Supplementary materials for “Significant mixed layer nitrification in a natural iron-fertilized bloom of the Southern Ocean”

Authors: F. Fripiat1, M. Elskens1, T.W. Trull2, S. Blain3, A.-J. Cavagna1, C. Fernandez3, D. Fonseca-Batista1, F. Planchon4, P. Raimbault5, A. Roukaerts1, and F. Dehairs1

1Analytical, Environmental and Geo – Chemistry; Earth Sciences Research Group, Vrije Universiteit Brussel, Brussels, Belgium

2 Antarctic Climate and Ecosystems Cooperative Research Centre, University of Tasmania, and CSIRO Oceans and Atmosphere Flagship, Hobart, Australia

3Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Océanographie Microbienne (LOMIC), Observatoire Océanologique, Banyuls/mer, France

4Laboratoire des Sciences de l’Environment Marin, Institut Européen de la Mer, Plouzané, France

5Aix-Marseille Université, CNRS/INSU, IRD, Mediterranean Institute of Oceanography UMR 7294 (MIO), Marseille, France

Corresponding Author : François Fripiat, Analytical, Environmental and Geo- Chemistry ; Earth Science Research Group, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels ; telephone number: +32 (0)2 629 32 71; email address: ffripiat@vub.ac.be

## S1. Nitrite interference on the combined Nitrite+Nitrate isotopic measurement

This study reports measurements of δ15N and δ18O of the combined pool of NO3-+NO2- and interprets the seasonal decoupling between N and O isotopes in the mixed layer as a significant input of nitrate by nitrification (i.e., early spring Δ(15-18) minus late summer Δ(15-18) = 0.53  0.30 ‰). We assume a negligible bias induced by the small and invariant NO2- contribution to the NO3-+NO2- pool (1.2 ± 0.5 %). In the discussion, the NO3-+NO2- δ15N and δ18O values are taken to reflect the NO3- δ15N and δ18O values. However, several recent studies highlighted that the inclusion of NO2- in measurements of the isotopic composition of NO3-+NO2- can have a significant impact on the results even if nitrite is present at extremely low levels [Rafter et al., 2013; Fawcett et al., 2015; Smart et al., 2015]. The sensitivity differs in regard of N and O isotopes and can, therefore, have an impact in the assessment of the decoupling between N and O isotopes as follows:

- *O isotopes*: During the reduction of NO3- and NO2- to N2O in the denitrifier method, O atoms can be transferred to the subsequent nitrogen oxide pool (ultimately to N2O) or lost as water with an isotopic fractionation [Casciotti et al., 2002]. For NO3-, this effect is corrected with a calibration curve based on analyses of NO3- isotopic reference materials [Casciotti et al., 2002]. Because NO2- reduction to N2O represents a smaller fractional loss of oxygen atoms than NO3-, the N2O generated from NO2- by the denitrifier method is ~25 ‰ lower in δ18O than N2O generated from NO3- with the same initial δ18O [Casciotti et al., 2007; Smart et al., 2015]. For the given NO2- contribution to the NO3-+NO2- pool (1.2 ± 0.5 %), this methodological bias is the same for both early spring and late summer samples (underestimation of NO3- δ18O by ~0.3‰). In addition to this methodological bias, ambient NO2- δ18O is likely to differ from NO3- δ18O. Though we are not aware of any studies reporting direct measurements of NO2- 18O for the surface ocean, ammonium oxidation leads to the production of NO2- with δ18O values between -3 and 14 ‰ [Casciotti et al., 2010; Buchwald and Casciotti, 2013]. Such range implies a further underestimation of the NO3 δ18O of 0.0  0.1 ‰ (~ 0.3  0.1 ‰ including the methodological bias).

