RECRUITMENT OF MICROBES TO SEEDS OF AN ETHNOBOTANICALLLY IMPORTANT RESTORATION PLANT (*PROSOPIS VELUTINA*): LAND USE HISTORY AND STUDENT ENGAGEMENT

by

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As members of the Master's Committee, we certify that we have read the thesis prepared by Desirae Kissell titled Recruitment of microbes to seeds of an ethnobotanically important restoration plant (*Prosopis velutina*): land use history and student engagement and recommend that it be accepted as fulfilling the dissertation requirement for the Master's Degree.

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Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

I hereby certify that I have read this thesis prepared under my direction and recommend that it be accepted as fulfilling the Master's requirement.

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DEDICATION

To the original and traditional stewards of the Sonoran Desert, Mvto.

To my grandmother, Mikki Aganstata, my mother, Cecilia Tyndall, and my father, Daniel Kissell: Thank you for always supporting me in my education and cheering me on.

I love you.

To my fiancé, Gerardo Abella, thank you for your constant support, encouragement and love.

I can't wait to see where life takes us, I love you.

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ABSTRACT

Changes in regional priorities, cultural practices, soil quality, and climate can lead to the abandonment of agricultural lands. Revegetation of post-agricultural lands is vital to prevent further soil degradation and encourage re-establishment of native species. Historically mesquite forests were an abundant riparian plant community in the American Southwest; however, mesquite forests have declined and continue to decline for diverse reasons. Restoration of degraded lands with velvet mesquite has the potential not only to improve soil health but to assist in preserving the heritage of cultural landscapes. Indigenous communities in Southwestern Arizona traditionally have relied heavily on velvet mesquite for many applications. Through the adoption of a western diet, a decrease in consumption of indigenous foods, and an underlying genetic susceptibility, these indigenous communities have the highest prevalence of Type 2 diabetes in the nation. Products of plants like mesquite have hypoglycemic effects, controlling blood glucose levels and increasing insulin sensitivity, thus helping to control diabetes. This thesis focuses on use of velvet mesquite for the restoration of abandoned agricultural land from a social-ecological resilience perspective. I first describe a field experiment in which I evaluated microbial recruitment of seeds to velvet mesquite, with results that provide insight into how microbial communities relevant to seed success may vary seasonally and between post-agricultural and riparian soil. I then translate the concepts of my research through an outreach module to engage young, diverse students in plant-microbiome science. With this outreach module, I aim to dispel current stereotypes about scientists while contributing to equitable and inclusive scientific communication.

INTRODUCTION

Before urbanization, mesquite forests were the abundant riparian plant community in the American Southwest (Minckley & Clark, 1984). Mesquite forests have declined, and continue to decline, as a result of groundwater pumping (Stromberg et al., 1992) and land-use changes such as woodcutting, land clearing, and agricultural development (Rea, 1993; Valdés-Casillas et al., 1998; Hinojosa-Huerta et al., 2005). Much of Arizona's mesquite forests were lost to agriculture after World War I, with a substantial increase in habitat conversion after World War II when much of Arizona transitioned from rural to urban (Comus, 2000).

Native to the Sonoran Desert, velvet mesquite (*Prosopis velutina*) is a drought-deciduous, medium-sized tree that can reach heights up to 18 m, though it mostly occurs as a multi-stemmed shrub due to apical meristem damage (Bovey, 2016). This quick-growing desert legume is named for the velvety texture of its foliage. Mesquite produces sizable amounts of flowers and seeds: up to 6,000 flowers and 140,000 seeds per tree per season (Simpson et al. 1977). The flowers are yellow, slender, dense, cylindrical catkins, that emerge primarily in the spring and often again in the summer. Catkins give rise to green fruit in pod form, ranging from 9 to 15 cm in length. The pods ripen two months after flowering, and fruit drop occurs from late summer to winter. Dry, mature pods are tan and contain several small (5-10mm) brown seeds (Bovey, 2016).

Velvet mesquite is drought resistant and can grow in alkaline and highly saline soils (Bovey, 2016). Its deep and extensive roots system can extract and redistribute deep soil micronutrients to upper soil layers. As a legume, mesquite engages in a symbiosis that leads to fixation of nitrogen, which increases organic soil carbon and fertility (Bovey, 2016). Velvet mesquite serves as an important nurse tree for flora and fauna (Taylor, 2008); many insects,

small mammals, birds, and other wildlife in the Southwest depend on mesquite for protection, food, and survival (Kingsolver et al., 1977).

Indigenous use of velvet mesquite

Historically, velvet mesquite was used heavily by the Indigenous communities of the Southwestern United States. The fruits (pods) of velvet mesquite, were once a staple food of the Indigenous desert people (Bovey, 2016). The thick and spongy pericarps (i.e., the edible tissue around the seed) of velvet mesquite are high in sucrose (32.1% of pericarp content) (Becker & Grosjean, 1980) and the seeds contain large amounts of protein (44.1% of seed content) (De Lumen, 1986). Because velvet mesquite blooms once in the spring and again in midsummer it can produce reliable crops, even during drought years (Stromberg, 1993). One mesquite tree is capable of producing over 10kg of pods per season (Rea, 1979). Annually, the women of Indigenous peoples would gather millions of kilograms of pods (Grossman, 1873). Velvet mesquite also provided fuel, shelter, weapons, tools, dyes and paints, medicines, cosmetics, baskets, furniture, clothing, rope, glue, and many other everyday items for Indigenous communities (Bell & Castetter,1937; Rea, 1991; Bovey, 2016).

Between 1875 and World War II, the indigenous way of life changed, and the use of staple foods like mesquite nearly disappeared (Rea, 1997). This deviation away from traditional foods, combined with an underlying genetic susceptibility, explains why more than half of indigenous desert peoples, such as the Tohono O'odham people, develop Type 2 diabetes by age 35 today (Bennett et al., 1971; Nabhan, 1991). Products of many desert plants like mesquite have hypoglycemic effects, slowing digestion, and the release of glucose into the bloodstream (Nabhan, 1991).

Revegetation of abandoned agricultural lands

Globally, arid and semi-arid lands account for more than 40% of the Earth's surface, with a projection of an increase of 20% by the year 2100 due to human activity and human-induced climate change at local, regional, and global scales (Maestre, 2015). Crop production in dryland conditions often worsens soil quality (Mainguet & De Silva, 1998), over time reducing productivity and leading to long-term declines in agricultural yields, food security, and plant biodiversity (Tan et al., 2005; Østergård et al., 2009). Croplands often are abandoned and are left to recover on their own when they are no longer productive (Munroe et al., 2013). Effects of land degradation can have an impact on the economy, eventually leading rural people to relocate to urban areas, thus severing the strong connections that people hold to the land; resulting in profound changes in social structure, cultural identity, and political stability (Dregne 1977; McCarthy, 2001). Thus land degradation not only harms the environment but also can lead to changes in the landscape, reducing the ability of the land to support people (Winslow et al., 2006).

There is a need for the restoration of degraded land agricultural lands to restore biodiversity, soil fertility, water retention, and carbon sequestration (Cramer et al., 2008). The process of natural colonization, plant succession, and recovery of degraded lands can be slow, often taking decades or longer (Dobson, 1998; Cramer et al., 2008). Natural land recovery in arid and semi-area areas often take longer than in mesic environments due to extreme temperatures, drought, and poor soil fertility (Virginia & Bainbridge, 1987). Restoration of abandoned agricultural lands to create resilient ecosystems typically requires seeding or transplanting of

native species, with the important benefits of improving plant-soil-water relations and controlling invasive species (Chambers et al., 2014).

Incorporating microorganisms into revegetation efforts has emerged as a potential tool for accelerating the recovery of desert ecosystems (Requena et al., 2001; Bashan et al., 2012). Indigenous leguminous plant species were demonstrated to establish more effectively in severely eroded soil when paired with microbial symbionts (Bashan et al., 2012), suggesting that revegetation of abandoned agricultural lands with velvet mesquite and symbionts is a promising system. Currently, the seasonal and spatial variation of microbial communities that interact with mesquite, particularly at the seed stage, are not documented. This need motivated the first portion of this thesis, wherein my colleagues and I developed and deployed a field experiment to evaluate microbial recruitment to mesquite seeds in proximate riparian versus post-agricultural lands. This experiment is described in Appendix A of this thesis.

Engaging diverse young students in science

The United States has been unable to achieve its STEM workforce goals and thus lags in STEM education in comparison to many more-and less-developed nations (Estrada et al., 2018). To fill this gap, it is important encourage younger Americans to pursue careers in STEM. Unfortunately, science and engineering instruction are typically absent from early childhood classrooms, and particularly so in programs that serve children from low-income families, many of whom are underrepresented minorities (Bustamante et al., 2018). These talents and potential of such under-represented minorities thus can be overlooked, underdeveloped, and under-utilized (Hossain & Robinson, 2012). Additionally, unequal gender and racial/ethnic representation in STEM has fostered unfavorable stereotypes with regard to "what a scientist should look like"

(Grossman & Porche, 2013), making it more difficult for students of under-represented groups to visualize themselves as scientists in the future. It is suggested that elementary teachers should incorporate more hands-on, inquiry-based activities into math and science lessons to ignite students' interests at an early age (DeJarnette, 2012). Engaging students' interests in classrooms that serve under-represented students is needed urgently.

To contribute to such efforts my colleagues and I developed a hands-on, customizable lesson plan with a reproducible, low-cost science experiment for elementary and middle school science teachers to incorporate into their classrooms. The module incorporated themes of my research and focused on building an understanding of how microorganisms engage with plants through symbiosis. I presented this module to a diverse classroom of sixth-grade students in Tucson, AZ, USA. The details of the module and all of its components are provided in Appendix B of this thesis.

EXPLANATION OF THESIS FORMAT

The broad goals of this thesis were twofold. First, I sought to evaluate the abundance, diversity, and composition of microbial assemblages (fungi and bacteria) recruited in soil to seeds of velvet mesquite, with the downstream aims of understanding how these symbionts may influence survival, germination, and early growth of mesquite and identifying those that may aid in propagation and productivity of plants important to southern Arizona. Second, I incorporated concepts of my research into a learning module for young and diverse students. I have organized this work into two appendices.

In Appendix A, I investigate the recruitment of soilborne microorganisms to seeds of velvet mesquite, an ecological and ethnobotanically important plant in the Sonoran Desert

bioregion. This thesis argues for the use of velvet mesquite in restoration practices to return to a point in time when this riparian plant species was abundant, specifically to encourage the incorporation of mesquite back into the indigenous peoples' diets. This study aimed to characterize microbial communities associated with velvet mesquite in the context of land disturbance, contrasting abandoned agriculture lands to a more natural riparian zone. This work was developed with undergraduate Ryan Valdez and represents the first major collection of seed-associated microbes affiliated with mesquite in the Tucson basin. We identify seasonal patterns of infection (i.e., visible microbial growth in culture after surface-sterilization and plating) before, during, and after the North American monsoon; characterize microbes with DNA barcoding; and relate microbial infection to germination success, highlighting recommendations for revegetation practitioners in the area. I will prepare this chapter for publication in a peer-reviewed journal (e.g., *Journal of Arid Environments*).

In Appendix B, I describe an outreach learning module that I developed with Ashton B. Leo to engage underserved and underrepresented sixth-grade students at Walter Douglas Elementary School (Flowing Wells Unified School District). The learning module was developed to align with the teacher's existing lesson plans focused on microscopes, single-celled organisms, and biodiversity. I provide a customizable lesson plan with step-by-step instructions for a hands-on activity, two PowerPoints, a pre/post-test, and a classroom handout. I have also provided my perspectives on implementation, how to conduct the activity with a small budget, and how the module may be improved for future use. I plan to distribute this module through the Arnold lab website and aggregations of teaching materials for microbiome science, which will be released via the NSF Genealogy of Life website (mycophygolife.org) in early 2020. I also will explore venues to share this work in peer-reviewed outlets relevant to teaching in the sciences.

APPENDIX A

Recruitment of microbes to seeds of an ethnobotanically important restoration plant, *Prosopis velutina,* in degraded versus riparian soil

Recruitment of microbes to seeds of an ethnobotanically important restoration plant (*Prosopis velutina*) in degraded versus riparian soil

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Abstract

Changes in regional priorities, cultural practices, soil quality, and climate lead to abandonment of agricultural lands. Revegetation of post-agricultural lands is important to prevent further soil degradation and encourage re-establishment of native species. Rehabilitation of such lands typically requires labor-intensive practices like direct seeding and transplanting. Due to extreme temperature, drought, poor soil fertility, and changes in communities of microbial symbionts, restoration in arid and semi-arid desert ecosystems is particularly challenging. In southern Arizona, planting of nitrogen-fixing legumes such as mesquites (*Prosopis* sp.) can positively affect chemical and physical properties of eroded desert soils. We characterized microbial communities that recruit to velvet mesquite in post-agricultural soil and less-impacted, riparian soils. Seeds of velvet mesquite (*P. velutina*) were placed in mesh bags and buried in riparian and post-agricultural soil in Tucson, Arizona in three seasons. Seeds were retrieved after 10 days, surface-sterilized, and evaluated for microbial infection and germination. We characterized bacteria and fungi with molecular analyses of barcode loci. Fungi that recruited to seeds of velvet mesquite varied seasonally and as a function of soil type. Higher fungal loads were associated with lower germination frequency, but causality was not established. Bacteria varied less over time and as a function of soil type, and assays suggest that they may not be essential for seed survival or germination in soil. We conclude that for revegetation with mesquite, seeds should be deployed when seasonally appropriate (monsoon, potentially with fungicide; or pre-monsoon, with or without fungicide), but that antibacterial treatments are not needed. Such approaches may maximize success by tribes or land managers seeking to remediate damaged lands with native species. ***Keywords:*** Ascomycota, *Bacillus*, germination, land abandonment, *Prosopis velutina*, restoration, revegetation

Introduction

As climate change and intensive use impact soils worldwide, agricultural land abandonment is on the rise (Munroe et al., 2013; Plieninger et al., 2014; Queiroz et al., 2014). The likelihood of abandoned land recovering to a pre-disturbance state may be limited due to land degradation (Jackson et al., 1991), a factor that contributes to land abandonment (Munroe et al., 2013). Land abandonment can also be caused by changing economic conditions such as changing commodity prices, production costs, and declining farm profitability (Lubowski et al., 2006; Plieninger et al., 2014). Land abandonment has been long viewed as an opportunity for habitat regeneration (Queiroz et al., 2014) with the assumption that such lands are capable of returning to historical vegetation state with little to no effort (Jackson et al., 1991; Grantz et al., 1998; Benayas et al., 2007). However, recent studies have established that it can take decades to centuries for land to recover to its original vegetation quality, if at all (Cramer et al., 2008; Munroe et al., 2013).

Natural recovery of land in arid and semi-arid regions often takes longer than in mesic environments due to extreme temperatures, drought, and poor soil fertility (Virginia and Bainbridge, 1987). Experiments in the Sonoran and Chihuahua Deserts have documented difficulties involved in restoring arid environments (Bainbridge and Virginia, 1990; Cox et al., 1982). Restoration of abandoned agricultural lands to create resilient ecosystems typically requires seeding or transplanting of native species, improving plant-soil-water relations, and controlling invasive species (Chambers et al., 2014).

