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Biogeosciences Discussions

Interactive comment on "Use of flow cytometry and stable isotope analysis to determine phytoplankton uptake of wastewater derived ammonium in a nutrient-rich river" *by* Calla M. Schmidt et al.

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General comment : This study aims to trace the dissolved inorganic nitrogen source primarily used by phytoplankton in a river impacted by an heavy anthropogenic nutrient (ammonium) enrichment. The authors report an interesting dataset of stable nitrogen isotope ratio measurement in several inorganic and organic nitrogen pool. Moreover, they used a novel and elegant method (combination of flow cytometry cell sorting with stable isotope analysis) in order to distinguish (healthy) phytoplankton cells from the bulk particulate organic matter. The manuscript is well-written and the results

reported by the authors are appropriately discussed, overall I greatly enjoyed reading this manuscript.

Specific comment:

*p4, line 26 : No results from a 15N-labeled nutrient uptake experiment are described in this manuscript, hence I would suggest to remove this from the material & method section.

AC: This text has been removed.

*p5, line 1-5 : Please, provide more information about the methodologies used to measure NO2-, NO3- and NH4+ concentration (the chemistry behind).

AC: The following information will be added to the methods section:

"Nitrite concentration was determined by the Greiss reaction in which sulfanilamide and N-(1-Naphthy1) ethylenediamine dihydrochloride (NNED) reacts with nitrite in aqueous acidic solution to form an intensely pink diazo dye with an absorption maximum at 540 nm (Bendschneider and Robinson, 1952). To determine nitrate concentration, nitrate was reduced to nitrite by passage through a column containing copperized cadmium filings (Wood, Armstrong and Richards, 1967), and then the concentration of nitrate plus nitrite was determined in the manner described above. NH4+ concentrations were measured spectrophotometrically using a 10-cm path length cell according to the phenolhypochlorite method described in Solorzano (1969)."

*p5, line 6 : I don't understand to what "70_m" is related. As you certainly know, nominal pore size of of the GFF filters is 0.7 _m. typo ?

AC: Typo corrected, GFF filters had a nominal 0.7 μm pore size.

*p8, line 6-19 : I would suggest to plot the d15N-NO3- and d15N-NH4+ data in a way similar to figure 3 (ie. Data plotted against travel time). These data are interesting, but it is difficult to visualize the trend when looking at table 1 only.

AC δ15N-NO3- and δ15N-NH4+ are in fact plotted against travel time in Figure 5. A reference to figure 5 will be added to the paragraph to improve clarity.

*p9, line 25 : typo : fluorescence, and not florescence.

AC: Typo corrected.

*p10, line 2 : Higher importance of labile POM mineralization in the +EFF parcel seems indeed plausible. It is a bit unfortunate that you did not measure heterotrophic bacteria abundance, but did you measure dissolved oxygen concentration, or any other variable related to ecosystem metabolism (for instance, community respiration) ? They might be helpful to directly put in evidence a putative higher importance of heterotrophic metabolism in the +EFF parcels.

AC: Thank you for the helpful suggestion. We did measure dissolved oxygen (DO) concentration during the study, which reveals that DO was consistently lower in the parcels containing effluent (see figure S3D from Kraus et al. 2017, included below), thus providing additional support for the argument that heterotrophic metabolism differed between +EFF and –EFF parcels.

This point will be clarified in the manuscript:

"It is also possible that the presence of effluent caused an increase in heterotrophy by zooplankton and bacteria in the +EFF parcels, resulting in δ15N-POM values greater than δ15N-PHY downstream of the WWTP. Heterotrophic bacterial abundance was not measured during this study, but dissolved oxygen concentration was monitored, and in both October and June experiments dissolved oxygen concentrations were lower in the parcels containing effluent (Kraus et al., 2017)."

*p10, line 16 and below : I understood reading your paper that the diatom "health status" was decreasing downstream, then could it be hypothesized that a change in the composition of the phytoplankton assemblage downstream of the location where the effluent enter the Sacramento river explains the gradual increase in the contribution

of NH4+ ? Did you look at the phytoplankton composition (and assess its variability) at several location during the travel of the two parcels of water downstream ?

