Effect of iron limitation on the isotopic composition of cellular and released fixed nitrogen in *Azotobacter vinelandii*

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Most biological nitrogen transformations have characteristic kinetic isotope effects used to track these processes in modern and past environments. The isotopic fractionation associated with nitrogen fixation, the only biological source of fixed nitrogen (N), provides a particularly important constraint for studies of N cycling. Nitrogen fixation using the 'canonical' Mo-nitrogenase produces biomass with a $\delta^{15}N$ ca. -1‰. If the 'alternative' V- and Fe-only nitrogenases are used biomass $\delta^{15}N$ can be between -6‰ and -7‰. These values are assumed to be relatively invariant and to reflect the isotope effect of nitrogen fixation expressed at the cellular level. However, field and laboratory studies report wide ranges of diazotrophic biomass $\delta^{15}N$ (from -3.6‰ to +0.5‰ for Mo-based nitrogen fixation). This variation could be partly explained by the release of dissolved organic N (DON) that is isotopically distinct from biomass. The model nitrogen fixer *Azotobacter vinelandii* secretes siderophores, small molecules that aid in Fe uptake and can comprise >30% of fixed N. To test whether siderophores (and other released N) can decouple biomass $\delta^{15}N$ from the isotope effect of nitrogen fixation we measured the $\delta^{15}N$ of biomass and released N in Fe-limited *A. vinelandii* cultures. We report that biomass $\delta^{15}N$ was elevated under Fe-limitation with a maximum value of +1.2‰ for Mo-based nitrogen-fixation. Regardless of the nitrogenase isozyme used, DON $\delta^{15}N$ was also 2-3‰ lower than biomass $\delta^{15}N$. Siderophore nitrogen had a slightly higher $\delta^{15}N$ than the rest of the DON pool but was produced in large enough concentrations to increase biomass $\delta^{15}N$. The low $\delta^{15}N$ of siderophores (relative to biomass) is consistent with what is known for the $\delta^{15}N$ of amino acids used in siderophore biosynthesis, and indicates that other amino-acid derived siderophores should also have low $\delta^{15}N$.