Land degradation and intensive agriculture often impact microbial diversity in soil, often contributing to low densities of mutualistic microbial symbionts (Ding et al., 2013) or altered microbial communities relative to unimpacted soils (Hamzazai et al., 2019; see also Leo, 2019).

When symbioses fail to occur, successful revegetation is less likely (Requena et al., 2001; Herrera et al., 1993).

Microbial symbionts can be endophytic, living within plant tissues, causing no apparent symptoms of any diseases (Wilson, 1995), or free-living in the soil and then colonizing plant structures such as seeds (Lehman, 2015). These symbiotic associations influence the host plant's ecophysiology, nutrition, growth rates, resistance to biotic and abiotic stressors, plant survival, and distribution (Singh, 2011).

Seeds interact with a diversity of symbionts. It is hypothesized that some seeds use chemical exudates or other characteristics to recruit beneficial microbial associations for protection against pathogens (Dalling et al., 2010). In some cases, seeds may come equipped with symbiotic microorganisms, inherited from the mother plant via vertical transmission (Shahzad, 2018). While seed-borne microbes have been poorly explored, they are believed to promote plant growth and reduce stress (Truyens et al., 2015), and thus are important aspects of revegetation strategies.

Revegetation of post-agricultural landscapes with native plant species is important for soil remediation, dust control, and re-establishment of ecosystem function. In this area, native species such as nitrogen-fixing legumes like velvet mesquite (*Prosopis velutina*) can have a beneficial effect on the chemical and physical properties of desert soils (Virginia, 1986). Velvet mesquite has value in semi-arid and arid areas of the southwestern USA due to its resistance to drought, salinity, and alkalinity (Fagg & Stewart, 1994). Moreover, nitrate concentrations in mesquite woodlands in the low desert are comparable to those in the best agricultural lands, while nearby soils lacking mesquite stands are nitrogen deficient (Rundel et al., 1982). Bashan et al. (2012) demonstrated that plant-growth-promoting microorganisms and native legumes species

could aid in the restoration of eroded desert soils, motivating the present study. Revegetation of post-agricultural landscapes in southern Arizona with a native plant species such as velvet mesquite, offers an alternative crop, one with economic impacts, ecological relevance, and cultural meaning.

Here, we examined the microbial community that recruits to seeds of velvet mesquite under field conditions. We focused on post-agricultural soils (hereafter, 'degraded') and soils in a relatively natural riparian zone, both located in Tucson, AZ (USA). In our experiment we deployed seeds to soils and then measured germination in vitro, while also employing a culture-based approach and molecular sequencing to evaluate microbial communities.

Materials and Methods

The field experiment was carried out at the University of Arizona Campus Agricultural Center (CAC) in Tucson, Arizona, in 2018. The climate of the area is characterized as semi-arid with mean annual precipitation of 303 mm and a mean annual temperature of 21.6°C (U.S Climate Data, 2019). At CAC we focused on two sites: the edge of a riparian strip (32°16'47.2" N, 110°56'14.4" W) and a nearby, fallow agricultural field (32°16'47.3" N, 110°56'14.9" W). The two sites were separated by about 5 m by a small dirt track. Within each site, we established three plots (2 m x1 m) at ca. 3 m intervals. The experiment described below was repeated three times in one year (April, July, and November), framing the North American monsoon, which occurs in the region from July through September (Adams & Comrie, 1997).

Field experiment

Jim Koweek of Arizona Revegetation & Monitoring Co. provided seeds of velvet mesquite (*Prosopis velutina* var. *juliflora*) in March of 2018, and pods containing seeds of that species in September of 2018. Each set of seeds and pods represented multiple maternal trees in the Tucson region (Koweek, personal communication). During collection, seeds and pods were treated with Sevin® insecticide dust (Garden Tech, Atlanta, GA, USA). Before use in this study, seeds were rinsed with sterile water to remove the dust. If needed, seeds were removed from pods by crushing the dried pods by hand. Seeds from March 2018 were used in deployments in April and July, and seeds from September 2018 were used in the deployment in November.

As with many leguminous plants, the seed coat of velvet mesquite seed is hard and impervious to water (Glendening & Paulsen, 1955), requiring scarification for germination. Seeds were treated using an adapted protocol from Vilela and Ravatta (2001). Briefly, we soaked seeds in 1 N H_2SO_4 for 15 minutes, rinsed seeds in sterile water three times for 2 minutes, soaked seeds in sterile water for 15 minutes, and then allowed seeds to surface-dry under sterile conditions (see also Valdez, 2019). Surface-sterilized seeds were placed into mesh bags (10 seeds/bag, occasionally 7-11 seeds/bag), which we constructed from 50 μm mesh (Duda Energy LLC, USA) and sterilized by autoclaving on the gravity cycle (Leo, 2019; Hamzazai et al., 2019). After filling, the bags were wrapped in aluminum foil and stored in plastic bags until deployment on the following day.

We deployed 18 seed bags into each plot. We buried each bag at a depth of ca. 5 cm in the soil and at a distance of ca. 5-8 cm from each other (Leo, 2019; Valdez, 2019). Two additional bags per plot served as controls: one field control that was exposed to the air for about one minute and placed back in foil, and a lab control, which never left the laboratory (Leo, 2019; Hamazazi et al., 2019; Valdez, 2019). Overall, 3585 seeds were included in this experiment.

Soil chemistry analysis

Soil samples were collected at a depth of 5 cm from each corner of each plot at each deployment. Soil samples from a given plot at a given timepoint were pooled, resulting in 18 soil samples for analysis. Soil samples were dried at 21.5°C for three days and sieved through a 2 mm mesh. We sent 300 g per sample for chemical analysis by Motzz Laboratories (Phoenix, AZ, USA). Soil characteristics that were evaluated are listed in Table 1 and the legend for Figure 1.

Seed processing

We retrieved seed bags from the field 10 days after deployment. Bags were returned to the laboratory and processed promptly. Seeds were removed from the bags and then surface-sterilized by agitating sequentially in 95% ethanol for 10 seconds, 10% consumer bleach (0.5% NaOCl-) for 2 minutes, and 70% ethanol for 2 minutes (Arnold et al., 2007) and were placed in a biosafety cabinet to surface-dry under sterile conditions (Leo, 2019; Valdez, 2019). All seeds from an individual bag were placed into an individual 100 mm Petri dish containing 2% malt extract agar (MEA) (Fröhlich & Hyde, 1999). Plates were sealed with Parafilm and stored in a dark cabinet at room temperature (ca. 21.5°C) for 10 days.

Plates were checked daily for microbial growth and seed germination. Emergent microbes were isolated into pure onto 60 mm plates with 2% MEA. Living vouchers of bacteria were made by transferring cells to 50% glycerol (Shaffer et al., 2017). Living vouchers of fungi were made by transferring mycelia to sterile water (Shaffer et al., 2017).

We used analysis of variance (ANOVA) to evaluate isolation frequency (the proportion of seeds from which a fungus or bacterium was isolated in culture) as a function of soil type

(degraded, riparian) and season (pre-monsoon/April, monsoon/July, post-monsoon/November). We used linear regression to relate the germination frequency of seeds to isolation frequency. We compared molecular data from barcode loci (below) to the NCBI GenBank database to estimate taxonomic placement of microbes, which we analyzed qualitatively.

Molecular methods

We extracted bacterial DNA as described by Shaffer et al. (2017). Briefly, cells were placed into 20uL of Y-PER™ Yeast Protein Extraction Reagent (Thermo Scientific™, Rockford, IL, USA) (Packeiser et al., 2013). The polymerase chain reaction (PCR) was used to amplify the diagnostic barcode region of the bacterial 16S ribosomal RNA (16S rRNA) following Shaffer et al. (2017).

Fungal DNA was extracted with the Sigma Extract-n-Amp Plant Kit (Sigma-Aldrich, St. Louis, MO, USA) per the manufacturer's recommendations. PCR was used to amplify the diagnostic barcode locus, the nuclear ribosomal internal transcribed spacers (ITS rDNA) and partial large subunit (LSU rDNA), following Shaffer et al. (2017).

PCR success was confirmed via gel electrophoresis on 1% agarose gel with SYBR Green I stain (Molecular Probes, Invitrogen; Carlsbad, CA, USA). Positive amplicons were cleaned with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) per manufacturer's instructions and submitted to the University of Arizona Genetics Core for normalization and bidirectional Sanger sequencing using the Applied Biosystems BigDye Chemistry Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

Sequences were assembled automatically, bases scored, and quality scores assigned by *phred* and *phrap*, coordinated by Mesquite v. 2.01+ (http://mesquiteproject.org/; Ewing and Green, 1998; Ewing et al., 1998). Assembled sequences were edited manually in Sequencher v.

5.1 (Gene Codes Corporation, Ann Arbor, MI, USA). The resulting sequences were compared against known sequence data in GenBank via the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990), which provided an estimate of taxonomic identification.

Germination trials

Because we used seeds from two seed lots, we conducted a germination trial to examine if the lots differed intrinsically in germination frequency. Three one-week trials were conducted in mid-2019 with a total of 277 velvet mesquite seeds (N = 140 from seed lot 1 and N = 137 from seed lot 2). We did not observe any variation in germination frequency among trials (ANOVA, $F = 0.7182$, DF = 2, 9, P = 0.5136). Therefore we combined the data from the three trials to compare germination frequency between seed lots. We found no significant difference in germination frequency between the seed lots ($t = -0.80$, DF = 10, p = 0.4400). Seeds germinated similarly on tabletops versus in a dark cabinet (data not shown). On average ca. 30-40% of seeds germinated in all cases. We observed higher germination rates in control and deployed seeds (ca. 58% overall, Supplementary Information), potentially reflecting a longer germination time in the field experiment and use of seeds for the field experiment closer to the original collection date (germination trials to evaluate seed lot differences were conducted 8-14 months after the deployments, seeds were stored at room temperature).

Results and Discussion

Over the full experiment, 302 microbial cultures (isolates) were obtained from seeds of velvet mesquite. The isolation frequency for the entire experiment was 8.4% (4.9% of seeds yielded a bacterial isolate, and 3.5% of seeds yielded a fungal isolate).

A total of 30 isolates (ca. 10% of the isolate library) came from control seeds that were never deployed into soil (i.e., 5 fungal isolates and 25 bacterial isolates). These could represent bacteria that were carried by the seeds themselves, as in the case of maternally transferred endophytes (Kandel et al., 2017). The remaining isolates (90% overall) were obtained from seeds that were deployed into soil.

Microbes from control seeds

The majority of the bacteria obtained from control seeds were found in deployment 1. For the control seeds in that deployment, the bacterial isolation frequency was 15%, and 18 of the 25 bacterial isolates from control seeds were found then (i.e., 72% of bacterial isolates from control seeds were found in control seeds from deployment 1). For deployments 2 and 3, isolation frequency for bacteria in control seeds was 2.9%. As the same seed lot was used in deployments 1 and 2, it is possible that the bacteria observed so frequently in control seeds for deployment 1 were laboratory contaminants. However, the bacteria observed in these control seeds represented multiple species, suggesting that a single laboratory contaminant was not an issue (Supplementary Information). Moreover, the bacteria found in the control seeds for deployment 1 represent species of Firmicutes that were often observed in seeds deployed into soil (Supplementary Information). These observations, coupled with the decrease in the isolation frequency for control seeds in deployments 2 and 3, argue for mistaken transfer of soil bacteria from deployed seeds to control seeds in the April experiment rather than vertical transmission or lab contaminants per se. We therefore interpret the lower isolation frequency of bacteria in seeds from deployments 2 and 3 as more realistic than the higher rate observed in control seeds for

deployment 1. For seeds deployed into soil, bacterial isolation frequency overall was 4.4%, indicating that infections increased with soil exposure except in the first deployment.

The fungal infection frequency in control seeds was 1.4%. All of the fungi isolated from control seeds were observed in deployments 2 and 3. Fungal infections increased four-fold with soil exposure (6.5% overall for seeds exposed to soil). The fungi identified from control seeds were diverse and included species with airborne conidia or yeasts that appress closely to surfaces (Supplementary Information). The lack of any fungi in control seeds from deployment 1, coupled with the rare occurrence of fungi that as a whole were diverse in control seeds and also were found in soil-deployed seeds, again argues for inadvertent transfer within the lab rather than strong evidence for the maternal transfer of endophytes or a problem with laboratory contaminants.

Overall, the observation that 90% of the isolates obtained in culture came from soil-exposed seeds, and the inference that 10% of cultures were potentially transferred to control seeds, led us to retain control seeds in our broad analyses evaluating seasonality and soil type effects, below.

Differences in soil chemistry: degraded vs. riparian soils

Our soil analyses confirmed that soils differed markedly in the degraded vs. riparian plots (Table 1, Figure 1). In general, riparian soils were characterized by a lower pH, higher electrical conductivity, and higher quantity of magnesium, potassium, zinc, iron, manganese, copper, nickel, phosphate, sulfate, and boron than the degraded soils ($p \leq 0.05$ in all cases; t-tests; data not shown). Riparian and degraded soils did not differ markedly in calcium, sodium, nitrate, exchangeable sodium percentage, free lime, or cation exchange capacity (Table 1). Valdez

(2019) described the vegetation in this area and noted that the riparian plots had higher stem density and richness than the degraded plots, with such vegetation differences potentially corresponding to differences both in soil chemistry and in microbial assemblages and activity (see below).

Alkalinity of soils is often a challenge in revegetation and farming in Arizona (Schalau, 2002). High pH can be associated with iron deficiency and deficiencies in several other vital micronutrients (Schalau, 2002). The soils in our plots generally were relatively fertile compared to expected standards for amendments (e.g., Young, 2007): the soils were not deficient in most nutrients (Table 1), though levels of iron were below desirable levels of 4.0 ppm (Young, 2007). However, the particularly high pH in the post-agricultural soil was notable, as was the observation in the field that soils in those plots had minimal organic material. In future work, we recommend quantifying organic carbon and water retention, soil compaction, and physical characteristics, as the nutrient profiles – while significantly different – do not speak to soil degradation per se in the post-agricultural sites, other than the elevated pH.

More generally, desert soils frequently have poor soil fertility with low levels of inorganic nitrogen and plant-available phosphorus (Bainbridge & Virginia, 1990). There is a possibility that the relatively high levels of metals we observed reflect that riparian soils indicate that this area is a sink for contaminants (Liu et al., 2016). Soil pH often is negatively correlated with metal contents of soils (Navas & Machín, 2002; Du Laing et al., 2017), consistent with the lower pH observed here in riparian soils.

