

Interactive comment on “**Peru upwelling plankton respiration: calculations of carbon flux, nutrient retention efficiency and heterotrophic energy production**” by **T. T. Packard et al.**

Anonymous Referee #1 Received and published: 19 January 2015 In this manuscript the authors use microbial ETS activity as a proxy for the export and degradation of particulate organic carbon from the epipelagic to the deep ocean. This relationship is described using a new metric, the nutrient retention efficiency (NRE), which is defined as the ratio of nutrient regeneration in a layer to the nutrients introduced into that layer. The paper is very well written, contains some interesting ideas/concepts and the topic is of high relevance for Biogeosciences. I therefore suggest that the manuscript is accepted for publication after some minor edits.

Minor comments

RESPONSE TO ANONYMOUS REVIEWER #1:

We thank the reviewer #1 for the encouraging remarks and we will do our best to respond constructively to his /her comments.

*Comment #1. Page 16179, line 14-15: Add references for the statements on the “studies of Jenkins” and the “vertex program”.*

*Authors’ Response and changes in the manuscript:* The following references to Jenkins and the VERTEX program (Martin et al., 1987) have been added.

Jenkins, W.J.: Oxygen utilization rates in North Atlantic subtropical gyre and primary production in oligotrophic systems, *Nature*, 300, 246–248, 1982.

Jenkins, W.J.: The use of tracers and water masses to estimate rates of respiration, *Heterotrophic Activity in the 340 Sea*, 391-403., eds Hobbie, J.M., Williams, P.J.Le B. (Plenum Press, New York), 1984.

Martin, J.H., Knauer, G.A., Karl, D.M., Broenkow, W.W.: VERTEX: carbon cycling in the northeast Pacific, *Deep-Sea Res*, 34 (2), 267-285, 1987.

---

*Comment #2. Page 16180, line 24: Spell out “HEP”.*

*Authors’ Response:* HEP is “heterotrophic energy production”, we have spelled it out in our revised manuscript.

*Authors’ Changes in the manuscript:*

Here, we address his concern by calculating Heterotrophic Energy Production (HEP) in a C-Line section (Fig. 2d).

---

*Comment #3. Page 16182, line 18-20: Please add a more detailed description of the ETS method used.*

*Authors’ Response:* We have replaced these lines with the text below:

Please note that the theoretical background, history, and ocean application of the ETS methodology have been described in two reviews (Packard, T.T. Measurement of electron transport activity of marine microplankton. In: *Advances in Aquatic Microbiology*, P.J. LeB. Williams and H.W. Jannasch (eds.), Academic Press, New York, pp. 207-261, 1985. and Packard, T.T. Oxygen consumption in the ocean: Measuring and mapping with enzyme analysis. *Mapping Strategies in Chemical Oceanography*, A. Zirino (ed.), American

Chemical Society, Washington, D.C. pp. 177-209, 1985.). The second reference is included in the manuscript. (These references have been added.)

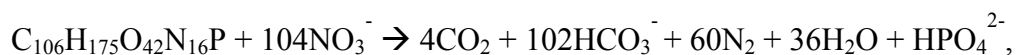
*Authors' Changes in the manuscript:* Section, “2.3 ETS activity, respiratory O<sub>2</sub> consumption, CO<sub>2</sub> production, and NO<sub>3</sub><sup>-</sup> reduction,” now reads, “Respiratory ETS activity in the Ez was measured according to Kenner and Ahmed (1975) as described in Packard and Williams (1981). In deeper waters it was measured according to (Packard et al., 1971) and multiplied by 3.35 to render the two data sets comparable as explained in Christensen and Packard (1979). The calculations for potential respiration and respiration were calculated from the combined ETS data set according to Packard and Christensen (2004) and Packard and Codispoti (2007). Table 2 explains the calculation in detail.”.

---

*Comment 4. Page 16182, line 25-26: This part is not clear for me; so please explain in more detail.*

*Authors' Response* Chen and Strous (2013) state: “Denitrification and aerobic respiration depend on the same core respiratory machinery“. However specifically, the in vivo denitrification rates have been described in Codispoti and Packard (1980) and in Codispoti et al. (2001). In detail the calculations are as follows:

