

## Reply to the Review of the Referee #1 (Bourbonnais)

(**RC**: Referee Comment; **AR**: Author's Responds)

First of all, thank you very much for thoroughly reviewing our manuscript and for the helpful comments and suggestions. We try to include as many comments and suggestions as possible which help us to improve our manuscript.

*Abstract*

### Page 1, line 20

**RC**:  $N^* < -1 \mu\text{M}$  is not a strong N deficit relatively to other regions of the ocean where N deficit is close to  $40 \mu\text{M}$  (see Bourbonnais et al., 2015). What is the analytical error on their  $N^*$  estimate? Also, indicate depth of the minimum  $N^*$ .

**AR**: I agree with you that in comparison with other regions an  $N^*$  of  $< -1 \mu\text{M}$  is not really a strong N deficit, but with lowest values of  $-4 \mu\text{M}$  within the IDW (Page 14, lines 4-5) these values are significant in our study area. The analytical error on our  $N^*$  estimate based on the relative error of nitrate and phosphate analyses was below 1.5 % for duplicate sample measurements (Page 5, lines 12-13). The  $N^*$  Minimum is located within the RSPGIW and the IDW at a core depth of  $\sim 1500 \text{ m}$  and ranged from  $\sim 1000 \text{ m}$  until  $\sim 1600 \text{ m}$  (Page 5, lines 12-13). This information will be added to the abstract.

### Page 1, lines 23-24

**RC**: Indicate how the contribution from  $N_2$ -fixation was estimated (i.e., using N/P and Redfield ratio assumptions).

**AR**: We are using a simple calculation for a first estimate of the input of new nitrate into the surface layer by  $N_2$ -fixation by using the deviation of the N/P-Redfield-ratio. We will clarify this in the revised version.

*Introduction*

### Page 2, line 12

**RC**: The transition is awkward. Rewrite.

**AR**: We modified the sentence to: "To study the marine nitrogen cycle, we use nitrate and phosphate concentrations as well as the isotopic signature of nitrate (Deutsch et al., 2001; Deutsch et al., 2007; Gruber and Sarmiento, 1997; Lehmann et al., 2005; Sigman et al., 2005)."

### Page 2, lines 20-22

**RC**: One important caveat is that  $N^*$  cannot be used to derive rates of  $N_2$ -fixation in region where denitrification co-occurs, as the  $N^*$  signatures associated with denitrification and  $N_2$ -fixation are overprinting each other's. One advantage of measuring the dual isotopic composition of nitrate is that it allows disentangling these different overprinting processes, because, as stated later in the

manuscript, N<sub>2</sub>-fixation is associated with negative N to O nitrate isotope anomalies. On the other hand, denitrification is not expected to produce such N to O nitrate isotope anomalies because N and O are equally fractionated during this process. This point should be better emphasized in the introduction (and better exploited in their discussion).

**AR:** You are right that in regions where denitrification and N<sub>2</sub>-fixation simultaneously occur N\* cannot be used alone. However, in our study area no denitrification takes place and we just see a signal in intermediate and deep waters coming from the Arabian Sea, where denitrification take place. We use the positive surface N\* signatures as a first evidence for N<sub>2</sub>-fixation and confirm these signatures with the distinct upward decrease of N-isotope values compared to strongly elevated  $\delta^{15}\text{N}$  values in subsurface waters (~500 m, elevation of ~-2-3.5 ‰). I agree that dual isotope measurements of nitrate will help to improve the weakness associated with the N\* approach and we will rewrite and add a section on dual isotopes.

**Page 2, lines 28-29**

**RC:** Change for: "lighter isotopes are preferentially assimilated, leaving the substrate enriched in <sup>15</sup>N and <sup>18</sup>O."

**AR:** This sentence will be rewritten as you noted.

**Page 3, line 1**

**RC:** Add references here, e.g., Knapp et al., 2008 and Bourbonnais et al., 2009.

**AR:** We will add these references.

**Page 3, line 4**

**RC:** Which depth range corresponds to  $\delta^{15}\text{N}_{\text{deep}}$  and  $\delta^{18}\text{O}_{\text{deep}}$ ?

**AR:** For  $\delta^{15}\text{N}_{\text{deep}}$  and  $\delta^{18}\text{O}_{\text{deep}}$  we use the mean of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  within the water depth below 2000 m. When we will still use the tracer  $\Delta(15,18)$  (see explanation below) we will add this information in the introduction part and in the discussion section on Page 18, line 1.