AC: This isotopic study was conducted in parallel with a larger investigation into this very important question and the results are reported in Kraus et al. 2017. To summarize: Whole-water samples for phytoplankton enumeration were collected at each sampling point during downstream travel (15 points in October, 9 points in June). Patterns in the phytoplankton assemblages were examined using nonmetric multidimensional scaling (NMDS) ordinations constructed from Bray–Curtis similarity matrices (square root- transformed abundance data). Potential differences between the +EFF and -EFF parcels were tested for significance using analysis of similarity (ANOSIM). The results of this analysis showed a significant separation of phytoplankton assemblages between October and June, but no statistical difference between +EFF and –EFF parcels in October or June.

Given this finding we concluded it was unlikely that changes in community composition cause the greater increase in %NH4 in +EFF parcels compared to –EFF parcels. To clarify this point, we will add the following sentence to the discussion section on pg 9 line 19:

"Patterns in the phytoplankton assemblages were examined and potential differences between the +EFF and -EFF parcels were tested for significance using analysis of similarity (ANOSIM). The results of this analysis showed no statistical difference between the assemblages present in +EFF and –EFF parcels in October or June (Kraus et al. 2017)."

*p10, line 16 and below : Beside NH4+ and NO3-, N2 fixation could also be a significant N source in systems where cyanobacteria are abundant. Do you know what was the contribution of cyanobacteria to the phytoplankton assemblage ? Could you explain why you rule out any contribution of N2 fixation as a N source in the Sacramento river?

AC: We are not aware of any studies that have looked at N2 fixation in the Sacramento

River. However, it is generally believed that N2 fixation is not a significant process in large rivers like the Sacramento River, which is a deep (4-10 m), swift moving, channelized river lined with rip rap, which also has high concentrations of DIN. We consulted with several colleagues on this topic. For example Vitousek et al. 2002 states "The few studies of nitrogen fixation in flowing water systems suggest that cyanobacteria and N fixation are limited by some of the same factors limiting other lotic algae: low light in small, heavily shaded streams (Horne & Carmiggelt 1975) and high current velocity (small to mid-sized streams) or high turbidity (large rivers). Indeed, planktonic cyanobacteria do not occur at all in most streams."

Vitousek, P. M., Cassman, K., Cleveland, C., Crews, T., Field, C. B., Grimm, N. B., Howarth, R. W., Marino, R., Martinelli, L., Rastetter, E. B. and Sprent, J. I.: Towards an ecological understanding of biological nitrogen fixation, Biogeochemistry, 57–58(McKey 1994), 1–45, doi:10.1023/A:1015798428743, 2002.

Additionally, for the purposes of this study, the source of the DIN in the river is less important than the fact that there are large differences in NH4 concentration in the presence versus absence of effluent. In the stable isotope mixing model calculations we assume that phytoplankton in the +EFF parcel use NH4 or NO3 with no contribution from nitrogen fixation. We feel that this is a valid assumption given that a) this environment is unlikely to foster nitrogen fixation in general, and b) the +EFF plume (where we used the mixing model) would be even less likely to support nitrogen fixation given the elevated NH4 concentration.

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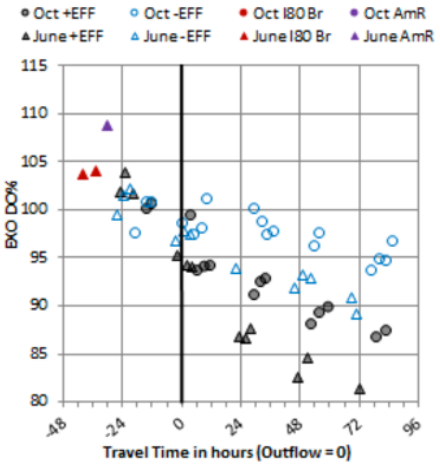

Fig. 1. Dissolved oxygen in parcels with effluent and without effluent. Figure reproduced from figure S3D in Kraus et al. 2017