Ben Gurion University of the Negev
Faculty of Natural Sciences
Department of Life Sciences

Germination analysis of natural and ecologically-restored sites in phosphate mining fields in Zin valley, Israel

Thesis submitted in partial fulfillment of requirements for the Master of Science degree

By

Tom Zylberberg

Under the supervision of Prof. Yaron Ziv and Dr. Guy Rotem

February 2020

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Thesis submitted in partial fulfillment of requirements for the Master of Science degree, Ben-Gurion University in the Negev, February 2020

Abstract

Ecological systems are severely damaged through the anthropogenic procedure of mining. Phosphate mining occurs over 200 km^2 of the Negev desert, Israel. However, the effects of the ongoing restoration efforts of the mines have not been studied. Plants and their seed banks have a major role in ecosystem processes, hence calling for main consideration in studying ecological restoration. I focused on three mining sites, restored in different years, at Zin valley, comparing the plant community and germination success of restored plots to adjacent natural plots. I hypothesized that (1) there is a lack of seed bank in the restored plots; (2) the altered soil composition at the restored plots inhibits germination. I set up two greenhouse experiments using soil samples collected from the different mining sites: (1) Comparison between natural and restored habitats, treated with planting mixture or vermiculite; (2) Addition of native seeds to test their germination potential on restored soil. Results indicated that lack of seed bank is the major limiting factor for restoring the plant community and that soil composition doesn't appear to hinder germination. Abundance was significantly lower in restored plots compared to natural plots for the youngest and intermediate mining sites. Species richness was likewise significantly lower, yet only within the vermiculite treatment. Community composition also differed significantly. For the oldest mining site, no significant differences in abundance or community composition were found. Species richness was found to be significantly lower in restored plots compared to natural plots only with the addition of planting mixture. When comparing restored plots of various restoration years, community composition was found to be significantly different. However, this result is misleading, since significant differences were found between the abundance and community composition of the natural habitats of the various mining sites. My results indicate a complex picture of vegetation reestablishment following the mining disturbance. Particular restoration efforts should focus on improving pre-mining planning to meet site-specific needs, preservation of the topsoil, active seeding in restored plots to allow vegetation reestablishment in a quicker manner, and monitoring efforts of the entire process. Generally, my study increased our knowledge of vegetation restoration efforts of hyper-arid deserts and phosphate-mining sites. Future research should focus on dispersal patterns of desert plants and above/belowground interactions. My study sheds light on the constraints of vegetation growth in disturbed, hyper-arid areas and is the basis for further experiments that test prospective practices for restoration of the phosphate mining fields to be implemented in the future.

Acknowledgements

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Introduction

In the last 100 years, humans have transformed over a third of the existing ecosystems into agricultural fields or cities, or have severely degraded ecosystems through processes of fragmentation, unsustainable harvest, pollution, climate change, or exotic species invasions (Millennium Ecosystem Assessment 2005). These processes result in a severe loss of biodiversity and ecosystem functions, and can also impact human health and food security (Suding 2011; Newbold *et al.* 2015).

Many conceptual and integrative frameworks combine theory with pragmatic practice (Hobbs & Norton 1996; Hobbs & Harris 2001; King & Hobbs 2006; Hobbs *et al.* 2014; Nilsson *et al.* 2016; Larios *et al.* 2017; Miller *et al.* 2017) to minimize the effect of biodiversity loss and the decrease in ecosystem functionality. The scientific field of restoration ecology, through its applied practice, attempts to resolve environmental problems that arise from ecosystem degradation related processes (Perring *et al.* 2015). In recent years, there is an increased interest in restoration ecology and numerous ecological restoration projects are implemented around the world (Aronson & Alexander 2013; Bendor *et al.* 2015; Suding *et al.* 2015; Kollmann *et al.* 2016; Hagger *et al.* 2017).

The Society of Ecological Restoration International (henceforth SERI) defines ecological restoration as “the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed” (SERI 2004). The intention is to promote the self-sustainability of an ecosystem both biotically and abiotically without further assistance. The SERI Primer (2004) provides a list of nine attributes to be used as guidelines to measure restoration success. However, due to financial and time constraints, most studies do not deal with all nine attributes, but rather focus on one or several of them (Ruiz-Jaen & Aide 2005). Many studies focus on the first attribute, which asserts that the restored area “contains a characteristic assemblage of the species that occur in the reference ecosystem and that provide appropriate community structure” (SERI 2004), as this is the first step to understand the extent of the degradation caused by the disturbance (Balaguer *et al.* 2014; Miller *et al.* 2017; Shackelford *et al.* 2018).

‘Reference ecosystem’ relates to a nearby undisturbed natural habitat which reflects the natural conditions that have characterized the disturbed area before the disturbance occurred (i.e. a control group). The reference ecosystem is often considered as the desired “end-point” or target that restoration practitioners want to reach (Ruiz-Jaen & Aide 2005; Wortley *et al.* 2013; Lima *et al.*

2016). Benayas *et al.* 2009 conducted a meta-analysis of 89 restoration studies done worldwide. The meta-analysis concluded that most restored areas have negative measurements of biodiversity and ecosystem services (i.e. “the benefits people obtain from ecosystems”; Millennium Ecosystem Assessment 2005) when compared with undisturbed reference areas (i.e. the desired target). However, when compared with degraded, untouched areas (i.e. unrestored), the restored areas show an increase in biodiversity and ecosystem services measurements. Therefore, this meta-analysis highlights the encouraging effects of restoration, even when the chosen reference site is perhaps an unattainable goal.

A disturbance is defined as a discrete event or a sequence of discrete events, either natural or anthropogenic, in space and time that alter the structure of populations, communities and ecosystems of aquatic or terrestrial environments (Willig & Walker 1999). An example of a serious anthropogenic disturbance is the practice of surface mining, including strip mining, open-pit (i.e. open-cast) mining and mountaintop-removal mining (Lima *et al.* 2016). Mining accounts for about 20% of global natural resources, with the major products consisting of fossil fuels, metallic and non-metallic ores, and construction and fertilizer materials (Ramani 2012). Direct impacts of mining on the ecosystem include the removal of soil, vegetation, and animals and alteration of the surface hydrology, while indirect effects can include pollution and fragmentation (Cooke & Johnson 2002; Wong 2003). Therefore, it is quite clear why many restoration efforts are taking place at mining sites (Sengupta 1993; Lei *et al.* 2016; Mattiske 2016).

