LOCKING DOWN THE DUST:

EXPLORING THE ASSOCIATION BETWEEN *COCCIDIOIDES*

POSADASII AND BIOLOGICAL SOIL CRUSTS

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ABSTRACT

LOCKING DOWN THE DUST: EXPLORING THE ASSOCIATION BETWEEN

COCCIDIOIDES POSADASII AND BIOLOGICAL SOIL CRUSTS

MARIEKE RAMSEY

Valley fever, or coccidioidomycosis, is the disease caused by two endemic soil-inhabiting

fungal pathogens, *Coccidioides immitis* and *C posadasii*, found throughout arid regions of the

southwestern United States, Mexico, Central, and South America. Humans and other animals

contract the disease through the inhalation of infectious spores (arthroconidia) from the

environment, and 40% of exposed individuals develop symptoms ranging from mild pneumonia

to severe disseminating disease that could lead to death if untreated. There has been little effort

to reduce exposure to Valley fever through environmental remediation and restoration of natural

soil communities. Biological soil crusts (biocrust) are communities of cyanobacteria, lichens,

mosses, liverworts, and many other microorganisms that live in and bind the top mineral soil

layer in dryland ecosystems. These communities provide critical ecosystem functions including

C and N fixation, soil stability against wind and water erosion, hydrological cycling, and thermo-

tolerance. Restoration of biocrust in areas of high *Coccidioides* endemism could reduce Valley

fever incidence via several mechanisms: 1) stabilizing the soil surface with biocrust should

reduce the potential of *Coccidioides* arthroconidia to aerosolize and reduce fungal burden in the

airborne dust; (2) directly decreasing the abundance of *Coccidioides* by changing the soil habitat

to be less favorable for its growth, and/or (3) decreasing the abundance of *Coccidioides*

indirectly by creating antagonism or competition from a more diverse and active soil

microbiome. Our overarching research objective is to determine if soil remediation with biocrust

can reduce the occurrence of *Coccidioides* spp. in the air and/or soil by stabilizing the soil surface, reducing associated dust, and increasing below-ground microbial competition.

For the current study, I took the following approaches to address the above questions. In the field, biocrust restoration plots were sampled as well as neighboring rodent burrows for the detection of *Coccidioides* spp. All restoration plots had no detection of *Coccidioides* spp. while 10% of rodent burrows surrounding the restoration site were positive, indicating *Coccidioides* spp. is found in the area, and soils are more likely negative where biocrust is present. An *in vitro* preliminary experiment was conducted to test if biocrust reduced arthroconidia aerosolization between three treatment groups: no biocrust, disturbed biocrust, and full biocrust coverage. These findings provide evidence that restoration of natural dryland communities can decrease the human fungal pathogen burden in soil and dust. Management practices that reduce the disruption of soil surfaces and/or restore disturbances with biocrust may reduce the abundance of airborne soil pathogens, and thus reduce the spread of endemic pathogen ranges.

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INTRODUCTION

Coccidioidomycosis, otherwise known as Valley fever, is a fungal disease caused by *Coccidioides immitis* and *Coccidioides posadasii*. Both species are soil-dwelling fungi that are endemic to the arid and semi-arid deserts of the western United States (Kollath et al., 2019; Barker et al., 2019), Mexico, and parts of Central and South America. Genetically distinct populations of *C. immitis* inhabit Central and Southern California, Northern Mexico and has been detected recently in southeastern Washington state (Kollath et al., 2019; Barker et al., 2019; Tiexeria & Barker, 2016; Gorris et al., 2019; Chow et al., 2021). For *C. posadasii*, genetically isolated populations are found in Arizona, parts of Mexico, New Mexico, Texas, Central, and South America (Kollath et al., 2019; Barker et al., 2019; Tiexeria & Barker, 2016; Gorris et al., 2019). Valley fever has gained attention in the United States due to both a steady increase in cases primarily in southern Arizona and the Central Valley of California and a newly discovered novel genotype identified in the state of Washington, which is significantly outside the current defined endemic area. It is estimated in the endemic area of the North American Southwest (California, Nevada, Arizona, Utah, and New Mexico) that incidences were 122.7 cases per 100,000 people (Benedict et al., 2019).

Valley fever is an environmentally acquired disease (i.e., not communicable) and infection dynamics are inherently influenced by environmental changes such as climate and land use (Gorris et al.,2018; Gorris et al., 2019). The fungal pathogen is endemic in regions with heavy intermittent rains and hot, arid summers (Johnson et al., 2014; Coopersmith et al., 2017; McCotter et al., 2019) and is found primarily in alkaline soils with high surface salinity (Elconin et al., 1964; Swatek, 1970; Lacy and Swatek, 1974). *Coccidioides* is a dimorphic fungus

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spending part of its life within in the saprobic cycle in soils as mycelia with alternating spore-containing cells known as arthroconidia (Figure 1, Kollath et al., 2019; Barker et al., 2019; Diep & Hoyer, 2020). *Coccidioides* growth and abundance are influenced by environmental conditions, proliferating during wet periods (Kollath et al., 2019; Barker et al., 2019; Gorris et al., 2019; 2019; Diep & Hoyer, 2020). When water is limited and soils are dry, environmental disturbances (e.g., livestock grazing, damage from vehicles, extreme dusting events, etc.) allow spores to be discharged into the atmosphere. These conidia that are suspended in the air are the causative agent for infection when inhaled. Conidia enter the lungs and, in the lungs, begin to differentiate into a spherule phase, developing into an endospore that replicates through endosporulation (Johannesson et al., 2006). Endospores mature and burst causing the release of more spores inside the host. This is known as the parasitic lifestyle of the disease.
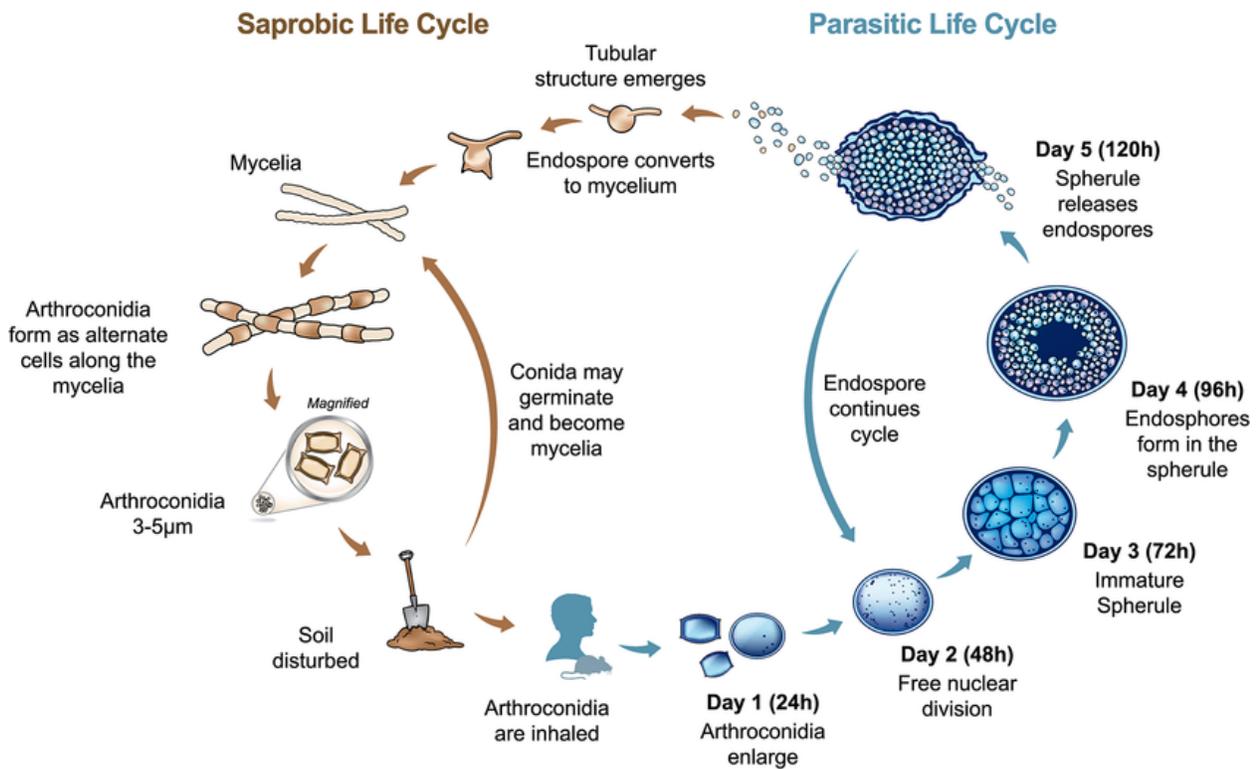
Life Cycle of *Coccidioides*

Figure 1. Life cycle of *Coccidioides*. Figure from: Lewis ERG, Bowers JR, Barker BM (2015)

Dust Devil: The Life and Times of the Fungus That Causes Valley Fever. PLoS Pathog 11(5):

e1004762. https://doi.org/10.1371/journal.ppat.1004762

In 60% of cases, the infection remains asymptomatic or presents mild flu-like symptoms

and is cleared by the host with no medical intervention, and the case is likely not diagnosed or

reported to state or federal health services (Saubolle et al., 2007). In 40% of cases, patients

present mild to severe flu-like symptoms that can become chronic. Less than 5% of these

symptomatic cases develop severe disseminated infection (Saubolle et al., 2007). Dissemination

can cause a wide variety of symptoms, including life-threatening meningitis. Patients of Asian,

non-white Hispanic, or Black ethnic backgrounds, pregnant women in the third trimester, and

immunocompromised patients appear to be at significantly higher risk for disseminated disease compared to other populations (Saubolle et al., 2007; Hector et al., 2011; Huang et al., 2012; Noble et al., 2016; Benedict et al., 2019).