**Page 3, lines 14-16**

**RC:** Be more specific about the new findings from this study. Which specific gaps were filled comparatively to previous studies?

**AR:** We will explain more precisely that our findings filled the gaps between the mentioned studies relating to nutrient distribution, nitrate isotope measurements and water mass analyses. First, in this region, we linked the different water masses of different origin with their isotopic signature. We will clarify our new findings in the revised version.

## *Materials and Methods*

### **Page 6, line 13**

**RC:** Why using a single point correction only?

**AC:** We will correct the method section, because we indeed do not use a single point correction but rather a two-point correction referred to IAEA-N3 ( $\delta^{15}\text{N-NO}_3^- = +4.7 \text{ ‰}$  and  $\delta^{18}\text{O-NO}_3^- = +25.6 \text{ ‰}$ ) and USGS-34 ( $\delta^{15}\text{N-NO}_3^- = -1.8 \text{ ‰}$  and  $\delta^{18}\text{O-NO}_3^- = -27.9 \text{ ‰}$ ) for  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$ .

### **Page 6, line 15**

**RC:** What was blank size?

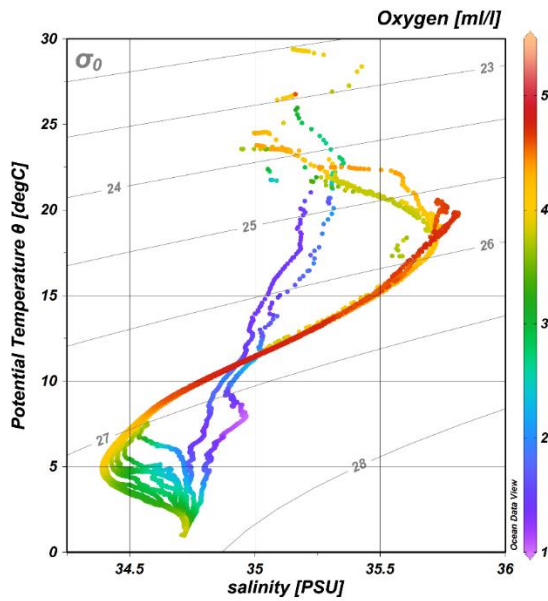
**AR:** The standard deviation for IAEA-N3 was generally better than 0.2 ‰ for  $\delta^{15}\text{N-NO}_3^-$  and 0.3 ‰ for  $\delta^{18}\text{O-NO}_3^-$ , which is within the same specification for  $\delta^{15}\text{N-NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  for at least duplicate measurements of the samples.

## *Results*

### **Page 6, section 3.1**

**RC:** It would be helpful to show T-S diagrams at this point rather than later in the discussion.

**AR:** We thought about the best position of the Sigma-theta-Salinity and Sigma-theta-Oxygen diagrams within our manuscript. In the end, we decided to show these diagrams with the distinct classification of the different water masses and the resultant water mass distribution model in a separate discussion section because of the high portion of discussion rather than just the presentation of results. In our water mass analyses, we use many different sources, describing water masses in the world's ocean and when available from expeditions in the Indian Ocean, but they are quite rare and no water mass model existed for our study area. Therefore, we decided to present the water mass distributions in an own discussion section and not as a part of the results. Consequently, the diagrams with the clear water mass classification along their density surfaces belong more to the discussion section. However, it would be a good opportunity to show a typical T-S diagram (see example below in addition to the salinity and oxygen color sections in Figures 2 a and b) in the results. These will give a first overview about the differences between northern and southern water masses and introduce the Figures and detailed explanation in the discussion part. This might be a good consensus.