There are numerous guidelines and conceptual models that apply an overarching framework concretely for mine restoration practices (Tischew & Kirmer 2007; Bielecka & Król-Korczak 2010; Grant *et al.* 2016; Lima *et al.* 2016). Most agree that the restoration efforts should be integrated from the onset of any mining project and should be monitored even on completion. However, restoration procedures vary regionally, and many countries have unique restoration strategies, due to different social, economic, and regulatory standards (Bielecka & Król-Korczak 2010). Correspondingly, many restoration cases are site-specific and may require novel applications (Stuble *et al.* 2017).

In Israel, regulatory standards by the government date back to the British mandate, yet restoration guidelines are inconsistent and incoherent (Milgrom 2008). Few restoration projects are taking place, and range through various disturbances (e.g. fires, construction, mining) and environments (e.g. Mediterranean-forests, sand dunes, deserts). Specifically for the case of mining, closure and rehabilitation of quarries and mines are subject to the Mining Regulations (Quarries Rehabilitation Fund) of 1978, yet a national survey of abandoned quarries suggests that most of them are abandoned without any restoration attempts, which causes an array of hazards (Milgrom 2008). Consequently, restoration efforts in Israel have been understudied and literature on the topic is very scarce.

Presently, Rotem-Amfert Negev LTD (part of the ICL-fertilizers Concern) operates the largest phosphate open-pit mines in the northeastern part of the Negev desert of Israel (over a total area of 200 km^2). Open-pit mining requires the removal of topsoil (defined here as the first 80 cm of soil), overburden soil, and waste rock by bulldozers to expose the phosphorus rock layer. Once removed, the phosphorus layer is moved by large trucks to a treatment facility. In its beginning, the company focused on post-mining landscape-oriented restoration, sometimes many years after the mining process has finished, without hardly any ecological consideration. Yet for the past decade, as a result of government pressure, the company has shifted to an ecological focus and has been employing a practice called “reclamation-oriented mining”. During this practice, a mining field is divided into strips. From the first strip, the upper 50 cm (i.e. topsoil) and the layer of overburden are excavated and set aside in separate piles next to the strip. Subsequently, the company removes and transfers the phosphorus layer. Afterwards, the company proceeds to an adjacent strip (i.e. second strip) where the topsoil is removed and placed in a new pile. Then, the overburden of the second strip is removed and used as backfill for the site of the first strip, along with overburden from the first strip, and the topsoil is returned to the top (leftover overburden soil and rocks are transferred away from the site). After that, a rototiller is driven over the entire strip and the ground is shaped to fit the overall landscape topography. This is considered a restored plot and the whole procedure can take several years to complete.

As stated above, there is much emphasis on removing and returning the topsoil. Rotem-Amfert Negev LTD defines the topsoil as the upper 50 cm of the soil since this is the smallest amount of soil a bulldozer can extract. However, the literature defines topsoil as “the upper 5-10 cm of the soil profile prior to extraction operations” (Kneller *et al.* 2018) because this soil depth contains the seed bank, soil microorganisms, and most nutrients, and is more susceptible to environmental conditions and changes than the rest of the soil profile (Gerasimova & Lebedeva-Verba 2010). Because of this, topsoils are of great importance to the natural habitat and ecosystem functioning and are a major focus of research (Abella *et al.* 2015; Luna *et al.* 2016; Merino-Martín *et al.* 2017; Kneller *et al.* 2018).

Although the topsoil is reinstated at the end of the restoration practice, the removal of the topsoil during mining results in soil disturbance and, consequently, causes a reduction in organic matter, seeds and minerals and creates a homogeneous landscape. Also, the pile of topsoil can remain heaped for years before it is reestablished, and the effects of the long storage time on the soil are unclear. Although some studies show that restoration processes improve over time even when left to natural processes (i.e. 'passive restoration'; Bradshaw 1997; Cooke & Johnson 2002; Suding 2011; Zahawi *et al.* 2014; Lima *et al.* 2016), other studies suggest that a disturbance can be so severe that recovery is just unattainable without active measures (Suding *et al.* 2004; Jones & Schmitz 2009; Miller *et al.* 2017).

Typically after severe disturbances, community composition varies significantly from the original community and recovers over time in a successional manner (Connell & Slatyer 1977). There have been numerous attempts to connect the basic ecological theory of succession with the more pragmatic restoration theory (Suding *et al.* 2004; Tischew & Kirmer 2007; see an extensive review in Walker *et al.* 2007). More specifically, whereas both restoration and succession can concentrate on species structure and composition or ecosystem function, they differ because succession is generally restricted to a given ecosystem while restoration may address broader spatial scales that include adjacent ecosystems, catchments, and landscapes (Walker *et al.* 2007).

Plants are the most notable organisms that recover in a successional manner. Plants are also one of the most sensitive organisms to a mining disturbance, since not only is the aboveground vegetation removed, but also the reserve seed bank in the soil. Plants are a crucial part of the ecosystem. Specifically, plants provide food for herbivores and their roots support many soil organisms, among them bacteria and worms. Additionally, many perennials provide shelter and shade to surrounding organisms. It is assumed that the recovery of fauna and ecosystem processes depend and follow the establishment of vegetation (Suding 2011). Therefore, many restoration endeavors focus on vegetation structure, seed bank analyses, and revegetation attempts as objectives and measures of success (Ruiz-Jaen & Aide 2005; Banerjee *et al.* 2006; Palma & Laurance 2015; Perring *et al.* 2015; Buisson *et al.* 2017; Zirbel *et al.* 2017; Shackelford *et al.* 2018).

Many of the endeavors mentioned above depend on the process of plant germination. Germination is an irreversible process where the plant grows out of the seed and attempts to establish in a given area (Fenner & Thompson 2005). Abiotic factors that govern germination include water availability and frequency, along with temperature, different soil composition and microtopography. Specifically in desert plants, germination occurs when the proper range of temperature and relative humidity exists, and usually requires at least 12 mm of rain to begin (Gutterman 1993). In this regard, hyper-arid deserts are considered extreme, hostile environments because of fluctuating high and low temperatures and scarce water availability (Gomaa & Xavier Picó 2011). Accordingly, vegetation is scarce in such areas, and typically aggregates in depressions or wadis.

Two key factors that may critically affect germination after a mining disturbance are: (1) the existence of proper soil properties that allow for germination, and (2) the availability of seed bank (Cooke & Johnson 2002). Considering the first factor, mining processes could lead to changes in soil composition, reduction in soil fertility, loss of biodiversity and soil pollution and may potentially be severe enough to avert germination (Cooke & Johnson 2002). Considering the second factor, many seeds can be lost to predation or dispersal, given that the topsoil is removed and stored in large piles for several years (Gutterman 1993; Fenner & Thompson 2005). Also, while excavating and returning the topsoil to the mined plot, seeds may be damaged or buried too deep in the soil to allow for germination (Heerdt *et al.* 1996).