Although there is still debate around the exact role of dust and amount of airborne conidia, Valley fever is linked to dust emissions where higher airborne particulate matter is linked to a higher incidence of disease (Kollath et al., 2022). PM10 has been shown to be positively correlated with increased Valley fever cases and there is evidence that dust events and soil-disturbing activities lead to an increase in infection (Kollath et al., 2022; Tong et al., 2017; Tong et al., 2022; Freedman et al., 2018; Laws et al., 2018).

Coccidioides spp. has also been shown to be associated with desert mammals and their burrow systems. Multiple studies have shown that the probability of detecting the fungus in the environment is greatly increased when collecting soil from within rodent burrows as opposed to outside of the burrow (Kollath et al., 2020; Barker at al., 2012; Emmons, 1943) supporting an endozoan hypothesis (Taylor & Barker, 2019). This evidence suggests that burrowing rodents or the burrows themselves may be an important reservoir for the pathogen in the environment.

Current estimates of dryland coverage (~41% of terrestrial ecosystems) predict drylands are expected to expand in global area by 11-23% by 2100 (Huang et al., 2016) and are one of the most sensitive ecosystems to climate and human activities (Reynolds et al., 2007). Projected climate models predict an increase in annual temperature and shifts in precipitation patterns that favor an expansion of *Coccidioides* spp. environmental niche and widen the defined endemic range (Gorris et al., 2019; Gorris et al., 2018). This can impact disease dynamics, especially in

fragile areas where degradation of the natural environment is occurring, human population is growing, and urbanization is increasing.

Biocrusts are diminutive communities that are composed of lichens, bryophytes, cyanobacteria, fungi, liverworts, and other microorganisms. They bind and live in the top layer of mineral soil predominantly in drylands. Often inhabiting interspaces between and under plant canopies, it is estimated that up to 70% of the soil surface within some dryland ecosystems are colonized by biocrusts (Rutherford et al., 2017). Biocrusts create a thin cohesive layer that interacts with the soil surface minerals (Weber et al., 2022; Bowker et al., 2016) and enhance soil quality by (1) increasing fertility through N and C fixation (Coe et al., 2012; Antoninka et al., 2022; Savies et al., 2016), (2) reducing wind and water erosion through aggregating soil particles (Gao et al., 2020; Borrelli et al., 2017; Rodriguez-Caballero et al., 2012; Mazor et al., 1996; Evans et al., 1993), and (3) redistribution and enhanced containment of soil water to benefit neighboring vegetation (Chamizo et al., 2016; Bowker et al., 2013).

Biocrusts can tolerate stressors that are common within dryland ecosystems due to the ability to suspend metabolic activity during periods of no moisture and reactivate in response to minor rain events (Coe et al., 2012; Antoninka et al., 2022). However, biocrusts are vulnerable to specific global change stressors like reduced rainfall and greater evapotranspiration due to higher temperatures/lower moisture (Coe et al., 2012). The decrease in the length of hydration events increases metabolic respiration compared to reactivating metabolic activity and net primary productivity (Coe et al., 2012). Recent studies have indicated that increased aridity associated with climate change will reduce the diversity of soil microorganisms causing a shift in community composition (Ferrenberg et al., 2015; Delgado-Baquerizo et al., 2018; Maestre et al.,

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2015). Climate manipulation treatments suggest that climate change may have dramatic effects on biocrust community composition by eliminating key species of mosses and lichens, which are large contributors to biogeochemical and hydrological functions in drylands (Rutherford et al., 2017; Ferrenberg at al., 2015; Maestre et al., 2015; Reed et al., 2012; Escolar et al., 2012). This climate-induced loss of mosses and lichens in favor of early successional cyanobacteria-dominated biocrusts also reduces the characteristically dark, textured soil surface. Thus, a shift in biocrust community composition could cause rapid alteration of dryland albedo and energy balance by returning energy to the atmosphere that was once absorbed by the dark biocrust surfaces (Rutherford et al., 2017; Benlap, 1995; Matthias et al., 2000; Nash, 1996).

In addition to climate change stressors, land use changes with physical disturbances (e.g. livestock grazing, damage from vehicles) have been shown to reduce the diversity and abundance of lichens and mosses in biocrust communities which play an important role in ecosystem function (Rutherford et al., 2017; Chamizo et al., 2016; Bowker et al., 2013). This can lead to decreased water availability to neighboring vascular plants through decreased water infiltration into the soil (Maestre et al., 2015). The loss of biocrust can disrupt the net primary productivity, and fertility of soil through soil erosion, as well as the loss of the biota that perform these functions (Rutherford et al., 2017; Bowker et al., 2013; Coe et al., 2012). A shift in biocrust community successional states could cause rapid alteration of dryland albedo (Rutherford et al., 2017; Bowker et al., 2013; Coe et al., 2012; Antoninka et al., 2022) and increase soil loss from wind and water erosion (Coe et al., 2012). This soil that is transferred into the atmosphere may harbor soil-dwelling pathogens like *Coccidioides* spp., and this could influence disease

dynamics. Implementing restoration strategies would be one way to combat the expansion of *Coccidioides* as fragile dryland ecosystems expand.

There is also a link between established biocrust communities and a reduction in the amount of animal burrows present. It was found that in the Arava Valley, burrowing geckos (*Stenodactylyus doriae* and *Acanthodactylus* spp.) strongly preferred fragile early successional crust for burrowing compared to compacted late successional crusts (Zaady & Bouskila, 2002). The establishment of biocrust communities could be an important deterrent of *Coccidioides* in the environment by reducing the number of burrowing animals in an area and therefore reducing the amount of the pathogen load.