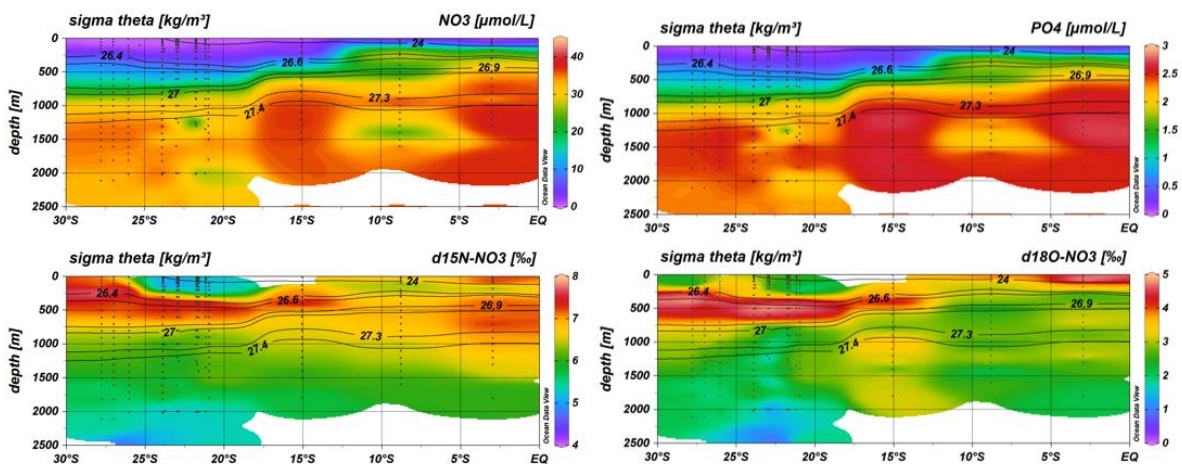


Example for a T-S-diagram

**Page 7, lines 6-16 and Page 8, lines 2-13**

**RC:** Figure 5 (panels a, b, c, d) should be presented in this section and Table 2 moved to the supplementary materials. Figure 5 (panels e, and f) should be presented in this section and Table 3 moved to the supplementary materials.

**AR:** If we move Figure 5 to the results we will have to remove the overlay of water mass boundaries in the panels because they were added as a consequence of the water mass discussion section. Above we explained why we decided to present our water mass analyses as a part of the discussion. An opportunity to leave Figure 5 (a-f) in section 4.2.1 and to accommodate with your remarks is to add only nitrate and phosphate, and N and O isotope color sections (see example below; like Figure 2a and b for salinity and oxygen) to the results (3.2) and move Table 2 and 3 to the supplementary materials.



Example for nitrate, phosphate and N and O isotope transects

## *Discussion*

### **Page 9, line 6-7**

**RC:** What is new in their water mass distribution model (Figure 4)?

**AR:** This is the first water mass distribution model for this region, for further explanation see response above for “page 6, section 3.1”

### **Page 9, lines 25-26**

**RC:** Change for “... because of respiration and the absence of effective ventilation...”

**AR:** We will rewrite the sentence as you mentioned.

### **Page 9-12:**

**RC:** It would be useful to include the nitrate isotopic composition (end-members) for the different water masses, either in Figure 4, or in a Table.

**AR:** This is a good annotation. A type of endmembers are shown in Table 3 where the mean  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  are presented for different latitudes (because the water mass distribution changes along the transect) and for different water depth representing the different water masses. We can modify this Table and add the water masses for a better overview. Then we can move this Table to the beginning of section 4.2.1, after the water mass discussion part. It would be better to represent this new “end-member Table” for nitrate isotopes in section 4.2.1 rather than in section 3.1, because for the first time we connect the water masses and the results of the nutrient and isotopic measurements in section 4.2.1. This Table would then nicely correspond to Figure 5(a-f).

### **Page 12, lines 20-21**

**RC:** The  $\text{NO}_3^-/\text{PO}_4^{3-}$  should however increase if  $\text{N}_2$ -fixation is significant.

**AR:** Enhanced N/P ratios in N-fixing organisms has been reported and would introduce these enhanced N/P ratios also to the water mass as the N-fixers are mineralised. This process is reflected in enhanced N/P ratios. The way we calculated the contribution from  $\text{N}_2$ -fixation is thus a minimum estimate of N contribution from  $\text{N}_2$ -fixation. If part of the P was also from N-fixers and if the N/P ratio of N fixers was known, their contribution could have been better estimated. However, we are not sure about the N-fixers N/P ratio. We will, however, examine this carefully in the revised version and improve this part including the dual isotope approach.

### **Page 13, lines 10-11**

**RC:** How does the mean  $\text{NO}_3^-/\text{PO}_4^{3-}$  ratio changes along the latitudinal transect? What are the implications for  $\text{N}_2$  fixation?