Seed banks are described as the reserve of viable seeds present in the soil profile and on the soil surface (Roberts 1981). Soil seed banks play a functional role in population dynamics, adaptivity and evolution of the plant species, especially in desert ecosystems, and effect communities and coexistence. Soil seed banks can have diverse durations, seasons, depths, quantities, and states of dormancy or germination potential (Thompson & Grime 1979; Roberts 1981; Saatkamp *et al.* 2014). In unpredictable environments such as deserts, persistent seed banks that can remain dormant for years until the right conditions arrive are evolutionarily promoted and are vital for the survival of plant species (Gutterman 1993; Saatkamp *et al.* 2014). Formation of a persistent seed bank requires the burial of the seeds, since on the soil surface seeds are more likely to be predated on or to germinate because of light signals (Fenner & Thompson 2005). This tends to make persistent seeds smaller in size. However, in deserts, seeds that are buried below 7 cm in the soil can be considered lost from the desert seed bank as they are not involved in seed bank dynamics (Kemp 1989; Gutterman 1993).

Seed bank analysis usually relies on two methodologies: (1) seed separation methods (also referred to as seed extraction) that include flotation or sieving; and (2) seedling emergence methods that include soil samples kept under optimal conditions for germination, either in greenhouse or field experiments (Roberts 1981). Specifically, for better detection of small-sized seeds such as desert seeds and for studies employed on a large spatial scale, the seedling emergence method is more suited (Brown 1992; Heerdt *et al.* 1996). These methods demonstrate the germination potential of the seeds present in the soil and are thus analogous to the seed bank in the field. Nevertheless, the germinated plants that sprout by these methods provide an underestimation of the entire seed bank and plant community (Thompson & Grime 1979).

While general knowledge of restoration, deserts, and plants is relatively abundant, the interdisciplinary integration of this data is still lacking. There are several studies that specifically address phosphate-mining restoration projects (Chambers *et al.* 1994; Brown 2005; Yang *et al.* 2014; Gillespie *et al.* 2015; Toktar *et al.* 2016; Ngugi *et al.* 2018), yet none are in hyper-arid areas. However, studies about restoration in arid lands are becoming more abundant, with the realization that drylands have enormous social, cultural, and economic impacts (Bainbridge 2007). More recently, SERI has launched a new initiative to highlight the newest studies in a specific journal on the subject called: *Restoration Ecology: Arid Lands*. Restoring desert ecosystems that have been disturbed could potentially combat desertification and improve ecosystem services. To the best of my knowledge, this is one of a handful of studies examining vegetation restoration in a hyper-arid desert. This study aimed to enrich the research on vegetation of hyper-arid desert ecosystems in general, and particularly after mining disturbances, both conceptually and practically.

Research Goals

Considering the above, the main objective of this study is to evaluate the plant community in restored phosphate mining sites as an indication for proper ecological restoration. This knowledge will assist to increase understanding of the area and promote better restoration practices in the future. I examine the plant community using three community measures (abundance, species richness and composition) and at two scales. At the spatial scale, I compare between communities of a restored habitat and an adjacent natural habitat (i.e. reference) within the same mining site. Additionally, I compare between control restored soil and restored soil with an added seed bank. At the temporal scale, I compare between communities of the various restored mining sites that were restored in different years.

Specifically, this study has three main goals. The first goal is to better understand the vegetation patterns in the field. I hypothesize that the natural habitat would be less disturbed and more heterogenous than the restored habitat in each mining site. I therefore predict a significantly higher species richness and a significantly different species prevalence in the natural habitat.

The second goal is to explore the difference in germination potential at a spatial scale. I posit two non-mutually exclusive hypotheses regarding seed establishment: (1) The seed bank hypothesis states that in the restored habitat, relative to the adjacent natural habitat, the seed bank (i.e. germination potential) is poorer and less abundant. Therefore, I predict significant differences in all the community measures of the restored habitat due to lack of seeds in the soil; (2) The soil composition hypothesis suggests that restored soils' characteristics prevent germination and limit vegetation growth. Therefore, I predict significant differences in all the community measures of the restored habitat due to the inability of plants to germinate and establish in the soil.

To accomplish this goal, I set a greenhouse experiment with control soil samples and soil samples with added supplementary treatments (i.e. enriched soil samples) from natural and restored habitats. Accordingly, if the difference between the habitats is due to low seed bank, I expect to see similar community measures within the restored habitat when comparing control soil samples to enriched soil samples. However, if this difference is due to soil composition, then I expect to see an increase in community measures in the enriched soil samples of the restored habitat when comparing to the control. If this difference is the result of both hypotheses, then I expect that while the enriched soil samples of the restored habitat will have higher measures than the control restored soil samples, it will still be less than the adjacent natural habitat. This will indicate a decrease in the seed bank in addition to the decreased germination due to soil characteristics.

To further test these hypotheses, I conduct a greenhouse experiment comparing control restored soil with restored soil with an added seed bank and between enriched soil treatments. If the difference between the samples is due to low seed bank, I expect to see similar community measures when comparing the control and enriched soil samples with the added seed bank.

However, if this difference is due to soil composition, then I expect to see an increase in community measures in the enriched soil samples with the added seed bank. I predict that plant community measurements will increase with an added seed bank, and that these measurements will be even higher in enriched soil samples when compared with the control.

The third goal is to explore the difference in germination potential on a temporal scale. Given that the study area is under similar climatic and lithological conditions, and that the restoration in the various mining sites was done using the same method, any difference found in the restored habitat between the different sites could be attributed to the time since restoration occurred. Hence, I hypothesize that the plant community in the restored habitat is in the process of ecological succession. Accordingly, I predict significant differences in all the different community measures between the various restored sites, with the older site being richer than the younger restored site. This will happen with both control and enriched soil samples.

Methods

Research area

The research took place in Zin mining fields, located at Zin river valley (**Figure 1**). The valley is located in the northeastern Negev highlands, a mountainous chain containing a series of ridges separated by large valleys, within which phosphate has been deposited approximately 50 million years ago. The area is characterized by a hyper-arid desert climate with about 50 mm of annual rainfall (Zin factory meteorological data). The rainy season is infrequent and irregular, and can start anywhere between October to December, ending anywhere between March to May (Gutterman 1993). The hottest summer months of July and August reach an average temperature of about 30˚ C, while the coldest months of December and January reach an average of 15˚ C. During the winter period, the average minimum is around 4˚ C. The soil composition in the study area consists of a phosphorous layer, covered by different layers of shallow marine sediments such as marlstone and limestone. The top layer is covered with Reg soils, which form a well-developed desert pavement with high salinity (Singer 2007).