The potential link between biocrust and soil-borne pathogens has not been explored thoroughly. The ecology and distribution of *Coccidioides* make it a model disease system to investigate the role of biocrust on soil-borne pathogens in dryland environments. Here we investigate how the importance of healthy biocrust in dryland systems could lead to the reduction of *Coccidioides posadasii*, impacting human health. We hypothesize that diverse healthy intact soil communities such as biocrust stabilize surface soil and can reduce the potential of infectious *Coccidioides* arthroconidia to aerosolize. In addition, we hypothesize that biocrust decreases the abundance of *Coccidioides* directly by reducing the favorable habitat, or indirectly by creating competition or though antagonistic or synergistic interactions.
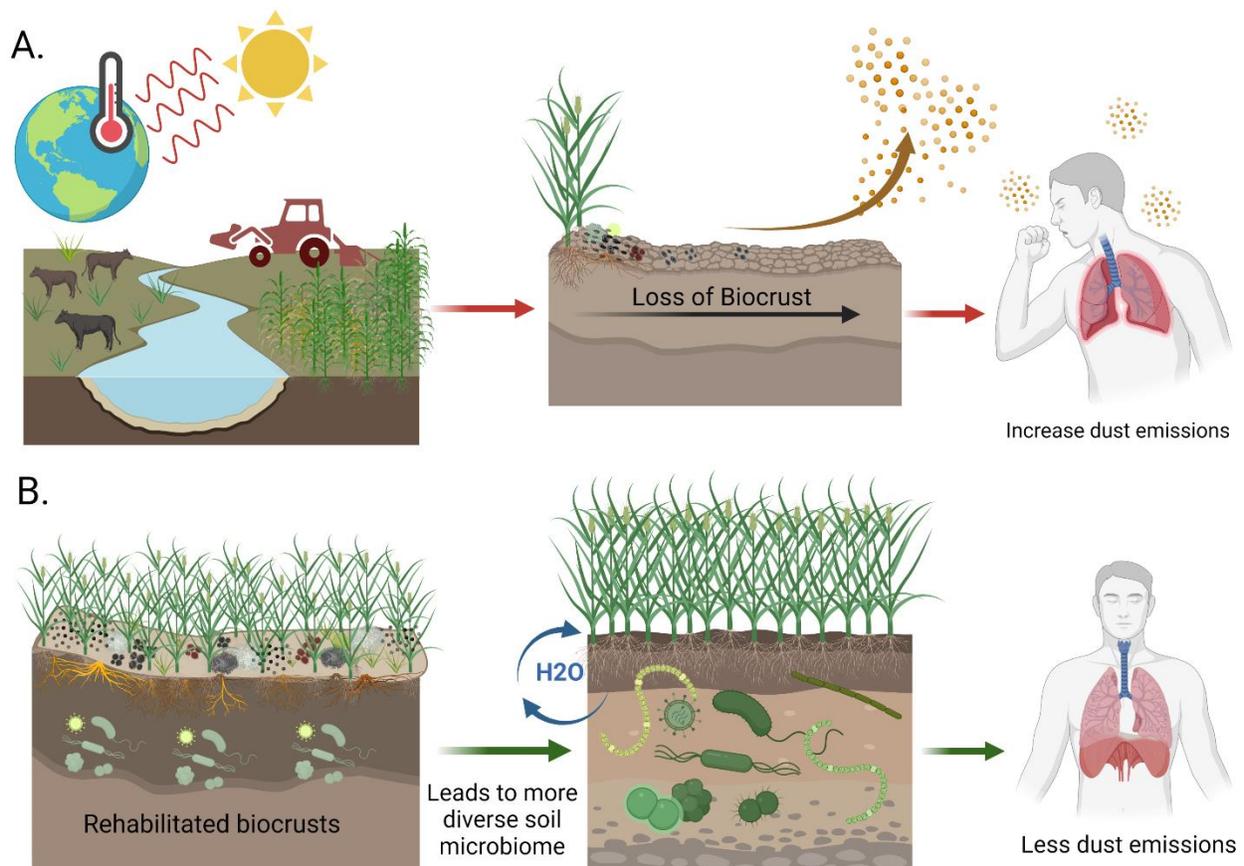
Figure 2. Overview of climatic and anthropogenic stressors on biocrust communities vs. the benefits of healthy intact biocrust communities. A) Climatic shifts and anthropogenic disturbances deplete biocrust communities and loosen soils allowing soil pathogens to aerosolize and impact disease dynamics. B) Rehabilitation of biocrust communities provide increase nutrient cycling and promote a healthy soil microbiome leading to an abundant plant community while also locking down soil which may be harboring pathogens.
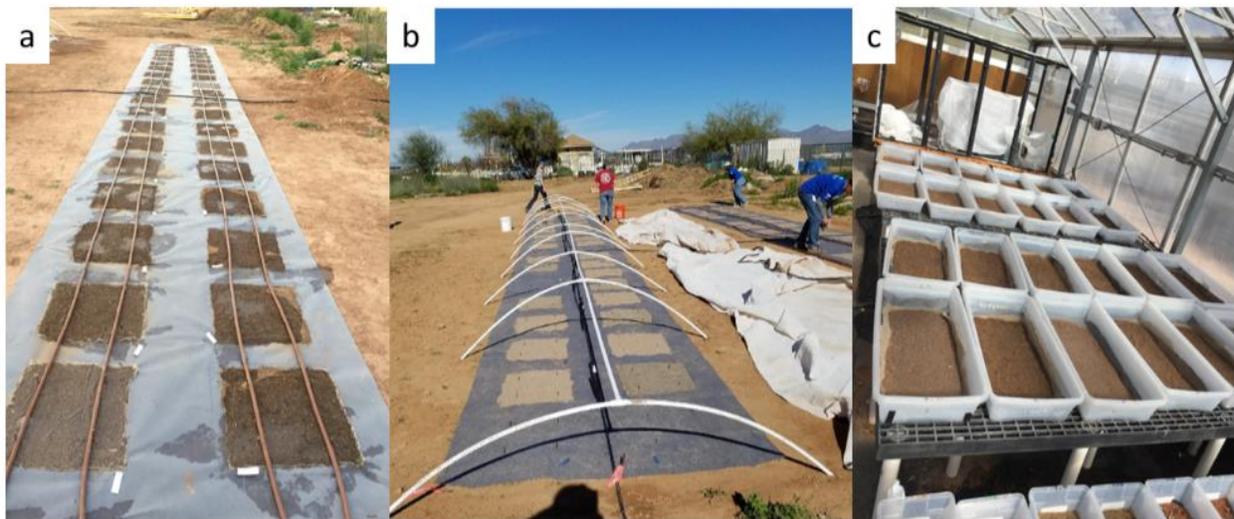
METHODS

Field Experiment

Surface soil samples were collected in Arizona at the McDowell Sonoran Conservancy within and surrounding a biocrust restoration site in north Scottsdale in September 2021 (pre-winter) and in April 2022 (post-winter). Soil was collected using a garden trowel or kitchen spoon depending on soil depth and put directly into sterile 120-milliliter (4 oz.) specimen containers (Thermo Scientific™ Samco™ Wide-Mouth Bio-Tite™); trowels and spoons were sterilized with HYPE-WIPE™ bleach towelettes between soil samples.

Twenty randomly selected animal burrows surrounding the biocrust restoration transect. Samples from burrows were taken from inside animal burrows as higher *Coccidioides* detection rates are associated with rodent burrows (Kollath et al., 2020). Soil collected from inside animal burrows were taken from approximately 10 centimeters into the burrow entrance (depending on the size of the burrow). This depth was determined by the depth of the burrow, the surface soil samples were taken at 10cm depths.

Sixty biocrust plots were sampled in September 2021 (pre-winter) and in April 2022 (post-winter). These biocrust restoration plots varied in three cultivation methods: two field methods and greenhouse cultivation using live native soil or bare sand addition and with and without addition of a base layer of jute (added to keep soil in place) (Figure 3). A mixed community inoculum salvaged Fraesfield trailhead in the Sonoran Desert of Arizona was used. Composite soil samples of about 5cm depths were taken from the four corners of each biocrust restoration plot (Figure 4). Due to the lack of rodent burrows surrounding the restoration site and difficulty in detecting the pathogen via random surface soil sampling compared to targeted sampling, more biocrust restoration plots were sampled to increase the chance of observing positivity from surface soil sampling. Biocrust plots were surveyed for percent coverage within

the center 25cm x 25cm of the plot using a grid point intercept method with 36 points (Figure

5B). Cyanobacteria, moss, and lichen cover were estimated, and plant cover measurement were

recorded using standard cover classes. A wet aggregate stability test was conducted on the plots

to test soil stability (Herrick et al., 2006) (Figure 5C).

Figure 3. Biocrust cultivation methods. Three cultivation methods: two field methods and

greenhouse cultivation using live native soil or bare sand addition and with and without addition

of a base layer of jute (added to keep soil in place). A mixed community inoculum salvaged

