Referee #1, comment #1

The authors present a ms that utilizes sets of linear equations (as a matrix model) to describe nitrogen transformations in seawater from 25 meter depth. The authors argue that 'conventional methods' for calculating rates, including nitrification, do not consider that multiple nitrogen processes are occurring simultaneously. The authors present the model, and then illustrate 2 manipulations where enriched $^{15}NH_4^+$ was added to determine what nitrogen pools it ends up in. They use the program STELLA to estimate parameters of their model. They conclude that NH_4^+ regeneration is likely an important process through isotope dilution, that their model can give differing results from the 'traditional model', and that DON is likely very important. They were able to solve for multiply occurring processes. This is an interesting ms and the community will be interested in the approach. The authors, however, need to address a number of comments to make this a more significant ms.

Author response:

Thanks for reviewer's appreciation of the merit of our method.

Referee #1, comment #2

The recognition that there are multiple nitrogen transformations is an important one, and the coupling of the model to an enrichment assay is a strong approach. Although I appreciate what the authors are doing here, the statement that they are the first to do needs to be amended, given the recent publication of Pfister et al. in BGS. Biogeosciences, 13, 3519-3531, 2016, http://www.biogeosciences.net/13/3519/2016/ ("To our knowledge, this is the first and most convenient method designed to quantitatively and simultaneously resolve complicated nitrogen transformation rates, albeit with some uncertainties."). Thus, throughout the discussion it would be appropriate to see their model compared with the differential equation model used by Pfister et al. to model multiple nitrogen transformations. The authors also need to compare their conclusions with the above study. For example, I note that this study follows processes in seawater only, while Pfister et al. includes benthic species. It would be useful for the authors to comment on the comparisons.

Author response:

This is a very constructive suggestion. Indeed, we missed the paper by Pfister et al. (2016) while we submitted our manuscript in July. Pfister et al. (2016) applied similar approach to resolve the N cycle processes in a tidal pool. In this version, we introduced the paper by Pfister et al. (2016) in Introduction. As suggested also by Reviewer #2, we made discussions and comparisons with their method for tidal pool.

The similarity is the coupled monitoring of changes in isotopic composition and concentration in multiple pools (NH_4^+, NO_2^- and NO_3^-). However, dissimilarities include：(1) we focused on water column and all operationally defined nitrogen pools were measured, (2) the benthic biomass, which equals to particulate organic nitrogen in our case, were not measured in their tidal pools; thus, system level mass conservation cannot be made. Accordingly, their case did not allow discussions of DON release, which is an important process in water column; (3) they applied ODE to derive the mean rate constant by fitting parameter combination over the monitoring time course; by contrast, we use matrix (or linear programming in old version) to obtain rate/rate constant for the first two data points and then predict latter on changes.

We thoroughly revised our manuscript basing on three reviewers' comments. In this version, we modified our model structure slightly (see the reply for Reviewer #2) and discussed more in term of model structure and method for rate derivation. The rates derived by using ODE were also added into our tables for comparison. Meanwhile, we reorganized the manuscript in sequence from simple (low nutrient assay) to complex case (high nutrient assay). We believe that such a re-arrangement will be easier for readers to understand our method.

We attempt to resolve rates in water column, more specifically, in sun-lit ocean where intensive substrate competition occurs, thus, we modified our original statement to "This is a convenient method in euphotic zone to quantitatively and simultaneously resolve complicated nitrogen transformation rates, albeit with some uncertainties.".

Referee #1, comment #3

The ms would benefit from more direct discussion about the comparison of the models presented in this paper and other models and approaches. Nitrogen processing rates don't seem to differ much based on methodology (Table 3), with values at least being within a similar range. I find this surprising, especially given the authors' recognition of error sources (L576). The abstract states: "comparisons with conventional labeling methods are discussed" (L28) and this is too vague. Similarly, the Conclusions could be stronger and more direct.

Author response:

The significance of our method is to resolve multiple rates by adding one single tracer in one bottle for incubation. This cannot be achieved by conventional methods. As mentioned above, Pfister et al. (2016) did not include DON in their discussion according to under-identified benthic biomass.

We highlighted the significance of our method; however, we do not criticize traditional methods. For example, the traditional method for nitrification was usually conducted in the dark or deep water, thus, the consumption of substrate (ammonium) and product (nitrate) by phytoplankton was minimized presumably. In the dark, the traditional approach is ok; however, bias could be significant in the euphotic zone where phytoplankton competition appears. Meanwhile, similar rate values (less biased) can be obtained by traditional methods when one or two specific processes dominate the system.