*Calculation of denitrification from ETS activity.* Microbial respiratory nitrate reduction to nitrogen gas (denitrification) occurs in the water column between 47 and 400 m below the sea-surface between stations C3 to C12 (Garfield et al., 1979; Codispoti and Packard, 1980; Table 2; Fig.5). Accordingly, the calculation of respiratory CO<sub>2</sub> production in these waters from the ETS activity measurements has to be done differently than it is in the oxic waters that support aerobic respiration. In the first place the stoichiometric relationship between the nitrate reduction to nitrogen gas and the INT reduction to formazan in the ETS assay is 5:1, mole-per-mole, because the formation of 1 mole of N<sub>2</sub> in denitrification requires 10 electrons (10e<sup>-</sup> + 10H<sup>+</sup> + 2NO<sub>3</sub><sup>-</sup> → N<sub>2</sub>) while the reduction of INT to formazan require only two electrons (2e<sup>-</sup> + 2H<sup>+</sup> + INT → Formazan + HCl). Thus, an ETS activity expressed as 1 mol Formazan h<sup>-1</sup> m<sup>-3</sup> is stoichiometrically equivalent to a potential denitrification rate (nitrate respiration) of 1/5 mol N<sub>2</sub> h<sup>-1</sup> m<sup>-3</sup>. For ETS activity expressed as mol e<sup>-</sup> h<sup>-1</sup> m<sup>-3</sup>, the stoichiometric equivalent is a potential denitrification rate of 1/10 mol N<sub>2</sub> h<sup>-1</sup> m<sup>-3</sup>. The possibility of using ETS activities to calculate denitrification was first proposed in Packard (1969), but was not applied until Devol (1975) and Codispoti and Packard, 1980, and not explained in detail until Packard et al, (1983). Codispoti and Packard (1980) used this conceptual model to calculate denitrification in the oxygen minimum zone in the Peru Current Upwelling System. To convert ETS activities directly to in vivo denitrification rates (R<sub>N<sub>2</sub></sub>) we used the method of Codispoti and Packard (1980) that has recently been corroborated by Dalsgaard et al (2012). This approach uses a ratio, ETS/R<sub>N<sub>2</sub></sub>, of 2.4 ml O<sub>2</sub> L<sup>-1</sup>h<sup>-1</sup>/(gN m<sup>-3</sup> yr<sup>-1</sup>) where ETS was expressed in oxygen units rather than the currently preferred electron units. When inverted and converted to equivalent molar, volume, and time units, this inverted ratio, R<sub>N<sub>2</sub></sub>/ETS, becomes 105 mol e<sup>-</sup> per mol N<sub>2</sub>. Then, using a molar ratio, C/N<sub>2</sub>, of 106/60 from Gruber and Sarmiento et al. (1997) as given in Eq. 4 of Codispoti et al, 2001,



we calculated the respiratory CO<sub>2</sub> production rate:  $R_{CO_2} = (106 \text{ mol C}/60 \text{ mol N}_2) \times \text{ETS activity (mol e}^- \text{ h}^{-1} \text{ m}^{-3}) / (105 \text{ mol e}^- \text{ (mol N}_2)^{-1})$ .

New References: Chen, J., Strous, M. Denitrification and aerobic respiration, hybrid electron transport chains and co-evolution. *Biochim. Biophys. Acta.* 1827, 136–144, 2013 and Packard, T.T. The estimation of the oxygen utilization rate in seawater from the activity of the respiration electron transport system in plankton. Ph.D. Thesis, University of Washington, Seattle, pp. 115 (1969).

*Authors' Changes in the manuscript:* Before the text from line 25 (pg 16182) to line 2 (pg 16183) read: “The calculation of in vivo denitrification rates ( $R_{N_2}$ ) is from Codispoti and Packard (1980) according to Codispoti et al. (2001). The approach has recently been corroborated by Dalsgaard et al. (2012).  $R_{CO_2}$  was calculated as  $R_{CO_2} = [106/60 \text{ mol C (mol N}_2)^{-1} \times \text{ETS activity (mol e}^- \text{ h}^{-1} \text{ m}^{-3})] / [105 \text{ mol e}^- \text{ (mol N}_2)^{-1}]$  using the C-N conversion of Gruber and Sarmiento (1997). We changed it to: ”The calculation of in vivo denitrification rates is based on the fact that the ETS for respiratory oxygen consumption and denitrification differ only in the terminal electron acceptor (Packard, 1969; Chen and Strous, 2013). Accordingly we calculate in vivo denitrification rates ( $R_{N_2}$ ) as before in Codispoti and Packard (1980) and Codispoti et al. (2001). The approach has recently been corroborated by Dalsgaard et al. (2012).  $R_{CO_2}$  was calculated from  $R_{N_2}$  by the expression:  $R_{CO_2} = [106/60 \text{ mol C (mol N}_2)^{-1} \times \text{ETS activity (mol e}^- \text{ h}^{-1} \text{ m}^{-3})] / [105 \text{ mol e}^- \text{ (mol N}_2)^{-1}]$  using the C-N conversion of Gruber and Sarmiento (1997).”.