Fraesfield trailhead in the Sonoran Desert of Arizona was used in all three cultivation methods.
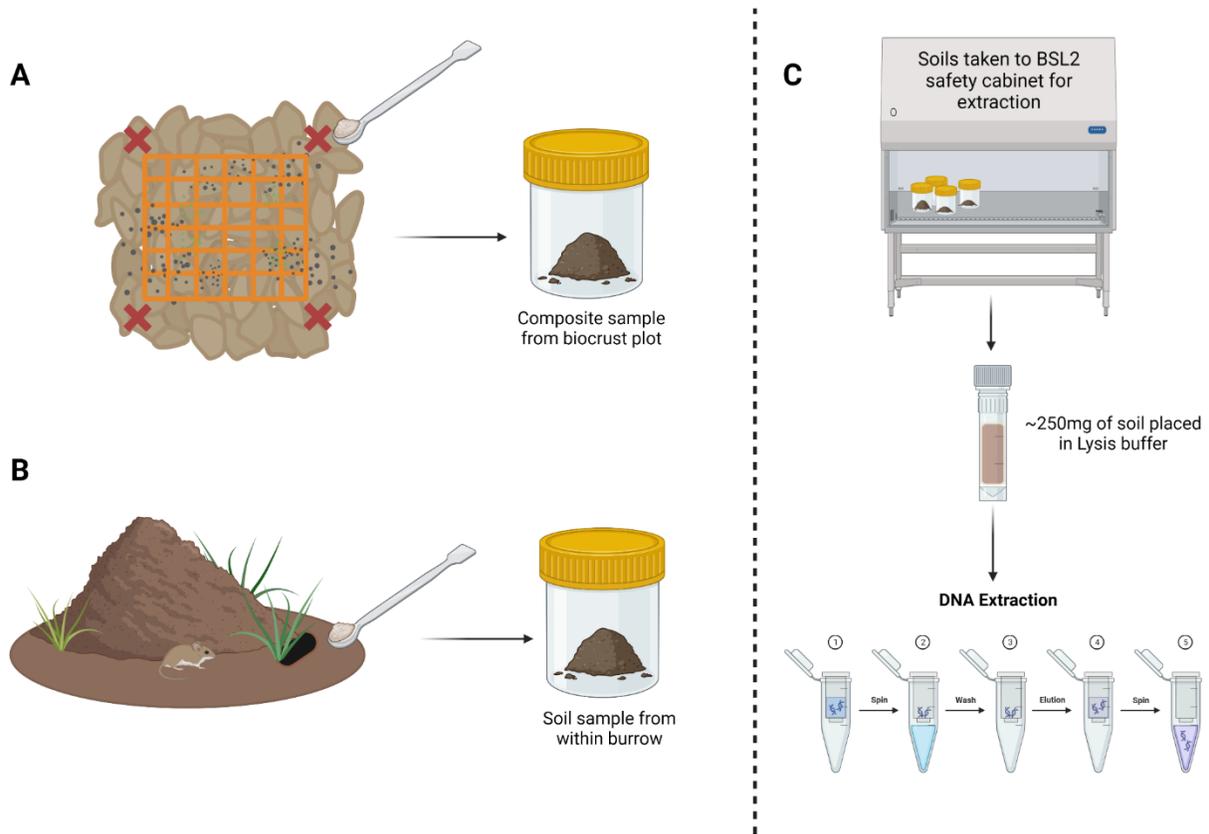
Figure 4. Workflow of soil collection and DNA extraction. A) Composite samples were taken at 5cm depths surrounding the biocrust plots from the four corners of the survey grid indicated by the red X's. B) Soil samples from the rodent burrows were taken at 10cm depths from inside the burrow. Both samples from the biocrust plots and rodent burrows were stored in sterile collection cups. C) Soils extracted for DNA inside a BSL2 safety cabinet following the QIAGEN DNeasy PowerSoil extraction kit methods.
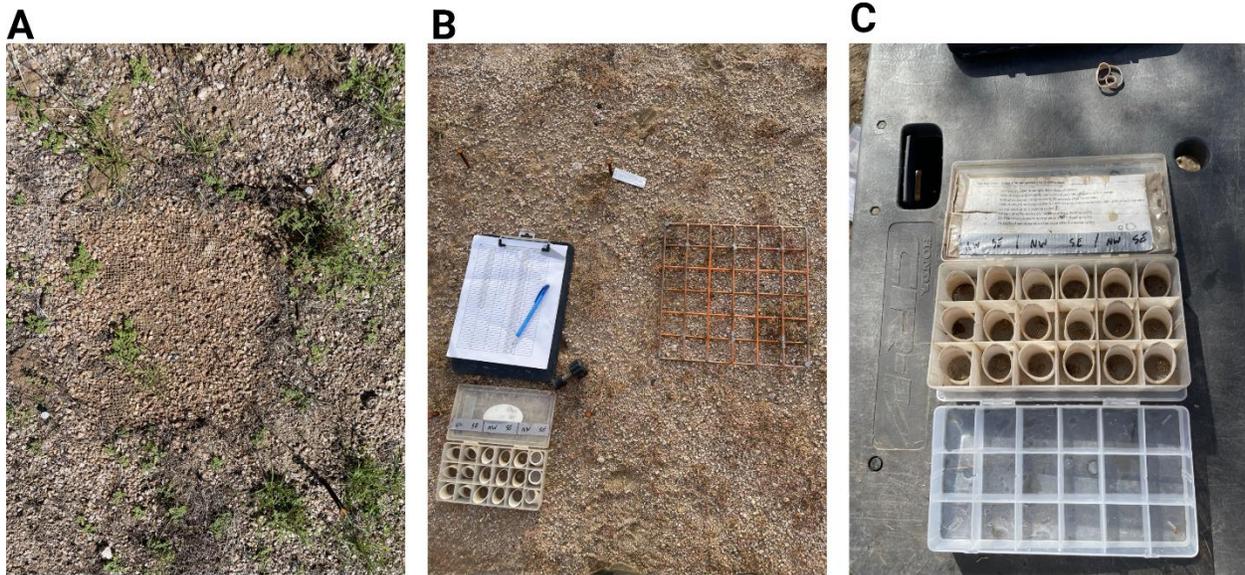
Figure 5. Field biocrust plots. A) Synthetic light gray landscape fabric placed across soil surface to keep vegetation out (used as the base layer in the greenhouse units), 1L of substrate (native soil collected from near the biocrust collection area and sieved to remove rocks or commercial play sand (Quikrete graded and washed coarse sand)), and fine woven jute fabric (only in jute treatment; Viagro 100% natural burlap) cut to cover the 0.25m^2 area. B) Percent coverage and community estimate of biocrust plot using 36-point point grid. C) Wet aggregate stability test.

Greenhouse Biocrust Cultivation

Within the Northern Arizona University Greenhouse Complex, salvaged crumbled biocrusts from the Sonoran Desert were placed over commercial play sand in nested basins (85cm X 30cm) with water wicking over sand and biocrust form below. Inoculated basins received 375mL of homogenized biocrust. The bottom container has a drain hole and a ¼ inch irrigation tube entering the basin. The top basin has small wicking holes drilled into it. The bottom is

covered with a wicking weed cloth. The wicking system used in the nested basins can be

described in Doherty et al. 2015. Watering ran at 6am, 12pm and 4pm for 30 second intervals for

3 months to keep the plots hydrated. Synthetic light grey landscape fabric was placed over the

basins to reduce UV stress. The greenhouse was maintained at 20C$\pm$2C, with a relative humidity

varying between 20-45%.
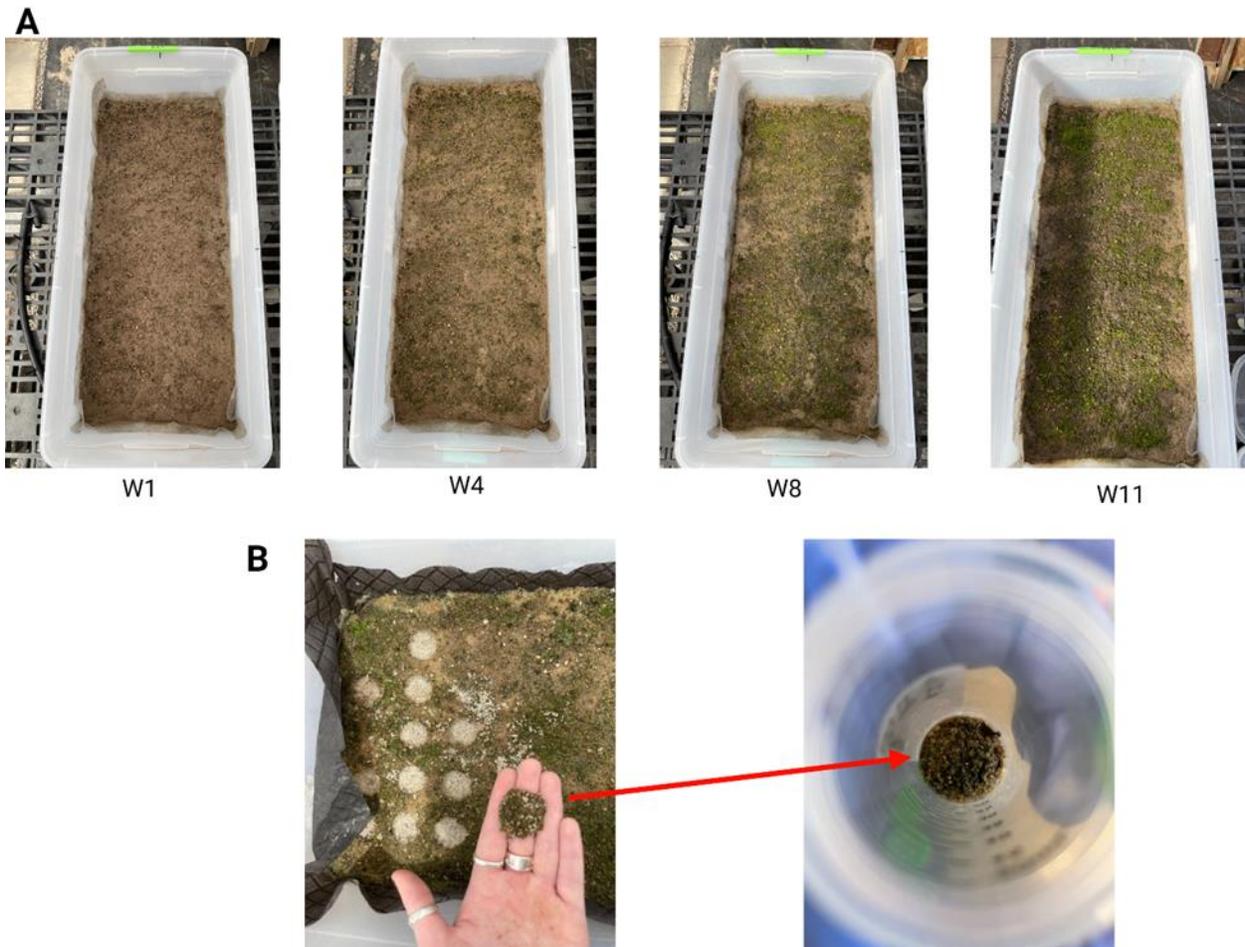
Figure 6. Greenhouse grown biocrust for *in vitro* experiment. A) Progression of basin biocrust

grown in Northern Arizona University Greenhouse Complex from week 1 to week 11 (A).

