

## **A multi-windowed view of nitrogen fixation in the lab and field**

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The requirement for bioavailable fixed nitrogen is a fundamental constraint for all life on Earth, making biological nitrogen fixation (BNF) one of the most important evolutionary breakthroughs. All BNF is catalyzed by the metalloenzyme nitrogenase in a complex reaction that reduces atmospheric N<sub>2</sub> into bioavailable ammonium. Environmental BNF has been automatically attributed to canonical Mo-based nitrogenases despite long standing knowledge of two other metalloenzyme forms: those containing catalytic V or Fe-only in the active site. A key area of missing information is the contribution of “alternative” V and Fe-only nitrogenases, as N budgets often depend on the type of nitrogenase. Here, I present recently developed methods based on genes and stable isotope fractionation to distinguish between Mo, V, and Fe-only nitrogenases and the results of our first field surveys. Genetic data indicate alternative nitrogenase presence in diverse taxa and environments; isotopic data show remarkable levels of alternative BNF in a variety of modern samples, even in those from environments that should preclude alternative nitrogenase usage. To better understand why alternative nitrogenases exist, we have initiated investigations on the physiological roles of the nitrogenases using cultures and model microbial communities. We are also studying the mechanistic basis for variations in the fractionation of BNF. Our results affect N budgets based on natural abundance stable isotopes or traditional acetylene reduction assays, reveal new interactions between N and trace metal cycling, and invite reconsideration of the role of canonical and alternative nitrogen fixation in the environment.