Isolation frequency as a function of season

Microbial isolation frequency was sensitive to the interaction of soil type and season (Table 2), as illustrated for fungi (Figure 2) and bacteria (Figure 3). Few fungal infections were observed pre-monsoon, and only in the riparian soils (Figure 2). Fungal infections increased in frequency in the monsoon season, and they increased substantially in the post-monsoon season, with relatively high rates of infection in both the degraded and the riparian soils (Figure 2). We interpret this as evidence of the activation of soilborne fungi with soil moisture after the monsoon (Lockwood & Filonow, 1981). Activation of soilborne fungi is due to moisture needed for growth, particularly in the degraded soil. These patterns are reflected further by seasonal- and soil-type differences in fungal communities, and potentially by cold stress to the seeds in November.

Bacteria were particularly common in seeds in the pre-monsoon season, in part reflecting their appearance in control seeds in deployment 1 (Figure 3). Bacterial infections diminished in number in the second and third deployments and did not differ markedly with soil type. As described below, the identity of common bacteria differed in different soils and in different seasons, such that even if the frequency of bacterial isolation did not change, the composition of the isolated bacteria did change with soil type and season.

Germination as a function of season and soil type

Germination frequency of mesquite seeds differed as a function of soil type, season, and their interaction (Table 2). In general, 58% of seeds germinated in the experiment (Table 3). The overall germination frequency decreased from pre-monsoon through the monsoon and post-monsoon periods (Table 3). The lowest germination success in the experiment was for seeds that interacted with soil in deployment 3 (Table 3). We ascertained that this did not appear to reflect

intrinsic differences in the germination percentage of seed lots 1 and 2 as described in the Methods section. We ascribe this pattern instead to cooler soil temperatures in November. We expect maximum germination of mesquite seeds at temperatures of 27-29°C (Lyons & Rector, 2009). We did not measure soil temperature in our deployments, but regional data suggest that soil temperatures in November 2018 were much cooler than optimum for mesquite germination (https://cals.arizona.edu/azmet/data/0118em.txt: average soil temperature at 10 cm, Tucson, AZ:12.8°C). While all seeds were incubated at the same temperature in the laboratory regardless of deployment, exposure to warm soil in the earlier deployments (estimated with the same website as 20.9°C in mid-April 2018 and 27.8°C in mid-July 2018) may have initiated germination, whereas exposure to cooler temperatures in November did not do so at the same rate of success.

The most striking difference in germination observed in our experiment was in the monsoon season (July), when germination frequency for seeds exposed to riparian soils was markedly higher than the germination frequency of control seeds or seeds in the degraded soil (Table 3). This would represent the most natural conditions for native mesquite (i.e., monsoonal germination in riparian zones) and may speak to the best-matched microbial community or soil conditions for seed success or seedling health.

Germination and microbial infections

We observed a negative association between seed germination frequency and fungal isolation frequency (Figure 4), but not bacterial isolation frequency (Figure 5). When 40-50% of the seeds in a bag were infected by fungi, germination was effectively zero (Figure 4). It is possible that these represent pathogenic fungi, which could be tested with inoculation experiments for

verification. It also is possible that inviable seeds were colonized by saprotrophic fungi (i.e., fungi that live on dead tissue rather than infecting as pathogens). The fungi we observed include fungi with both saprotrophic and pathogenic lifestyles (Onyike & Nelson, 1993; Latge, 2003), meriting further study with inoculation experiments, especially under realistic field conditions.

Bacterial infections were not associated with reduced germination in seeds exposed to soil (Figure 5). There was a trend for increased germination of control seeds when bacteria were more common; in future work, we can inoculate seeds with these bacteria to see if they represent beneficial strains of the species that were common in the soil more generally. It is possible that they are beneficial, but they are poor competitors in dominating seeds or that their benefits are negated by fungal infections, a matter for further study. Overall, we did not observe a correlation between the isolation frequency of fungi and bacteria ($R^2 = 0.02$), suggesting that seeds were not simply colonized at random by microbes as a whole.

Microbial communities

Among the identified bacterial isolates, *Bacillus* was the predominant genus, and the most abundant species were identified tentatively as *B. subtilis*, *B. velezenis*, and *B. amyloliquefaciens*. Only four identified isolates represented other taxa: *Streptomyces coelicoflavus, Siccibacter colletis, Brevibacterium* sp.*,* and *Paenibacillus jamilae* (Supplementary Information). *Streptomyces coelicoflavus, Siccibacter colletis,* and *Brevibacterium* sp. were observed only once, with the caveat that not all bacterial isolates were sequenced. Thus, the primary associates of velvet mesquite seeds were *Bacillus* spp., representing multiple species. These are Gram-positive bacteria in the Firmicutes that, in some plants, can impart physiological benefits. Some *Bacillus* spp. have been shown to elicit induced systemic responses (ISR), trigger plant growth

(Kloepper et al., 2004), increase plant stress tolerance, and reduce the toxic effects of salinity (Radhakrishnan et al., 2017). A species within one of the *B. subtilis* subgroups, *B. mojavensis,* was observed only in germinated control seeds that never left the lab. *Bacillus mojavensis* has been demonstrated to inhibit the growth of *Fusarium moniliforme*, a pathogen of maize (Bacon & Hinton, 2001). We recommend that inoculation experiments be conducted in the future to investigate the physiological effects of *Bacillus* spp. on seeds and seedlings of velvet mesquite.

Among the 90 identified fungal isolates, the majority represented Ascomycota (86.7%), with a minority representing Mucoromycota and Basidiomycota (Supplementary Information). The majority of the isolates represented Eurotiomycetes (51.0%), Sordariomycetes (16.7%), and Dothideomycetes (15.5%), as is typical for seed-associated fungi in previous studies in this region (Hamzazai et al., 2019; Leo, 2019; Valdez, 2019). *Aspergillus* was the most abundant genus (Supplementary Information). This genus was in both soil types and control seeds, but it was especially prevalent in the degraded soil, and it was not observed in the pre-monsoon deployment (Supplementary Information). Fungi representing Chaetomiaceae only occurred in the pre-monsoon season. *Fusarium* only occurred in the post-monsoon season and was found in both degraded and riparian soil, though it was somewhat more abundant in riparian soil (Supplementary Information).

Overall we observed the highest germination frequency for seeds deployed into riparian soil in the monsoon season, and seeds deployed into both soil types in the pre-monsoon season (Table 3). We did not sequence all isolates due to budget constraints, such that conclusions regarding associations between microbial communities and germination success are premature. Given that caveat, in the monsoon, Dothideomycetes were relatively more common than in the post-monsoon, when Eurotiomycetes were far more common. In the pre-monsoon,

Eurotiomycetes also were rare or not observed. In future work we will use inoculation experiments to evaluate the prediction that the Eurotiomycetes that dominated strongly when germination frequency was lowest (Supplementary Information) were particularly detrimental to seed success.

Conclusions

Overall, our results suggest that communities of fungi that recruit to seeds of velvet mesquite can vary seasonally and as a function of soil type, both in terms of abundance (isolation frequency) and composition. In general, higher fungal loads were associated with lower germination frequency, but causality is not yet established and should be disassociated from soil temperature via inoculation experiments. Bacteria varied less over time and under the different soil conditions, both in terms of abundance and composition, and our results suggest that they may not be essential for seed survival in soil. Therefore, we conclude that for revegetation with mesquite, seeds should be deployed when seasonally appropriate (monsoon, potentially with fungicide; or pre-monsoon, with or without fungicide). Under these conditions, seed germination in degraded and riparian soils of interest may be maximized by tribes or land managers seeking to remediate damaged lands.

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Table 1. Soil characteristics (mean and standard deviation, SD) as a function of land use at Campus Agricultural Center. Data represent 18 samples (9 from degraded soils and 9 from riparian soils). Characteristics that differed markedly between degraded and riparian soils are shown in bold, with * marking the significantly greater value. See Figure 1 for a comparison of soil traits in aggregate, as it is expected that many soil traits covary and measures of each may be correlated (Fujita et al., 2013).

Soil Characteristics	**Degraded**		**Riparian**	
	Mean	**SD**	**Mean**	**SD**
pH (SU)	**8.48**	0.08	8.10	0.15
Electrical conductivity (dS/m)	0.25	0.06	**0.34**	0.09
Calcium, Ca (ppm)	1688.89	105.41	1677.78	120.19
Magnesium, Mg (ppm)	91.44	19.26	**113.22**	11.33
Sodium, Na (ppm)	23.00	5.00	21.78	2.68
Potassium, K (ppm)	244.44	32.06	**283.33**	50.25
Zinc, Zn (ppm)	1.18	0.29	**4.57**	1.39
Iron, Fe (ppm)	2.48	0.031	**3.83**	0.81
Manganese, Mn (ppm)	5.77	1.57	**10.29**	4.25
Copper, Cu (ppm)	1.17	0.16	**1.68**	0.38
Nickel, Ni (ppm)	0.07	0.01	**0.13**	0.03
Nitrate-N, NO3-N (ppm)	11.54	5.16	15.88	7.93
Phosphate -P, PO4-P (ppm)	8.78	1.49	**19.89**	6.21
Sulfate-S, SO4-S (ppm)	4.44	1.50	**6.72**	2.79
Boron, B (ppm)	0.51	0.04	**0.88**	0.22
Free lime, FL	High	High	High	High
Exchangeable sodium percentage (%)	0.99	0.22	0.96	0.10
Cation exchange capacity (meq/100g)	9.92	0.69	10.16	0.80

Table 2. Relevance of soil type, season, and soil type by season interaction with respect to seed colonization by microbes and seed germination. (A) Isolation frequency for microbes was dependent on soil type and season, but not by soil type or season alone. (B) Variation in percent germination could be explained by the soil type by season interaction. Significant results are bolded and labeled with (*).

A. Isolation frequency

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Soil Type	2	2	0.03103406	1.2899	0.2766
Deployment	2	2	0.03884787	1.6147	0.2004
Soil Type*Deployment	4	4	0.21637550	4.4969	**0.0015***

B. Germination

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Soil Type	2	2	0.7654410	11.9448	**<.0001***
Deployment	2	2	4.6605268	72.7279	**<.0001***
Soil Type*Deployment	4	4	1.0128882	7.9031	**<.0001***

Table 3. Percent germination of mesquite seeds (± standard deviation) and sample sizes (number of seed bags) for each soil type and season.

Soil type	Pre-monsoon	Monsoon	Post-monsoon
Control	68.3 ± 13.4 (N = 12)	58.3 ± 33.0 (N = 12)	44.6 ± 18.7 (N = 12)
Degraded	73.9 ± 13.0 (N = 54)	55.9 ± 21.1 (N = 54)	30.3 ± 17.5 (N = 54)
Riparian	72.0 ± 15.7 (N = 54)	78.3 ± 13.8 (N = 54)	38.8 ± 20.7 (N = 54)

Figure legends

Figure 1. Soil characteristics differ between riparian and degraded sites. Data reflect hierarchical clustering based on pH, electrical conductivity, calcium, magnesium, sodium, potassium, zinc, iron, manganese, copper, nickel, nitrate, phosphate, sulfate, boron, free lime, exchangeable sodium percentage, and cation exchange capacity. Proximity of points in branching order indicates similarity. Two riparian plots clustered with the degraded plots: D2-RF and D3-RF. Means and standard deviations for each measure are shown in Table 1.

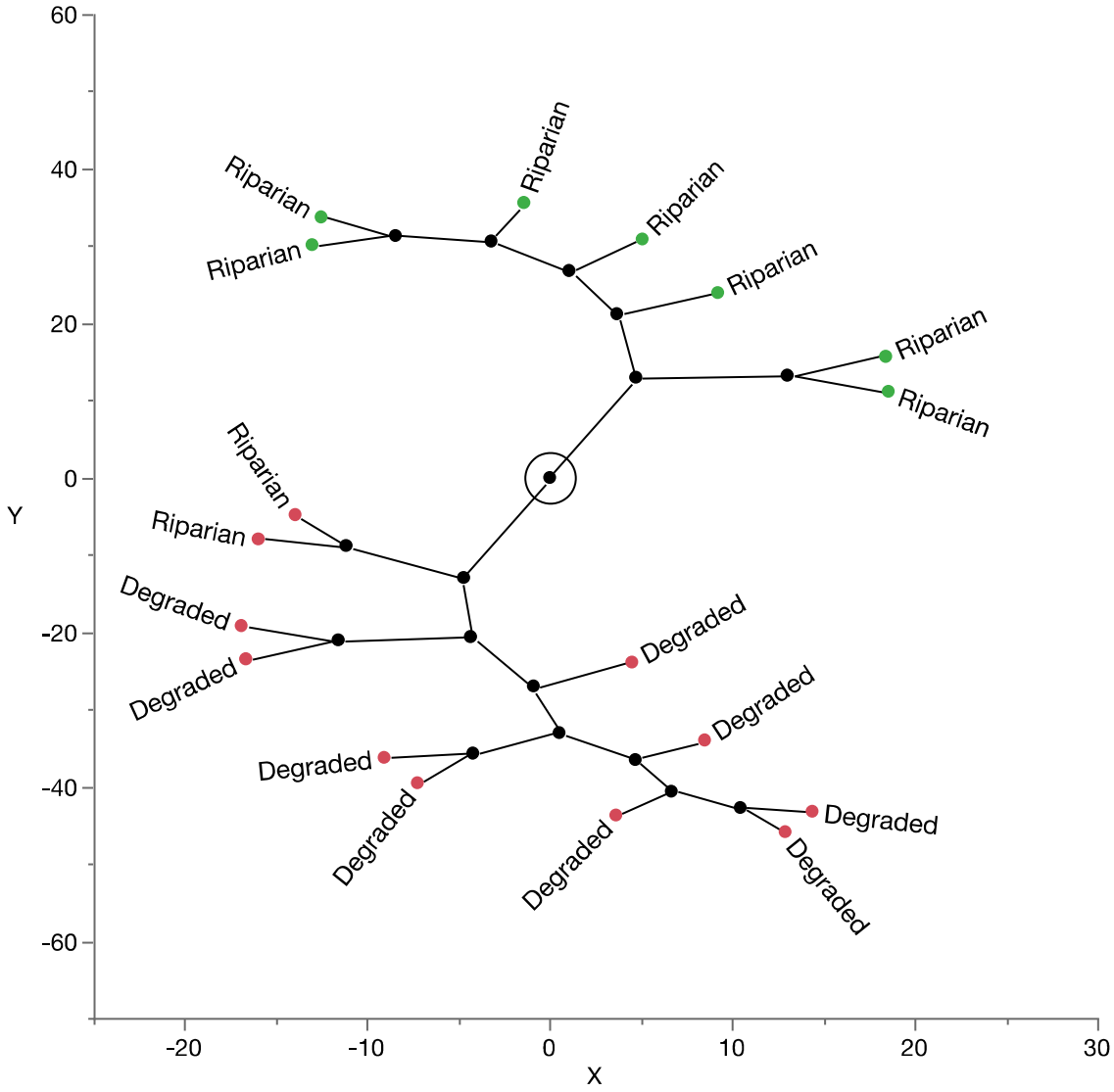
Figure 2. Fungal isolation frequency as a function of season and soil type. Error bars indicate standard error (SE).

Figure 3. Bacterial isolation frequency as a function of season and soil type. Error bars indicate standard error (SE).