Biocrust samples transferred from basin to vented 50mL centrifuge tube at week 11.

In Vitro Experiment- Trial 1

In 50-mL vented centrifuge tubes (CELLTREAT Scientific Products^{TM}), autoclaved desert soils were inoculated with three different biocrust coverage treatments; full-compacted coverage (n=7), disturbed/broken coverage (n=7), and no coverage (n=6). Crust communities were watered with a spray bottle weekly for viability after transferring from greenhouse basins as this is considered a disturbance of crust community. After a month incubation period, the 50mL centrifuge tubes, samples were taken into a level 3 biological safety lab and inoculated with 10^3 arthroconidia of *Coccidioides posadasii* Silveira strain below the crust layer into the soil with a 0.5mL syringe. A cellulose filter was placed above the surface layer for 14 days to capture aerosolized arthroconidia at room temperature (20-25C) in a biological safety cabinet. We quantified presence/absence of arthroconidia with qPCR on the filter after the 14-day incubation period.
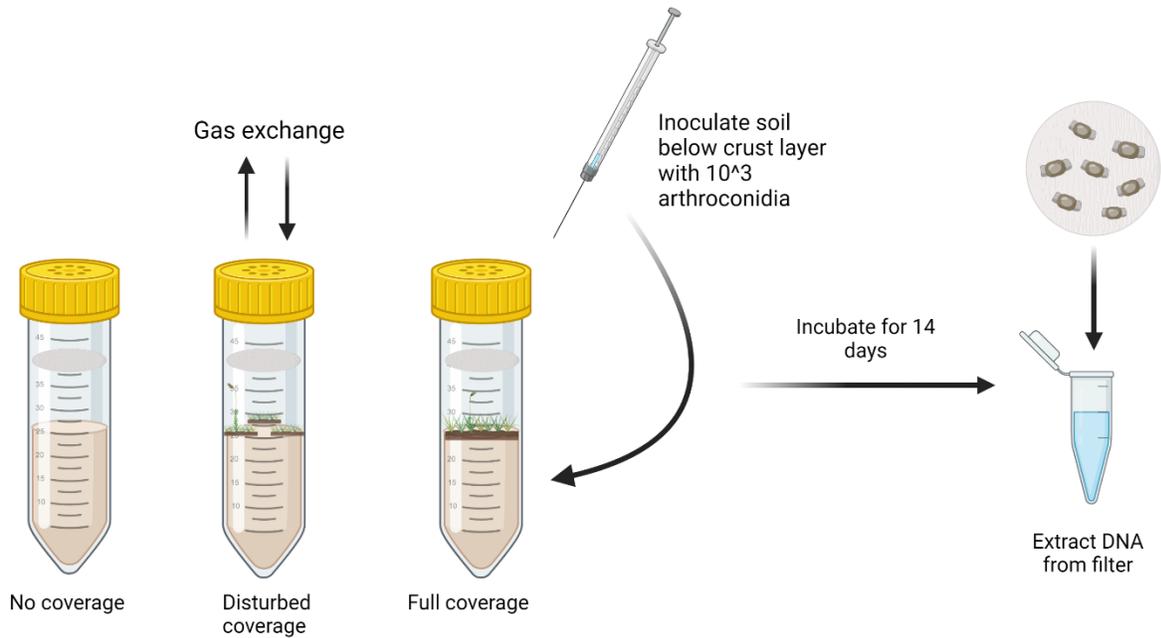
Figure 7. Methods workflow of *in vitro* trial 1. Treatment groups were inoculated with 10^3 arthroconidia of *C. posadasii* Silveira strain and incubated at room temperature in a biological safety cabinet for 14 days. Filter was washed for DNA after the 14-day incubation period.

In Vitro Experiment- Trial 2

In 50-mL vented cap CELLTREAT Scientific Products centrifuge tubes, autoclaved desert soils from McDowell Sonoran Conservancy and Bunyan Wash in Arizona were inoculated with three different biocrust coverage treatments; full-compacted coverage (n=8), disturbed/broken coverage (n=8), and no coverage (n=8) for a total of 48 samples. Crust communities were watered with a spray bottle weekly for viability after transferring from greenhouse basins as this

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is considered a disturbance of crust community. After 6-month incubation within the 50mL

vented cap centrifuge tubes, samples were taken into a level 3 biological safety lab for 4 weeks

and were inoculated with 10^3 arthroconidia of *Coccidioides posadasii* Silveira strain below the

crust layer into the soil with a 0.5mL syringe. A cellulose filter was placed above the surface

layer for 14 days to capture aerosolized arthroconidia at room temperature (20-25C) in a

biological safety cabinet. After 14 days, a new filter was placed above the crust layer and

incubated for an additional 14 days. We quantified presence/absence of arthroconidia with qPCR

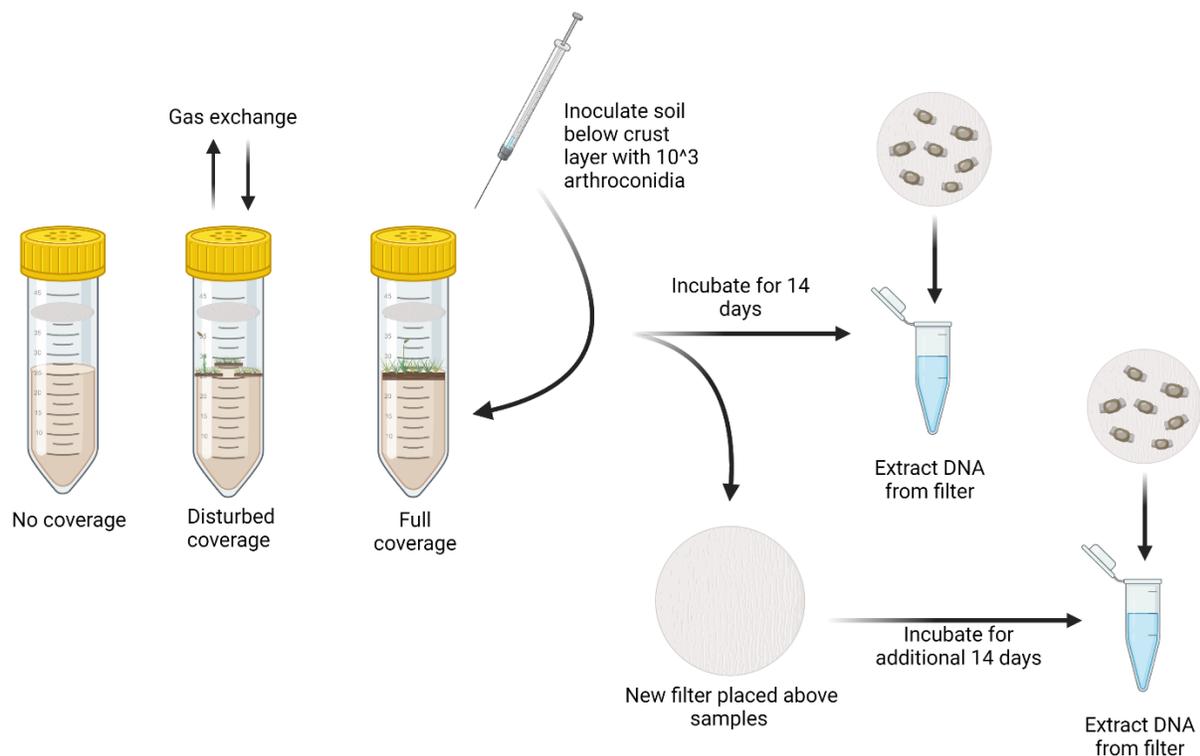on the filter after the 14-day incubation period.

Figure 8. Methods workflow of *in vitro* trial 2. Treatment groups were inoculated with 10^3

arthroconidia of *C. posadasii* Silveira strain and incubated at room temperature (20-25C) in a

biological safety cabinet for 14 days. Filter was washed for DNA after the 14-day incubation period and replaced with a new filter for another 14-day incubation period.

Soil DNA Extraction

DNA from soil samples was extracted using the DNeasy PowerSoil Pro kit (QIAGEN, Valencia, CA). Due to potential infectious propagules in the soil, DNA extractions were performed in a biological safety level two laboratory inside a biological safety cabinet. Appropriate PPE was used consisting of a front gown and gloves (primary and secondary). The manufacturer's protocol was followed except for an additional 10-minute heat step at 65° C was added before the homogenization step (Lauer et al., 2012). Two DNA extractions per soil sample were performed. Approximately 250mg of soil was placed into each tube with lysis buffer.

Cellulose Filter Extraction

DNA from the cellulose filters (labForce®) above crust samples was extracted using the DNeasy PowerSoil Pro kit (QIAGEN, Valencia, CA). Due to potential infectious propagules from the Silveira strain in the filters, DNA extractions were performed in a biological safety level 3 laboratory inside a biological safety cabinet. Appropriate PPE included scrubs, water-proof shows, gloves (primary and secondary), Tyvek suit, face shield with PAPR airflow, and long boot covers. The manufacturer's protocol was followed (Lauer et al., 2012). A single DNA extraction per filter sample was performed.

