10750-004-7102-2), we can say that the Appels site is uniformly fresh and salinity ranges from 2 to 25 psu at the Waarde site. There is less information available about the Rattekaai site in the Oosterschelde, but Gerringa et al. (1998) suggests the salinity is generally around 30 psu with less seasonal and daily variation.

For the title of sections 2.1 and 2.2, I suggest to replace “perturbation” by “Pulse disturbance” in order to keep the same expression along the entire manuscript.

This is a good suggestion. We will make this revision as recommended.