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Use of flow cytometry and stable isotope analysis to determine phytoplankton uptake of wastewater derived ammonium in a nutrient-rich river

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Abstract. Anthropogenic alteration of the form and concentration of nitrogen (N) in aquatic ecosystems is widespread. Understanding availability and uptake of different N sources at the base of aquatic food webs is critical to establishment of effective nutrient management programs. Stable isotopes of N (14N, 15N) are often used to trace the sources of N fuelling aquatic primary production, but effective use of this approach requires obtaining a reliable isotopic ratio for phytoplankton. In this study, we tested the use of flow cytometry to isolate phytoplankton from bulk particulate organic matter (POM) in a portion of the Sacramento River, California, during river-scale nutrient manipulation experiments that involved halting wastewater discharges high in ammonium (NH_4^+). Field samples were collected using a Lagrangian approach, allowing us to measure changes in phytoplankton N source in the presence and absence of wastewater derived NH₄⁺. Comparison of δ^{15} N-POM and δ^{15} N-phytoplankton (δ^{15} N-PHY) revealed that their δ^{15} N values followed broadly similar trends. However, after 3 days of downstream travel in the presence of wastewater treatment plant (WWTP) effluent, δ^{15} N-POM and δ^{15} N-PHY in the Sacramento River differed by as much as 7‰. Using a stable isotope mixing model approach, we estimated that in the presence of effluent between 40 and 90% of phytoplankton-N was derived from NH₄⁺ after 3 days of downstream transport. An apparent gradual increase over time in the proportion of NH_4^+ in the phytoplankton N pool suggests that either very low phytoplankton growth rates resulted in an N turnover time that exceeded the travel time sampled during this study, or a portion of the phytoplankton community continued to access nitrate even in the presence of elevated NH₄⁺ concentrations.