-----  
Comment 5. Page 16183, line 5: *Why was the data normalised?*

*Authors' Response:* This technique was used by John Martin's group in their VERTEX sediment trap paper (Martin et al. (1987). We used it in our Gulf of Maine carbon-flux-from-respiration paper (Packard and Christensen, 2004). It is critical, mathematically, as Daniel Bourgault from UQAM, Québec has pointed out (personal communication), because  $R_z = R_0 z^b$  is dimensionally unbalanced. The right-hand side of the equation has units of **nmol CO<sub>2</sub> min<sup>-1</sup> L<sup>-1</sup> m<sup>b</sup>**, while the left-hand side of the equation has units of **nmol CO<sub>2</sub> min<sup>-1</sup> L<sup>-1</sup>**. Only if depth is normalized ( $R_z = R_t (z/z_t)^b$ ) does the equation achieve balance with units of **nmol CO<sub>2</sub> min<sup>-1</sup> L<sup>-1</sup>**.

*Authors' Changes in the manuscript:* In section 2.5 we added this text: “Only if depth is normalized does the equation achieve balance with units of nmol CO<sub>2</sub> min<sup>-1</sup> L<sup>-1</sup>”. In addition, in ACKNOWLEDGEMENTS, we credited D Bourgault for confirming the importance of this normalization.

---

Comment 6: Page 16183, line 16-17: The assumption that no degradation of DOC is taking place is not supported by the literature; how would a more labile DOC pool impact your results?

*Authors' Response:* Before reading the new paper by Arrieta et al. (2015) we responded in the following way. “*We know that DOC is being degraded by microbes and partially supporting their respiration, but below the euphotic zone we assume it is less than the POC degradation. Carlson et al. (2010) argue from recent measurements that in the North Atlantic the DOC fuels 5-29% of ocean respiration while POC fuels 71-95%, the balance. We will address this issue in our response to the third reviewer. In all cases, our calculations of carbon flux, (but not our calculations of respiration, NRE, and HEP) will be elevated by the amount of DOC*

*based respiration. This means that, our calculation of POC flux is really an estimation of total carbon flux of which the POC flux is the larger part and the DOC flux is the smaller part.”* After reading Arrieta et al. (2015) we have changed the text in the following way: (*Authors’ Changes in the manuscript:*) **Conceptually, planktonic RCO<sub>2</sub> in a seawater cube is considered as equivalent to the difference between the total FC through the top of the cube and total FC<sub>2</sub> through the bottom of the cube, where total carbon flux refers to the sum of the DOC and the POC carbon flux. We deduce, on the basis of (Craig, 1971; Carlson et al., 2010; Hansell et al., 2012), that R based on DOC and lateral POC flux, compared to the R based on the vertical flux of labile POC, is less than 30% of the total R. Note that if organic matter, in any form, is resistant to oxidation (Arrieta et al., 2015) its flux through the water column will not be detected by respiration measurements. The flux will be transparent to our ETS measurements. However, the dissolved organic matter in the ocean, at least, appears to be oxidizable (Arrieta et al., 2015). In all cases, to a first approximation, one can express our conceptual model by the expression, RCO<sub>2</sub> =FC<sub>1</sub> -FC<sub>2</sub>. In other words, in the vertical, one-dimensional case, the changes in the FC between depths in a water column are equal to the RCO<sub>2</sub> between those depths.**

-----

New Reference to be included in the paper:

Carlson, C. A., Hansell, D. A., Nelson, N. B., Siegel, D. A., Smethie, W. M., Khatiwala, S., Meyers, M. M., Halewood, E. Dissolved organic carbon export and subsequent remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin. *Deep-Sea Res. II*, 57, 1433-1445, 2010.

Hansell, D. A., Carlson, C. A., Schlitzer, R. Net removal of major marine dissolved organic carbon fractions in the subsurface ocean. *Global Biogeochem. Cycles*, 26 (GB1016), doi:10.1029/2011GB004069, 2012.

-----

Comment 7. *Page 16183, line 21: What is a “small seafloor C burial? Please be more precise.*

Authors’ Response: Most of the POC deposited on the seafloor supports benthic respiration, but a small fraction (< 1%) gets buried and becomes part of the geological record. For millions of years it is removed from the ocean carbon economy. The recent Biogeoscience paper by Dale et al. (2014) supports our finding of high carbon burial under the OMZ of the Peruvian continental slope waters.