- *N isotopes*: Since every N atom in both NO2- and NO3- is transferred into N2O with the denitrifier method, the interference from NO2- is only dependent on the ambient NO2- δ15N and its relative contribution to the NO2-+NO3- pool (i.e., no methodological bias). The N atoms of NO2- in the mixed layer are mainly produced from ammonium oxidation (AmO) and are consumed by nitrite oxidation (NiO) and nitrite uptake (NiU). NO2- δ15N will thus depend on the contribution of isotope effects (i.e., 15ε (‰) = ((14k/15k)-1) \* 1000; where nk is the rate coefficient for the nN-containing reactant), the relative importance of which are weighted by the relative contribution of their fluxes to ammonium and NO2- removals as follows [Fripiat et al., 2015]:

(1)

where org. N is organic N, R remineralization, AmU ammonium assimilation, AmO ammonium oxidation, NiU nitrite uptake, NiO nitrite oxidation, and where x and y are AmU/Amo and NiU/AmO, respectively. For a given system with relatively constant physical and biogeochemical properties, we can reasonably assume that the isotope effects associated with the different processes should not varied significantly with time. By taking the optimum guess for the combination of isotope effects (2 ‰ for the remineralization; 5 ‰ for NH4+ assimilation; 15 ‰ for NH4+ oxidation; 0 ‰ for NO2- assimilation; -13 ‰ for NO2- oxidation), NO2- δ15N lies between -8.6 and -23.1‰ [Hoch et al., 1992; Waser et al., 1997; Pennock et al., 1998; Casciotti, 2009; Casciotti et al., 2003; Santoro and Casciotti, 2011; Möbius, 2013; Vo et al., 2013; and reference therein]. Given this range and the relative NO2- contribution to the NO2-+NO3- pool (1.2 ± 0.5 %), such interference implies an underestimation of the NO3- δ15N of 0.3  0.1 ‰. We also tested the range of nitrite 15N given by Smart et al. [2015] in the winter Southern Ocean (-20 to -40 ‰). This range induce an underestimation of the NO3- δ15N of 0.5  0.1 ‰.

Since (i) we are looking to the seasonal changes in both δ15N and δ18O, and (ii) there is no significant change in the contribution of NO2- into the NO3-+NO2- pool (1.2 ± 0.5 %), an interference from an invariant NO2- δ15N and δ18O will have no effect on our assessment of the decoupling between N and O isotopes (i.e., early spring Δ(15-18) minus late summer Δ(15-18) = 0.53 ‰). It is only if NO2- δ15N and δ18O vary significantly between early spring to late summer that a bias in our assessment could occur. By taking the range for both NO2- δ15N and δ18O and the methodological bias for O isotopes, the seasonal decoupling between N and O isotopes is 0.56  0.22 ‰ (i.e., early spring Δ(15-18) minus late summer Δ(15-18)). Such range is indistinguishable to the average and precision given by the observations (0.53  0.30 ‰), implying a negligible impact of nitrite interference on the estimation of the contribution of nitrification to nitrate assimilation. The single-box model is optimized by fitting the model outputs to the observations by taking into account their associated uncertainties. Although that an underestimation of NO3- 15N and 18O is likely, this is not clearly seen with the initial vertical profile (20th October) showing the near full range in nitrite concentration but with no significant variations with depth in both NO3-+NO2- 15N and 18O (Fig. S1).

## S2. Vertical 1-dimensional box model: surface vs. subsurface nitrification

We use a time-dependent 1-dimensional vertical box model (0-250 m; 50 layers; Fig. S2) to qualitatively assess, from NO3- δ15N-δ18O distribution, if nitrification occurs only below the mixed layer (case a) or both in the mixed layer and below (case b).

The model simulates the change in NO3- concentration, δ15N, and δ18O for each layer, in the timeframe given by the observations (~20th October to ~13 February). Three processes, that present contrasting vertical distribution are considered (Fig. S2): (i) vertical mixing between each layer (with mixing rates in the mixed layer higher than in the underlying water column), (ii) NO3- assimilation in the mixed layer only (with an isotope effect of 5 ‰ for both 15N and 18O) [Granger et al., 2004; DiFiore et al., 2010], and (iii) nitrification, either considered to represent a fraction of NO3- assimilation in the mixed layer (mixed layer nitrification) or to decrease with depth from a subsurface maximum at the base of the mixed layer (subsurface nitrification) [Berelson, 2001]. Recently nitrified NO3- δ15N and δ18O have the isotopic composition of the PN (average for both KEOPS 1 & 2; 1.9 ‰) and the ambient water plus 1.1 ‰ (0.6 ‰ over the Kerguelen plateau, taking H2O δ18O in the Antarctic surface waters = -0.5 ‰) [Archambeau et al., 1998; Casciotti et al., 2008; Sigman et al., 2009; Rafter et al., 2013], respectively. The mass and isotopic balance for each layer (i) is given by:

 (2)

 (3)

 (4)

where S↑, S↓, NaU, and Nitr are the upward mixing fluxes (representing a fraction of each layer which is mixed upward at each time step), the downward mixing fluxes (representing a fraction of each layer which is mixed downward at each time step), NO3- assimilation, and nitrification, respectively. S↑ equals S↓ to respect the water balance (set at 0.25 in the mixed layer, the maximum in term of water balance, and 0.05 below in the control run). The model is run for N time steps (i.e., 1000) of length dt, consuming at each time step an amount of ∫NaU/N (= NaU) and adding nitrate from nitrification ∫Nitr/N (= Nitr), where ∫NaU and ∫Nitr are the integrated NO3- assimilation and nitrification, respectively. We tested two scenarios: (a) nitrification below the mixed layer only and (b) nitrification in the mixed layer and below.

For case (a), ∫NaU equals more or less the observed seasonal NO3- drawdown, 7.1 and 6.3 ± 1.5 µmol l-1, respectively. Exported PN (=∫NaU) is remineralized and nitrified, following an attenuation with depth (using the average oceanic depth attenuation coefficient, 0.82, in a power law) [Berelson, 2001]. For case (b), ∫NaU equals case (a) plus the nitrification in the mixed layer. We took a contribution of nitrification to NO3- assimilation in agreement with the best fit (0.7) as detailed for the single box model (see section “Surface contribution of nitrification to NO3- assimilation”). Such rationale implies the same seasonal NO3- drawdown in both cases (a and b). Nitrification below the mixed layer is parameterized as for case (a). In both cases, the computed vertical net NO3- supply (upward – downward) into the mixed layer is 0.2 mol m-2 (with upward and downward fluxes at 7.2 and 7.0 mol m-2, which represents already a significant mixing rate in comparison with other rates, e.g., nitrate uptake, ammonium uptake, PN export, …), similar to the estimated vertical NO3- diffusive flux (0.2 ± 0.2 mol m-2; see section “Biogeochemical properties”).

The model sensitivity to the different parameters is shown in Figure 6. The Vertical Δ(15-18) distribution is highly sensitive to the assigned nitrified NO3- δ18O (from -1.5 to 1.3 ‰) [Buchwald et al., 2012]. On the other hand, the model is neither very sensitive to the depth attenuation coefficient (from 0.6 to 1.3, i.e., the oceanic range) [Berelson, 2001], nor the vertical mixing rates (S↑ and S↓ below the mixed layer from 0.005 to 0.1; representing a net nitrate supply varying from 0.03 to 0.3 mol m-2 and an upward fluxes from 0.15 to 14.5 mol m-2). This range for the vertical mixing rates is unrealistically large in comparison with other fluxes (e.g., nitrate and ammonium uptake). This was motivated to see how fast a subsurface signal can be propagated into the mixed layer. The small variations in the net supply result from the small vertical nitrate gradients (5.2 ± 3.4 μmol l-1; between 0-100 and 140-160 m depth stratum), implying nearly equal upward and downward fluxes.

