Referee´s comments on "**Nitrate dual isotopes and nitrification rates in central Chile**" by G. Choisnard et al.
egusphere-2025-3514

General Comments:

This paper analyzes measurements of nitrate dual isotopes and bulk nitrification rates along a transect offshore of Concepción, Chile, during the upwelling season, providing insights into the spatial extent of potential nitrogen processes occurring in the water column that may explain the observed isotope signatures. Although the paper is relatively well written and potentially interesting for scientists working in the region, I have several concerns that, in my opinion, prevent its publication in its current form. In addition, the manuscript presents several important formatting inconsistencies that suggest limited rigor in its preparation. For example, Table 1 is not included, and nitrification rates are reported in the text but are not shown in the corresponding figure

My first concern relates to the general objective of the paper. In the introduction, no clear working hypotheses are formulated, and the stated objective is reduced to a single sentence: *"to explore the N cycling along a transect offshore of Concepción during austral summer"*. This framing gives the impression of a rather exploratory approach without a well-defined scientific rationale. I strongly recommend revisiting the introduction to more clearly articulate the aim of the study and to formulate explicit working hypotheses that provide a stronger conceptual foundation for the paper

The authors state in the abstract that nitrification is a low-oxygen requirement process. However, nitrification strictly requires oxygen, which acts as the electron acceptor during the oxidation of ammonium, although nitrifiers are known to function under low dissolved oxygen conditions. The authors should provide further explanation regarding the incubation setup and clarify whether specific precautions were taken to avoid oxygen contamination of water samples collected from hypoxic–suboxic zones. Such methodological considerations are particularly relevant, as they could help explain the increasing pattern of nitrification rates with depth observed at some stations

I consider another important issue of the manuscript to be the approach used for data analysis and interpretation. I strongly recommend including a plot of the estimated Ekman transport for the different sampling days, taking into account the synoptic wind behavior in the study area, to better evaluate the influence of wind-driven upwelling versus downwelling conditions on nitrification rates. Based on the data presented, it remains unclear whether sampling was conducted during an active upwelling pulse, a cessation phase, or wind-reversal conditions. To address this, an analysis of Ekman transport intensity (or an upwelling index) would help illustrate how wind forcing modulated variability in water column structure, biological processes, and geochemical signatures. For instance, during active and intense upwelling pulses, one would expect a less stratified water column with stronger influence of ESSW. In contrast, during relaxation or reversal of upwelling-favorable winds, one would expect a phytoplankton response to nutrient injection and stronger coupling between surface and subsurface processes through the downward flux of organic matter, which represents the main source of remineralized ammonium fueling nitrification. Moreover, features such as the homogeneous δ15N–NO2– profile observed at St 14 could potentially be explained by an intense upwelling pulse establishing similar conditions along the water column

Dissimilatory nitrite reduction to ammonium (DNiRA) has been demonstrated to be an important process that can sustain both anammox and nitrification rates, including the ammonium utilized for N_2O production at the St 18 (*Galán, A., Thamdrup, B., Saldías, G. S., & Farías, L. (2017). Vertical segregation among pathways mediating nitrogen loss (N2 and N2O production) across the oxygen gradient in a coastal upwelling ecosystem. Biogeosciences, 14(20), 4795-4813*). I wonder how the $\delta^{15}N$ signal left by this process in the NO_2^- (NO_3^-) pool is expressed, and how such isotopic signatures could contribute to the interpretation of the results presented here (e.g., *Tang, F. H., & Maggi, F. (2012). The effect of 15N to 14N ratio on nitrification, denitrification and dissimilatory nitrate reduction. Rapid Communications in Mass Spectrometry, 26(4), 430-442*).

Galán et al. (2017) also present $\delta^{15}N$ profiles in the NO_3^- pool across the upwelling season development that help to explain multiple N-cycling processes, including the first step of nitrification (nitrite production from ammonium oxidation). I suggest that the authors consult this study to better contextualize the results presented here.

I suggest reporting oxygen saturation values, as surface oxygen concentrations appear lower than historically reported (*see De La Masa et al., 2024*), particularly at the time-series stations (St 18) and the coastal station (St 14; 60 μmol L^{-1}). At St 14, oxygen measurements at depth approached the detection limit of the STOX sensor, suggesting that the entire water column may have been influenced by bottom waters, potentially affected from sediments. This influence could be further assessed using Ekman transport estimates.

Please discuss the potential influence on nitrification rates of possible oxygen contamination during sampling with the pump–CTD system (St 14, 18, and 26) compared to regular rosette (e.g., Niskin) sampling.

In the estimation of deviations in NO_3^- dual isotopes using the parameter Δ(15-18), the authors assume that assimilation and denitrification exert similar isotope effects on $\delta^{15}N$- and $\delta^{18}O–NO_3^-$. Please clarify the rationale for this assumption, which is repeated in the discussion (Lines 309–310), given that denitrification is known to have a larger enzymatic isotope effect than assimilation (Brandes et al., 1998; Sigman et al., 2005).

