

Potential Ecosystem Services of Free-Living, Nitrogen-Fixing Bacteria in Land Reclamation



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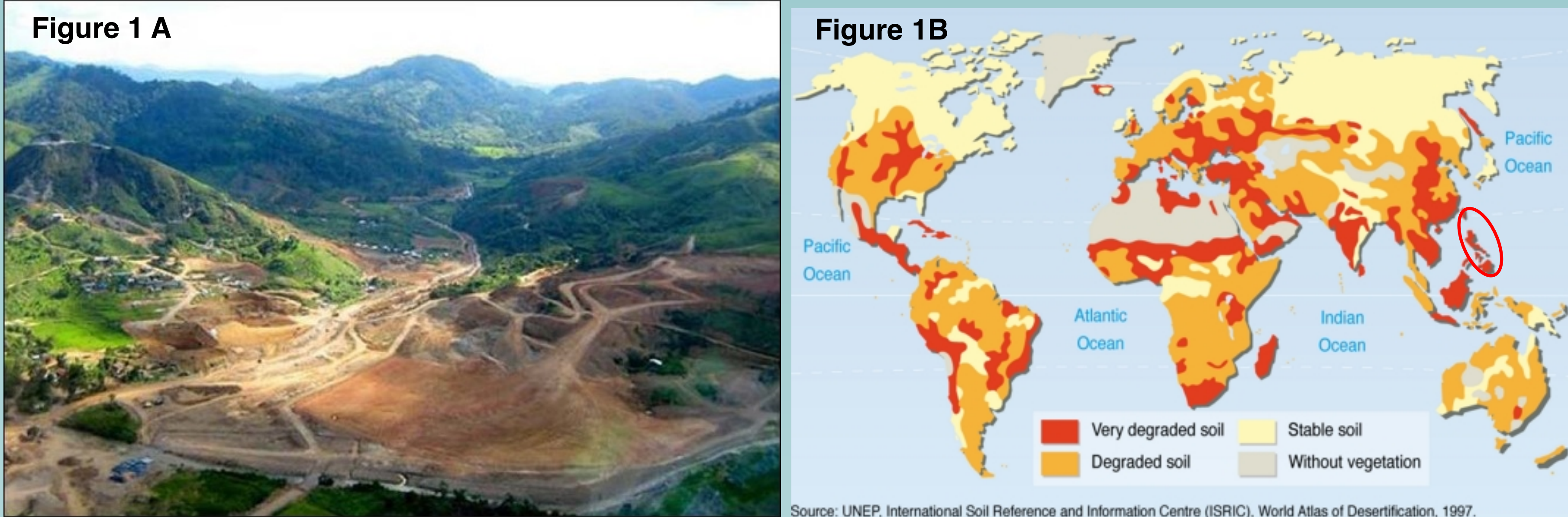
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Soil Degradation Is a Global Problem

Collaborative research between Penn State and the University of the Philippines aims to evaluate use of free-living N₂-fixing bacteria as soil amendments to remediate degraded soils.



Human activities such as deforestation, mining, overgrazing, and intensive agriculture accelerate natural rates of soil loss. Open-pit mining in the Philippines (Fig. 1A) leaves extensive land areas unvegetated and vulnerable to accelerated erosion (source: <http://filipinofreethinkers.org/wp-content/uploads/2011/12/Open-pit-Mining-in-the-Philippines.jpg>)

All soils in the Philippines are rated as very degraded in the Global Survey Map of Human-induced Soil Degradation (GLASOD) constructed by the United Nations Environment Program, 1988-91 (Fig. 1B). Globally, an estimated 38% of all agricultural soils are very degraded.

Biological Crusts Help Initiate Natural Soil Formation Processes



In Pennsylvania and other parts of Appalachia, USA, more than 300,000 ha of minelands abandoned before the passage of the Surface Mining Control and Reclamation Act in 1977 remain in inadequate reclamation status.

Areas affected by overland flow from abandoned mine discharges can be more severely affected, such as the 50-year-old barrens in Clearfield Co., Pennsylvania (Fig. 2A). There, biological soil crusts (BSCs) form on wet surfaces, where C and N contents are 1% and 0.1%, respectively (Fig. 2B).

BSC are communities of phototrophs (green algae, mosses, lichens) growing in association with free-living N₂ fixers (Belnap and Lang, 2001). Incorporation of BSCs as a "reclamation amendment" with lime, compost, and a first-year oats nurse crop supported rapid establishment of native successional plants within two years (Fig. 2C).