## S2. Single box model: contribution of nitrification to NO3- assimilation

We use a time-dependent geochemical one-box model of the mixed layer to constrain the relative rate of nitrification from the observed NO3- and PN concentrations, NO3- and PN δ15N, and NO3- δ18O. The model simulates the change in the concentration of the fixed N pools (NO3-, NO2-, NH4+, PN) and their isotopic composition (δ15N for all of them and δ18O for NO3-), in the timeframe given by the observations (~20th October to ~13 February). We tested the range given in the literature for the different isotope effects: 5 ‰ for NO3- assimilation (1), 0-2 ‰ for the remineralization (PN,DON 🡪 NH4+)[Möbius, 2013; and reference therein]; 0-5 ‰ for NH4+ assimilation[Hoch et al., 1992; Pennock et al., 1998; Vo et al., 2013], 15-22 ‰ for NH4+ oxidation [Casciotti et al., 2003; Santoro and Casciotti, 2011], 0 ‰ for NO2- assimilation [Waser et al., 1997], and 0 - -13 ‰ for NO2- oxidation [Casciotti, 2009]. NO3- assimilation discriminates N and O isotopes to the same extent (15ε ≈ 18ε) [Granger et al., 2004, 2010; Karsh et al., 2012]. Recently nitrified NO3- δ18O has the isotopic composition of the ambient water plus 1.1 ‰ (0.6 ‰ over the Kerguelen plateau, taking the H2O δ18O of the temperature minimum layer) [Archambeau et al., 1998; Casciotti et al., 2008; Sigman et al., 2009; Rafter et al., 2013]. The mass and isotopic balance is given by:

 (5)

 (6)

 (7)

 (8)

 (9)

 (10)

 (11)

 (12)

 (13)

where NiO is nitrite oxidation, S vertical nitrate diffusivity, NaU nitrate assimilation, AmU ammonium assimilation, AmO ammonium oxidation, R remineralization, and E export of PN. The model is run for N time steps of length dt, transforming at each time step an amount of the integrated rate (e.g., NaU = ∫NaU/N). Since the integrated rates for NaU, AmU, E, and S are known (see section “Surface contribution of nitrification to NO3- assimilation”) and since we assume steady state conditions for both NH4+ and NO2- (consumption = production), this leaves us with two unknowns, AmU/R and NiU/AmO ratios (Fig. 5). We solve the model differential equations by varying AmU/R and NiU/AmO, targeting the best agreement between the observations {NO3-(i), PN(i), δ15NNO3-(i), δ18ONO3-(i), δ15NPN(i)} at the collapse of the bloom (KEOPS1) and the model counterparts {NO3-(m), PN(m), δ15NNO3-(m), δ18ONO3-(m), δ15NPN(m)}. To measure this agreement, we use the minimum cost function, searching for the lowest standardized residual (SR) [Elskens et al., 2007]:

 (14)

where σconc, σδ15N and σδ18O express the standard deviation for the concentration (1 µmol l-1), δ15N (0.2‰), and δ18O (0.2‰), respectively. Standardized residuals account for the different units of the two variables, in this case concentration and δ15N-δ18O. One typical example of a sensitivity test for one configuration of both isotope effects and rates is shown in Fig. S3. Satisfying solutions correspond to combinations of AmU/R and NiU/AmO ratios that result in a SR lower than ~30. Results from all simulations that fulfill this criterion have a final simulated NO3- concentration, NO3- δ15N, NO3- δ18O, and PN δ15N within the error range (2sd) of the observations (KEOPS1). Adding DON to the PN pool, assuming that it is an actively cycling N pool, allows the final simulated PN concentration to fall also within the error range (2sd) of the observations.

We tested the sensitivity to the prescribed integrated rates (NaU = 2.2 ± 1.0 mmol m-2, AmU = 0.5 ± 0.3 mmol m-2, E = 0.2 ± 0.2 mmol m-2, and S = 0.2 ± 0.2 mmol m-2; see section “Surface contribution of nitrification to NO3- assimilation”), as well as for the values for each isotope effect (348 simulations in total). The contribution of nitrification to NO3- assimilation is the most sensitive to NaU, with lower contribution at lower rates. The best fits (within the error range of the observations; 147 simulations) are always found with high NiO/NaU ratio (min. SR; 0.4 to 0.8; Fig. S4), low AmU/R ratio (min.SR; 0.1 to 0.5; Fig. S4), and low NiU/NiO ratio (min.SR; 0.0 to 0.5). The average (± sd) of these simulations for final NO3- concentration, PN concentration, NO3- δ15N and δ18O, and PN δ15N, are given in Figs. 2a and 2b. We tested further the sensitivity to the NO3- supply terms (Fig. S5), from 0 to 1.0 mol m-2 yr-1. From this analysis, the model cannot find a good fit with nitrate supply above 0.6-0.8 mol m-2 yr-1.