Experimental field design and soil sampling

Experimental plots were located in three mining sites: Gov, Hagor, and Saif. Mining sites differ in years since restoration – 2007, 2010, and 2015 in Gov (i.e. oldest site), Hagor (i.e. intermediate site), and Saif (i.e. youngest site), respectively. Within each site I set up four 100×50 m plots –

two natural plots (i.e. reference) and two restored plots (**Figure 1**). The intermediate site has one additional natural plot. I divided each plot into 50 quadrates of 10×10 m each. In each plot, I collected top soil (defined here as the first 10 cm) from three quadrates (**Figure 2**). The sampled soil was taken to the university campus for greenhouse experiments.

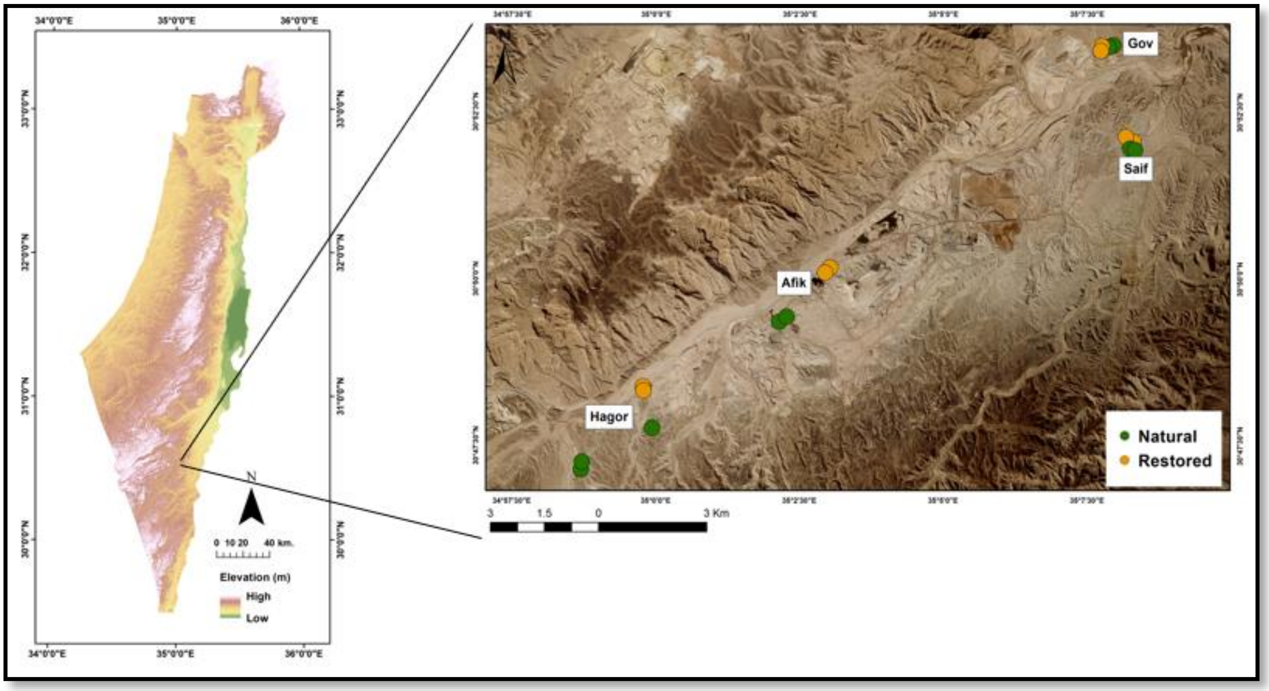

Figure 1: Map of research area. Each mining site consists of two natural (green) and two restored (yellow) plots.

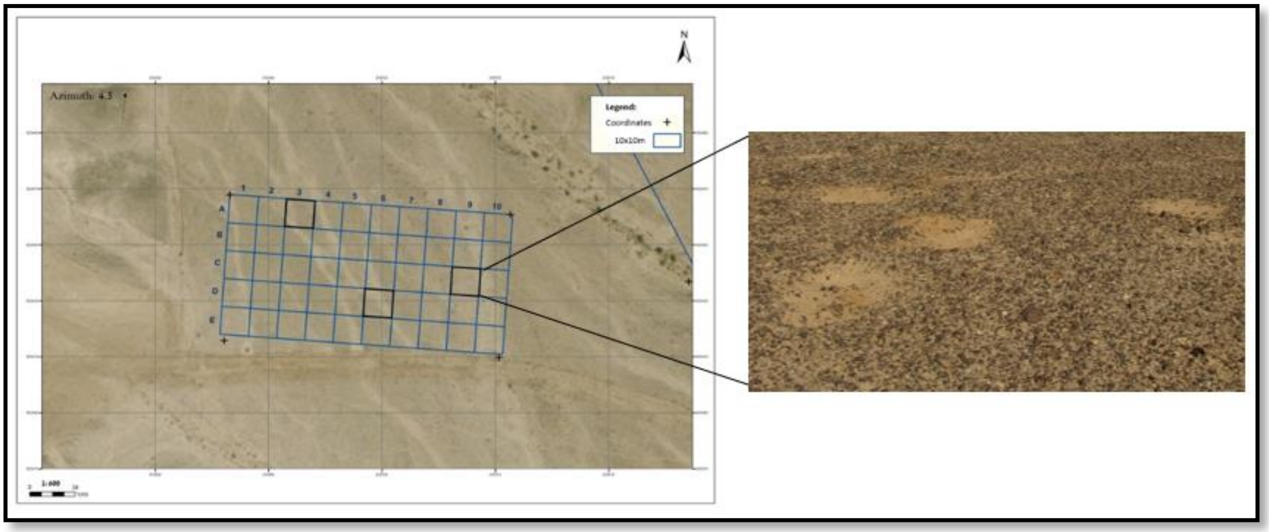

Figure 2: Scheme of sample collection within each plot. Three selected 10×10 m quadrates are marked in black, where topsoil samples were collected and later composited.

A vegetation survey of all natural and restored plots from the three mining sites was conducted on March 2019. In the survey, the entire 100×50 m plot was checked for plant species richness. Plants were identified on site or documented by photos and identified in the lab (**Figure 3**).

