

Reviewer #3, Comment #1

The manuscript would be stronger if a wider range of environments with varying relative importance of the different processes were examined. At present, the manuscript really just addresses two incubation experiments taken from high light environments.

Author response:

The coastal case, in fact, we sampled two layers with different light intensity, 80% and 2% sPAR, and bottles were incubated in neutral density-screened incubator to simulate original light. In the old version, we did not present entire data since the scope of this paper is to provide a convenient method.

According to this suggestion, we presented additional data. We saw higher rates of ammonium, nitrite and nitrate uptake for the high light layer. While nitrite and ammonium oxidation were both low compared with phytoplankton uptake. The overall low ammonium oxidation rate was likely attributable to the low temperature in winter. The amount of ammonium uptake by microbes was similar to that by phytoplankton in both cases underscoring the importance of ammonium flow to < 0.7 μm particle fraction.

We do not add further cases we have. Our next paper will be focusing on the application of this method to discuss the temperature and light effect on multiple processes in an estuary along salinity gradient.

Reviewer #3, Comment #2

The manuscript should include a deeper discussion of the results beyond just the new method, extending to the actual ecology of the processes being examined. For example, the finding that varying the remineralization rate does not affect the nitrification rate seems significant, though potentially an artifact of the samples chosen for investigation (see #1 above).

Author response:

We agree with reviewer, the results might be very different in other environments. We included the layer with 2% sPAR for discussion. According to this comment, we modified the model structure (see reply to comment #4 by Reviewer #2) to discuss the missing ammonium. In old version, the unbalanced nitrogen was assigned as a leakage to DON from PN. As indicated in our manuscript, PON was operationally defined (on GF/F filter pore size of 0.7 μm). The nitrogen leakage, in fact, had been observed elsewhere. As pointed out by Laws (1985), the leakage from PON to DON or bacterial ammonium uptake (<0.7 μm , absence on filter) may account for the vanishing $^{15}\text{NH}_4^+$ on PON. In this version, we separated the missing nitrogen into account for the vanishing ammonium in incubation bottle. Thus, variable remineralization rate (variable $r_{\text{NH}_4^+}$) was assigned to test the dilution effect.

Basing on our observational data, the continuously decreasing ammonium over time was obvious, suggesting that remineralization was insufficiently high to maintain the ammonium at steady state. Such rapid drop in ammonium was supportive of low remineralization rate deduced from

time course extrapolation. As indicated by Pfister et al. (2016), benthic mussels play a critical role in ammonium supply in tidal ponds. In our both cases, micro-zooplankton in sampled water may not present in high abundance. Limited zooplankton (animals) in sampled water is likely the key for insignificant remineralization. More discussions will be made for ecological implications.

Specific comments

There is an over emphasis on the novelty of this work being ‘abandoning inhibitors’, as most stable isotope labeling papers in the last decade have not used inhibitors to actually calculate rates, but rather to inform specific groups of organisms that might be contributing to a specific process. This is the case for many of the papers incorrectly cited in lines 61-63.

Author response – We do not mention ‘abandoning inhibitors’ in this version. References were carefully checked and cited accordingly.

80% surface light intensity is a very high light intensity for trying to measure nitrification. I would suggest noting in the discussion that the contribution from nitrification to ¹⁵N uptake might be considerably different at lower (e.g. 1-10%) surface irradiance. This is somewhat alluded to in lines 381-384, but the implications could be discussed more explicitly.

Author response – We totally agree with this comment. We provide low light case in this version. However, nitrification rate was still low due to low temperature in winter. We described the light effect on nitrification and referred to papers about light inhibition and substrate competition (Smith et al. 2014; Peng et al. 2016). We also explicitly stated these flows or rates in low light environment could be very different from results we presented in this study.

Line 539: Are rates (nmol L⁻¹ h⁻¹) or rate constants (h⁻¹) being compared here? Clarify language. Also, the phrase ‘their nitrate uptake rate’ is confusing . . . I think what is meant is ‘nitrate uptake calculated using their method’

Author response – Corrected. All units in tables were carefully checked. Both rate values and rate constant will be presented clearly.

Line 562: The discussion about the relevance of this research to PNM dynamics is not warranted based on the results presented here.

Author response – We removed PNM relevant discussions.

Table 3: The column title ‘Santoro et al.’ should be clarified to say ‘Rate calculation of Santoro et al’ and units should be clarified for all columns (see comment above about rates versus rate constants). Table 2 has the NO_x uptake rate constant (k) as 0.059 h⁻¹, but this same value is listed as a rate (nM h⁻¹) in Table 3.

Author response – The column title is corrected. We carefully checked for rate and rate constants throughout the manuscript and tables. The units and associated descriptions are now consistent.

Line 57: ‘manily’ should be ‘mainly’.

Author response – Corrected.

Line 182: is sulfamic, not sulfanilic meant here?

Author response – Corrected.

Line 482: 'resut' should be 'result'.

Author response – Corrected.