**AR:** The change of N/P ratio along the latitudinal transect is presented in Figure 5c and demonstrates the oligotrophic regime in the subtropical gyre. Implications for  $\text{N}_2$ -Fixation are: (1) Elevated  $\text{N}^*$  values of  $>2 \mu\text{M}$  in surface

waters south of  $\sim 15^{\circ}\text{S}$ . (2) We observe distinctly lower  $\delta^{15}\text{N}$  values ( $<4.5\text{-}5.0\text{‰}$ ) in the surface waters compared to the subsurface values within the SAMW with values of  $>7\text{‰}$  and highest values of  $\sim 8\text{‰}$ . This leads to a  $\delta^{15}\text{N}$  difference of  $2\text{-}3.5\text{‰}$ , which is similar to the difference in other studies, i.e. Bourbonnais et al. (2009) with a difference of  $3\text{‰}$  (from  $5\text{‰}$  to  $2\text{‰}$ ). Our surface  $\delta^{15}\text{N}$  values are also slightly lower than the average  $\delta^{15}\text{N}$  values of depth water nitrate ( $>2000\text{ m}$ ;  $5.5\text{‰}$ ). (3) We estimated in your simple calculation the input of new nitrate into the surface layer by  $\text{N}_2$ -fixation and demonstrated the increase of the N/P ratio of completely assimilated nitrate (Figure 9a).

**Page 14, line 1 and Page 18, line 16**

**RC:** Bourbonnais et al. (2009) is incorrectly referenced here.

**AR:** We apologize for the incorrectly referenced study. We will correct this.

**Page 15, lines 5-6**

**RC:** Add references to support this statement.

**AR:** We will add references.

**Page 16, lines 17-18**

**RC:** Why nitrate utilization is unlikely? It is too deep?

**AR:** It is to our knowledge a clear mixing signal that causes the moderate slopes of  $\delta^{15}\text{N}/\ln(\text{NO}_3)$  in both the gyre region and in the Subantarctic and we think that nitrate utilization is unlikely at this depth. Sigman et al. (2000) also described the mixing of different end-members along the SAMW from the Antarctic with higher  $\delta^{15}\text{N}$  (up to  $13\text{‰}$ ) values and lower  $\delta^{15}\text{N}$  ( $<6\text{‰}$ , Liu et al., 1996) values towards lower latitudes.

**Page 18, lines 21-24**

**RC:** Bourbonnais et al. (2009) report a range of  $2$  to  $5\text{‰}$  for the  $\delta^{15}\text{N}$  of nitrate in surface waters of the subtropical northeast Atlantic Ocean. Using a simple isotopic mass balance, they estimated that  $\text{N}_2$  fixation could account for up to  $40\%$  of the export production in this region.

**AR:** This agrees with our  $\delta^{15}\text{N}$  values, which are between  $4.5$  and  $5.0\text{‰}$  in surface waters and we estimated that  $\text{N}_2$ -fixation could account for  $\sim 30\%$  of the export production. We will clarify this in the revised version.

**Page 18, lines 29-31**

**RC:** It is peculiar to note that the  $\Delta(15,18)$  anomalies observed in this studies are at least half of the anomalies observed in the subtropical northeast Atlantic Ocean by Bourbonnais et al. (2009) ( $\Delta(15,18)$  of  $-7$  to  $0\text{‰}$ ). Why would that be if the estimated contribution from  $\text{N}_2$  fixation is supposedly in the same range (accounting for  $30\text{-}40\%$  of new supplied nitrate) for these two regions? The  $\text{N}^*$  observed by Bourbonnais et al. (2009) was also up to  $\sim 3.5\text{ }\mu\text{mol/kg}$ .

**AR:** We will carefully examine this in the revised version using the suggested literature and include a discussion on the dual isotopes. We will reconsider the use of tracer  $\Delta(15,18)$  because of the diverse source waters. Better would be the tracer  $\Delta(15-18)$  from Rafter et al. (2013), who used only the difference between N and O isotope signatures which is more useful in regions characterised by a variety of water masses. We will consider this in the revised version.

**Page 19, lines 8-9**

**RC:** In equation (6), the nitrate to phosphate ratio ( $\text{NO}_3^-/\text{PO}_4^{3-}$ ) is divided by the measured phosphate concentrations, not multiplied.

**AR:** You are right, the equation is incorrect and  $\text{N/P}_{\text{cal}}$  must be multiplied by the phosphate concentrations. Sorry for this mistake.

**Page 19, lines 1-23**

**RC:** This approach requires many assumptions. One likely invalid assumption is assuming a Redfield ratio of 16. The Redfield ratio is variable in marine microalgae (see Geider et al., 2002).  $\text{N}_2$  fixers also have higher N/P ratios (e.g., Letelier et al., 1998). Finally, this approach does not take into account inputs from atmospheric depositions.

**AR:** Because  $\text{N}_2$ -fixers have higher N/P ratios, we calculated the assimilated nitrate by representing the deviation from the Redfield stoichiometry of 16:1 and therefore the higher N/P ratios of the assimilated nitrate are an evidence for  $\text{N}_2$ -fixation in surface waters (see comment above). We believe that we have presented a minimum estimate by our calculation but will re-examine our approach and try to find a better way to estimate the N-contribution by nitrogen fixers. We will check the literature on atmospheric deposition but we think that it is quite small in the study area as sinking particles and sediment have only little lithogenic material.

