# Abstract

Biological soil crusts (BSC) are key components of ecosystem productivity contributing significantly to the nitrogen (N) budget of arid ecosystems. BSC N2-fixation is attributed to heterocystous cyanobacteria and BSC diazotrophs from early-successional and mature crusts have been extensively studied by profiling BSC nifH gene content. Published surveys of BSC *nifH* gene content show *nifH* genes PCR amplified and sequenced from BSC are predominantly cyanobacterial. Early successional crusts possess few N2-fixing cyanobacteria but can fix N2 at rates comparable to mature crusts where heterocystous cyanobacteria are more common suggesting microorganisms other than cyanobacteria may mediate N2-fixation during the critical 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. The median abundance (of abundances greater than zero) of 15N-responsive OTUs in SSU rRNA gene collections from environmental BSC samples is less than 5 in 10,000 sequences. 15N-responsive Firmicutes OTUs were predominantly *Clostridiaceae*. Proteobacterial 15N-responsive OTUs were predominantly *Gammaproteobacteria* and proteobacterial OTUs that most conclusively incorporated 15N from 15N2 into biomass shared at least 95% 16S rRNA gene sequence identity with isolates in genera possessing known N2-fixers 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.