Figure 3: Plant identification during field survey (left) and specimen taken back to the lab for identification (right).

Greenhouse experiments

Soil samples collected from the field were placed in a greenhouse on raised tables under a fixed irrigation system (**Figure 4A**). I placed one liter of each soil sample in aluminum trays above a two mm vermiculite layer. Besides trays with untreated soil samples (i.e. control), I added two different treatments: (1) one liter of vermiculite; (2) one liter of HR2 planting mixture (**Figure**

4B). Vermiculite is a hydrated magnesium aluminum silicate mineral which resembles mica (chemical composition in **Appendix table 1**) and has various uses including construction, industry, horticulture and agriculture (**Figure 4C**; The Vermiculite Association 2019). I chose vermiculite for this study due to its ability to air the soil and increase water and nutrient retention. The nutrients it helps retain are only ones found in the natural soil composition, since vermiculite does not decompose and so does not add any new nutrients. I used vermiculite size 3 as it reflects the optimal condition for soil improvement (The Vermiculite Association 2019). The HR2 planting mixture is a fertilizer used to induce plant growth (chemical composition in **Appendix table 2**; **Figure 4D**). Like vermiculite, it helps aeration and increases water retention. However, it also supplies nutrients, mainly organic matter, to the soil. The liter of different treatments and liter of soil were mixed homogenously and then placed in the trays. The trays were examined on a regular basis for germination. Once germination occurred, plant samples were documented and identified.

Figure 4: Trays of soil samples in the greenhouse under a fixed watering system (A). Examples of soil samples with different treatments in aluminum trays (B). One treatment was vermiculite (C), the other treatment was HR2 planting mixture (D).

Two separate experiments were carried out in the greenhouse. The first experiment (i.e. greenhouse experiment 1) was conducted in order to test germination potential in natural and restored soils and between various soil treatments. Greenhouse experiment 1 was composed of 468 trays of soil samples from both reference and restored plots from all sites. From each quadrate there were 12 trays, meaning four repetitions of every treatment (i.e. four trays of control, four trays of vermiculite and four trays of planting mixture).

The second experiment (i.e. greenhouse experiment 2) was conducted to check whether restored soil composition would hinder seed germination once native seeds were added artificially. Greenhouse experiment 2 was composed of 522 trays of soil samples from solely restored plots from all mining sites. Seeds of 10 native desert plant species, acquired from the Israeli Gene Bank, were placed on 432 of the trays (**Appendix table 3**). A total of 50 seeds were placed on each tray (i.e. five seeds from 10 separate species). From each quadrate there were 24 trays, meaning eight repetitions of every treatment (i.e. control, vermiculite and planting mixture) with actively added seeds. There were also five repetitions of control soil samples without artificial seeding. The soil was not sterilized, since studies show that this can alter soil composition (Jenneman *et al.* 1986; Trevors 1996; Shaw *et al.* 1999) as well as the required local microorganism community, hence making it necessary to keep soil composition as similar to natural as possible for future applied use of the data from the experiment. A summary of all the different experiments is presented below (**Figure 5**).

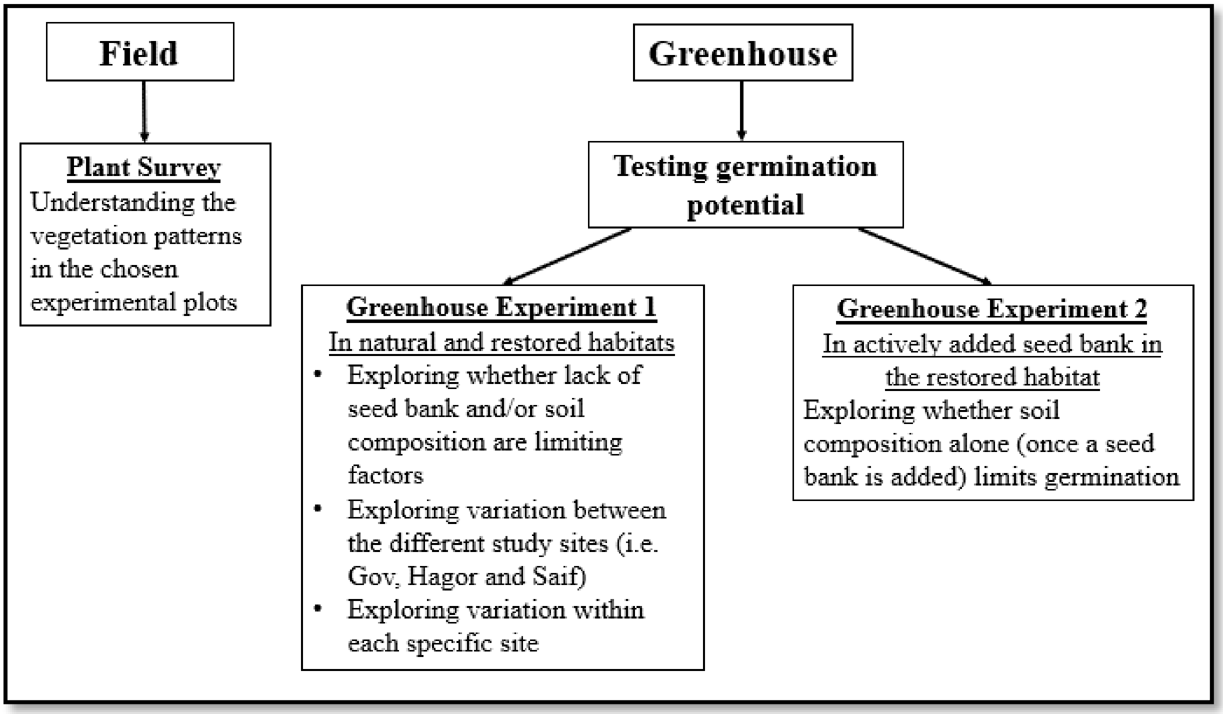

Figure 5: Schematic summary of the study.

Statistical analysis

To test for the effects of habitat and treatment on abundance and richness within each site, I used generalized linear mixed models (GLMMs), followed by Dunn's test of multiple comparisons. The GLMMs included abundance or richness as response variables, and habitat and treatment as fixed explanatory variables. To account for spatial dependency, I included the sampling plots as a random factor, nested in the habitat. A negative binomial link function was used in the abundance model. A Poisson link function was included in the species richness (count data) model. These two statistical analyses were performed using R 3.5.3 (R Core Team 2019) with the *lme4* (Bates *et al.* 2015) and *dunn.test* (Dinno 2017) packages. A similar analysis was performed to test for the effects of site and treatment on abundance with an actively added seed bank in the restored habitat.