Molecular Detection of Coccidioides spp. by qPCR

A real-time qPCR-based assay was used to detect *Coccidioides* DNA extracted from the collected soil samples and cellulose filters (Litvintseva et al., 2014) These TaqMan-based assays are highly sensitive and target a repetitive region of DNA that is only known to occur in the two species within the *Coccidioides* genus (Bowers et al., 2019). The CocciDX assay targets a 106-bp sequence present in multiple copies within a transposable element in the genome of both *Coccidioides* species and is specific to this genus (Saubolle et al., 2018; Litvintseva et al., 2014). The assay was performed on the Applied Biosystems QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific). Each reaction was performed in 20 μl-volume containing a mixture of 2X TaqMan Environmental Master Mix 2.0 (Thermo Fisher Scientific), 1x of CocciDX at 100μM concentration of Cocci Assay oligo/probe mix, and two μl of DNA template. PCR cycling conditions were performed as follows: Two minutes of activation step at 50° C, 10 minutes at 95° C for denaturation step, followed by 45 cycles of 15 seconds at 95°C and one minute at 60° C. Each sample was performed in triplicate and the average Cycle threshold (Ct) value was taken if amplification occurred. Control samples included purified DNA from *Coccidioides posadasii* strain Silveira as a positive control and molecular grade sterile water was used as negative no-template DNA control. Soils were considered positive if the Ct value was < 40 and negative for a Ct value >40. A sample was considered "low positive" if Ct value ranged from 38-40, "moderately positive" if Ct value ranged from 35-37, and "high positive" if Ct value ranged form 25-34. Any Ct value <25 is considered "very high positive" detectability.
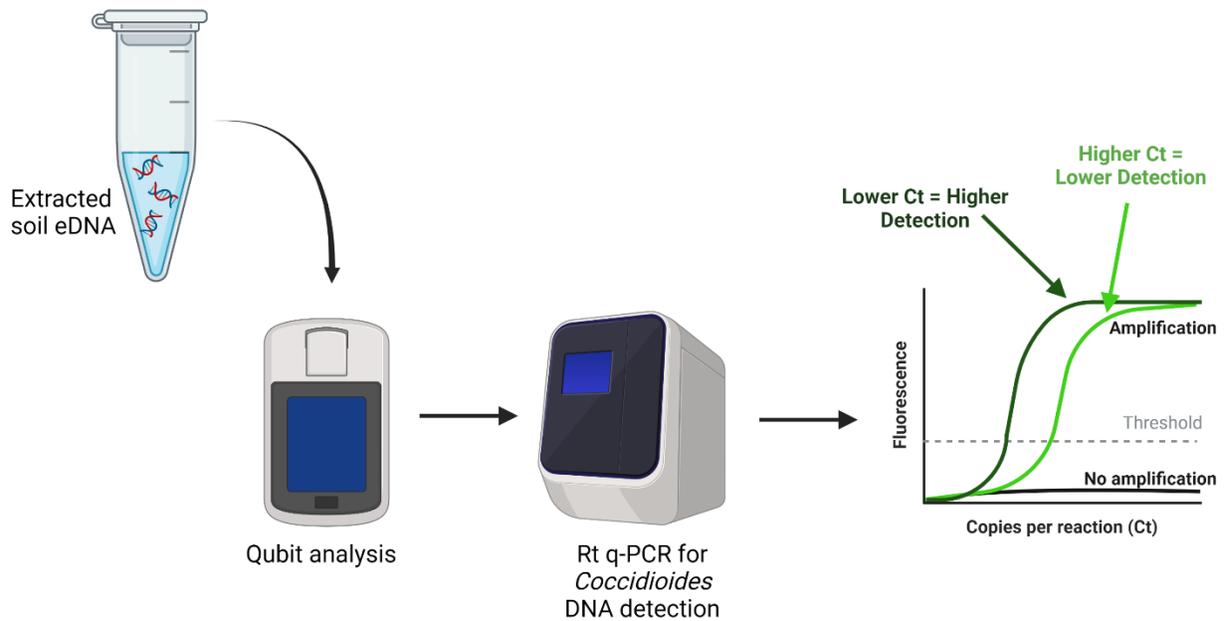
Figure 9. Schematic of molecular detection of *Coccidioides posadasii* through real-time qPCR.

RESULTS

Field Experiment

Of the 120 samples taken from biocrust plots pre- and post-winter, *Coccidioides* was not detected in any of the composite samples (0% positivity) (Figure 10). However, 2 of the 20 rodent burrows sampled surrounding the biocrust plot had a positive read for *Coccidioides* spp. (10% positivity) (Figure 10). Indicating, in this specific area there is a negative correlation between biocrust abundance and the presence of *Coccidioides* at the soil level. This also furthers the hypothesis that burrowing animals have an association with *Coccidioides* within the soil.

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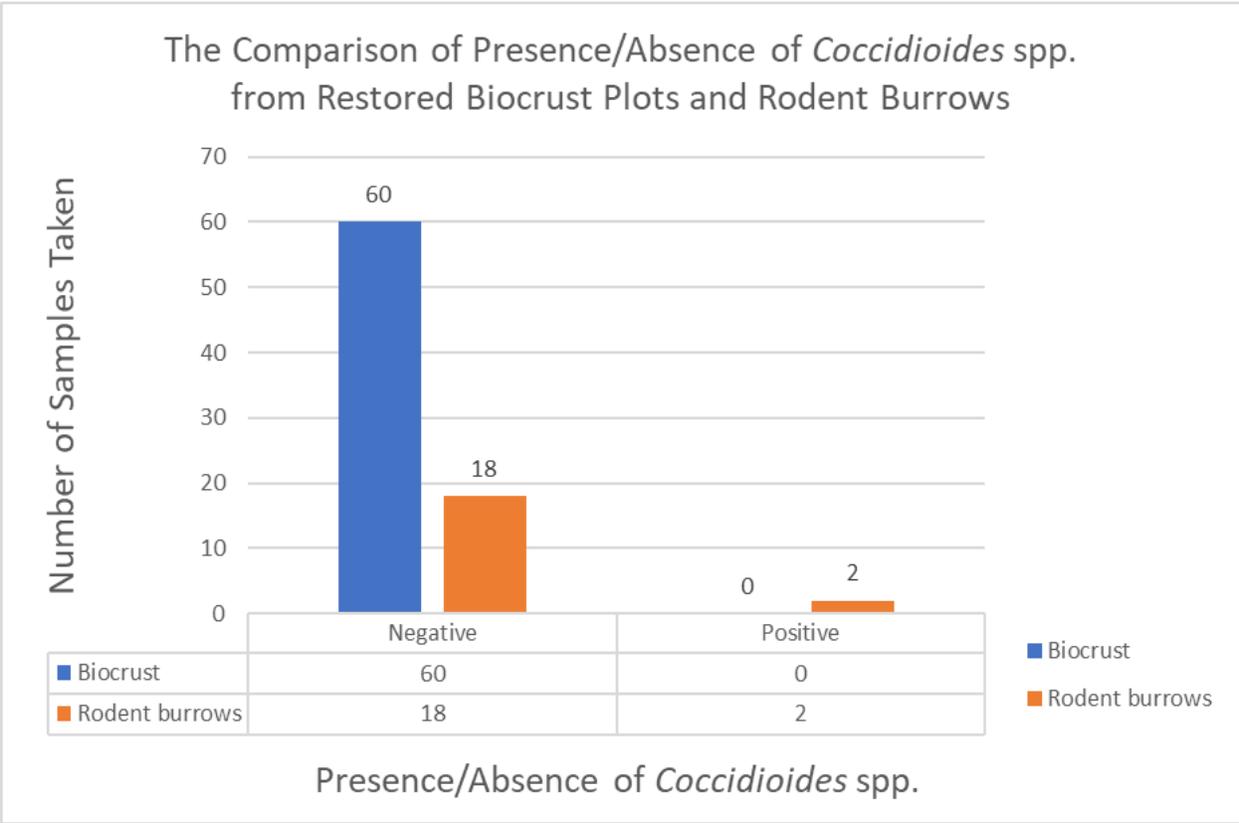
Figure 10. The comparison of presence/absence of Coccidioides spp. from soil samples taken

