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# *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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The manuscript by Schmidt et al. provides an interesting case study of the application of a very promising and little-used technique, by using flow cytometry as a sample preparation step to analyse d15N of phytoplankton to study nitrogen cycling in river systems . The ms is well written and data interpretation is generally solid.

I have relatively minor suggestions for improvement:



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-While the focus of the manuscript is evidently on nitrogen cycling, it might be interesting to also report and discuss d13C values for the flow cytometry sorted phytoplankton samples – I assume these were analyses in the same run ?

AC:Unfortunately we were not able to measure $\delta$13C on the sorted samples. The peak focusing required to measure $\delta$15N for these small samples saturated the $\delta$13C signal. For this particular study, where we had a very limited amount of sample, we prioritized running duplicates for $\delta$15N over analyzing $\delta$13C. As described at the end of the manuscript, it would be ideal to change the field sampling approach in future studies to allow for sorting of more material. This should allow for isotopic analysis of specific populations within POM and it would also allow the method to be optimized for $\delta$13C analysis.

-the authors sampled following a Lagrangian approach – but it should perhaps be mentioned somewhere that the residence time of particles in a river system is expected to be higher than the water travel time in a river system, and some discussion on how that might affect the interpretation of results.

AC:We understand this concern. A single value for parcel travel time is best considered as an average residence time that represents a distribution of particle travel times. Because the focus of the study is on comparison of +EFF and –EFF conditions we took care to track parcel locations using surface current-following drifters, which track the velocity of a neutrally buoyant particles, and we also verified our position within each parcel by measuring changes in water quality that could be attributed to presence and absence of effluent. Since the parcels were $\sim$15 km long, we feel confident that our data represents particles exposed to these two different conditions even if we have slightly underestimated or overestimated the travel time of some particles.

This will be further clarified in the manuscript with the following text:

“. . .field sampling was conducted using a Lagrangian sampling approach during October 24 to 29, 2013, and May 30 to June 4, 2014 (hereafter referred to as the “October”





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and “June” experiments). During both October and June, sampling was coordinated with ∼20 hour WWTP effluent discharge holds, creating a ∼15 km stretch of effluent-free river to allow comparison of two parcels of river water; one containing effluent high in NH4+ (+EFF) and one without effluent (-EFF). During both experiments, +EFF and -EFF parcels were tracked using small drifters and a high-speed mapping boat equipped with a custom designed flow-through instrument package that continuously displayed surface-water measurements of specific conductance (a conservative tracer), to assure that samples were collected from within the same parcel of water as it travelled ∼70 km downstream (Fig. 1).”

-page 5, line 7: I assume this should be 0.7 _m GFF filters (not 70 _m) ?

AC: Typo will be corrected, GFF filters had a nominal 0.7 $\mu$m pore size.

-Methods: while the authors refer to Polissar et al. (2008) for the ‘micro-EA’ setup, the latter does not use a GasBench as interface, and the authors provide no details on the trapping/focussing of the eluting gases. Some more details would be of interest to readers who wish to setup a similar configuration.

AC: We used the same approach for trapping and focusing nitrogen as described in Pollisar et al. 2008, the use of the gasbench interface only allowed for automation of the trapping procedure.

This will be further clarified in the methods (pg 6 lines 24-32) with the following text,

“Sorted cells were transferred to 20 mL glass vials and dried down under vacuum using a centrifugal evaporator. Dried phytoplankton samples were redissolved in 20 $\mu$L ultra high purity deionized water and transferred into tin capsules. Capsules were dried overnight at 60 °C and then crushed into small cubes. $\delta$15N analysis of sorted phytoplankton was conducted using elements of a coupled Carlo Erba CHNS-O EA1108-Elemental Analyzer and Thermo Finnigan Gasbench II system with automated cryo-trapping system that is connected to an isotope ratio mass spectrometer (Thermo





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Fisher Scientific) at the UC Santa Cruz Stable Isotope Laboratory facility. The elemental analyzer and gas bench were configured to run small samples using the methods described in Polissar et al. (2009). In this configuration, samples as small as 35 nmol N could be analyzed with a precision of 0.5 ‰Ṗhytoplankton samples analyzed for this study ($\delta$15N-PHY) ranged in size between 50 and 100 nmol N. Analysis of duplicate samples (sorted and analyzed independently) indicated a precision of 0.8 ‰ for the entire method.

-The nutrient concentration profiles clearly suggest that nitrification could be an important process affecting nutrient cycling in this system – it would be worth discussing this aspect and checking if there are data that might shed some light on this. Are there dissolved oxygen data available ? Have others measured nitrification rates in this system?

AC: Yes, other authors have investigated nitrification in the Sacramento River and we do discuss this and include reference to this literature on page 8, lines 14-19:

“Due to low concentrations of NH4+ upstream of the WWTP, it was only possible to measure $\delta$15N-NH4+ in the +EFF parcels downstream of the WWTP. In the +EFF parcels $\delta$15N-NH4+ increased from 7.9 ‰ to 9.7 ‰ in October and from 8.0 ‰ to 10.7 ‰ in June with downstream travel. We also observed that $\delta$15N-NH4+ increased while NO3- concentration increased and $\delta$15N-NO3 values decreased during transit in parcels containing effluent, which suggests nitrification was occurring. This observation is consistent with high rates of nitrification previously reported in the Sacramento River (Hager and Schemel, 1992; Parker et al., 2012a; O’Donnell 2014; Damashek et al., 2016).”

This is also a timely question as a paper estimating nitrification rates in this portion of the Sacramento River was just accepted for publication in Water Resources Research. We will add this additional reference to the manuscript:

Kraus, T.E.C. K. O’Donnell, B.D. Downing, J.R. Burau, B.A. Bergamaschi, in press.

Using paired in situ high frequency nitrate measurements to better understand controls on nitrate concentrations and estimate nitrification rates in a wastewater impacted river. Water Resources Research.

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