California State University, Fresno
Department of Biology presents

Evidence for foliar endophytic nitrogen-fixation in a widely distributed subalpine conifer

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Old-growth temperate and boreal coniferous forests accumulate more nitrogen (N) in soil and vegetation than can be explained by known sources of N, limiting our ability to understand and predict carbon (C) and N cycling across 15 % of the Earth's land surface. While novel N input pathways that may contribute N input to some high latitude forests have been suggested (e.g. N_2 fixation by cyanobacteria in feather moss and weathering of N-rich bedrock), these sources are not universally present, or do not account for all the N present in forests. Thus, there is an incentive for evaluating novel sources of biological nitrogen fixation in these ecosystems, especially in association with vegetation. A consistent association between subalpine conifers and acetic acid bacteria (AAB) related to the genera *Gluconacterobacter, Glucanobacer, Asaia,* and other AAB that fix N_2 in crop plants, indicates native foliar endophytes may supply subalpine forests with N. Distinct taxa in the AAB dominate the endophyte community (20-80%) across individual trees, host species (*Pinus flexilis, Pinus contorta, Picea engelmanii*), sites (Rockies and Sierras), and years, suggesting a long-standing association, selective uptake by the plant, and potentially, mutualism.

To investigate whether endophytes have access to foliar N_2, we incubated *P. flexilis* twigs with $^{13}N_2$ enriched air and imaged radioisotope distribution in needles, the first experiment of its kind using ^{13}N. We used the acetylene reduction assay to test for nitrogenase activity within *P. flexilis* twigs four times from June - September. We found evidence for N_2 fixation in *P. flexilis* foliage. The association between *P. flexilis* and the likely responsible bacterial group is stable, N_2 diffused readily into needles, and nitrogenase activity was positive across sampling dates. We propose that foliar endophytes represent a low cost, evolutionarily stable N_2 fixing strategy in established coniferous forests. This novel source of biological N_2 fixation has fundamental implications for understanding forest N budgets.

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