As mentioned in Elskens et al. (2005), none model was perfect. For example, in a simple system without phytoplankton and light there will be no need to apply our approach. As mentioned in manuscript already, the traditional estimate for nitrification does not work under simulated *in situ* light in the euphotic zone since the end-product, nitrate, drops quickly due to intensive phytoplankton consumption. Such drop in end-product violates the assumption "end-product increase" in traditional method. Thus, dark incubation is required to limit phytoplankton uptake. The incubation in the dark, of course, does not represent "*in situ*" condition. The advantages of our method are (1) to explore the transformation of pathways for *in situ* condition, particularly, in euphotic ocean and at around the transition zones (e.g., nitracline and thermocline in the field) and (2) to examine responses of multiple metabolic pathways via manipulation experiments (e.g., pH, temperature and light).

Finally, the rate numbers for ammonium, nitrate uptakes and nitrification in Table 3 revealed difference. Ours values are 3-20% higher than those by traditional methods. Moreover, nitrate uptake rate by our method was ~6 times higher (in Table 3) than that derived from the equation suggested by Santoro et al. (2010) although nitrification rate was within a similar range. In this

version, we pointed out explicitly the reasons for the offset between ours and conventional methods. We enhanced comparisons among methods in Discussion part and made a stronger statement in Conclusions. We found the sentence “comparisons with conventional labeling methods are discussed” to be improper in the Abstract. We eliminated this sentence.

Referee #1, comment #4

The ms would benefit from adhering more strongly to a clear separation of methods, results, discussion. The paragraph starting L395 is a good example where this needs to be done. It might help to shorten the ms too.

Author response:

Follow this suggestion, the manuscript was reorganized. Examples were given together now in Methods from the simple to the complex case. Details for matrix solution and sensitivity test were now mentioned in Methods first and then appeared in Results. Yet, the entire length was expanded due to additional data presentation (we added 2% light incubation for comparison as requested), methodology comparison (we added results from ODE as requested; see reply to reviewer #2) and in-depth discussions.

Referee #1, comment #5

Finally, although I greatly appreciate the enrichment assay, it appears to be done once. I cannot be sure based on the description given, but it appears unreplicated and that does limit the interpretation the authors can make. Starting L314, more detail is needed including how many incubations, and whether they were replicates or uniquely treated. Having the high and low nutrient assay immediately next to each other in the methods would also lend better comparison. As is, it is looks like these assays are unreplicated and water was collected at different depths, etc.

Author response:

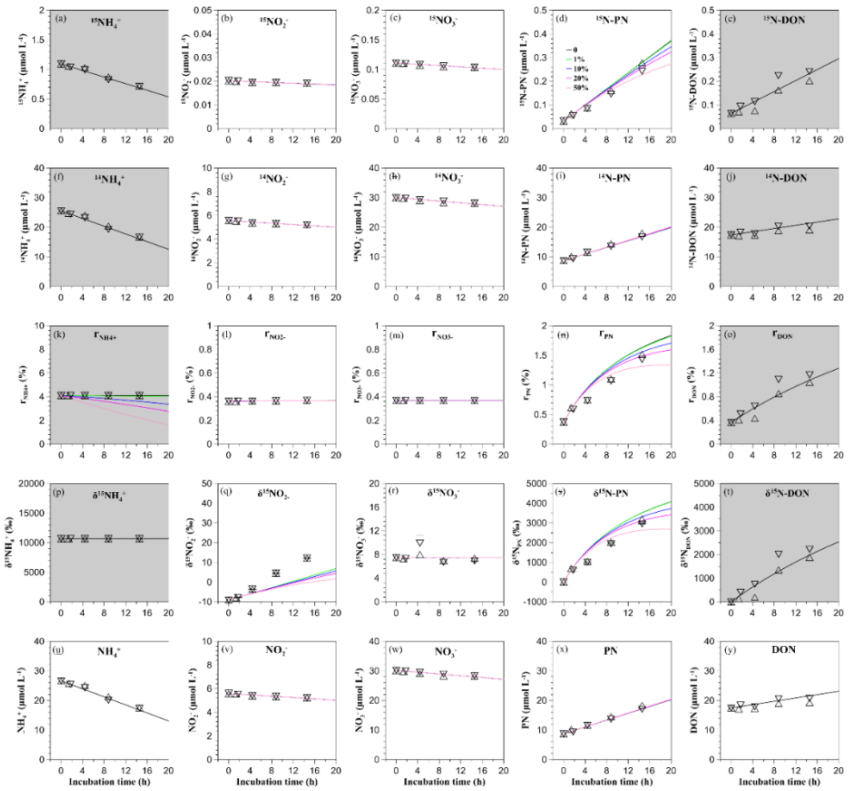
We do have replicates. We added descriptions for replicates in this version. In the old version, two data points were representative of replicates. We now used regular and inverse triangles, which give a clearer image of data distribution (see panels below). Moreover, we provided a new case incubated under 2% light.