from restored biocrust plots and rodent burrows.
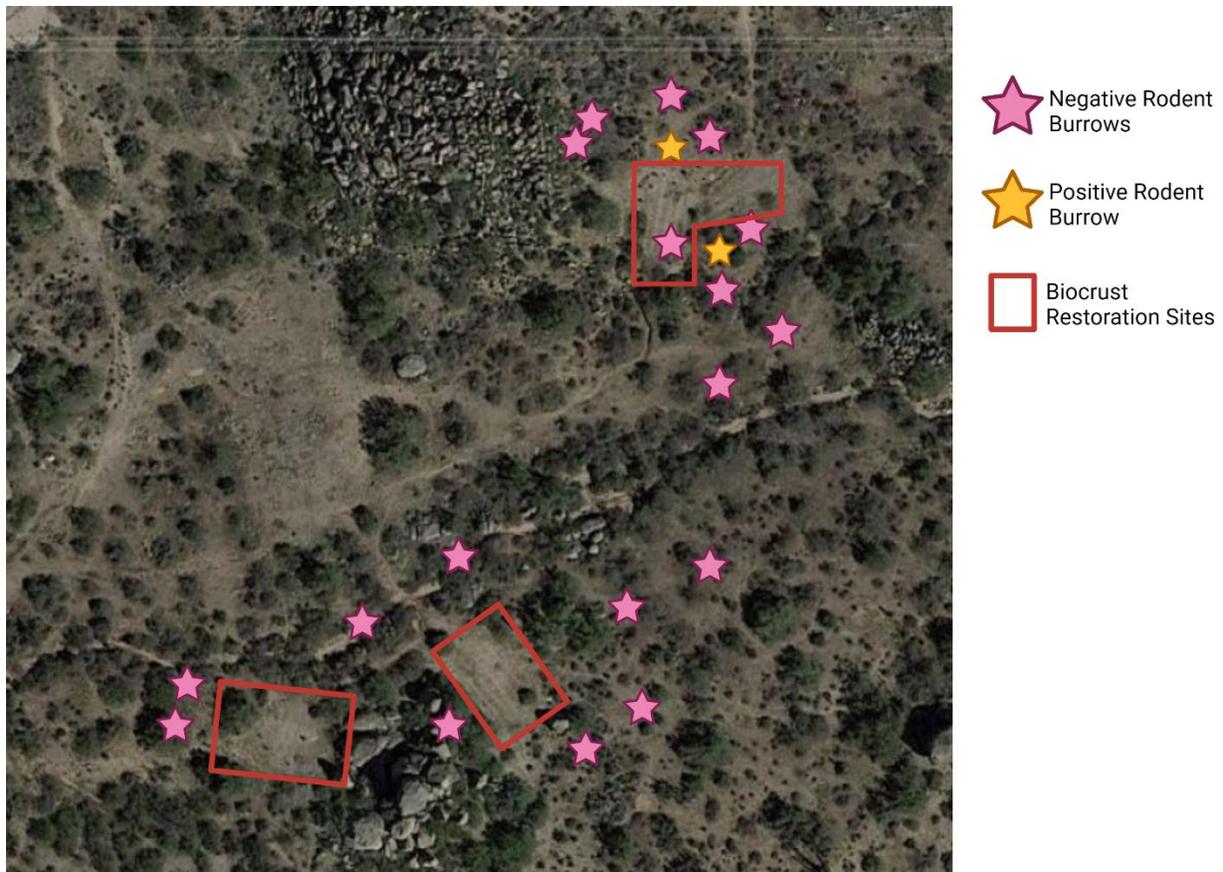
Figure 11. Field site in north Scottsdale. Pink stars indicate location of sampled rodent burrows that were negative for the presence of *Coccidiodes* spp.; Yellow stars indicate location of sampled rodent burrows that were positive for the presence of *Coccidiodes* spp.; Red outlines indicate location of the three biocrust restoration sites that encompasses the 60 biocrust plots that were sampled from for presence/absence of *Coccidiodes* spp.

In Vitro Experiment- Trial 1

No significant difference was found among all three treatment groups. There was a detection of arthroconidia transfer to the cellulose filter in all three treatment groups. Overall, for the no coverage group 4 out of the 7 centrifuge tubes had a positive read (57.14%), 5 out of the 7

disturbed/broken had positivity (71.43%), and 5 of the 7 full coverage group (71.43%) were

positive (Figure 9). However, Ct values did vary between treatment groups. Only the no crust

treatment group had moderate detection (Ct 35-37) of Silveira on the air filter (Figure 9). The

disturbed and full treatment groups either had low detection (Ct 38-40) or no detection (>40) at

all on the air filters. Although the results are not significant, this experiment showed that the

experimental design works and a decrease in detection from the disturbed and full treatment

groups compared to the no crust treatment group.

	No	Disturbed	Full
S1	Yellow	Green	Green
S2	Green	Green	Green
S3	Green	Yellow	Yellow
S4	Yellow	Yellow	Yellow
S5	Orange	Yellow	Yellow
S6	Orange	Yellow	Green
S7	Orange	Yellow	Yellow

Key:	
No detection (>40)	Green
Low (Ct 38-40)	Yellow
Moderate (Ct 35-37)	Orange
High (Ct 25-34)	Red
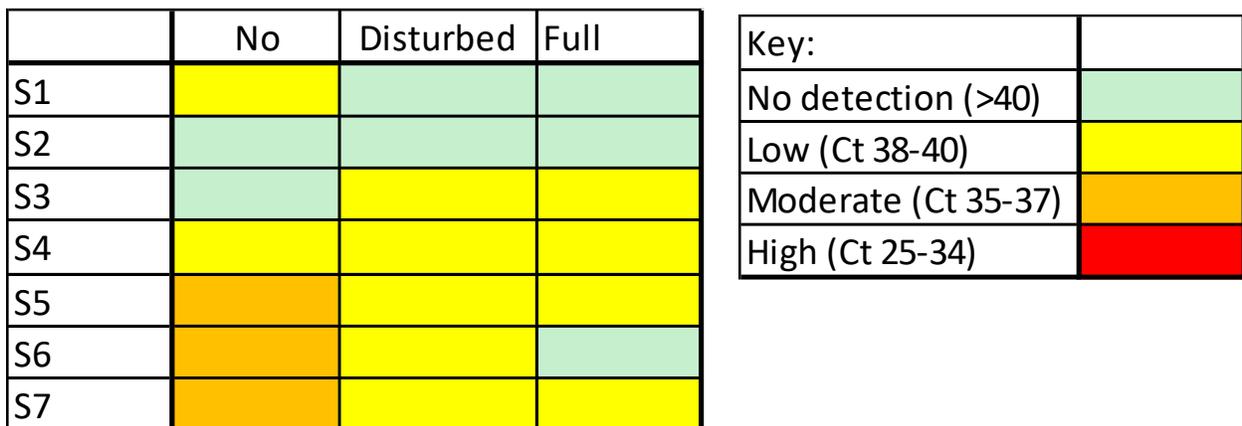
Figure 12. Heat map of *Coccidioides* qPCR Ct results. Green represents no arthroconidia

detection on the air filter, yellow representing low detection (Ct 38-40), orange representing

moderate detection (Ct 35-37), and red representing high detection (Ct 25-34).

In Vitro Experiment- Trial 2

Of the total 48 cellulose air filters, there was no detection of conidia on any filters, including the

treatment group with no greenhouse grown biocrust for both soil types. There was no observed

fluctuation of conidia aerosolization observed between the first 14 days and second 14-day

incubation period.

DISCUSSION

Climate change and increased soil disturbance due to anthropogenic disturbances in endemic areas has been hypothesized as the cause of increased morbidities due to Valley fever (Coulson et al., 2017). Biocrust communities have been determined to reduce global atmospheric dust emissions by preventing aerosolization of soil particulate matter by entangling them within hyphae and rhizines as well as secreting extracellular polymeric substances which aggregate soil particles (Rossi & De Philipps, 2015). Stable soils keep dust that may be harboring human pathogens, like Valley fever, out of the atmosphere. To date, no one has attempted to address Valley Fever through environmental remediation and restoration of natural soil communities.

In this study, we analyzed biocrust plots and soils surrounding the biocrust plots for the presence of *Coccidioides* spp. These biocrust plots varied in percent coverage and were dominated by cyanobacteria and lichen communities with trace amounts of bryophytes; representative of natural occurring biocrust in the Arizona arid ecosystem. Of the 60 pre-winter samples taken from biocrust plots was determined to be negative for the presence of *Coccidioides* spp. and remained negative post-winter. However, *Coccidioides* was detected in animal burrows surrounding the biocrust transects. This implies that a location that is known to be positive for *Coccidioides* within the soil, is negative in areas with intact biocrust communities within the overall positive location. Alternatively, differential sampling depth may influence the presence of *Coccidioides,* and future work will address these variables.

