

# Abstract

Biological soil crusts (BSC) are key components of ecosystem productivity contributing significantly to the nitrogen (N) budget of arid ecosystems. N<sub>2</sub>-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 N<sub>2</sub>-fixing cyanobacteria but can still fix N<sub>2</sub> at rates comparable to mature crusts and this suggests that microorganisms other than cyanobacteria mediate N<sub>2</sub>-fixation during the early stages of BSC development. DNA stable isotope probing (DNA-SIP) with <sup>15</sup>N<sub>2</sub> revealed that *Clostridiaceae* and *Proteobacteria* are the most common microorganisms that assimilate <sup>15</sup>N in early successional crusts. Specifically, 34 OTUs were found to incorporate <sup>15</sup>N from <sup>15</sup>N<sub>2</sub> during experimental incubations. <sup>15</sup>N-responsive OTUs were members of the *Firmicutes* (19 OTUs), *Proteobacteria* (12 OTUs), *Actinobacteria* (2 OTUs) and *Gemmatimonadetes* (1 OTU). Thirty-eight percent of <sup>15</sup>N-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<sup>-4</sup>). <sup>15</sup>N-responsive OTUs that belong to *Firmicutes* were predominantly *Clostridiaceae*. Proteobacterial <sup>15</sup>N-responsive OTUs were predominantly *Gammaproteobacteria* and proteobacterial OTUs that conclusively incorporated <sup>15</sup>N<sub>2</sub> into biomass shared at least 95% 16S rRNA gene sequence identity with isolates commonly associated with N<sub>2</sub>-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.