Instead of discussing the biogeochemical significance of specific processes, the scope of this paper is to propose a convenient method in the field for multiple rate measures. We agree with reviewer that replicates will be helpful indeed if we attempt to probe ecosystem biogeochemistry, however, not necessarily be helpful for a new method establishment. The rate uncertainty, in fact, was largely sourced from heterogeneity of water sample and analytical errors for isotopic composition and concentration, rather than the estimator itself.

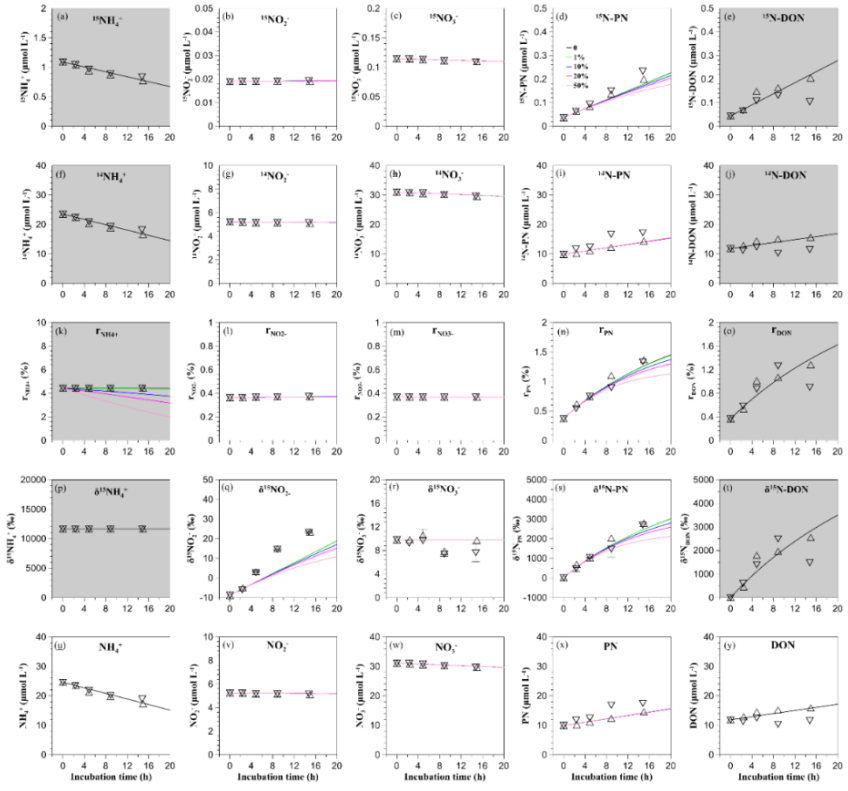
The two assays, in fact, are for two very different yet commonly seen conditions. The simple one is for oligotrophic ocean (nitrite and nitrate were pulled together in one nitrogen pool, NOx). The complex one is for estuary and coastal water where nitrite concentrations are relatively high. According to this suggestion, we illustrated both assays together in the Methods and reorganized the manuscript from the simple case to the complex case. To convince readers the applicability of our method, we presented additional data in this version to discuss these N transformation pathways under different light conditions (see panel (a) and (b) below). All the fluxes were derived from the matrix solution for the first two time points. The full time courses were generated via equation-based simulation by using Stella. We successfully and precisely predicted

the observed time courses further illustrating the performance of our method.

(a) High nutrient case – 80% sPAR



(b) High nutrient case – 2% sPAR



Referee #1, specific comments:

Line 50, explain what is meant by the 'inventory method'

Author response:

We followed B. B. Ward (2011). The description is now "The inventory method (monitoring substrate and/or product changes over time) is often used …".

Line 57 "mainly"

Author response:

Corrected.

L105, 210 – be specific about what 'new method' means

Author response:

We changed to "isotope matrix" method.

L106, is STELLA a model or a method? What is meant by "The method was also validated using the STELLA model".