Figure 4. Top**,** linear regression model depicting relationship between velvet mesquite germination frequency and fungal infection frequency. Red line indicates line of best fit, pink shaded area represents confidence interval. Bottom, linear regression model depicting relationship between velvet mesquite germination frequency and fungal infection frequency in response to soil treatments. Blue line indicates line of best fit, light blue shaded area represents confidence interval.

Figure 5. Top**,** linear regression model depicting relationship between velvet mesquite germination frequency and bacterial infection frequency. Red line indicates line of best fit, pink shaded area represents confidence interval. Bottom, linear regression model depicting relationship between velvet mesquite germination frequency and bacterial infection frequency in response to soil treatments. Blue line indicates line of best fit, light blue shaded area represents confidence interval.

Figure 1.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Degraded

Figure 2.

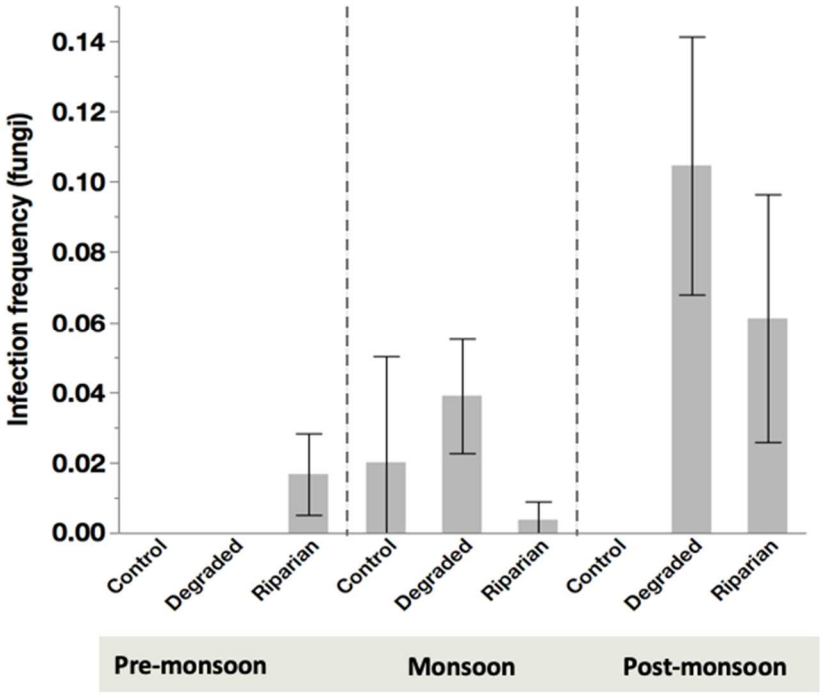

Infection frequency (fungi)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Control
Degraded
Riparian
Control
Degraded
Riparian
Control
Degraded
Riparian
Pre-monsoon
Monsoon
Post-monsoon

Figure 3.

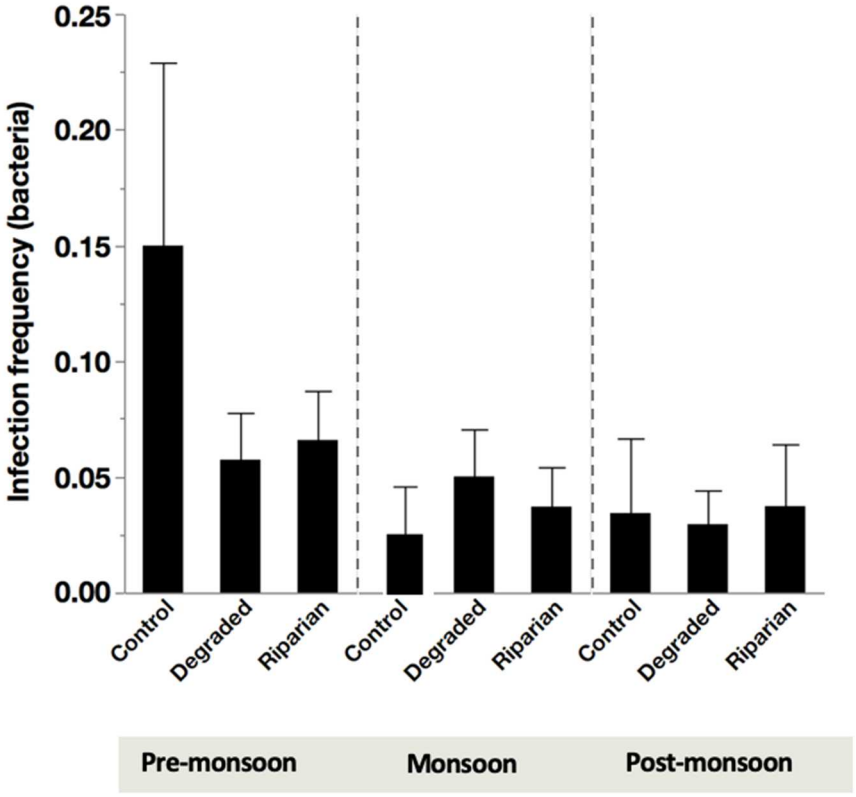
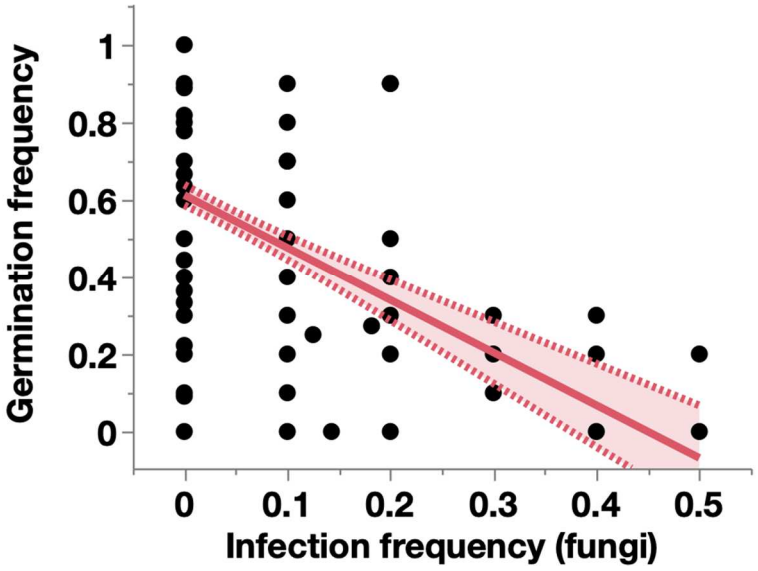

Infection frequency (bacteria)
0.25
0.20
0.15
0.10
0.05
0.00
Control
Degraded
Riparian
Control
Degraded
Riparian
Control
Degraded
Riparian
Pre-monsoon
Monsoon
Post-monsoon

Figure 4.


Germination frequency
1
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
0.5
Infection frequency (fungi)

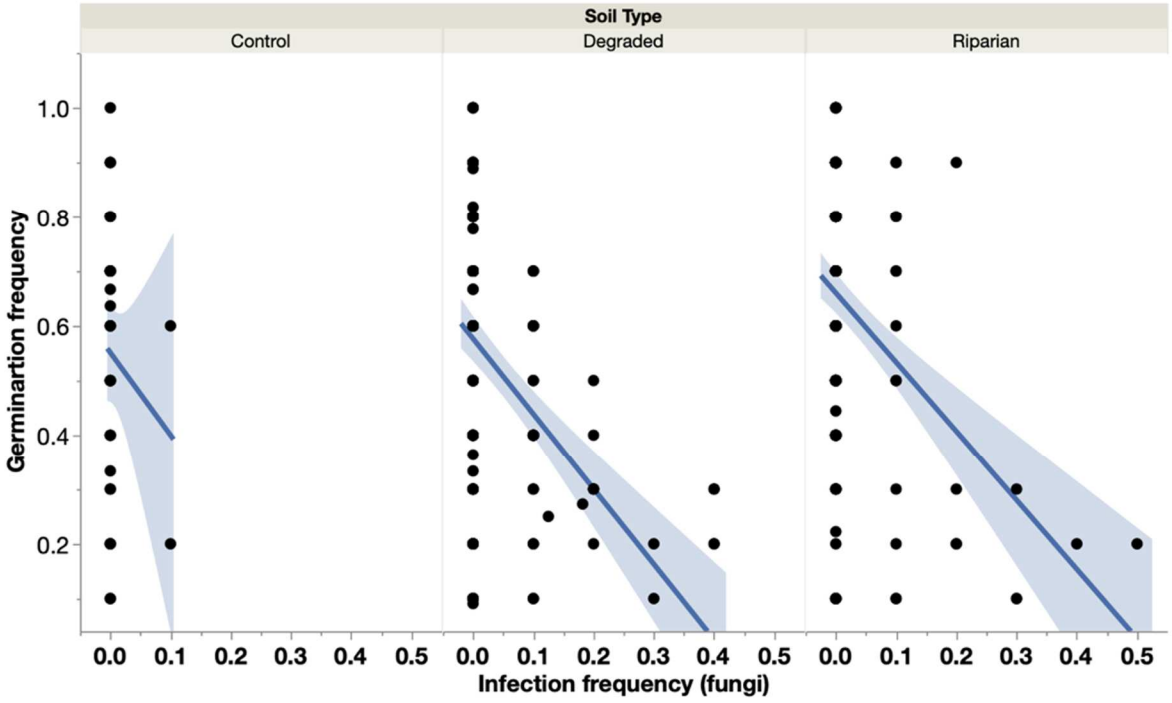
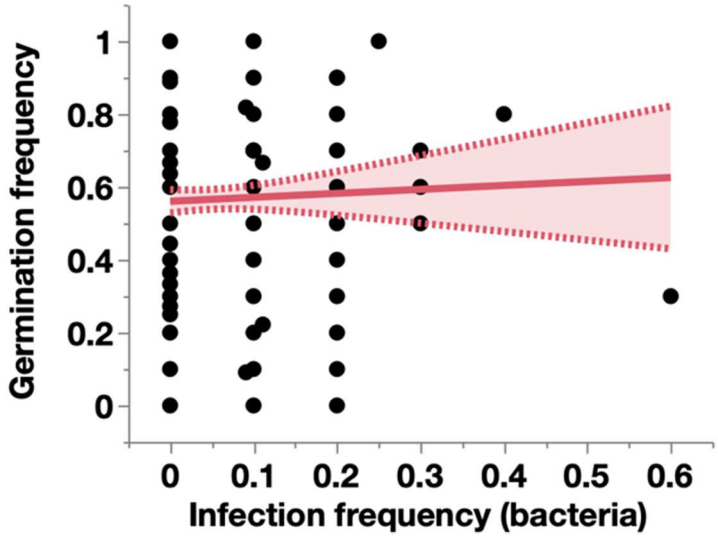
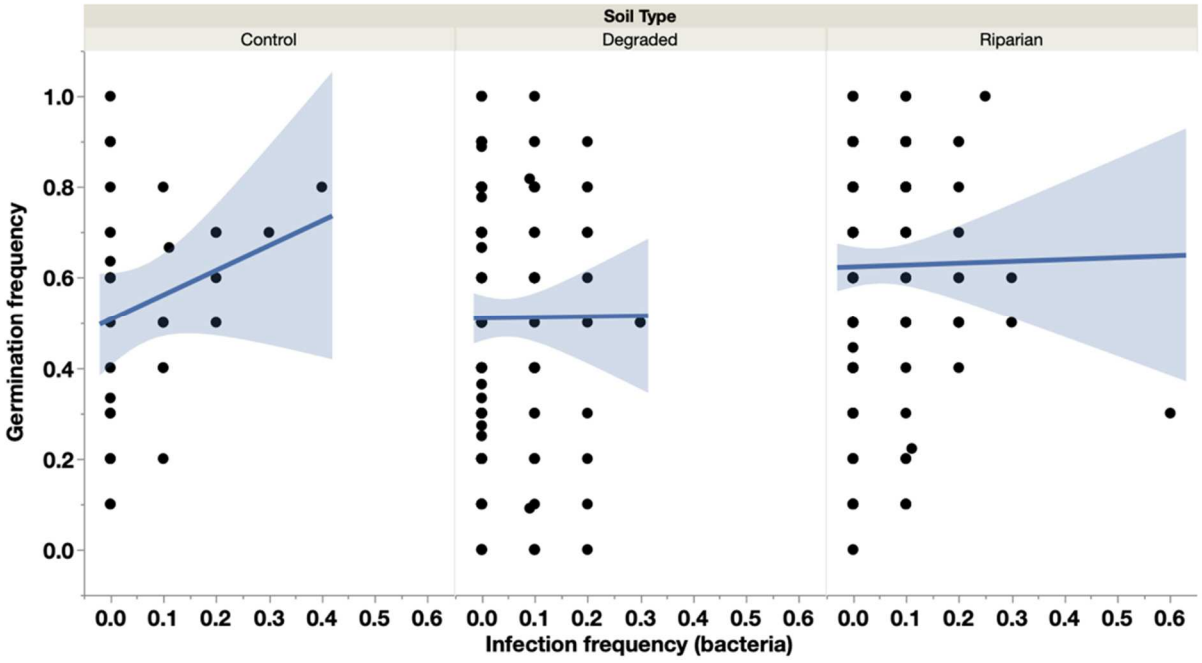

Soil Type
Control
Degraded
Riparian
Germinartion frequency
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.3
0.4
0.5
Infection frequency (fungi)

Figure. 5


Germination frequency
1
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Infection frequency (bacteria)


Soil Type
Control
Degraded
Riparian
Germination frequency
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Infection frequency (bacteria)

Supplementary Information

Supplemental File 1. List of bacterial isolates that were identified by 16S rRNA sequencing. Columns indicated isolate code, season (1- pre-monsoon, 2 - monsoon, 3- post-monsoon), soil type (control, degraded or riparian), plot (A, B, C, D, E, F, FC, or LC) and accession number and identity of the top BLAST match from http://blast.ncbi.nlm.nih.gov. (LC= lab control, FC= field control)

Supplemental File 2. List of fungal isolates that were identified by ITS rDNA sequencing. Columns indicated isolate code, season (1- pre-monsoon, 2 - monsoon, 3- post-monsoon), soil treatment (control, degraded or riparian), plot (A, B, C, D, E, F, FC, or LC), and accession number and identity of the top BLAST match from http://blast.ncbi.nlm.nih.gov. (LC= lab control, FC= field control)

Supplemental File 3. List of data collected for all seed bags deployed. Columns indicate plot (A, B, C, D, E, F, FC, or LC), soil type (control, degraded or riparian), bag number, season (1 - pre-monsoon, 2 - monsoon, 3 - post-monsoon), total number of seeds in bag, number of seeds that germinated, and number of seeds that showed signs of infection. (LC= lab control, FC= field control)

Supplemental Information

Supplemental File 1. List of bacterial isolates that were identified by 16s rRNA sequencing. Columns indicated isolate code, season (1- pre-monsoon, 2 - monsoon, 3- post-monsoon), soil type (control, degraded or riparian), plot (A, B, C, D, E, F, FC, or LC) and accession number and identity of the top BLAST match from http://blast.ncbi.nlm.nih.gov. (LC= lab control, FC= field control)

