# Abstract

Biological soil crusts (BSC) are key components of ecosystem productivity contributing significantly to the nitrogen (N) budget of arid ecosystems. N2-fixation in BSC is generally attributed to heterocystous cyanobacteria. Published surveys of BSC *nifH* gene content show that *nifH* genes PCR amplified and sequenced from mature and early successional BSC are predominantly cyanobacterial. Early successional crusts, however, possess few N2-fixing cyanobacteria but can still fix N2 at rates comparable to mature crusts and this suggests that microorganisms other than cyanobacteria mediate N2-fixation during the early stages of BSC development. DNA stable isotope probing (DNA-SIP) with 15N2 revealed that *Clostridiaceae* and *Proteobacteria* are the most common microorganisms that assimilate 15N in early successional crusts. Specifically, 34 OTUs were found to incorporate 15N from 15N2 during experimental incubations. 15N-responsive OTUs were members of the *Firmicutes* (19 OTUs), *Proteobacteria* (12 OTUs), *Actinobacteria* (2 OTUs) and *Gemmatimonadetes* (1 OTU). Thirty-eight percent of 15N-responsive OTUs have been observed previously in published SSU rRNA gene surveys of BSC though at low abundance (median non-zero abundance of 5 x 10-4). 15N-responsive OTUs that belong to *Firmicutes* were predominantly *Clostridiaceae*. Proteobacterial 15N-responsive OTUs were predominantly *Gammaproteobacteria* and proteobacterial OTUs that conclusively incorporated 15N2 into biomass shared at least 95% 16S rRNA gene sequence identity with isolates commonly associated with N2-fixation including *Pseudomonas*, *Klebsiella*, *Shigella*, and *Ideonella*. The low abundance of non-phototrophic diazotrophs in BSC may explain why they have not previously been characterized. Diazotrophs play a critical role in BSC formation and characterization of these organisms represents a crucial step towards understanding how anthropogenic change will affect the formation and ecological function of BSC in arid ecosystems.