**Page 19, line 21**

**RC:** This is confusing, as  $\delta^{15}\text{N-NO}_3\text{-fix}$  (i.e. supplied from  $\text{N}_2$  fixation) should be about 0‰. I suggest removing the “fix” subscript.

**AR:** We agree with this remark, that  $\delta^{15}\text{N-NO}_3\text{-fix}$  is confusing; we will remove the subscript “fix”.

**Page 19, lines 20-23**

**RC:** Overall, the dual nitrate isotopic data could be better exploited in their discussion and used in an isotopic box model to derive an independent assessment of the contribution from  $\text{N}_2$  fixation (see examples from Knapp et al., 2008 and Bourbonnais et al., 2009).

**AR:** Our simple estimation on  $\text{N}_2$ -fixation is the first try to get an impression on the input of new nitrate into the system of the subtropical gyre in the South Indian Ocean. For a box model we need to combine or water column analyses with the result of suspended matter samples and particle flux samples from sediment

traps. For this study, we first wanted to demonstrate the diversity of water masses in the less explored subtropical gyre of the South Indian Ocean and second, to highlight their varying influence on the nutrient and isotopic composition, which is likewise less investigated in this region. Our simple estimation on N<sub>2</sub>-fixation is a first approach on the input of new nitrate into this special oligotrophic region. We will include the dual isotopes to strengthen our point on N-fixer contribution.

**Page 20, lines 9-10**

**RC:** Bourbonnais et al. (2009) did not observe significant positive  $\Delta(15,18)$  anomalies in the subtropical northeast Atlantic Ocean. Which make me wonder what is the propagated (analytical) error associated with their  $\Delta(15,18)$  measurements. In other words, is their calculated positive  $\Delta(15,18)$  significantly different from 0?

**AR:** See above: We will consider to use the tracer  $\Delta(15-18)$  instead of  $\Delta(15,18)$  and agree that a  $\Delta(15,18)$  of +0.5 or -0.5 ‰ is not a significant amplitude.

**Page 20, line 16**

**RC:** N<sub>2</sub>-fixation have been shown to occur at lower temperatures in temperate regions (see Moisander et al., 2010).

**AR:** The sudden change in  $\delta^{15}\text{N}$  and N\* is difficult to explain in the gyre as nutrients are not increasing. We have no data on micronutrients but find it unlikely that these change significantly within the gyre. Therefore, the only feasible explanation seems to be the temperature drop. However, we will stress the contradictory literature in the revised version.

*Tables*

**Table 1**

**RC:** It is not necessary since the information is already presented in Figure 1. I recommend moving it to the supplementary materials.

**AR:** Table 1 will be moved to the supplement.

**Table 2 and 3**

**RC:** Should be moved to the supplementary materials as this information is already in Figures 3 and 5.

**AR:** See comment to Page 7, lines 6-16 and Page 8, lines 2-13



## *Figures*

### **Figure 1**

**RC:** It is difficult to see the shaded arrow representing the South equatorial current.

**AR:** We will highlight the shaded arrow by adding a contour line.

### **Figure 6**

**RC:** What is the  $r^2$  and error on the slope?

**AR:**  $r^2$  is 0,99. We will add the  $r^2$  and the error of the slope in the revised version.

### **Figure 7b**

**RC:** Which processes cause the positive  $\Delta(15,18)$ ?

**AR:** See Sigman et al (2005): Nitrification/Remineralisation cycle in deeper waters leads to a slightly positive in  $\Delta(15,18)$ . We will add more information on that in the revised version.

## *Technical comments*

### **Page 1, lines 30-31 and Page 2, line 2**

**RC:** This sentence is repetitive. Replace by something like: "The South Indian Ocean is dominated by a subtropical anticyclonic gyre (refs), the Indian Ocean subtropical gyre" (IOSG), one of the major subtropical gyres in the world's ocean. The IOSG has been, thus far, sparsely investigated." Use the IOSG acronym defined earlier.

**AR:** We will rewrite the sentence as you mentioned and define the "IOSG" acronym earlier in the text.

### **Page 3, line 10**

**RC:** Remove "Therefore" at the beginning of sentence.

**AR:** We will remove the "therefore".

### **Page 12, line 7**

**RC:** Change for "nutrient distribution and N cycle processes"

**AR:** We will change the headline as you mentioned.