Isolate #	Soil Treatment	Season	Plot	Bag #	Accession #	Top BLAST Match
MSEB001	Degraded	1	FC	C	MF662519.1	*Bacillus siamensis*
MSEB002	Degraded	1	B	17	CP030097.1	*Bacillus amyloliquefaciens*
MSEB003	Riparian	1	E	13	MH569338.1	*Bacillus subtilis*
MSEB004	Riparian	1	F	7	MH109383.1	*Bacillus megaterium*
MSEB005	Riparian	1	D	17	CP030097.1	*Bacillus amyloliquefaciens*
MSEB007	Riparian	1	E	1	MH569338.1	*Bacillus subtilis*
MSEB008	Control	1	LC	E	MH580205.1	*Bacillus subtilis*
MSEB009	Control	1	LC	D	NR118290.1	*Bacillus mojavensis*
MSEB010	Control	1	LC	D	NR117946.1	*Bacillus amyloliquefaciens*
MSEB013	Control	1	FC	F	MF662520.1	*Bacillus velezensis*
MSEB014	Control	1	FC	B	NR113265.1	*Bacillus subtilis*
MSEB015	Riparian	1	F	17	MH569338.1	*Bacillus subtilis*
MSEB017	Riparian	1	E	11	MH569338.1	*Bacillus subtilis*
MSEB019	Degraded	1	C	11	NR112686.1	*Bacillus subtilis*
MSEB020	Degraded	1	C	15	MF425821.1	*Bacillus velezensis*
MSEB021	Degraded	1	B	15	NR117946.1	*Bacillus amyloliquefaciens*
MSEB022	Degraded	1	B	13	NR075005.2	*Bacillus velezensis*
MSEB023	Riparian	1	D	9	MF662520.1	*Bacillus velezensis*
MSEB024	Riparian	1	D	14	MG755242.1	*Bacillus subtilis*
MSEB026	Riparian	1	D	17	MF662520.1	*Bacillus velezensis*
MSEB027	Riparian	1	D	17	MH580205.1	*Bacillus subtilis*
MSEB028	Riparian	1	D	15	MH542292.1	*Bacillus subtilis*
MSEB029	Riparian	1	D	18	MF662426.1	*Bacillus subtilis*
MSEB031	Degraded	1	A	10	MH542292.1	*Bacillus subtilis*
MSEB032	Degraded	1	A	11	MH569338.1	*Bacillus subtilis*
MSEB033	Riparian	1	F	6	MH017383.1	*Bacillus amyloliquefaciens*
MSEB035	Riparian	1	D	2	JQ936563.1	*Bacillus sp.*

MSEB036	Degraded	1	C	13	MF662520.1	*Bacillus velezensis*
MSEB038	Degraded	1	A	6	NR113265.1	*Bacillus subtilis*
MSEB039	Degraded	1	C	18	MH542292.1	*Bacillus subtilis*
MSEB040	Degraded	1	C	12	MH569338.1	*Bacillus subtilis*
MSEB042	Degraded	1	B	18	MF983545.1	*Bacillus subtilis*
MSEB043	Degraded	1	B	14	MF662426.1	*Bacillus subtilis*
MSEB044	Degraded	1	B	15	MH393328.1	*Bacillus sp.*
MSEB045	Riparian	1	E	6	NR117946.1	*Bacillus amyloliquefaciens*
MSEB047	Riparian	1	F	10	MH569338.1	*Bacillus subtilis*
MSEB048	Control	1	LC	A	NR117946.1	*Bacillus amyloliquefaciens*
MSEB049	Degraded	1	C	6	MF662519.1	*Bacillus siamensis*
MSEB050	Control	1	FC	F	NR117946.1	*Bacillus amyloliquefaciens*
MSEB051	Control	1	FC	C	NR104873.1	*Bacillus subtilis*
MSEB053	Degraded	1	C	11	MH569338.1	*Bacillus subtilis*
MSEB054	Riparian	1	E	15	NR075005.2	*Bacillus velezensis*
MSEB055	Control	1	LC	F	MG755242.1	*Bacillus subtilis*
MSEB056	Riparian	1	F	12	MH569338.1	*Bacillus subtilis*
MSEB057	Riparian	1	F	11	NZCP012600.1	*Bacillus gobiensis*
MSEB060	Riparian	1	F	15	MF662426.1	*Bacillus subtilis*
MSEB062	Degraded	1	B	11	NR113265.1	*Bacillus subtilis*
MSEB063	Degraded	1	A	13	MH569338.1	*Bacillus subtilis*
MSEB064	Riparian	1	D	2	FJ392727.1	*Bacillus subtilis*
MSEB066	Riparian	1	D	16	MH569338.1	*Bacillus subtilis*
MSEB067	Riparian	1	E	5	MF662520.1	*Bacillus velezensis*
MSEB068	Control	1	FC	C	MH569338.1	*Bacillus subtilis*
MSEB069	Control	1	FC	C	MH569338.1	*Bacillus siamensis*
MSEB072	Riparian	1	D	6	FJ392727.1	*Bacillus subtilis*
MSEB073	Riparian	1	D	9	NR117729.2	*Paenibacillus polymyxa*
MSEB074	Riparian	1	D	7	MH569338.1	*Bacillus subtilis*
MSEB075	Riparian	1	F	3	MF662520.1	*Bacillus amyloliquefaciens*
MSEB076	Degraded	1	C	5	NR117729.2	*Bacillus subtilis*
MSEB077	Degraded	1	A	16	MH569338.1	*Bacillus subtilis*
MSEB078	Degraded	2	B	7	NR116240.1	*Bacillus velezensis*
MSEB083	Riparian	2	F	18	NR116240.1	*Bacillus velezensis*
MSEB084	Degraded	2	A	10	NR116240.1	*Bacillus velezensis*
MSEB086	Degraded	2	C	14	NR112686.1	*Bacillus subtilis*
MSEB088	Riparian	2	D	2	NR113265.1	*Bacillus subtilis*
MSEB091	Degraded	2	A	12	NR112686.1	*Bacillus subtilis*
MSEB095	Riparian	2	F	3	NR117946.1	*Bacillus amyloliquefaciens*
MSEB096	Riparian	2	F	3	NR113265.1	*Bacillus subtilis*

MSEB098	Riparian	2	E	4	NR117946.1	*Bacillus amyloliquefaciens*
MSEB099	Degraded	2	B	2	NR102783.2	*Bacillus subtilis*
MSEB100	Riparian	2	D	6	NR104873.1	*Bacillus subtilis*
MSEB103	Degraded	2	B	7	NR112686.1	*Bacillus subtilis*
MSEB109	Riparian	2	D	7	NR113265.1	*Bacillus subtilis*
MSEB110	Riparian	2	E	2	NR113265.1	*Bacillus subtilis*
MSEB111	Riparian	2	E	10	NR075005.2	*Bacillus velezensis*
MSEB118	Degraded	2	A	6	NR117946.1	*Bacillus amyloliquefaciens*
MSEB121	Riparian	2	E	10	NR104919.1	*Bacillus tequilensis*
MSEB122	Riparian	2	D	3	NR104873.1	*Bacillus subtilis*
MSEB124	Degraded	2	B	9	NR113265.1	*Bacillus subtilis*
MSEB125	Degraded	2	B	10	NR112686.1	*Bacillus subtilis*
MSEB126	Riparian	2	E	1	NR075005.2	*Bacillus velezensis*
MSEB127	Riparian	2	D	8	JX994100.1	*Bacillus subtilis*
MSEB128	Riparian	2	D	13	KJ526844.1	*Bacillus licheniformis*
MSEB129	Degraded	2	B	18	NR112686.1	*Bacillus subtilis*
MSEB132	Riparian	2	D	3	NR113265.1	*Bacillus subtilis*
MSEB133	Degraded	2	B	2	NR102783.2	*Bacillus subtilis*
MSEB134	Riparian	2	F	4	NR113265.1	*Bacillus subtilis*
MSEB148	Riparian	2	F	16	NR113265.1	*Bacillus subtilis*
MSEB149	Degraded	1	B	14	NR117946.1	*Bacillus amyloliquefaciens*
MSEB150	Riparian	1	D	12	NR113265.1	*Bacillus subtilis*
MSEB151	Degraded	2	C	3	NR113945.1	*Bacillus safensis*
MSEB152	Degraded	1	A	1	NR113265.1	*Bacillus subtilis*
MSEB153	Degraded	1	A	10	NR104873.1	*Bacillus subtilis*
MSEB154	Degraded	2	A	3	NR157609.1	*Bacillus haynesii*
MSEB155	Degraded	2	B	11	KY465508.1	*Bacillus sp.*
MSEB157	Degraded	1	A	1	NR041175.1	*Streptomyces coelicoflavus*
MSEB158	Degraded	2	A	16	NR115953.1	*Bacillus aryabhattai*
MSEB159	Riparian	1	F	16	NR113265.1	*Bacillus subtilis*
MSE3002	Degraded	3	A	10	MK241859.1	*Siccibacter colletis*
MSE3012	Degraded	3	B	1	MH577800.1	*Bacillus licheniformis*
MSE3020	Degraded	3	B	7	MN099359.1	*Bacillus subtilis*
MSE3033	Control	3	FC	B	Identified by partner	*Paenibacillus jamilae*
MSE3034	Control	3	LC	B	MN004828.1	*Bacillus mojavensis*
MSE3042	Degraded	3	C	7	MN176501.1	*Bacillus safensis*
MSE3043	Degraded	3	C	8	Identified by partner	*Brevibacterium sp*
MSE3049	Degraded	3	C	12	EU780733.1	*Bacillus subtilis*
MSE3051	Degraded	3	C	13	KU161292.1	*Bacillus aryabhattai*

MSE3058	Degraded	3	C	17	Identified by partner	*Bacillus sp.*
MSE3070	Riparian	3	D	4	KY026605.1	*Bacillus licheniformis*
MSE3092	Riparian	3	D	16	MN176503.1	*Bacillus subtilis*
MSE3097	Riparian	3	D	17	MN176501.1	*Bacillus safensis*
MSE3102	Riparian	3	E	3	MN176503.1	*Bacillus subtilis*
MSE3107	Riparian	3	E	16	JN585723.1	*Bacillus subtilis*
MSE3108	Riparian	3	F	2	MK100762.1	*Bacillus circulans*
MSE3115	Riparian	3	F	12	KR999944.1	*Bacillus subtilis*
MSE3116	Riparian	3	F	13	MK318796.1	*Bacillus megaterium*

Supplemental File 2. List of fungal isolates that were identified by ITS rDNA sequencing. Columns indicated isolate code, season (1- pre-monsoon, 2 - monsoon, 3- post-monsoon), soil treatment (control, degraded or riparian), plot (A, B, C, D, E, F, FC, or LC), and accession number and identity of the top BLAST match from http://blast.ncbi.nlm.nih.gov. (LC= lab control, FC= field control)

Isolate #	Season	Soil Treatment	Plot	Bag #	Accession #	Top BLAST Match
MSEF001	1	Riparian	D	10	GQ505450.1	*Chaetomium floriforme*
MSEF002	1	Riparian	D	12	KX664371.1	*Chaetomium sp.*
MSEF003	1	Riparian	D	12	KF673618.1	*Chaetomium bostrychodes*
MSEF004	1	Riparian	F	14	GU183132.1	*Chaetomium sp.*
MSEF005	1	Riparian	F	17	EU167573.1	*Stagonosporopsis cucurbitacearum*
MSEF006	1	Riparian	F	10	GU183132.1	*Chaetomium sp.*
MSEF007	1	Riparian	D	6	GU183132.1	*Chaetomium sp.*
MSEF008	1	Riparian	D	5	GU183132.1	*Chaetomium sp.*
MSEF009	1	Riparian	E	12	GU183132.1	*Chaetomium sp.*
MSEF010	2	Degraded	B	16	KC128815.1	*Yarrowia lipolytica*
MSEF013	2	Control	LC	A	KC128815.1	*Yarrowia lipolytica*
MSEF014	2	Control	LC	A	MG923591.1	*Yarrowia lipolytica*
MSEF016	2	Degraded	B	17	MK032762.1	*Aspergillus flavus*
MSEF018	2	Riparian	F	10	MH109325.1	*Leptosphaerulina australis*
MSEF019	2	Control	LC	E	KC128815.1	*Rhodotorula sp.*
MSEF020	2	Degraded	A	8	MH109325.1	*Aspergillus niger*
MSEF021	2	Degraded	A	4	MH109325.1	*Rhodotorula sp.*
MSEF022	2	Control	LC	B	GQ505450.1	*Aspergillus tubingensis*
MSEF023	2	Degraded	A	17	JQ759516.1	*Rhodotorula sp*
MSEF027	2	Degraded	A	1	MH109325.1	*Penicillium chrysogenum*
MSEF028	2	Degraded	A	2	KC128815.1	*Aspergillus niger*
MSEF029	2	Control	FC	B	MK280840.1	*Penicillium citrinum*
MSEF031	2	Degraded	B	13	MH474332.1	*Aspergillus tubingensis*
MSEF032	2	Degraded	B	4	GU183132.1	*Aureobasidium sp.*
MSEF034	2	Degraded	A	5	KP335252.1	*Rhizopus oryzae*
MSEF035	2	Riparian	D	15	GQ923988.1	*Curvularia spicifera*
MSEB107	2	Degraded	B	18	KM458840.1	*Cryptococcus albidus*
MSEB117	2	Degraded	A	9	GQ505450.1	*Rhodotorula mucilaginosa*
MSE3003	3	Degraded	A	12	MH109325.1	*Aspergillus niger*