Permutational multivariate analysis of variance (PERMANOVA) (Anderson 2017), based on Bray-Curtis similarity matrix was used to test for the combined effect of habitat and treatment on community composition within each site, and the combined effect of site and treatment on community composition with an induced seed bank in the restored habitat. The relative species abundance data was square-root transformed. I performed a type III PERMANOVA on the unrestricted raw data with 99999 permutations. Then, a pairwise comparison test was used to determine differences between the different levels of treatments (i.e. control, vermiculite and planting mixture) and sites (i.e. Gov, Hagor and Saif). This statistical analysis was performed using PRIMER v.7 with PERMANOVA+ (Anderson *et al.* 2008; Clarke & Gorley 2015).

Results

Field vegetation survey

In total, 46 plant species were surveyed across the study area (**Appendix table 4**). All the natural plots and the two restored plots of the oldest site cluster together at 50% similarity, while the intermediate restored plots and the youngest restored plots cluster individually (**Figure 6**). Plant species prevalence did not significantly differ between sites in the natural habitat (PERMANOVA: Psuedo-F=1.912, P(MC)=0.138) yet did differ significantly between sites in the restored habitat (Psuedo-F=10.685, P(MC)=0.003; **Table 1**). Comparisons within each site showed no significant difference between the natural and restored habitats in the oldest site (Psuedo-F=1.914, P(MC)=0.238), while the intermediate (Psuedo-F=8.83, P(MC)=0.02), and youngest sites (Psuedo-F=17.806, P(MC)=0.025) did show a significant difference between their habitats (**Table 1**). Plant species richness did not differ across any analysis due to small sample sizes.

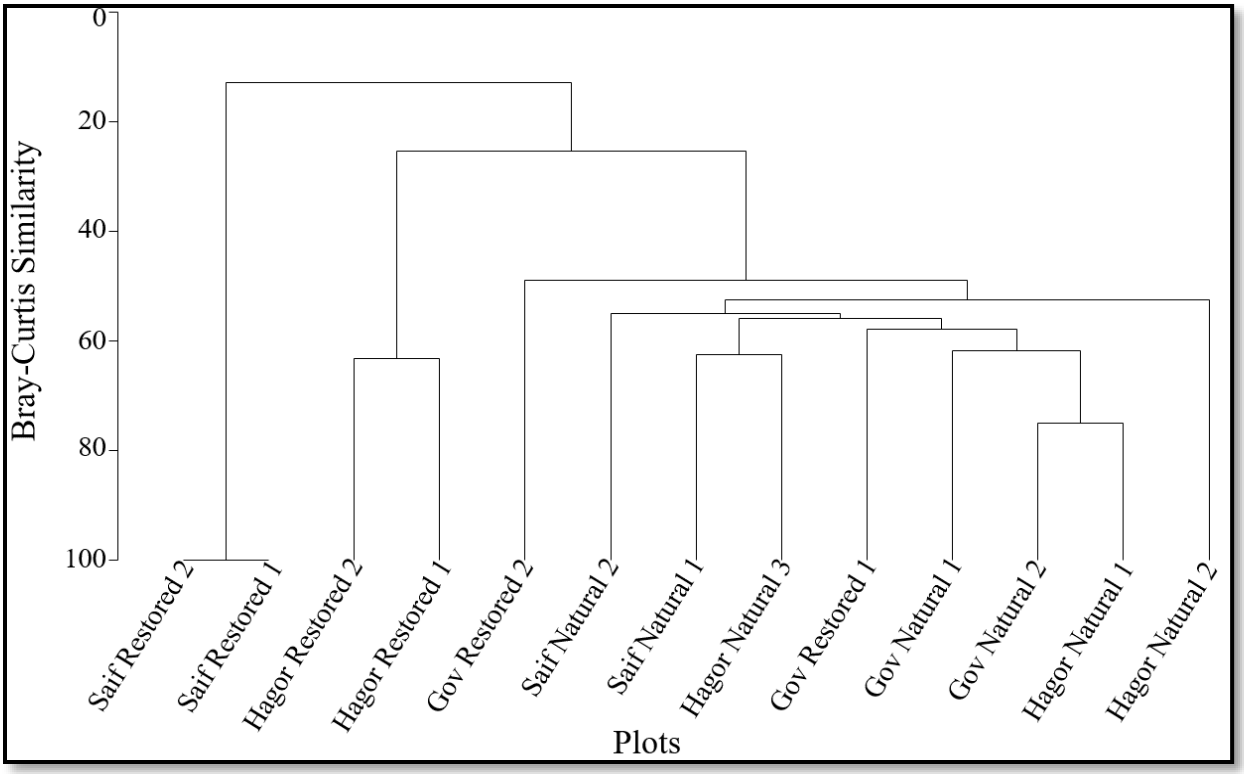

Figure 6: Relative similarity (Bray-Curtis index) dendrogram of species prevalence in the study plots. The legend signifies the site (Gov – restored 2007, Hagor – restored 2010, or Saif – restored 2015), the habitat (natural or restored), and the plot number.

Table 1: Results of permutational multivariate analysis of variance (PERMANOVA), testing for the effect of site (between natural and restored habitats) and habitat (within each mining site) on the prevalence of the plant community. Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

Source	df	SS	MS	Pseudo-F	Unique perms	P(MC)
Comparison of same habitat across different mining sites						
Natural Habitat						
Site	2	2818.40	1409.20	1.912	105	0.138
Gov, Hagor	1	1296.20	1296.20	1.810	10	0.211
Gov, Saif	1	1281.00	1281.00	2.038	3	0.209
Hagor, Saif	1	1624.90	1624.90	1.956	10	0.177
Plot (Site)	4	2948.80	737.19	No test		
Total	6	5767.20				
Restored Habitat						
Site	2	11652.00	5826.00	10.685	9	0.003**
Gov, Hagor	1	4025.30	4025.30	4.922	3	0.089
Gov, Saif	1	7245.00	7245.00	15.140	2	0.028*
Hagor, Saif	1	6207.80	6207.80	18.294	2	0.023*
Plot (Site)	3	1635.70	545.23	No test		
Total	5	13288.00				
Comparison of different habitats within mining site						
Gov (restored 2007)						
Habitat	1	1353.30	1353.30	1.914	3	0.238
Plot (Habitat)	2	1414.00	707.02	No test		
Total	3	2767.40				
Hagor (restored 2010)						
Habitat	1	6976.80	6976.80	8.830	10	0.020*
Plot (Habitat)	3	2370.40	790.14	No test		
Total	4	9347.30				
Saif (restored 2015)						
Habitat	1	7122.20	7122.20	17.806	2	0.025*
Plot (Habitat)	2	800.00	400.00	No test		
Total	3	7922.20				

Evaluation of restored mines and natural areas

Greenhouse experiment 1 – with soil of control and enriched samples from the restored plots and natural reference plots – provided a total of 1797 seedlings from 28 species (one identified to the family level, one identified to the genus level, 20 identified to the species level, six unidentified; **Appendix table 4**).