In the discussion of nitrification rates (Section 4.4), the authors suggest that the low rates relative to previous studies may be due to an intense upwelling event that dilutes the nitrifying community. However, low nitrification rates could also result from the limited availability of the labeled substrate added, as 50 nM of $^{15}N–NH_4^+$ represents only ~1% of the maximum ammonium concentration observed (~5 µM at ~25 m in St 26). According to Damashek et al. (2016), approximately 10% of the ambient ammonium is typically recommended for ^{15}N amendment incubations, which aligns with general best practices for such experiments (e.g., *Thamdrup, B., Dalsgaard, T., Jensen, M. M., Ulloa, O., Farías, L., & Escribano, R. (2006). Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile. Limnology and Oceanography, 51(5), 2145-2156*).

Specific comments:

Line 28. Change to "the marine **C** and N budgets".

Lines 41-42. Sentence is unclear; please revise for clarity.

Lines 59-60. Check the phrasing, as it appears contradictory.

Line 50. I suggest change "Dominated" to "Influenced".

Line 56. There appears to be an error in the values within the parentheses. It seems the authors intended to show the temperature range of SAAW in addition to salinity, but the numbers do not make sense.

Line 63-67. This sentence contains some inconsistencies. The ammonium concentrations reported at depth (up to 5 µM; e.g., Galán et al., 2014) at St 18, which are used to support N-loss processes, are higher than those previously reported in the oxycline (up to 2 µM; Farías et al., 2009). The vertical extent of N-cycling and N-loss processes can be further referenced in Farías et al. (2009, 2015), Fernández & Farías (2012), and Galán et al. (2017) (*Galán, A., Thamdrup, B., Saldías, G. S., & Farías, L. (2017). Vertical segregation among pathways mediating nitrogen loss (N_2 and N_2O production) across the oxygen gradient in a coastal upwelling ecosystem. Biogeosciences, 14(20), 4795-4813*).

Line 85. Include "e" in "thse pools".

Line 93. The authors mention Table 1, which is not included in the manuscript. I would expect this table to report the sampling dates to determine the time between water collections and to assess whether sampling occurred under the same upwelling or downwelling conditions.

Line 111. There are inconsistencies regarding isotope measurements and which samples were collected using the rosette (St 14 and 22) versus the pump–CTD system (St 18 and 26), relative to the information provided earlier (Lines 96 and 101).

Line 206. Clarify that $\delta^{15}N\text{-}NO_3^-{+}NO_2^-$ values increased in surface waters relative to values measured below the mixed layer.

Line 243. The sentence appears incorrect. It states, "Just below the mixed layer…", but negative $\Delta 15\text{-}18$ values are associated with surface samples.

Lines 245-247. The authors report that maximum nitrification rates at St 14 were found at 30 m depth, but this point is not shown in Figure 5b. Additionally, the depth and maximum rate reported for St 22 (60 m depth and 3.9 ± 0.3 nmol L^{-1} h^{-1}) appear incorrect; these values may be missing due to the axis break.

Line 256. Indicate that this stabilization of $\delta^{15}N\text{-}NO_2^-$ occurs for all stations except for the St 26.

Lines 295-300. I do not understand the rationale for this analysis. Why did the authors expect nutrient concentrations to match previous measurements? As noted at the end of the paragraph, the system is highly variable, with upwelling strength being the main driver of this variability. For this reason, I suggested estimating a parameter that quantifies upwelling intensity, such as Ekman transport or an upwelling index. This would help to interpret the observed variability in hydrography and biological activity.

Lines 331-341. I cannot follow the proposed argumentation. The authors explain possible mechanisms modulating the concentration and $\delta^{15}N$ observed in the primary and secondary NO_2^- maxima and propose processes such as nitrite oxidation (second step of nitrification) or nitrite reduction (during anammox or denitrification). However, both of these processes consume nitrite and cannot explain the observed accumulation. Please revise this paragraph for clarity and consistency with the observed data.

Lines 368-370. The argument presented here appears inconsistent. If partial denitrification leaves the system rich in nitrate and nitrite, it is unclear why the authors state that this process would limit primary production. Please revise this sentence for clarity and logical consistency.

Lines 386-388. Why do the authors compare the maximum nitrification rates measured in this study with nitrite oxidation rates from Fernández and Farías (2012)? The direct rates measured here using ^{15}N tracers do not discriminate between ammonium and nitrite oxidation (or at least these rates are not shown separately), and the only indication of these other processes is reflected in the $\delta^{15}N$ of NO_3^- or NO_2^-. Please clarify or justify this comparison

Figure 2. It is not possible to see the oxygen profile at St 14. Please include an inset to clearly display this profile.

Figure A1. Please check the rectangle area, as temperature and salinity ranges of the ESSW are higher than those currently shown (*Silva, N., Rojas, N., & Fedele, A. (2009). Water masses in the Humboldt Current System: Properties, distribution, and the nitrate deficit as a chemical water mass tracer for Equatorial Subsurface Water off Chile. Deep Sea Research Part II: Topical Studies in Oceanography, 56(16), 1004-1020*).

Figure A2. Please indicate samples below the mixed layer, where the primary NO_2^- maximum was observed, using a different symbol to distinguish them from surface samples.