MSE3004	3	Degraded	A	14	FJ037743.1	*Aspergillus niger*
MSE3005	3	Degraded	A	15	FJ037743.1	*Alternaria infectoria*
MSE3006	3	Degraded	A	6	MK267619.1	*Aspergillus niger*
MSE3007	3	Degraded	A	17	KX664401.1	*Aspergillus niger*
MSE3009	3	Degraded	A	18	MK685131.1	*Aspergillus japonicus*
MSE3010	3	Control	LC	A	MH109325.1	*Aspergillus niger*
MSE3015	3	Degraded	B	3	MH109325.1	*Aspergillus niger*
MSE3016	3	Degraded	B	4	MK828116.1	*Aspergillus tubingensis*
MSE3017	3	Degraded	B	4	KX664417.1	*Aspergillus flavus*
MSE3021	3	Degraded	B	8	MH109325.1	*Aspergillus niger*
MSE3022	3	Degraded	B	9	FN428912.1	*Aureobasidium pullulans*
MSE3023	3	Degraded	B	9	MH109325.1	*Aspergillus niger*
MSE3024	3	Degraded	B	11	KX664400.1	*Phoma sp.*
MSE3027	3	Degraded	B	12	MK139781.1	*Aspergillus niger*
MSE3028B	3	Degraded	B	12	MH109325.1	*Choanephora cucurbitarum*
MSE3037	3	Degraded	C	3	JQ759516.1	*Aspergillus flavus*
MSE3038	3	Degraded	C	3	MF599715.1	*Aspergillus japonicus*
MSE3039	3	Degraded	C	3	HQ130718.1	*Aspergillus flavus*
MSE3041	3	Degraded	C	4	MH109325.1	*Aspergillus niger*
MSE3044	3	Degraded	C	9	MH061335.1	*Aspergillus tubingensis*
MSE3046	3	Degraded	C	11	MK139781.1	*Fusarium brachygibbosum*
MSE3047	3	Degraded	C	12	MK267619.1	*Phoma aliena*
MSE3050	3	Degraded	C	13	KU847863.1	*Aspergillus tubingensis*
MSE3052	3	Degraded	C	14	KC128815.1	*Fusarium equiseti*
MSE3053	3	Degraded	C	15	KX664417.1	*Aspergillus niger*
MSE3054	3	Degraded	C	15	MK139781.1	*Aspergillus niger*
MSE3055	3	Degraded	C	15	KX664401.1	*Aspergillus niger*
MSE3057	3	Degraded	C	17	KT150681.1	*Aureobasidium sp.*
MSE3059	3	Degraded	C	18	MH109325.1	*Ambomucor seriatoinflatus*
MSE3062	3	Riparian	D	2	KX664307.1	*Ambomucor seriatoinflatus*
MSE3064	3	Riparian	D	3	GQ505450.1	*Fusarium sp.*
MSE3065	3	Riparian	D	4	MF319889.1	*Fusarium brachygibbosum*
MSE3066	3	Riparian	D	2	AY743664.1	*Fusarium brachygibbosum*
MSE3067	3	Riparian	D	3	MH582464.1	*Aspergillus japonicus*
MSE3069	3	Riparian	D	4	MG839500.1	*Fusarium sp.*
MSE3071	3	Riparian	D	4	MH582464.1	*Aspergillus japonicus*
MSE3072	3	Riparian	D	5	MH109325.1	*Fusarium brachygibbosum*
MSE3073	3	Riparian	D	5	GQ505450.1	*Aspergillus niger*
MSE3074A	3	Riparian	D	5	MK139781.1	*Aspergillus japonicus*
MSE3074B	3	Riparian	D	5	JQ759516.1	*Fusarium arcuatisporum*
MSE3075	3	Riparian	D	5	AY743664.1	*Aspergillus japonicus*

MSE3079	3	Riparian	D	11	EU489919.1	*Aspergillus japonicus*
MSE3080	3	Riparian	D	12	KC128815.1	*Didymella calidophila*
MSE3082	3	Riparian	D	13	KX664371.1	*Aspergillus niger*
MSE3083	3	Riparian	D	13	MH109325.1	*Mucor racemosus*
MSE3084	3	Riparian	D	14	MH109325.1	*Fusarium sp.*
MSE3085	3	Riparian	D	14	KC128815.1	*Aspergillus niger*
MSE3086	3	Riparian	D	15	MH109325.1	*Aspergillus japonicus*
MSE3088	3	Riparian	D	15	AJ271061.1	*Aspergillus tubingensis*
MSE3089	3	Riparian	D	15	MH582464.1	*Aspergillus niger*
MSE3090	3	Riparian	D	16	FN428912.1	*Aureobasidium pullulans*
MSE3091	3	Riparian	D	16	NR_149343.1	*Kwoniella heveanensis*
MSE3093	3	Riparian	D	16	MN177721.1	*Rhodosporidiobolus ruineniae*
MSE3094	3	Riparian	D	17	FJ037743.1	*Alternaria sp.*
MSE3095	3	Riparian	D	17	JQ759516.1	*Aspergillus niger*
MSE3096	3	Riparian	D	17	KP638739.1	*Fusarium brachygibbosum*
MSE3098	3	Riparian	D	18	FN428912.1	*Aureobasidium pullulans*
MSE3099	3	Riparian	D	18	MN153951.1	*Aspergillus niger*
MSE3100	3	Riparian	D	18	MF319893.1	*Aspergillus flavus*
MSE3117	3	Degraded	C	17	KM458840.1	*Aureobasidium pullulans*
MSE3119	3	Riparian	F	4	MK267619.1	*Aspergillus niger*

Supplemental File 3. List of data collected for all seed bags deployed. Columns indicated plot (A, B, C, D, E, F, FC, or LC), soil treatment (control, degraded or riparian), bag number, season (1- pre-monsoon, 2 - monsoon, 3- post-monsoon), total number of seeds in bag, number of seeds that germinated, and number of seeds that showed signs of infection. (LC= lab control, FC= field control)

Plot	Soil Type	Bag #	Season	Total #	# Germinated	# Infected
A	Degraded	1	1	10	5	2
A	Degraded	2	1	10	7	0
A	Degraded	3	1	10	8	0
A	Degraded	4	1	10	7	1
A	Degraded	5	1	10	9	0
A	Degraded	6	1	10	8	2
A	Degraded	7	1	9	7	0
A	Degraded	8	1	10	8	1
A	Degraded	9	1	10	7	0
A	Degraded	10	1	10	7	2
A	Degraded	11	1	10	9	1
A	Degraded	12	1	10	7	0
A	Degraded	13	1	10	7	1
A	Degraded	14	1	9	8	0
A	Degraded	15	1	10	7	0
A	Degraded	16	1	10	6	1
A	Degraded	17	1	10	8	1
A	Degraded	18	1	10	9	0
B	Degraded	1	1	9	6	0
B	Degraded	2	1	10	9	0
B	Degraded	3	1	10	8	0
B	Degraded	4	1	10	7	0
B	Degraded	5	1	10	5	0
B	Degraded	6	1	10	6	1
B	Degraded	7	1	10	7	0
B	Degraded	8	1	10	8	0
B	Degraded	9	1	10	8	0
B	Degraded	10	1	10	7	1

B	Degraded	11	1	10	7	2
B	Degraded	12	1	10	5	0
B	Degraded	13	1	10	8	1
B	Degraded	14	1	10	9	2
B	Degraded	15	1	10	6	2
B	Degraded	16	1	10	7	0
B	Degraded	17	1	10	8	1
B	Degraded	18	1	10	7	1
C	Degraded	1	1	10	8	0
C	Degraded	2	1	10	10	0
C	Degraded	3	1	10	6	0
C	Degraded	4	1	10	10	0
C	Degraded	5	1	10	8	1
C	Degraded	6	1	10	8	1
C	Degraded	7	1	10	5	0
C	Degraded	8	1	10	6	0
C	Degraded	9	1	10	9	0
C	Degraded	10	1	9	6	0
C	Degraded	11	1	10	5	3
C	Degraded	12	1	10	9	1
C	Degraded	13	1	10	8	1
C	Degraded	14	1	10	9	0
C	Degraded	15	1	10	5	1
C	Degraded	16	1	10	7	0
C	Degraded	17	1	10	7	0
C	Degraded	18	1	11	9	1
D	Riparian	1	1	10	9	0
D	Riparian	2	1	10	6	2
D	Riparian	3	1	10	5	0
D	Riparian	4	1	10	6	0
D	Riparian	5	1	10	6	1
D	Riparian	6	1	10	7	2
D	Riparian	7	1	10	7	1
D	Riparian	8	1	10	5	0
D	Riparian	9	1	10	5	2
D	Riparian	10	1	10	7	1
D	Riparian	11	1	10	10	0
D	Riparian	12	1	10	9	3
D	Riparian	13	1	10	9	0
D	Riparian	14	1	10	5	2

D	Riparian	15	1	10	9	1
D	Riparian	16	1	10	7	1
D	Riparian	17	1	10	6	3
D	Riparian	18	1	10	10	1
E	Riparian	1	1	10	5	1
E	Riparian	2	1	10	9	1
E	Riparian	3	1	10	7	0
E	Riparian	4	1	10	5	0
E	Riparian	5	1	10	7	1
E	Riparian	6	1	4	4	1
E	Riparian	7	1	10	7	0
E	Riparian	8	1	10	9	2
E	Riparian	9	1	10	4	0
E	Riparian	10	1	10	7	0
E	Riparian	11	1	10	6	1
E	Riparian	12	1	10	9	1
E	Riparian	13	1	10	8	1
E	Riparian	14	1	10	8	0
E	Riparian	15	1	10	8	1
E	Riparian	16	1	10	7	0
E	Riparian	17	1	10	6	0
E	Riparian	18	1	10	7	0
F	Riparian	1	1	10	8	0
F	Riparian	2	1	10	8	0
F	Riparian	3	1	10	9	1
F	Riparian	4	1	10	6	0
F	Riparian	5	1	10	7	0
F	Riparian	6	1	10	8	2
F	Riparian	7	1	10	7	1
F	Riparian	8	1	10	6	0
F	Riparian	9	1	10	8	0
F	Riparian	10	1	10	5	2
F	Riparian	11	1	10	9	1
F	Riparian	12	1	10	8	1
F	Riparian	13	1	10	5	0
F	Riparian	14	1	10	9	1
F	Riparian	15	1	10	8	1
F	Riparian	16	1	10	9	1
F	Riparian	17	1	10	5	2
F	Riparian	18	1	10	7	0

Control	Control	FC-D	1	10	5	1
Control	Control	FC-D	1	10	5	1
Control	Control	FC-D	1	10	8	4
Control	Control	FC-R	1	10	8	1
Control	Control	FC-R	1	10	9	0
Control	Control	FC-R	1	10	7	3
Control	Control	LC	1	10	6	2
Control	Control	LC	1	10	8	0
Control	Control	LC	1	10	5	2
Control	Control	LC	1	10	7	2
Control	Control	LC	1	10	7	0
Control	Control	LC	1	10	7	2
A	Degraded	1	2	10	4	2
A	Degraded	2	2	10	7	1
A	Degraded	3	2	10	4	1
A	Degraded	4	2	10	6	1
A	Degraded	5	2	10	3	2
A	Degraded	6	2	10	4	1
A	Degraded	7	2	10	4	1
A	Degraded	8	2	10	4	2
A	Degraded	9	2	10	2	1
A	Degraded	10	2	10	3	3
A	Degraded	11	2	10	6	0
A	Degraded	12	2	10	6	1
A	Degraded	13	2	10	7	0
A	Degraded	14	2	10	5	0
A	Degraded	15	2	10	4	0
A	Degraded	16	2	10	6	1
A	Degraded	17	2	10	7	1
A	Degraded	18	2	10	4	1
B	Degraded	1	2	10	5	0
B	Degraded	2	2	10	6	2
B	Degraded	3	2	10	4	1
B	Degraded	4	2	10	4	2
B	Degraded	5	2	10	6	1
B	Degraded	6	2	10	2	2
B	Degraded	7	2	10	1	2
B	Degraded	8	2	10	3	0
B	Degraded	9	2	10	7	3
B	Degraded	10	2	10	3	2

B	Degraded	11	2	10	4	1
B	Degraded	12	2	10	7	0
B	Degraded	13	2	10	5	1
B	Degraded	14	2	10	8	0
B	Degraded	15	2	10	4	1
B	Degraded	16	2	10	1	1
B	Degraded	17	2	10	6	1
B	Degraded	18	2	10	5	3
C	Degraded	1	2	10	5	0
C	Degraded	2	2	10	8	2
C	Degraded	3	2	10	6	1
C	Degraded	4	2	10	6	0
C	Degraded	5	2	10	8	0
C	Degraded	6	2	10	8	0
C	Degraded	7	2	10	8	0
C	Degraded	8	2	10	6	2
C	Degraded	9	2	10	8	0
C	Degraded	10	2	10	7	0
C	Degraded	11	2	10	8	0
C	Degraded	12	2	10	8	0
C	Degraded	13	2	10	10	0
C	Degraded	14	2	10	10	1
C	Degraded	15	2	10	7	0
C	Degraded	16	2	10	7	0
C	Degraded	17	2	10	7	0
C	Degraded	18	2	10	8	0
D	Riparian	1	2	10	7	0
D	Riparian	2	2	10	9	1
D	Riparian	3	2	10	4	2
D	Riparian	4	2	10	8	1
D	Riparian	5	2	10	7	0
D	Riparian	6	2	10	8	1
D	Riparian	7	2	10	9	1
D	Riparian	8	2	10	6	1
D	Riparian	9	2	10	7	0
D	Riparian	10	2	10	9	0
D	Riparian	11	2	10	8	0
D	Riparian	12	2	10	7	0
D	Riparian	13	2	10	8	1
D	Riparian	14	2	10	8	1

D	Riparian	15	2	10	7	0
D	Riparian	16	2	10	9	0
D	Riparian	17	2	10	8	0
D	Riparian	18	2	10	6	0
E	Riparian	1	2	10	7	1
E	Riparian	2	2	10	9	1
E	Riparian	3	2	10	6	0
E	Riparian	4	2	10	9	2
E	Riparian	5	2	10	7	0
E	Riparian	6	2	10	8	0
E	Riparian	7	2	10	9	0
E	Riparian	8	2	10	9	0
E	Riparian	9	2	10	9	0
E	Riparian	10	2	10	7	2
E	Riparian	11	2	10	9	0
E	Riparian	12	2	10	7	0
E	Riparian	13	2	10	9	0
E	Riparian	14	2	10	9	0
E	Riparian	15	2	10	8	0
E	Riparian	16	2	10	7	0
E	Riparian	17	2	10	4	0
E	Riparian	18	2	10	8	0
F	Riparian	1	2	10	10	0
F	Riparian	2	2	10	9	0
F	Riparian	3	2	10	6	2
F	Riparian	4	2	10	6	1
F	Riparian	5	2	10	10	0
F	Riparian	6	2	10	7	0
F	Riparian	7	2	10	9	1
F	Riparian	8	2	10	8	0
F	Riparian	9	2	10	7	0
F	Riparian	10	2	10	8	1
F	Riparian	11	2	10	8	0
F	Riparian	12	2	10	8	0
F	Riparian	13	2	10	9	0
F	Riparian	14	2	10	9	0
F	Riparian	15	2	10	9	1
F	Riparian	16	2	10	10	1
F	Riparian	17	2	10	5	0
F	Riparian	18	2	10	9	1

Control	Control	FC-D	2	10	1	0
Control	Control	FC-D	2	10	6	1
Control	Control	FC-D	2	10	9	0
Control	Control	FC-R	2	10	6	0
Control	Control	FC-R	2	10	7	0
Control	Control	FC-R	2	10	10	0
Control	Control	LC	2	10	9	4
Control	Control	LC	2	10	2	1
Control	Control	LC	2	10	1	0
Control	Control	LC	2	10	9	0
Control	Control	LC	2	10	7	2
Control	Control	LC	2	10	3	0
A	Degraded	1	3	10	4	0
A	Degraded	2	3	10	3	0
A	Degraded	3	3	10	3	0
A	Degraded	4	3	10	2	0
A	Degraded	5	3	10	6	0
A	Degraded	6	3	10	0	1
A	Degraded	7	3	10	3	0
A	Degraded	8	3	10	3	0
A	Degraded	9	3	10	4	0
A	Degraded	10	3	10	4	1
A	Degraded	11	3	10	7	0
A	Degraded	12	3	10	5	1
A	Degraded	13	3	11	4	0
A	Degraded	14	3	10	7	1
A	Degraded	15	3	10	6	1
A	Degraded	16	3	10	3	1
A	Degraded	17	3	10	3	1
A	Degraded	18	3	10	3	2
B	Degraded	1	3	10	5	1
B	Degraded	2	3	11	1	1
B	Degraded	3	3	8	2	1
B	Degraded	4	3	10	0	3
B	Degraded	5	3	9	6	0
B	Degraded	6	3	10	2	1
B	Degraded	7	3	10	2	1
B	Degraded	8	3	7	0	1
B	Degraded	9	3	11	3	2
B	Degraded	10	3	10	3	0