Comparison between mining sites

Species composition was compared within a habitat (i.e. natural or restored) across the different mining sites with various soil treatments. Species composition in the natural habitat showed that Gov plots clustered separately from the Hagor and Saif plots, while across the restored habitat, only one of the oldest restoration plots (Gov) clustered separately (**Figure 7**). Species composition of the natural habitat varied significantly between site (PERMANOVA: Psuedo-F=3.577, P(MC)=0.019), treatment (Psuedo-F=2.839, P(perm)=0.02), and plot (Psuedo-F=4.185, P(perm)<0.001), while the interaction between site and treatment was insignificant (Psuedo-F=1.312, P(perm)=0.244; **Table 2**). Gov mining site differed significantly from Hagor mining site (PERMANOVA pairwise comparison: Psuedo-t=2.227, P(MC)=0.022) and Saif mining site (Psuedo-t=2.902, P(MC)=0.017; **Table 3**). Planting mixture differed significantly from the control (Psuedo-t=1.701, P(MC)=0.047) and vermiculite (Psuedo-t=1.707, P(MC)=0.049) treatments (**Table 3**). In addition, a significant difference was found between the natural plots of Gov mining site (Psuedo-t=2.243, P(MC)=0.039) and the natural plots of Hagor mining site (between ‘Hagor Natural 1’ and ‘Hagor Natural 3’: Psuedo-t=2.709, P(MC)=0.029; **Table 3**). Differences in species composition between the restored habitat were insignificant, except for a marginally significant difference between the plots of Gov mining site (Psuedo-t=1.923, P(MC)=0.087; **Table 3**).

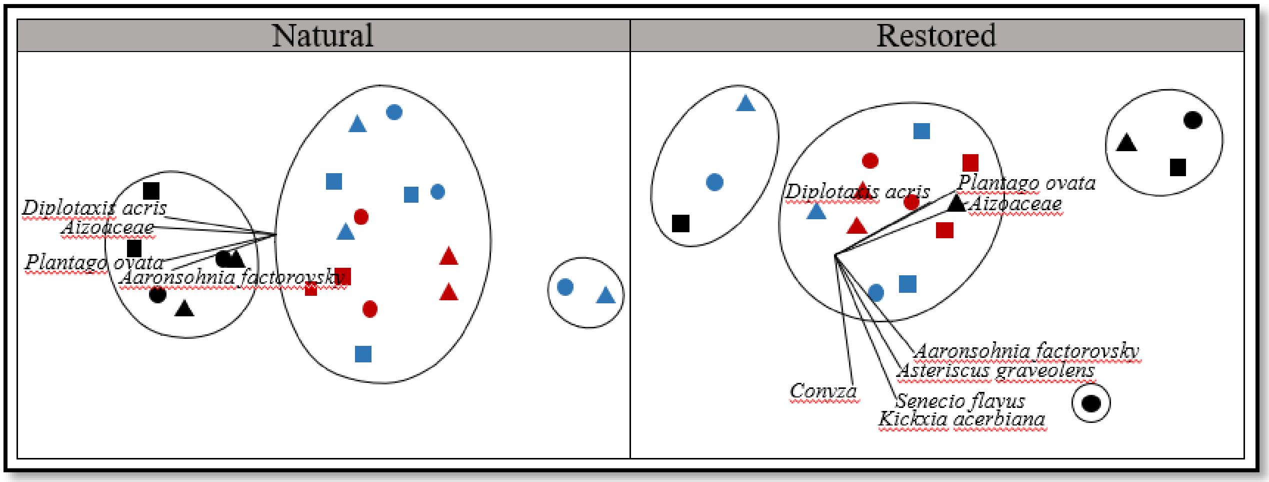

Figure 7: NMDS ordination based on Bray-Curtis similarities (calculated for square-root transformed abundances), presenting the plant community composition of samples from natural (left) and restored (right) plots for all study sites. Different colors represent the different mining sites – Gov (black), Hagor (blue), and Saif (red). Different shapes represent treatments – control (triangle), vermiculite (circle), and P.M (square). Stress values for NMDS of natural plots is 0.11 and for NMDS of restored plots is 0.09. The overlaid circles denote 50% similarity between clusters. Vectors denote plant species with a Pearson correlation >0.5.

Table 2: Results of permutational multivariate analysis of variance (PERMANOVA), testing for the effect of site and treatment on the composition of the plant community in natural and restored habitats. Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
Natural Habitat							
Site	2	11137.00	5568.40	3.577	0.068	105	0.019*
Treatment	2	2112.30	1056.20	2.839	0.020*	94289	0.025
Plot (Site)	4	6227.50	1556.90	4.185	0.000***	93495	0.001
Site×Treatment	4	1951.90	487.97	1.312	0.244	93340	0.254
Res	8	2975.90	371.99				
Total	20	24505.00					
Restored Habitat							
Site	2	9970.40	4985.20	1.666	0.067	15	0.202
Treatment	2	1197.70	598.84	0.475	0.909	94149	0.874
Plot (Site)	3	8979.40	2993.10	2.374	0.018*	93818	0.035
Site×Treatment	4	5731.70	1432.90	1.137	0.355	93184	0.376
Res	6	7564.00	1260.70				
Total	17	33443.00					

Table 3: Results of pairwise tests of PERMANOVA for factors site, treatment, and plot on the composition of the plant community in natural habitats. Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

