

Background:

Cryptic symbiotic microbes influence host adaptation by improving nutrient uptake or stress tolerance. Current technologies for increasing plant productivity, whether for food and fuel production or for restoration and remediation, often utilize approaches that *bypass*, rather than *leverage*, microbial influences. Such technologies are insufficient for reversing desertification and increasing vegetative production to meet the increased demands of expanding populations and changing climates. Improved understanding of host-microbe interactions across ecological gradients may facilitate technology development that harnesses microbial power to advance restoration, crop, and fuel production in extreme environments. The woody shrub genus, *Atriplex*, provides a useful model system in which to correlate microbial diversity with host adaptation to harsh arid and saline environments. *Atriplex* species contain diverse microbial endophyte communities and are valued for forage, restoration, and remediation worldwide.

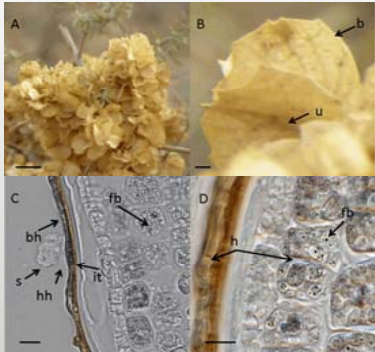


Figure 1: *A. canescens* produces abundant seeds (A), encased in hard utricles (B, u) surrounded by fibrous bracts (B, b). Seeds excised from the utricles (2C) reveal clear hyaline hyphae (hh) resting above aniline blue-staining hyphae (bh) surrounding the inner testa (it), or seed coat. Fungal bodies (fb) divide atop embryonic cells. Hyphae (h) are visible in the intercellular spaces of the developing embryo (2D).

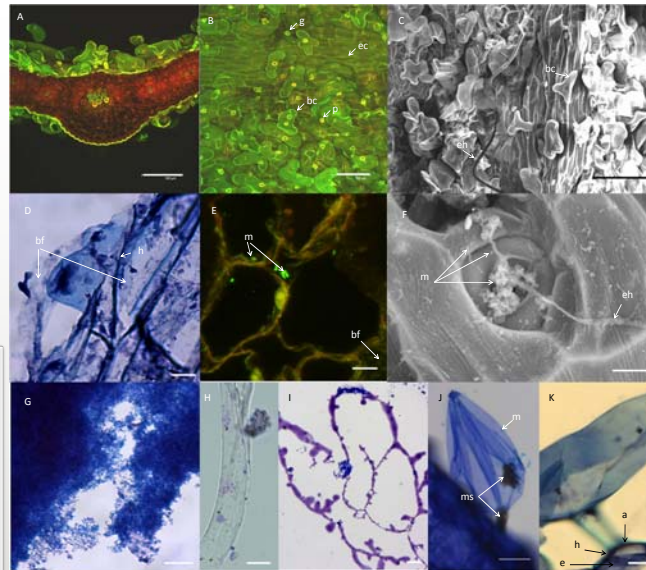


Figure 2. Micrographs of leaf (A-C and E-K) and root (D) sections of micropropagated *Atriplex*. A. Regenerated shoots stained with SYTO 9 and propidium iodide. Bladder and epidermal cells, and cells within vascular bundles fluoresce green. B. Leaf surfaces reveal zones of green fluorescing elongated cells (ec) interspersed between dense regions of bladder cells (bc) which penetrate the epidermis, creating yellow-collared penetration points (p). Guard cells (g) surrounding the stomatal pores fluoresce green. C. Leaf surface SEM shows bladder cells (bc) interspersed with regions of elongated cells. A single elongated hyphae (eh) is visible above the bladder cell region. D. An 8 μM trypan blue stained root section reveals a putative biofilm (bf) containing both hyphae (h) and yeast-sized microbial cells. The biofilm, which covers all cells, is most visible where it has been slightly raised by the growing tip of a lateral root initial (bf). Scale bar = 10 μm. E. Syto 9 and propidium iodide-stained leaf mesophyll cells reveal green-fluorescing microbial cells (m) concentrated near plant cell walls, and a lightly fluorescing biofilm (bf). Scale bar = 10 μm. F. SEM of a stomatal complex. An elongated hyphae (eh) extends across the pore. Microbial cells (m) are clustered within the pore and on the surfaces of surrounding guard cells (g). G. A 2 μm section excised from above the surface of an *A. torreyi* leaf reveals clusters of trypan blue stained, yeast like cells. Scale bar = 10 μm. H. A 2 μm section excised just above the leaf surface reveals a single fungal hyphae similar to the superficial hypha in 2C. Scale bar = 5 μm. I. A 2 μm section excised from above a leaf surface reveals a biofilm-like layer corresponding to the intracellular regions of the underlying leaf. Scale bar = 10 μm. J. Toluidine blue-stained, developing bladder cell contains microsclerotia (ms) in the stem and lateral region, and is covered with superficial microbial cells (m). Scale bar = 10 μm. K. Close up views of individual bladder cells could not clearly distinguish whether stem attachment points (a) were adjoined to the epidermal cells (e) or to associated hyphae (h).