*Authors’ Changes in the manuscript:* We do not feel that a change is needed on page 16183, but on pg 16169 we would like to include the Dale et al. (2014) reference. The text now reads: “This ratio is the NRE minimum at C8 (Fig. 2c) explaining why the high POC delivery, needed to sustain the high benthic R and C burial at this station, is sustained.” We have inserted at this point the following: “Recent studies of the organic carbon preservation on the upper parts of the Peruvian continental slope (Dale et al., 2014) support these calculations of high carbon burial (Fig 3a.) They find high burial rates at depths between 200-400m and attribute it to the anoxia overlying these sediments.”

New reference to be added: Dale, A. W., Sommer, S., Lomnitz, U., Montes, I., Treude, T., Gier, J., Hensen, C., Dengler, M., Stolpovsky, K., Bryant, L. D., Wallmann, K. Organic carbon production, mineralization and preservation on the Peruvian margin. *Biogeosciences Discuss.*, 11, 13067–13126, 2014.

---

Comment 8. *Page 16184, line 10: Spell out “NRE”.*

In the revised ms we have written “The Nutrient Retention Efficiency” at the start of the sentence on line 10.

---

Comment 9. *Page 16185, line 5-21: Move the “Ocean setting” section to the results.*

We have done this in the revised ms. A separate “Ocean setting” section has been eliminated.

---

Comment 10. *Results section Page 16186, line 9: This high respiration would not only be due to phytoplankton but also the associated heterotrophic bacteria.*

Microplankton to us includes all the microorganisms collected on our GF/F filters. Microplankton will include prokaryotes as well as eukaryotes. However, at this point we would keep the text as it is, but would explain our term “microplankton” earlier in the paper.

*Authors’ proposed change in the manuscript:* On Pg 16179 (lines 6-8) the text now reads, “but in accord with classical oceanographic understanding (Suess, 1980), zooplankton and microplankton (prokaryot) respiration balance vertical carbon flux.” We have changed the text to, “but in accord with classical oceanographic understanding (Suess, 1980), zooplankton and microplankton (prokaryote and eukaryote) respiration balance vertical carbon flux.”

---

Comment 11. *Page 16186, line 14: What impact would Anammox have on the calculations of anoxic respiration? Please explain and discuss this in more detail.*

The truth is we don’t know because no one has looked at ETS activity (NADH/NADPH dehydrogenase activity) in Planctomycete bacteria. However, according to Kartal et al. (2012) the anammox ETS contains the co-enzyme Q cycle and cytochrome bc<sub>1</sub> (ETS Complex III) so INT, the tetrazolium salt that we use to detect electron transport, should undergo reduction in anammox ETS (Smith and McFeter, 1997). In other words our assay should detect anammox if there is ammonium, nitrate, and nitrite data in these waters. In the water column OMZ between 47 and 400 m between stations C3 to C12 where denitrification could occur, the ammonium ranges from 0.55 to 2.04 μM, nitrate ranges from 9.39-36.02 μM, and nitrite ranges from 0.18 to 9.70 μM (Hafferty et al, 1978). We feel that our assay could detect some anammox, however Dalsgaard et al (2012) concludes that denitrification is more important than anammox in the Peruvian upwelling system. Consequently, we will assume anammox is minimal and interpret our measurements as nitrate respiration as calculated. In any case, because anammox is autotrophic our respiratory CO<sub>2</sub> production calculations will err on the high side due to any anammox.

-----  
Comment 12. *Discussion Page 16189, line 10: Delete one of the “and” after POC*

Thanks. The extra and has been eliminated.

---

13 *Tables*

*Table 2-6: Are all tables necessary? Could some of them not be combined?*

The tables are all necessary and if we combined them they would be too unwieldy.

*In Table 2: Please describe how you calculate potential R from the ETS*

*activity*. Specifically, multiply by 60, divide by 4, and nano mol/L is the same as micromole per meter cubed.

In more detail: We measure tetrazolium reduction to its formazan in the ETS assay. Two electrons are required for each molecule of tetrazolium to be reduced. After a few minutes in a spectrophotometer we know how many moles of formazan are produced (Beer-Lambert Law) and hence the “moles” of electrons flowing during this time through the ETS. The potential respiration is just the application of the logic that if 4 electrons (4e) are needed to reduce molecular oxygen (O<sub>2</sub>) to water (2H<sub>2</sub>O) and 2e are required for each molecule of tetrazolium to be reduced then 2 moles of formazan produced is equivalent to 1 mole of oxygen reduced to water. Thus we have potential respiration from measured tetrazolium reduction. This has been explained in Packard and Christensen (2004) and in Packard and Codispoti (2008).

