

05 June 2026

Dear Prof. Shen,

We sincerely thank you and the reviewers for the time and effort invested in evaluating our manuscript. We have carefully addressed all remaining comments and minor issues raised in the latest round of review, and the corresponding revisions are highlighted in blue throughout the manuscript.

We hope the revised version is now suitable for publication.

All the best,

Eyal Rahav on behalf of all co-authors

Comments from AE

Key word: consider replacing “Oligotrophic” with a more informative keyword.

Reply: We agree that ‘Oligotrophic’ is too broad as a standalone keyword. We have therefore replaced it with ‘Oligotrophic marginal sea’, which more precisely conveys both the trophic status and the oceanographic setting of the study.

Replace “Chlorophyll.a” with “Chlorophyll-a” or “Chlorophyll a” throughout the text.

Reply: Corrected throughout the text and Figures 2&4.

Figure legend: spell out “Sept.” in full (September) for consistency.

Reply: Corrected in Figures 1-3 as suggested.

Fig. 4 is a bit blurry – suggest replacing it with a higher-resolution version. Please also provide higher-resolution versions of the other figures, if possible.

Reply: We now provide a high-resolution Figure 4. We also enlarged the text’s fonts.

Review 1

I am satisfied with most of the responses, the text reads better, but remain 2 points unclear for me.

Reply: We thank the reviewer for his/her positive assessment of our manuscript. We have fully addressed the two remaining minor comments in the revised version (please see below).

1. “For surface samples with the lowest NO₃, sequential injections of multiple aliquots from the same filtrate were used to accumulate the 10-20 nmol N required for a reliable IRMS peak”. This is the first time I see such method. To accumulate 10 nmol, it may require more than 10 more vials of bacteria, it is difficult for me to imagine how the system work. Could you please add more description in supplementary materials? Adding, for example, some photos of the devices/schemes and example spectrum would be helpful. If possible, some raw isotopic data attached (same examples would be good enough) would be more convincing.

Reply: The isotope analyses were performed at a collaborating ‘service’ laboratory, and raw instrument files, device photographs, and example spectra are not available to us for inclusion as supplementary material. We have therefore addressed this point by expanding the Methods description to more clearly explain the sequential injection procedure: multiple small-volume aliquots from the same filtrate were introduced sequentially into the same denitrifier vial, allowing sufficient N mass to accumulate within the volume constraints of Sigman et al., (2001) and McIlvin and Casciotti (2011). The cumulative blank was estimated and subtracted based on injection number, and samples where the ¹⁵N signal could not be distinguished from the cumulative blank were excluded. We believe this description is sufficient for reproducibility purposes.

We revised the text to highlight that the injections went into the same vial (i.e., one bacterial culture vial receiving multiple injections): “...*For sequential-injection analyses of low-concentration surface samples, multiple small-volume aliquots from the same 1-L filtrate were introduced into the same denitrifier vial to accumulate sufficient N mass, and the cumulative bacterial blank was estimated based on injection number and subtracted accordingly...*” (Lines 139-142).

2. “...At the same time, near-zero ambient NO₂- or NO₃- at specific depths and months (e.g., 40 m for ammonia oxidation and 60 m for nitrite oxidation in July; Figure 1) should not be interpreted as the absence of substrate availability, but as evidence of tight coupling between substrate supply and demand over the incubation period...” (Lines 342-345).

I didn’t mean substrate but mean product. That is, when you analysed ammonia oxidation, you measured nitrite; when you did nitrite oxidation, you measured nitrate. So I actually mean it is difficult for me to see how to measure the rate with such a low product concentration.

Reply: We revised the relevant sentence to clarify that near-zero product pool concentrations at specific depths reduce the total N mass available for IRMS, increasing analytical uncertainty. While low product pools simultaneously reduce dilution of the ¹⁵N-labelled product (thus partially compensating through elevated atom% enrichment) rate estimates at these depths are now explicitly stated as conservative lower bounds on nitrification activity. We have revised this sentence to improve clarity: “...*At the same time, near-zero ambient NO₂- or NO₃- at specific depths and months (e.g., 40 m for ammonia oxidation and 60 m for nitrite oxidation in July; Figure 1) reflects extremely low product pool concentrations, which reduces the total N mass available for IRMS analysis. While this simultaneously reduces dilution of the ¹⁵N-labelled product, resulting in elevated atom% enrichment, rate estimates at these depths carry greater analytical uncertainty and should be treated as conservative lower bounds on nitrification activity...*” (Lines 343-348).