## References:

Archambeau A.-S., Pierre, C., Poisson, A. & Schauer, B. (1998), Distributions of oxygen and carbon stable isotope and CFC-12 in the water masses of the Southern Ocean at 30°E from South Africa to Antarctica: Results of the CIVA1 cruise, J. Mar. Syst., 17, 25-38.

Berelson, W.M. (2001), The flux of particulate organic carbon into the Ocean interior: A comparison of four U.S. JGOFS. Regional Studies, Oceanography, 14, 59-67.

Buchwald, C., Santoro, A.E., McIlvin, M.R. & Casciotti, K.L. (2012), Oxygen isotopic composition of nitrate and nitrite produced by nitrifying Cocultures and natural assemblages, Limnol. Oceanogr., 57(5), 1361-1375.

Buchwald, C., and K.L. Casciotti (2013), Isotopic ratios of nitrite as tracers of the sources and age of oceanic nitrite. Nature Geoscience, doi:10.1038/NGEO1745.

Casciotti, K.L., D.M. Sigman, M. Galanter Hastings, J.K. Böhlke and A. Hilkert (2002), A. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method, Anal. Chem., 74, 4905-4912.

Casciotti, K.L., Sigman, D.M. & Ward, B.B. (2003), Linking diversity and stable isotope fractionation in ammonia-oxidizing bacteria, Geomicrobiol. J., 20, 335-353.

Casciotti, K.L., J.K. Böhlke, M.R. McIlvin, S.J. Mroczkwski and J.E. Hannon (2007), Oxygen isotopes in nitrite: analysis, calibration, and equilibration. Anal. Chem. 79: 2427-2436, doi:10.1021/ac061598h.

Casciotti, K.L., Trull, T.W., Glover, D.M. & Davies, D. (2008), Constraints on nitrogen cycling at the subtropical North Pacific Station ALOHA from isotopic measurements of nitrate and particulate nitrogen, Deep-Sea Res. II, 55, 1661-1672.

Casciotti, K.L. (2009), Inverse kinetic isotope fractionation during bacterial nitrite oxidation, Geochim. Cosmochim. Acta*,* 73, 2061-2076.

Casciotti, K.L., M. McIlvin, and C. Buchwald (2010), Oxygen isotopic exchange and fractionation during bacterial ammonia oxidation. Limnol. Oceanogr. 55(2), 753-762.

Difiore, P. J., Sigman, D. M., Karsh, K.L., Trull, T.W., Dunbar, R.B. & Robinson, R.S. (2010), Poleward decrease in the isotope effect of nitrate assimilation across the Southern Ocean, Geophys. Res. Lett*.*, 37, L17601, doi:10.1029/2010GL04*4*090.

Elskens, M., de Brauwere, A., Beucher, C., Corvaisier, R., Savoye, N., Tréguer, P. & Baeyens, W. (2007), Statistical process control in assessing production and dissolution rates of biogenic silica in marine environments, Mar. Chem., 106, 272-286.

Fawcett, S.E., B.B. Ward, M.W. Lomas, and D.M. Sigman (2015), Vertical decoupling of nitrate assimilation and nitrification in the Sargasso Sea, Deep-Sea Res. I 103, 64-72.

Fripiat, F., D.M. Sigman, G. Massé. and J.-L. Tison (2015), High turnover rates indicated by changes in the fixed N forms and their stable isotopes in Antarctic landfast sea ice, J. Geophys. Res. Oceans 120, doi:10.1002/2014JC010583.

Granger, J., Sigman, D.M., Needoba, J.A. & Harrison, P.J. (2004), Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton, Limnol. Oceanogr., 49(5), 1763-1773.

Hoch, M.P., Fogel, M.L. & Kirchman, D.L. (1992), Isotope fractionation associated with ammonium uptake by a marine bacterium, Limnol. Oceanogr., 37(7), 1447-1459.