---

#### References for this discussion.

- Carlson, C. A., Hansell, D. A., Nelson, N. B., Siegel, D. A., Smethie, W. M., Khatiwala, S., Meyers, M. M., Halewood, E. Dissolved organic carbon export and subsequent remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin. *Deep-Sea Res. II*, 57, 1433-1445, 2010.
- Christensen, J.P. and T.T. Packard. Respiratory electron transport activities in plankton: comparison of methods. *Limnol. Oceanogr.*, 24(3), 576-583, 1979.
- Codispoti, L.A. , J A Brandes, J P Christensen, et al., The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter the anthropocene? *Scientia Marina*, 65(Suppl. 2), 85-105, 2001.
- Dalsgaard, T., Thamdrup, B., Fariás, L., Revsbech, N.P. Anammox and denitrification in the oxygen minimum zone of the eastern south Pacific. *Limnol. Oceanogr.* 57 (5), 1331-1346, 2012.
- Devol, A. H. Biological oxidations in oxic and anoxic marine environments: Rates and processes. Ph.D. thesis, Univ. Washington, Seattle, 208, 1975.
- Garfield, P., Packard, T.T., Codispoti, L.A. Particulate protein in the Peru upwelling system. *Deep-Sea Research*, 26/6A, 623-639, 1979.
- Jenkins, W.J., Oxygen utilization rates in North Atlantic subtropical gyre and primary production in oligotrophic systems. *Nature* 300, 246–248, 1982.
- Jenkins, W.J., The use of tracers and water masses to estimate rates of respiration. In: Hobbie, J.M., Williams, P.J.Le B. (Eds.), *Heterotrophic Activity in the Sea*. Plenum Press, New York, London, pp. 391–403, 1984.
- Kartal, B. de Almeida, N. M., Maalcke, W.J., Op den Camp, H. J. M., Jetten, M. S. M., Keltjens, J. T. How to make a living from anaerobic ammonium oxidation. *FEMS Microbiol Rev*, 1–34, 2012. DOI: 10.1111/1574-6976.12014.

- Martin, J.H., Knauer, G.A., Karl, D.M. and Broenkow, W.W. VERTEX: carbon cycling in the northeast Pacific. *Deep-Sea Res.*, 34 (2), 267-285, 1987.
- Packard, T.T. The estimation of the oxygen utilization rate in seawater from the activity of the respiratory electron transport system in plankton. Ph.D. Thesis, University of Washington, Seattle, 115, 1969.
- Packard, T.T. Measurement of electron transport activity of marine microplankton. In: *Advances in Aquatic Microbiology*, P.J. LeB. Williams and H.W. Jannasch (eds.), Academic Press, New York, pp. 207-261, 1985a.
- Packard, T.T. Oxygen consumption in the ocean: Measuring and mapping with enzyme analysis. *Mapping Strategies in Chemical Oceanography*, A. Zirino (ed.), American Chemical Society, Washington, D.C. pp. 177-209, 1985b.
- Packard, T.T. and L. A. Codispoti. Respiration, mineralization, and biochemical properties of the particulate matter in the southern Nansen Basin water column in April 1981, *Deep-Sea Research Part I*. 51 (3), 403-414, 2007.
- Packard, T.T. and P.J. LeB. Williams. Rates of respiratory oxygen consumption and electron transport in surface seawater from the Northwest Atlantic Ocean. *Oceanologica Acta*, 4(3), 351-358, 1981.
- Packard, T.T., P.C. Garfield and L.A. Codispoti. Oxygen consumption and denitrification below the Peruvian upwelling. In: *Coastal Upwelling: Its Sediment Record*, E. Suess and J. Thiede, (eds.), Plenum Press, New York pp. 147-173, 1983.
- Riley, G.A. 1951. Oxygen, phosphate, and nitrate in the Atlantic Ocean. *Bingham Oceanogr. Collection Bulletin*, 13 (3), 1-169.
- Smith, J. J., McFeters, G. A. Mechanisms of INT (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride), and CTC (5-cyano-2,3-ditolyl tetrazolium chloride) reduction in *Escherichia coli* K-12. *J. Microbiol. Methods* 29,161-175, 1997.
- Ward, B., Devol, A. H. Rich, J. J., Chang, B. X., Bulow, S. E., Naik, H., Pratihary, A., Jayakumar, A. Denitrification as the dominant nitrogen loss process in the Arabian Sea. *Nature* 461, 78-81, 2009.