B	Degraded	11	3	10	1	3
B	Degraded	12	3	10	2	3
B	Degraded	13	3	10	2	1
B	Degraded	14	3	10	4	2
B	Degraded	15	3	10	4	0
B	Degraded	16	3	10	5	0
B	Degraded	17	3	10	3	0
B	Degraded	18	3	10	2	0
C	Degraded	1	3	10	2	0
C	Degraded	2	3	10	5	2
C	Degraded	3	3	10	3	4
C	Degraded	4	3	10	2	0
C	Degraded	5	3	10	3	0
C	Degraded	6	3	10	1	0
C	Degraded	7	3	10	4	2
C	Degraded	8	3	10	5	1
C	Degraded	9	3	10	4	4
C	Degraded	10	3	10	3	1
C	Degraded	11	3	10	0	4
C	Degraded	12	3	10	4	6
C	Degraded	13	3	10	1	5
C	Degraded	14	3	10	2	4
C	Degraded	15	3	10	1	3
C	Degraded	16	3	10	1	1
C	Degraded	17	3	10	3	3
C	Degraded	18	3	10	2	3
D	Riparian	1	3	10	4	0
D	Riparian	2	3	10	2	2
D	Riparian	3	3	10	3	1
D	Riparian	4	3	10	2	6
D	Riparian	5	3	10	0	5
D	Riparian	6	3	10	3	0
D	Riparian	7	3	10	4	0
D	Riparian	8	3	10	5	0
D	Riparian	9	3	10	5	2
D	Riparian	10	3	10	1	1
D	Riparian	11	3	10	1	1
D	Riparian	12	3	10	2	1
D	Riparian	13	3	10	1	3
D	Riparian	14	3	10	2	2

D	Riparian	15	3	10	3	4
D	Riparian	16	3	10	3	2
D	Riparian	17	3	10	1	4
D	Riparian	18	3	10	3	3
E	Riparian	1	3	10	1	0
E	Riparian	2	3	10	5	0
E	Riparian	3	3	10	1	1
E	Riparian	4	3	10	5	0
E	Riparian	5	3	10	6	0
E	Riparian	6	3	10	4	0
E	Riparian	7	3	10	7	1
E	Riparian	8	3	10	6	0
E	Riparian	9	3	10	8	0
E	Riparian	10	3	10	1	0
E	Riparian	11	3	10	7	0
E	Riparian	12	3	10	6	0
E	Riparian	13	3	10	7	0
E	Riparian	14	3	9	2	1
E	Riparian	15	3	10	8	0
E	Riparian	16	3	10	4	1
E	Riparian	17	3	10	1	0
E	Riparian	18	3	10	5	0
F	Riparian	1	3	10	5	0
F	Riparian	2	3	10	2	1
F	Riparian	3	3	10	5	0
F	Riparian	4	3	10	5	3
F	Riparian	5	3	10	5	0
F	Riparian	6	3	10	5	0
F	Riparian	7	3	9	4	0
F	Riparian	8	3	10	3	6
F	Riparian	9	3	10	4	0
F	Riparian	10	3	10	5	0
F	Riparian	11	3	10	7	0
F	Riparian	12	3	10	3	1
F	Riparian	13	3	10	1	1
F	Riparian	14	3	10	5	0
F	Riparian	15	3	10	4	0
F	Riparian	16	3	10	6	0
F	Riparian	17	3	10	5	0
F	Riparian	18	3	10	6	0

Control	Control	FC-D	3	10	4	1
Control	Control	FC-D	3	10	2	1
Control	Control	FC-D	3	10	8	0
Control	Control	FC-R	3	10	5	0
Control	Control	FC-R	3	10	3	0
Control	Control	FC-R	3	11	7	0
Control	Control	LC	3	9	6	1
Control	Control	LC	3	10	4	1
Control	Control	LC	3	10	5	0
Control	Control	LC	3	10	2	0
Control	Control	LC	3	10	3	0
Control	Control	LC	3	9	4	0

APPENDIX B

MIDDLE SCHOOL STUDENT ENGAGEMENT IN STEM THROUGH PLANT MICROBIOMES

Middle school student engagement in STEM through plant microbiomes

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Abstract

Early exposure of students to Science, Technology, Engineering, and Mathematics (STEM) has shown to positively impact students' perceptions, contributing to the next generation of scientists. To engage young, diverse people in scientific activities, we developed a hands-on, customizable lesson plan with a reproducible, low-cost science experiment for educators. A learning module for elementary and middle school students was implemented at Walter Douglas Elementary School in Tucson, Arizona, in March 2018. The module focused on conceptually building an understanding of the plant microbiome and the content centered on ecosystem function through the lens of microbial symbioses. This learning module is intended to provide a framework for educators at the elementary and middle school level to introduce their students to STEM. The content includes a customizable lesson plan with step by step instructions for a hands-on activity, two PowerPoints, a pre/post-test, and a classroom handout. Through program assessment, we determined this lesson plan to be effective as the scores of the student's post-tests increased by 30.0% in comparison to the pre-test, making this a practical and economic learning module for educators.

Keywords: Science learning, STEM, lesson plan, native plants, plant microbiome, early exposure

Introduction

Underrepresentation of women and minorities in Science, Technology, Engineering, and Math (STEM fields) remains one of the biggest barriers in STEM participation. Studies have shown that scientists are often thought to be white, nerdy males who wear lab coats and work alone in a laboratory (Mead & Metraux, 1957; Eisenhart et al., 1996; Barman, 1999; Buck et al., 2002).

While the imagery of scientists has improved, these stereotypes have prevented populations that are traditionally underrepresented in science from seeing themselves as successful scientists. (Buck et al., 2002). Studies by Buck et al., (2002) recommend that elementary teachers should bring women and minority scientists in the classroom as a way of dispelling the current stereotype. Research by Barman, (1999) found that out of the 154 K-8 teachers involved in his study, very few of them felt confident in their ability to design and conduct formal research. This outreach chapter is intended to serve as a tool to assist educators in the classroom by providing a lesson plan and a relatively low-cost classroom experiment that can be tailored to the needs of the students.

Student Demographics

Walter Douglas Elementary is a Title I school in the Flowing Wells Unified School district located in Tucson, Arizona. Title I schools are provided with federal funding to help the schools that serve economically disadvantaged children (Gordon, 2004). Walter Douglas serves children from kindergarten through sixth grade and has a population of 598 students as of March 2019. Of these 598 students, the majority are of Hispanic ethnicity (83.6%), followed by white students (14.5%), African American (1.3%), and other (0.6%). The student body is comprised of 53.0% males and 47.0% females. Out of the 598 students, 93.3% are on free or reduced lunch, signifying that the majority of these students are from low income households (National Center for Education Statistics, U.S. Dept of Education). Most of the students (85.5%) at Walter Douglas are classified as underrepresented in STEM fields (Edwards, 1999). This outreach was developed for Jamie Camero's sixth grade science students. Mrs. Camero is the science teacher and the head of the sixth grade at Walter Douglas Elementary. At the time of development,

Ashton B. Leo was serving as Mrs. Camero's teaching assistant which helped to facilitate the design of this outreach project.

Learning Objectives

Early exposure to science has been shown to have a positive effect on students' perceptions of STEM (DeJarnette, 2012). Various studies have identified experiences occurring during the elementary school years to be highly impactful suggesting the best time to expose students to STEM is in elementary school (Tai et al., 2006; Russell et al., 2007). The learning objective of this module was to expose elementary students to science with a lesson conducted by a female, Indigenous scientist. Since 80% of teachers in Arizona are white and only 41.5% of students in Arizona are white (National Center for Education Statistics, U.S. Department of Education), we felt that having a member of an underrepresented minority group conducting the lesson would have the greatest impact.

As research into microbiomes increases, establishing new curriculum for young students on microbiome sciences could contribute to scientific literacy and possibly inspire the next generation of scientists. We developed a plant microbiome learning module equipped with a lesson plan and experiment that educators can easily incorporate in their classroom.

We challenged the sixth-grade students to think about microorganisms and their relationships with plants by using guayule plants (*Parthenium argentatum*), a native plant species which had been established in the classroom earlier that year. Native plants are adapted for the area where they naturally occur and play an important role in the ecosystem (Leimu & Fischer, 2008). Any plant species would be suitable for this experiment. If this lesson plan were

to be implemented in a classroom located on or near tribal lands, the authors recommend focusing on a culturally relevant plant species.

Module content

This plant microbiome module was implemented on March 22, 2019 and concluded on March 25, 2019. The module was presented to three groups of students and consisted of an interactive 15-minute PowerPoint (Supplemental 1), a lesson plan for the hands-on activity (Supplemental 2), pre/post assessment (Supplemental 3 &. 4), a worksheet (Supplemental 5), and a follow up presentation (Supplemental 6). The module was designed to coincide with the teacher's existing lesson plans about biodiversity (i.e. microscopes, plants, and microorganisms) (Arizona Department of Education, 2018).

In the hands-on activity, the students assembled into groups of no more than 5, with one guayule plant per group. Each student was provided a Petri dish with malt extract agar, a sterile cotton swab, and assigned a plant part to swab. The students swabbed the assigned plant part with the sterile swab, then used the swab to inoculate the Petri dish. The students labeled and sealed their Petri dishes and returned them to me for incubation. We returned to the classroom two days later, at this time the students were allowed to examine their Petri dishes and record their observations. We gave a short PowerPoint presentation (Supplemental 6) that included: revisiting the experiment, a review of key terms, the equipment that was used, and key morphological traits for identification of microorganisms. Photos of the microbial growth on the student's Petri dishes were used as the background of the second PowerPoint to showcase the student's efforts.

Assessment methods

To assess the effectiveness of this module, the students were given a pre- and post-test. The pretest (Supplemental 3) was administered at the beginning of the module, prior to the PowerPoint presentation. The goal of the assessment was to gauge the student's current knowledge of plant morphology, types of symbiosis, and the role of microorganisms in the ecosystem. After the review module concluded on my return visit, the students took the post-test (Supplemental 4). By comparing the scores of the pre-test to the post-test, we evaluated the students' improvement on understanding the concepts and evaluated the effectiveness of the module.

Three groups of students participated in the plant microbiome module for a total of 84 students. Overall 47 of the students were male, while 37 were female. The average of the pretests for the three classes was 50.9% (N=80, mean= 50.9% ± 2.1%), the students improved their scores on the post-test for an average of 81.6% (N=57, mean= 81.6% ± 1.8%). Due to absences in the classroom, not all students took both the pre and post-test. When we examined the test scores for those who took both tests, the pretest average was 54.0% (N=56, mean= 54.0% ± 2.5%) and the post-test average was 81.8% (N=56, mean= 81.8% ± 1.9%). Overall, the classes test scores improved by 29.3%.

The module concluded with an interview with the teacher, Jaime Camero. We interviewed Mrs. Camero via email to determine if the module if into the topic she was currently teaching and if the module was effective in sparking the students' interest. Regarding if the lesson fit, Mrs. Camero stated, "The lesson coincided with the curriculum I was teaching at that time, as the students were learning about single-celled organisms and their relationship with the

environment and contribution to biodiversity. The students were highly engaged and loved watching the microorganisms grow in the Petri dishes."

Perspectives on implementation

The videos selected for the PowerPoint presentation were selected with the age of the students in mind, videos that were educational, fun, engaging, and informative were chosen. The students responded positively to the selected videos. After assessing the pretests, we determined that it would be best to remove the term "symbiosis" in question 6 (Supplemental 3), we felt that this was confusing to the students.

For this outreach program we had a set budget offered by the teacher of $30 for supplies. However, this budget may not be suited for all classrooms. On future implementations of this project, we suggest identifying cheaper alternatives for each item used. For instance, cotton swabs can be sterilized in the microwave and glass Petri dishes can be purchased instead of plastic for reuse.

On the day we returned to the classroom, students examined their Petri dishes and were given the post-test. However, a majority of the students were not in their regularly assigned class period, the class periods were reversed that day to allow for makeup of missed activities. Many students are often excused from classes to make up activities or attend remedial classes (Jenkins & Heinen, 1989). This is one example of how some students miss important lessons and activities (Meyers et al.,1990) including ones of high interest. For example, there was one student who, when she had to leave the classroom, was visibly disappointed to be missing the activity. Overall, the students were visibly eager and fascinated with the microbial growth on their Petri dishes.

This module was created to increase teachers access to STEM activities that can be conducted with a variety of age groups however, there is room for improvement. Mrs. Camero felt the students could have benefitted from more discussion on how the module connected to their class lessons, this could be remedied by working closer with the teacher prior for the timing of the module.

Acknowledgments

We thank Jaime Camero and Walter Douglas Elementary School for the opportunity to engage the students, gain teaching experience and expose students to underrepresented STEM role models Ashton B. Leo thanks the College of Chemical and Electrical Engineering at The University of Arizona (Sustainable Bioeconomy for Arid Regions Fellowship).