The *in vitro* study was conducted to determine if biocrust could inhibit *Coccidioides posadasii* arthroconidia from aerosolizing. Although we did not see a significant difference in aerosolization between treatment groups during the first trial, it is important to note that the experiment is a preliminary study. Researchers are still studying the best methods for biocrust restoration in the desert ecosystem. There is no literature on biocrust cultivation within a vented cap centrifuge tube as well as challenging biocrust with soil-pathogens. Since Silveira strain of *Coccidioides posadasii* is a risk group 3 pathogen, samples had to be inoculated in a level 3 biological safety lab within a biological safety cabinet. Consequently, the biocrust communities could have been rapidly dried out during incubation in the biological safety cabinet where air flow is constant, influencing the efficacy of the experiment. In addition, the level 3 biological safety lab has artificial lighting with no source of natural lighting. Biocrust follows a circadian cycle that influences different metabolic activity by photosynthesizing during the day and undergoing respiration at night (i.e., Diel cycle) (Rajeev et al., 2013; Diamond et al., 2015). The constant stress of photosynthesizing could have influenced the biocrust community and cause cell death. In addition, *Coccidioides* has been observed to develop pigmentation when growing on a plate with circadian cycle present, developing dark rings of growth during the day and lighter rings at night (Heather Mead, personal observation). It is unclear how circadian rhythms influence the ecology of the organisms studied, but this observation should be further investigated.

For the second trial of the *in vitro* study, no conidia were detected on the filters for all treatment groups. This could have been due to the 6-month biocrust colonization period within the vented 50mL centrifuge tubes in the laboratory setting allowing the biocrust communities to

established longer than the previous trial, preventing aerosolization of conidia. Although the no crust treatment group had autoclaved soils, a biofilm could have formed during the 6 months permitting microorganisms to colonize the surface soil and preventing the transfer of conidia into the atmosphere and onto the filter. Conversely, there could have been an inaccuracy when inoculating soils with *C. posadasii* Silveira strain. If Silveira did not penetrate below the crust layer, the surface biocrust/biofilm could have outcompeted the fungal pathogen. It is hypothesized that *Coccidioides* is a poor competitor due to microbial antagonist studies and difficulties culturing directly from soils (Lauer et al., 2019; Kollath & Morales et al., 2023; Elconin et al., 1964). It is unknown whether these antagonistic relationships are due to direct competition for similar resources, secretion of toxic compounds, and/or if *Coccidioides* may be a preferred food source for microorganisms found in the similar ecological niche. In addition, the stock culture of Silveira used could have been non-viable causing no growth when infected into the soil. In future, a real-time qPCR on the DNA extracted from soil should be incorporated to ensure that soils were infected properly and *Coccidioides* is present.

In the future, we plan to repeat the *in vitro* study to increase the sample size, while also optimizing cultivation methods when crust samples are transferred into a lab setting. In addition, an analysis of the presence/absence of *Coccidiodes* in the soils to determine if biocrust is outcompeting *Coccidioides* at the soil level and/or trapping *Coccidioides* within the soil.

While our field data provide evidence that *Coccidioides* spp. have a lower probability of inhabiting soils occupied by intact biocrust soil communities, there is not enough evidence to say that the fungus is not present at a deeper soil horizon. Our data show that the probability of finding the organism within biocrust is significantly less than detecting it within burrow systems.

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A follow up field survey would be beneficial to analyze the abundance of rodent burrows and biocrust in the environment. Is there an association of rodents burrowing in areas of low or no biocrust? Or are these burrowing rodents actively destroying biocrust communities in the environment allowing *Coccidioides* to establish?

Finally, there are no current methods of bioremediation to inhibit the growth of *Coccidioides* in the soil. Kollath & Morales *et al.* 2023 and Lauer *et. al.* 2019 are the only current publications looking at possible microbial antagonists for *C. posadasii* and *C. immitis* respectively (Kollath & Morales et al., 2023; Lauer et al., 2019). Other publications only address the need for long-term dust mitigation efforts without solution around heavily disturbed soils that may harbor this fungal pathogen (Colson et al., 2017; Kollath et al., 2022) and/or increase surveillance of disease incidences (Gorris et al., 2019; Gorris et al., 2023). In addition, there are no fungicides developed to treat soils infected with *Coccidiodes*.

As the majority of fungal infections are environmentally acquired, understanding the ecology, and associated microbial communities of these pathogens is crucial to managing environmental point sources via biocontrol agents. The methods from this study (biocrust and pathogen interaction within a lab setting) may aid in future environmental pathogen research by providing non-invasive and non-detrimental effects on the environment. In the future, we look to optimize the cultivation methods and increase comparative field sampling at multiple known "hot spots" throughout Arizona.

STUDY LIMITATION

It is important to evaluate the limitations in this study. This study brings to light the possibility of combating disease prevalence through bioremediation of arid to semi-arid ecosystems. Only one field site was evaluated for presence/absence of *Coccidioides* around remediated biocrust communities. Although there was a difference in positivity between areas inhabited by biocrust and burrows, there can be variation in soil type, animal activity, biocrust abundance and community abundance, as well as climatic factors and anthropogenic disturbances that could be different in other known positive sites. More survey areas for *Coccidioides* must be analyzed to understand this relationship.

ADDITIONAL TABLES AND FIGURES

Sample	Ct	Ct	Ct	Ct Mean	Group Ct Mean
No Crust 1	Un	39.471	38.693	39.217	
No Crust 2	Un	Un	Un	Un	
No Crust 3	Un	Un	Un	Un	
No Crust 4	37.784	38.647	40.466	38.966	38.07586777
No Crust 5	37.934	Un	Un	37.934	
No Crust 6	36.54	36.49	37.412	36.814	
No Crust 7	37.873	37.697	36.775	37.448	
Disturbed Crust 1	Un	Un	Un	Un	
Disturbed Crust 2	Un	Un	Un	Un	
Disturbed Crust 3	Un	Un	39.839	39.839	
Disturbed Crust 4	38.419	38.6	Un	38.51	38.8832
Disturbed Crust 5	Un	38.447	Un	38.447	
Disturbed Crust 6	38.173	39.877	38.717	38.922	
Disturbed Crust 7	38.715	38.68	Un	38.698	
Full Crust 1	40.202	Un	Un	40.202	
Full Crust 2	Un	Un	40.574	40.574	
Full Crust 3	Un	Un	39.697	39.697	
Full Crust 4	40.202	38.458	37.805	38.822	40.06794419
Full Crust 5	Un	37.873	39.19	38.531	
Full Crust 6	42.875	Un	Un	42.875	
Full Crust 7	39.775	Un	Un	39.775	

Table 1. Ct values for all biocrust treatment groups for *in vitro* trial 1. Each filter DNA was run in triplicate on the QuantStudio and Ct mean was used to develop the result heat map.

McDowell Sonoran Conservancy Soil								
	14 day					28 day			
	Ct	Ct	Ct	Ct Mean		Ct	Ct	Ct	Ct Mean
No Crust 1	Un	Un	Un	Un		Un	40.356	Un	Un
No Crust 2	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 3	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 4	Un	Un	Un	Un		Un	Un	Un	Un
No crust 5	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 6	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 7	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 8	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 1	Un	Un	Un	Un		40.279	Un	Un	40.279
Disturbed 2	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 3	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 4	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 5	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 6	Un	Un	Un	Un		40.361	Un	Un	40.361
Disturbed 7	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 8	Un	Un	Un	Un		Un	Un	Un	Un
Full 1	Un	Un	Un	Un		Un	Un	Un	Un
Full 2	Un	Un	Un	Un		Un	Un	Un	Un
Full 3	Un	Un	Un	Un		Un	Un	Un	Un
Full 4	Un	Un	Un	Un		Un	Un	Un	Un
Full 5	Un	Un	Un	Un		Un	Un	Un	Un
Full 6	Un	Un	Un	Un		Un	Un	Un	Un
Full 7	Un	Un	Un	Un		Un	Un	Un	Un
Full 8	Un	Un	Un	Un		Un	Un	Un	Un

Table 2. Ct values for all biocrust treatment groups grown on McDowell Sonoran Conservancy soils for *in vitro* trial 2. Each filter DNA was run in triplicate on the QuantStudio.

	Bunyan Wash Soil								
	14 day					28 day			
	Ct	Ct	Ct	Ct Mean		Ct	Ct	Ct	Ct Mean
No Crust 1	Un	Un	Un	Un		Un	40.356	Un	Un
No Crust 2	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 3	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 4	Un	Un	Un	Un		Un	Un	Un	Un
No crust 5	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 6	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 7	Un	Un	Un	Un		Un	Un	Un	Un
No Crust 8	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 1	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 2	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 3	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 4	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 5	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 6	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 7	Un	Un	Un	Un		Un	Un	Un	Un
Disturbed 8	Un	Un	Un	Un		Un	Un	Un	Un
Full 1	Un	Un	Un	Un		Un	Un	Un	Un
Full 2	Un	Un	Un	Un		Un	Un	Un	Un
Full 3	Un	Un	Un	Un		Un	Un	Un	Un
Full 4	Un	Un	Un	Un		Un	Un	Un	Un
Full 5	Un	Un	Un	Un		Un	Un	Un	Un
Full 6	Un	Un	Un	Un		Un	Un	Un	Un
Full 7	Un	Un	Un	Un		Un	Un	Un	Un
Full 8	Un	Un	Un	Un		Un	Un	Un	Un

Table 3. Ct values for all biocrust treatment groups grown on Bunyan Wash soils for *in vitro* trial 2. Each filter DNA was run in triplicate on the QuantStudio.

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