Figure 3. Consensus sequences obtained from clustering tag-encoded pyrosequencing data from 165 sequences amplified from two micropropagated *Atriplex* species (ATCA, ATGR) reveals diverse bacterial endophyte and organelle sequences maintained *in vitro*. Red dots highlight reference sequences added for similarity comparisons

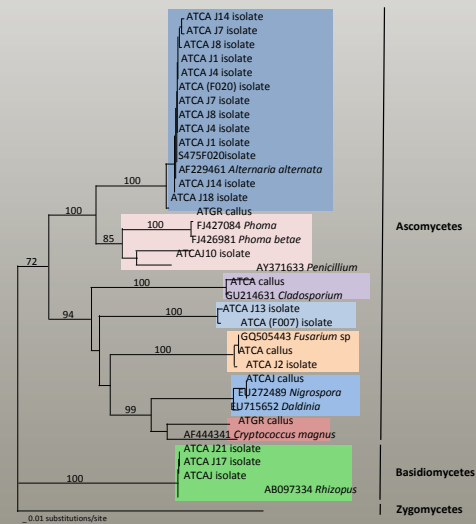
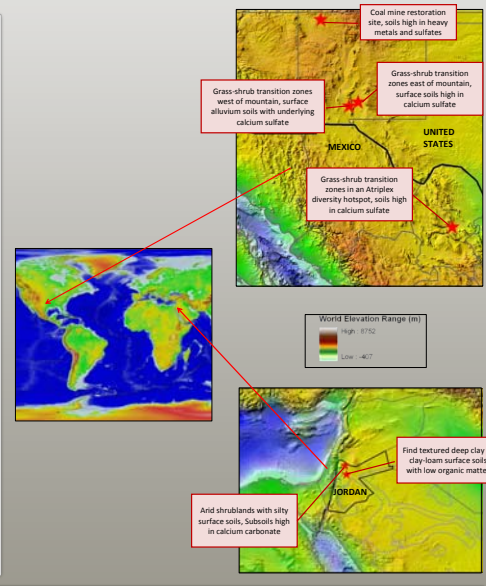


Figure 4. ITS phylogenies of fungal sequences amplified from seed borne isolates and micropropagated plant calli suggest several fungal species may also simultaneously, systematically colonize individual, asymptomatic plants, *in vitro*.

Future directions: Microbial profiles obtained from metagenomic analyses of seeds, leaves, roots, and rhizosphere samples obtained from shrubs collected along well defined ecological gradients will be compared to host plant genetics and environmental data to identify microbes that best correlate with either host plant genetics or with environmental factors such as drought tolerance.

Microbes that best correlate with the host plant, regardless of habitat will be subjected to phylogenetic comparisons with the host to explore potential co-evolutionary roles.

Taxa that correlate best with dry or saline habitats will be subjected to laboratory bioassays testing the osmotic stress tolerance conferred to host plants.



Acknowledgements: We thank Scott Dowd (Research and Testing Laboratory, Inc.) for pyrosequencing analysis, Shulei Sun (CAMERA) for data clustering, and Isaac Reyes-Vera, Helena Deswood, Emad Tahtamouni, and Ruth Sedillo for assistance with sample collection, micropropagation and DNA analysis. Funding has come from USDA-ARS project 6235-11210-006-00 and from International Arid Lands Consortium project 09R-05. Sampling plans are being developed and reviewed through ongoing discussions with research staff from the Jornada Experimental Range, White Sands National Monument, US Geological Survey, and Elizabeth Zacharias of the Harvard University Herbarium in the United States; Khalid Hameed and Sa'eb Khesat of Jordan University of Science and Technology, and Angelica Ruiz-Font and Sergio Trejo-Estrada of the Instituto Politécnico Nacional, Tlaxcala, Mexico.