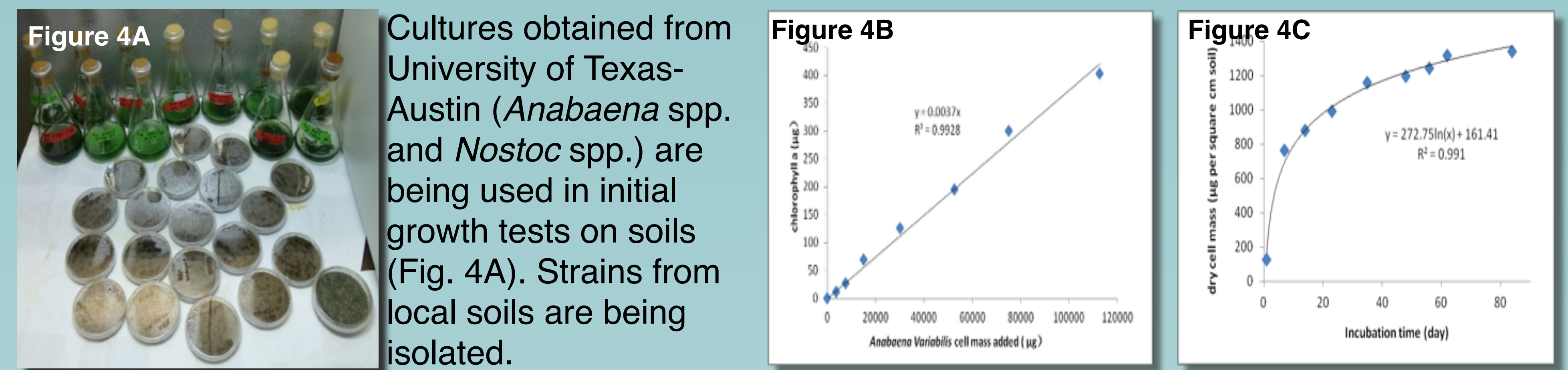
BSCs have long been recognized to stabilize soils in arid and semi-arid regions, where they may take decades to develop into stable structures.

BSCs have received much less attention in more humid, temperate regions, such as central Pennsylvania, where they appear in many agricultural fields, particularly in the fall after harvest (Fig. 3A).

Under greenhouse conditions, BSCs develop within weeks on soil surfaces (Fig. 3B)



Can Soil Surface Inoculation Achieve BSC Establishment?