Karsh, K.L., J. Granger, K. Kritee and D.M. Sigman (2012), Eukaryotic Assimilatory nitrate reductase fractionates N and O isotopes with a ratio near unity. Environ. Sci. Technol. 46, 5727-5735.

Möbius, J. (2013), Isotope fractionation during nitrogen remineralization (ammonification): implications for nitrogen isotope biogeochemistry, Geochim. Cosmochim. Acta*,* 105, 422-432.

Mosseri, J., B. Quéguiner, L. Armand, and V. Cornet-Barthaux (2008), Impact of iron on silicon utilization by diatoms in the Southern Ocean: A case study of Si/N cycle decoupling in a naturally iron-enriched area, Deep-Sea Res. II 55, 801-819.

Pennock, J.R., Velinsky, D.J., Ludlam, J.M., Sharp, J.H. & Fogel, M.L. (1996), Isotopic fractionation of ammonium and nitrate during uptake by Skeletonema costatum: Implications for δ15N dynamic under bloom conditions, Limnol. Oceanogr., 41(3), 451-459.

Rafter, P.A., DiFiore, P.J. & Sigman, D.M. (2013), Coupled nitrate nitrogen and oxygen isotopes and organic matter remineralization in the Southern and Pacific Oceans, J. Geophys. Res*.,* 118, 1-14, doi:1002/jgrc.20316.

Santoro, A.E. & Casciotti, K.L. (2011), Enrichment and characterization of ammonia oxidizing archaea from the open ocean: phylogeny, physiology and stable isotope fractionation, The ISME journal*,* 5, 1796-1808.

Sigman, D.M., DiFiore, P.J., Hain, M.P., Deutsch, C., Wang, Y. et al. (2009), The dual isotopes of deep nitrate as a constraint on the cycle and budget of oceanic fixed nitrogen, Deep-Sea Res. I, 56, 1419-1439.

Smart, S.M., S.E. Fawcett, S.J. Thomalla, M.A. Weigand, C.J.C. Reason, and D.M. Sigman (2015), Isotopic evidence for nitrification in the Antarctic winter mixed layer, Global Biogeochem. Cycles 29, doi:10.1002/2014GB005013.

Vo, J., Inwood, W., Hayes, J.M. & Kustu, S. (2013), Mechanism for nitrogen isotope fractionation during ammonium assimilation by Escherichia coli K12, Proc. Natl. Acad. Sci., 110(21), 8696-8701.

Waser, N.A.D., Harrison, P.J., Nielsen, B., Calvert, S.E. & Turpin, D.H. (1998), Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by marine diatom., Limnol. Oceanogr., 43(2), 215-224.



Figure S1: Observed vertical distributions of concentrations and isotopic compositions for the first profile (A3-1; 20th October). Profiles of NO2- concentration (black circles), NO3-+ NO2- δ15N (green circles), NO3-+ NO2- δ18O (purple triangles), and Δ(15-18) (green squares). Error bars = 1 sd for the replicates.



Figure S2: Conceptual scheme of the vertical 1-dimensional multi-layer box model (0-250m, 50 layers of 5m deep).



Figure S3: Standardized residuals in function of nitrification/nitrate assimilation (panel a) and ammonium assimilation/remineralization (panel b) ratios, for a typical optimization scheme, by varying both AmU/R and NiU/AmO ratios, with one configuration of isotope effects and prescribed flux rates. The lower the standardized residuals, the better the fit with the observations. The gray zone corresponds to the simulations within the error range (2 sd) of the observations (KEOPS1).



Figure S4: Nitrification/Nitrate assimilation (filled black circles) and Ammonium uptake/Remineralisation (empty black circles) ratios given by every simulation (n = 147) with model outputs within the error bars (± 2sd) of the observations (KEOPS1). The red circle represent the averages.



Figure S5: Model sensitivity to the vertical nitrate supply term (from 0 to 1.0 mol m-2 vegetative season-1). The X-axis is the nitrite oxidation (i.e., nitrification)/Nitrate assimilation ratio and Y-axis is the fit between the model outputs and observations (standard residual = difference between model outputs and observations). The area below the dashed line represents the simulation with the model outputs within 2sd of the observations.