Author response:

STELLA is user-friendly software for box model construction. We realized the appearance of Stella here in Introduction is improper, thus, this sentence was removed. The descriptions about STELLA will be in Methods.

L150, omit 'basically'

Author response:

Omitted.

L210, what is the 'incubation system'?

Author response:

We changed to incubation bottle.

L243, "approximately"

Author response:

Corrected.

L416, the depth of water collection for the experiment is unclear. Here it says 25 m, while elsewhere it states 3 m.

Author response:

We presented two samples collected from different locations. One was taken from the western North Pacific (low nutrient case) and the other was from a coastal bay in southern China (high nutrient case). In this version, we mentioned the two cases together in Methods to avoid confusion.

L419, the final enrichment value should be given.

Author response:

The description is now "…to achieve a final tracers concentration of 30 nM.."

Methods – What were the dissolved oxygen levels? Is the assumption that this is a well-oxygenated system and loss of ^{15}N as gas is irrelevant?

Author response:

Yes. We made assumptions for oxygenated water column and short term incubation. Such assumption is common and well accepted. In this version, we provided DO saturation values for all cases.

L482 'result'

Author response:

Corrected.

Though I could read eqns 5, 6, 7, they are reprinted poorly due to some 'translation' issue.

Author response:

We tried several times and even asked editorial office's help for file translation during our initial submission. The problem was due to version of software. We will work it out.

L552 – Is there good evidence for light inhibition? Many studies find high rates of nitrification with normal light.

Author response:

Light depresses nitrification efficiency by either direct inhibition on AMO or resource reallocation for damage recovery. Similar to previous studies, such as Merbt et al. (2012), Smith et al. (2014) and Peng et al. (2016), we found light inhibition also in coastal China seas although some recent evidences showed that some taxa of marine AOA hold genetic capabilities to reduce oxidative stress and to repair ultraviolet damage (Luo et al., 2014; Santoro et al., 2015). In the photic ocean, besides photo damage nitrifiers need to compete with phytoplankton for substrate. This is why the abundance of AOA/B increased downward in genera and also why we establish

this method to explore competing processes under in situ light condition.

According to this question and reviewer #3's suggestion, we presented additional data for the high nutrient case. For high nutrient case, actually, water from 80% sPAR and 2% sPAR were sampled for incubation. We measured fluxes for multiple pathways for different light environments, and then discussed effects of light on various processes.

The authors do not need to comment so much on inhibitors – which they did not use.

Author response:

We did not criticize the usefulness of inhibitor method since to block unwanted process is the only way to obtain a more accurate rate measure for specific process while using the traditional source-product method. Although inhibitor addition was not used in isotope labelling method, similar concept was applies to reduce the interference from unwanted pathway; such as nitrification rate measurement needs to be conducted in the dark to minimize ammonium and nitrate consumptions by phytoplankton. In this version, we made a clearer statement to convey proper information for inhibitor application.

Table 1 caption – explain "different r_{NH4+} variation". What seems to be meant here is that the authors are manipulating the values of r_{NH4+} to mimic the effects of isotope dilution as a consequence of regeneration.

Author response:

Yes, we did not measure isotopic compositions for NH_4^+. Thus, after obtaining fluxes (or rate constants) we set r_{NH4+} as variable to examine the significance of remineralization in short term incubation. Results showed that remineralization would be effective in our case when incubation is prolonged over 16 hours. According to the validation by consecutive observations, remineralization is limited in all our cases in the first few hours. We added more discussions about the sensitivity test for remineralization.

We mentioned in the manuscript that once the technique for isotopic composition of low concentration NH_4^+ is mature (open ocean case) or in any case r_{NH4+} time course was measured (coastal ocean case), all rates including remineralization can be obtained directly. Here in our case, we simulated the time courses of different nitrogen pools and assessed the importance of regeneration by manipulating r_{NH4+}.

Same for Table 2 caption. Table 3 – Provide a citation for the Traditional Rate Calculation (Dugdale and Wilkerson, 1986) and cite the equation numbers used for each.

Author response:

Reference was added.

Suppl. fig 1 and 2 are STELLA figs which can be confusing without equations. I did not get much out of these figs, other than the recognition that the authors used this model structure.

Author response:

We added equations into the two panels in this version.

Throughout the ms, the authors need to check that chemical terminology is reprinted accurately. Similarly, when subscripts are used.

Author response:

Thanks for reminding. We checked these terms carefully and will try our best to solve problems caused by format translation.