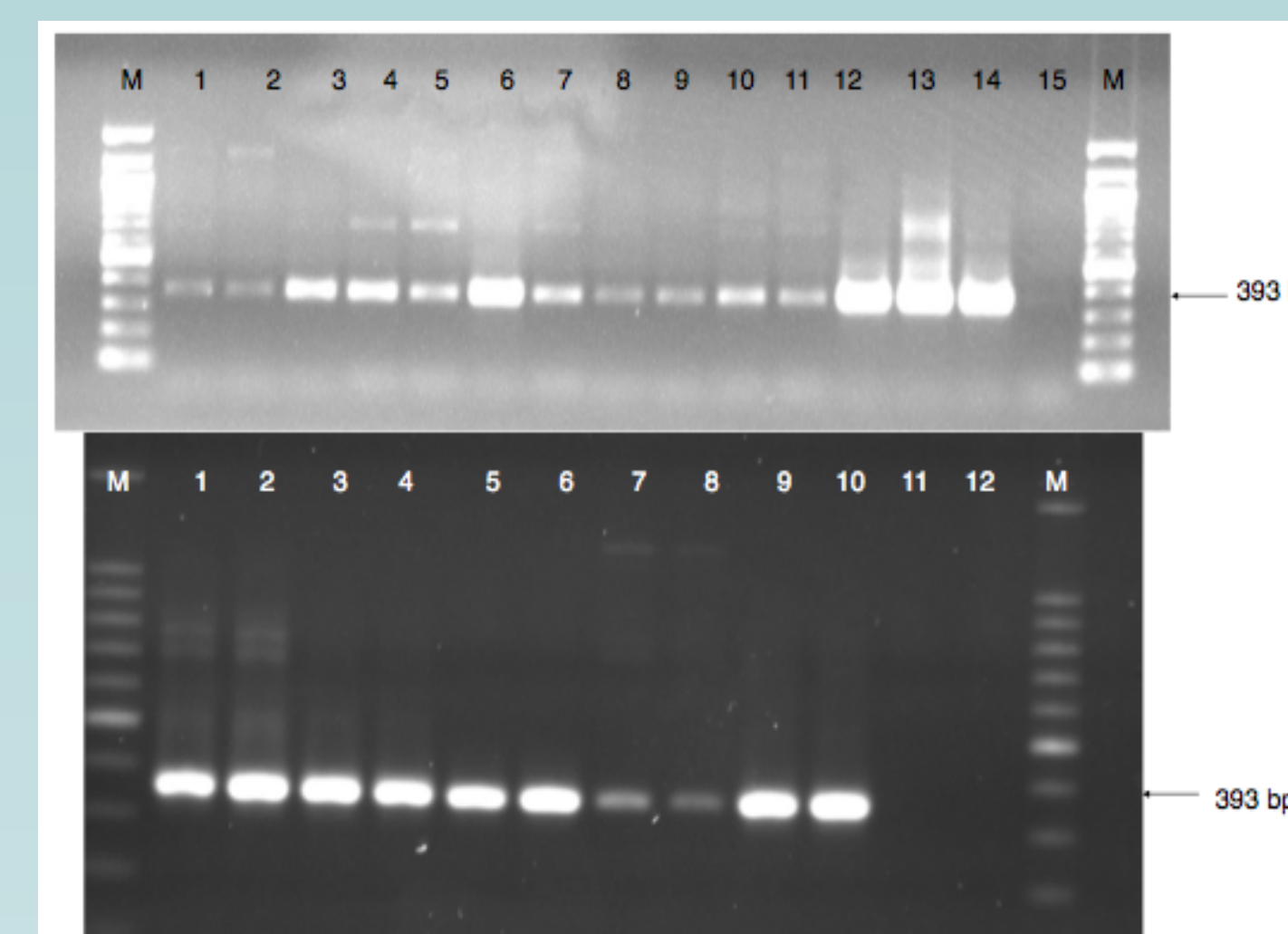


Cultures obtained from University of Texas-Austin (*Anabaena* spp. and *Nostoc* spp.) are being used in initial growth tests on soils (Fig. 4A). Strains from local soils are being isolated.

Standard curve of chlorophyll content from known amounts of cyanobacterial biomass (Fig. 4B) are used to measure biomass increase on soil surfaces under optimal conditions (Fig. 4C)

Assessing Native BSCs for Nitrogen Fixation Genes

We tested for the presence of *nifH* in natural BSCs from mine barrens, agricultural soils, and greenhouse pots.



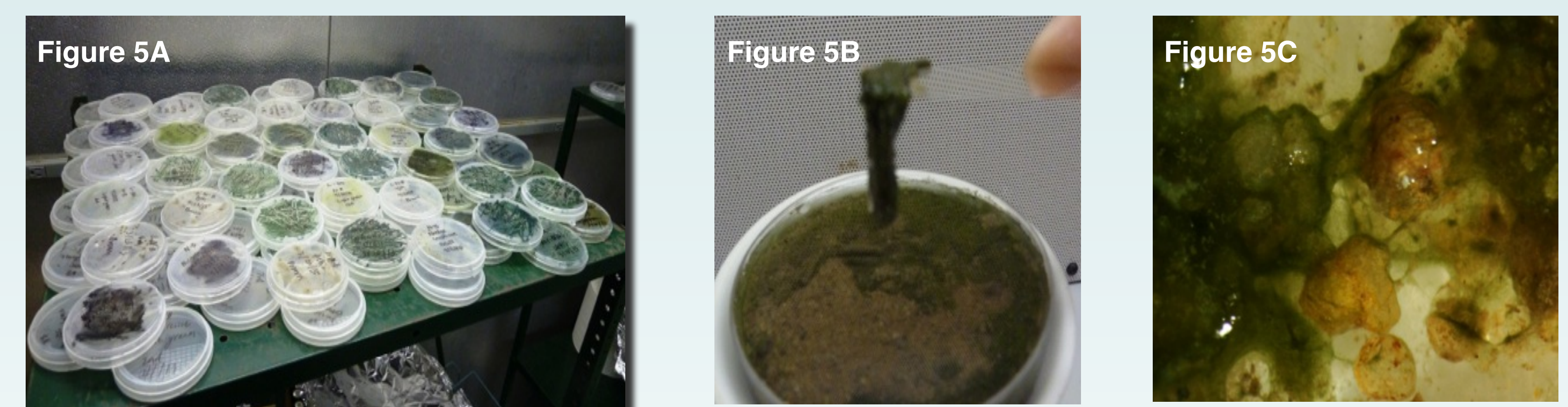
Upper panel: Amplification of the *nifH* gene using Poly et al. (2001) primers. A. Upper gel, Lanes 1-11 are PCR products generated using the *nifH* primers with DNA templates from AMD barrens, Lanes 12-14 are PCR products generated using the *nifH* primers with DNA templates from pure cultures of a local isolate, *Anabaena variabilis* and *Nostoc punctiforme* respectively. Lane 15, negative control reaction without DNA. Molecular marker used was the 100 bp DNA ladder.

Lower panel: Lanes 1-2 are PCR products generated from DNA template from pure cultures of *Nostoc punctiforme*, Lanes 3-4, *Anabaena variabilis*, Lanes 5-6, *Nostoc muscorum*, Lanes 7-8, *Anabaena cylindrica*, and Lanes 9-10, local cyanobacterial isolate. Lanes 11 and 12, negative control without DNA. Molecular marker used was the 100 bp DNA ladder. Arrows indicate the expected amplification products with size of approximately 393 bp.

Assessing Pure and Mixed Cultures for Soil Binding Properties

UTEX cultures and phototrophic isolates from local soils (Fig. 5A) are being grown on N-free medium for testing for polysaccharide production (Fig. 5B) and ability to coherent films on soil surfaces.

10× micrograph showing cohesion between *N. punctiforme* and soil particles in BSCs (Fig. 5C)



Future Research

Work is ongoing to sequence *nifH* gene products and to conduct real-time PCR estimation of *nifH* gene copies during growth of BSCs on soils. Nitrogen fixation by soil biofilms will be measured by ¹⁵N₂ uptake and also assessed with the use of natural N isotope abundance.

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