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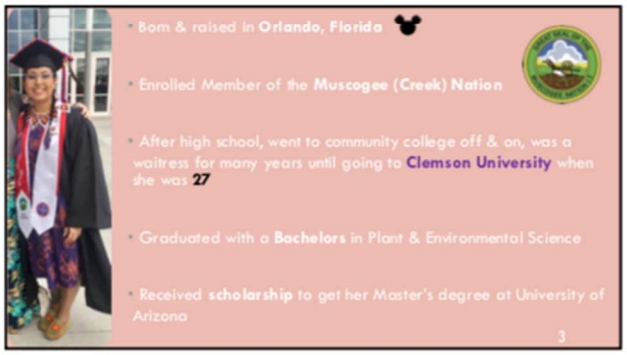
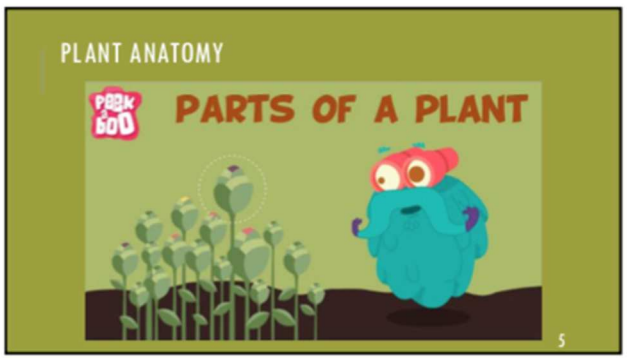
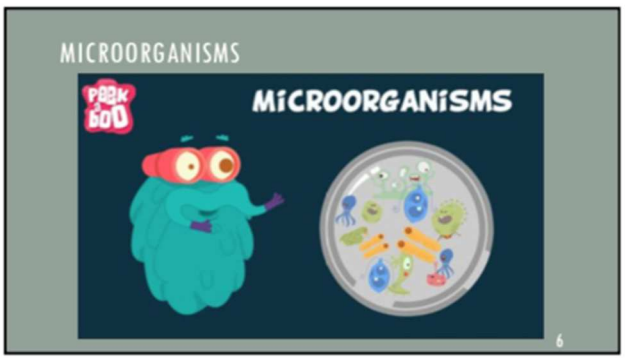
Supplemental File 1: Outreach_Powerpoint_ppt







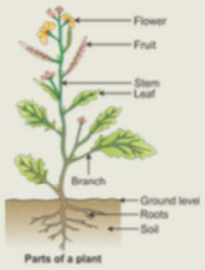
Video in slide 6: Rajshri Entertainment Private Limited, https://youtu.be/p3St51F4kE8
Video in slide 7: Rajshri Entertainment Private Limited, https://youtu.be/JZjzQhFG6Ec

PLANT MICROBIOMES

Every part of the plant has a group of microbes that live there

Microbes, like fungi and bacteria can live **on** and **inside** the plant

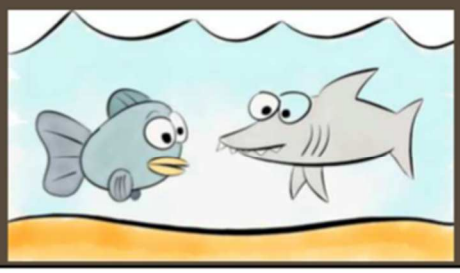
Some fungi and bacteria help the plant receive benefits



Parts of a plant

7

RELATIONSHIPS



8

RELATIONSHIPS

Symbiosis- unlike living things living together

Mutualism- both parties benefit

Commensalism- one benefits, one is neither harmed or helped

Parasitism- one benefits, other is harmed

9

EXPERIMENT TIME!

10

CULTURING PLANT MICROBES

We can grab microorganisms from the surface of plants by swabbing with a Q-Tip

Gently touch the round plate with the Q-Tip, the microorganisms are transferred to the plate

The microbes will grow on the plates, this is one way scientists are to identify who is the microbes are

11

DISCUSSION TIME!

What do you think will happen?

Why do you think that I came to the classroom today?

Does this fit in with anything else that you are learning right now?

12

Video in slide 8: Simple Science, Mark Drollinger, https://youtu.be/zTGcS7vJqbs

Supplemental File 2: Lesson_Plan.pdf

Microbes and Plants

Lesson Plan

Teacher: Ms. Desirae Kissell

Date: 03/22/2019

Overview & Purpose

This unit will expose students to plant microbes, soil microbes and symbiosis. This unit can be tailored to 5th- 12th grade students.

Keywords

Symbiosis, mutualism, microbiology, bacteria, fungi, microbes, native plants

Education Standards

To be completed by the teacher based on state/tribal/institutional guidelines and requirements.

Time Duration

Two class periods of 55 minutes each

Objectives

1. Students will be able to tell what microbes are, and what they look like in a controlled environment
2. Students will be able to name plant parts
3. Students will be able to explain the concept of symbiosis
4. Students will be able to explain that not all microorganisms are bad

Important Take Home Messages:

1. Microbes can live on and inside of plants without causing harm, sometimes provided benefit the plant
2. Microbes helping plants is a symbiotic and mutualistic relationship
3. Microbes can be friends not just enemies
4. Native plants have unique microbes that help the plant to live in that environment

Materials Needed

1. Disposable Petri Dishes, containing Malt Extract Agar (MEA) media
2. Native plants (i.e., guayule)
3. Sterile cotton tip applicators (Q-Tips)
4. Water
5. Soil
6. Controls: 1 control plate per class
7. Parafilm
8. *Optional:* Sharpies (to label)

Assessment

Steps to check for student understanding

1. Pre-test- given before presentation
2. Post-test- given at the end of the 2nd class (return visit)
3. Lab hand out

Cost

Sterile Cotton Swab, 100 count — $7.54, Amazon.com

- Q Tips can be used if sterilized
- Can use microwave for sterilization (2 min.)

35 x 10 mm disposable Petri Dish, Polystyrene
100 count — $16.29/ amazon.com

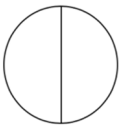
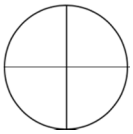
Handouts — Costs varies

Malt Extract Agar, 500ml recipe
Pour approximately 3.5 ml per 35 mm plate

Material	Cost	Amount needed	Cost per plate
Malt Extract	$12.54 for 453g	10g	$0.03
Agar 2%	$32.90 for 500g	10g	$0.07

Experiment

Students will break into groups; each will receive their own 35mm Petri dish. If using Petri dishes that are 60mm or larger, plates can be divided into quadrants (halves, thirds, fourths- based on materials available).

E.g. Plate in halves,  Plate in thirds,  Plate in fourths, 

Groups will be assigned a plant cultivar and a corresponding plant part to swab. Each student will have the opportunity to swab a plate.

Teachers: Tell your students "Be careful not to breathe, cough, or touch the plates, this could mess up the experiment!"

Day 1, Protocol:

1. Remove sterile Q TIP from foil packet
   a) Keep the packet closed when not in use (help decrease contamination)

2) Take one side of Q Tip and swab on assigned plant surface
   a) Do not allow the tip to touch anything else.
      i) If you cannot immediately swab the plate, put swab back in package

3) Take the Q Tip and **gently** streak the surface of the agar
   a) Be careful not to insert Q Tip into the media
   b) Place lid back on plate quickly

4) Once all sections of plate are swabbed, parafilm plate 2 times

5) Plates will be stored in a warm, dark place, store the plates **upside down,** so the bacterial growth remains undisturbed by any water droplets.
   a) The ideal temperature for growing bacteria is 70- 98°F (20-37°C).

6) Have students record their observations on the contents of each dish and draw conclusions about which locations had the most bacteria

***Teachers: Dispose of plates in biohazard safe manner ***

- U.S Environmental Protection Agency. Hazardous Waste Management for School Laboratories and Classrooms

*To dispose of environmental cultures without an autoclave, cover the agar surface with a 10% bleach solution and incubate for at least an hour at room temperature. After this incubation, plates can be disposed of in the regular waste

*It is recommended that you keep one section of the Petri dish free of bacteria sample to use as a control group. This allows you to know if the agar had been contaminated prior to the introduction of bacterial samples (can also use a plate as a control)

*Daily photos should be taken

*During the second lesson, have the students draw what they see on the plates and try to determine which microorganisms they have, e.g. bacteria, fungi, yeast

* Recommendation: Develop a PowerPoint that will highlight common species that students may encounter. You may tailor the PowerPoint according to the observations made during incubation period (predictions)

Unit Vocabulary

Symbiosis- the interaction between two different organisms living in close physical association, typically to the advantage of both

Mutualism- symbiosis that is beneficial to both organisms involved

Microbiology- The study of living organisms that are too small to see with the naked eye

Microorganism- organism that requires a microscope to be seen, especially a bacterium, virus, or fungus (*Often referred to as* ***microbe*** *for short)*

Bacteria- a member of a large group of unicellular microorganisms which have cell walls but lack organelles and an organized nucleus, including some which can cause disease

Fungus- any of a group of spore-producing organisms feeding on organic matter, including molds, yeast, mushrooms, and toadstools

Plant- a living organism of the kind exemplified by trees, shrubs, herbs, grasses, ferns, and mosses, typically growing in a permanent site, absorbing water and inorganic substances through its roots, and synthesizing nutrients in its leaves by photosynthesis using the green pigment chlorophyll

Native plants- plant that occurred naturally and has existed for many years in an area

- Tips for Microbe Identification:
    - **Bacteria**, https://microbiologyonline.org/teachers/observing-microbes/observing-bacteria-in-a-petri-dish
    - **Fungi**, https://microbiologyonline.org/teachers/observing-microbes/observing-fungi-in-a-petri-dish

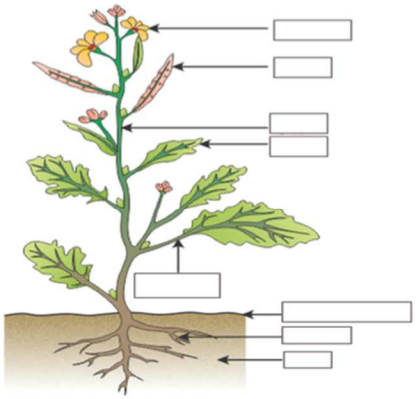
Supplemental 3: Pre-test

Name_____

Date _____

Class _____

Microbes and Plants Test



Parts of a plant

1. Fill in the blanks on the picture:

A. Ground Level **B**. Fruit **C.** Stem **D.** Soil
E. Leaves **F.** Branch **G.** Flower **H.** Roots

2. What is the technique used by scientists to grow microbes?

A. Gardening
B. Producing
C. Culturing
D. None of the above

3. Which of the following is not a microbe?

A. Virus
B. Bacteria
C. Fungi
D. Protist
E. They are all microbes

4. All microorganisms are harmful. True / False

5. Microbes, like fungi and bacteria can live on and inside the plant. True/ False

6. Write the letter of the correct match next to each problem

_____ mutualism	a. when both parties benefit
_____ parasitism	b. Interaction between to different organisms living together
_____ symbiosis	c. When one party benefits and the other is harmed
_____ commensalism	d. when one party benefits and the other Is neither harmed or helped

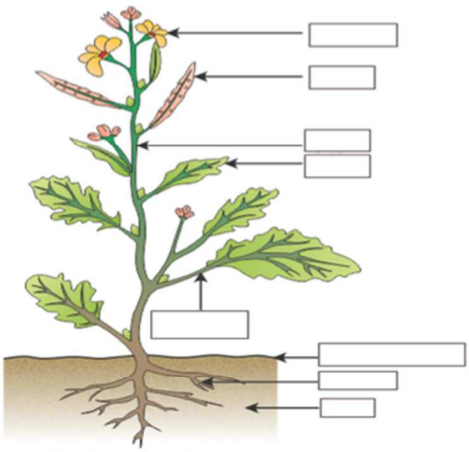
Supplemental File 4: Post-test

Name_____

Date _____

Class _____

Microbes and Plants Test



Parts of a plant

1. Fill in the blanks on the picture:

A. Ground Level **B.** Fruit **C.** Stem **D.** Soil
E. Leaves **F.** Branch **G.** Flower **H.** Roots

2. What is the technique used by scientists to grow microbes?

A. Gardening
B. Producing
C. Culturing
D. None of the above

3. Which of the following is not a microbe?

A. Virus
B. Bacteria
C. Fungi
D. Protist
E. They are all microbes

4. All microorganisms are harmful. True / False

5. Microbes, like fungi and bacteria can live on and inside the plant. True/ False

6. Write the letter next to the correct term.

_____ Parasitism

_____ mutualism

_____ commensalism

a. When both parties benefit **(+, +)**

b. When one party benefits and the other is neither harmed Or helped **(+, 0)**

c. When one party benefits and the other is harmed **(+, -)**

Supplemental 5: Handout for students' observations

SCIENCE EXPERIMENT

Title _____ Date _____

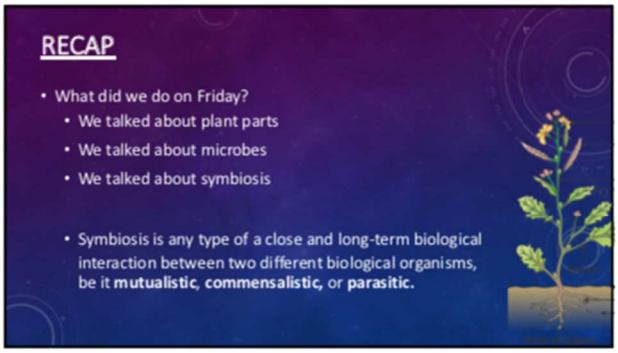
AIM: _____

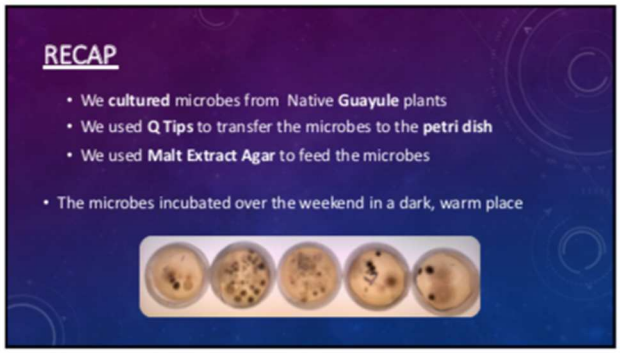
Equipment (What did you use?)

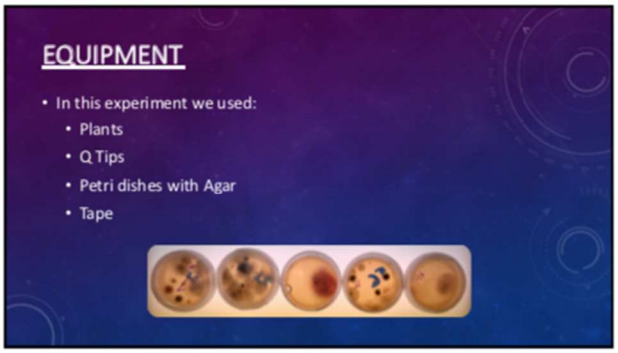
Method (What did you do?)

Results (What happened?)

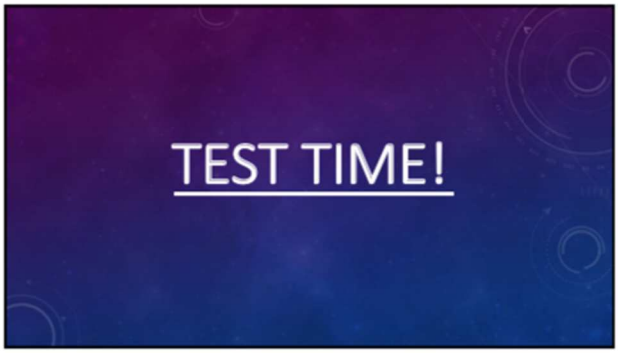
Supplemental File 6: Results_Presentation_ppt

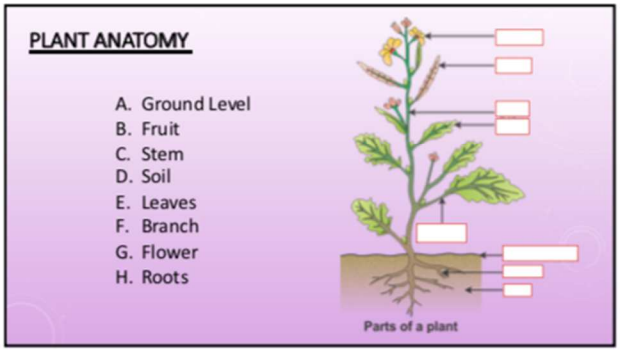






Results_Presentation_ppt





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