Groups	Pseudo-t	P(perm)	Unique perms	P(MC)
Natural Habitat				
Site				
Gov, Hagor	2.227	0.100	10	0.022*
Gov, Saif	2.902	0.332	3	0.017*
Hagor, Saif	0.927	0.700	10	0.513
Treatment				
Control, Vermiculite	1.581	0.086	95617	0.099
Control, Planting mixture	1.701	0.047*	95176	0.063
Vermiculite, Planting mixture	1.707	0.049*	95307	0.064
Plot (Site)				
Gov Natural 1, Gov Natural 2	2.243	0.017	60	0.039*
Hagor Natural 1, Hagor Natural 2	1.833	0.134	60	0.134
Hagor Natural 1, Hagor Natural 3	2.709	0.016	60	0.029*
Hagor Natural 2, Hagor Natural 3	2.176	0.033	60	0.051
Saif Natural 1, Saif Natural 2	1.531	0.183	60	0.192
Restored Habitat				
Site				
Gov, Hagor	1.216	0.334	3	0.288
Gov, Saif	1.397	0.334	3	0.203
Hagor, Saif	1.270	0.334	3	0.275
Treatment				
Control, Vermiculite	0.782	0.704	94676	0.647
Control, Planting mixture	0.654	0.827	94622	0.785
Vermiculite, Planting mixture	0.656	0.860	94507	0.811
Plot (Site)				
Gov Restored 1, Gov Restored 2	1.923	0.050	60	0.087
Hagor Restored 1, Hagor Restored 2	1.495	0.168	54	0.193
Saif Restored 1, Saif Restored 2	0.324	0.883	60	0.911

Comparison within mining sites

Abundance, species richness, and species composition were compared between natural and restored habitats and between different soil treatments within each specific mining site. Abundance was relatively higher in the Gov mining site than in the other two sites (**Figure 8**). In Gov mining site (restored 2007), abundance was not significantly different across habitat (GLMM: z=-0.702, P=0.483), soil treatment, or the interaction between the factors (**Table 4; Figure 8).** In Hagor mining site (restored 2010), abundance was significantly lower in restored plots compared to natural plots (z=-2.578, P=0.01), but insignificantly different between soil treatments or the interaction between the factors (**Table 4; Figure 8**). Likewise, the trend in Saif mining site (restored 2015) showed that abundance was significantly lower in restored plots (z=-3.482, P<0.001), but insignificant between soil treatments or the interaction between the factors (**Table 4; Figure 8**).

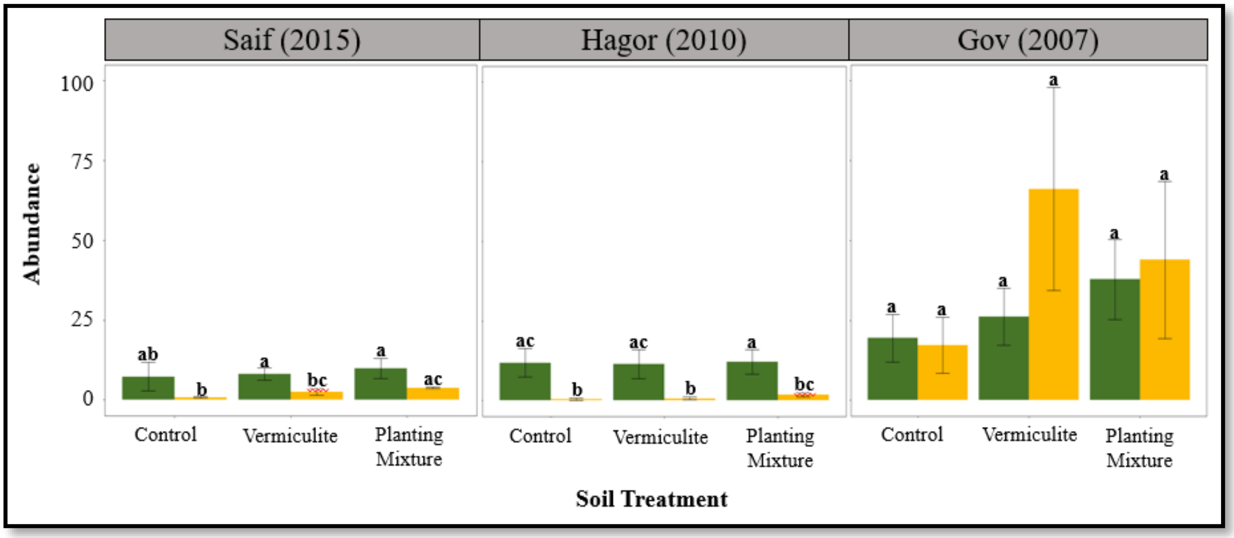

Figure 8: Abundance of germinated plants as a function of treatment and habitat in the different mining sites Saif, Hagor, and Gov. Habitat is represented by different colors: natural (green) and restored (yellow). Different letters indicate Dunn's significant differences for each site individually.

Table 4: Summary of generalized linear mixed model (GLMM) testing the effect of habitat and treatment on plant abundance for the different mining sites (Gov, Hagor and Saif). Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

	Abundance			
	Estimates	**SE**	**z value**	**P value**
Gov (restored 2007)				
Habitat				
Restored	-0.760	1.083	-0.702	0.483
Treatment				
Vermiculite	0.292	0.639	0.458	0.647
Planting mixture	0.694	0.640	1.083	0.279
Habitat×Treatment				
Restored×Vermiculite	1.573	0.941	1.671	0.095
Restored×Planting mixture	-0.123	0.934	-0.132	0.895
Constant term	2.951	0.758	3.895	0.000
Plot				
Variance	0.737	0.858		
Hagor (restored 2010)				
Habitat				
Restored	-2.753	1.068	-2.578	0.010**
Treatment				
Vermiculite	0.017	0.380	0.046	0.964
Planting mixture	0.403	0.395	1.021	0.307
Habitat×Treatment				
Restored×Vermiculite	0.208	0.958	0.217	0.828
Restored×Planting mixture	0.935	0.877	1.066	0.286
Constant term	1.920	0.573	3.350	0.001
Plot				
Variance	0.746	0.863		
Saif (restored 2015)				
Habitat				
Restored	-2.175	0.625	-3.482	0.000***
Treatment				
Vermiculite	0.108	0.459	0.235	0.814
Planting mixture	0.293	0.455	0.645	0.519
Habitat×Treatment				
Restored×Vermiculite	0.991	0.803	1.235	0.217
Restored×Planting mixture	1.233	0.786	1.569	0.117
Constant term	1.992	0.326	6.110	0.000
Plot				
Variance	0.000	0.000		

Species richness was overall relatively low (**Figure 9**). In Gov mining site (restored in 2007), richness was insignificantly different between habitat or the interaction between habitat and soil treatment. However, there was a significant difference for the planting mixture treatment (GLMM: z=2.117, P=0.034; **Table 5; Figure 9**). In Hagor mining site (restored in 2010), richness was significantly lower in restored plots compared to natural plots (z=-2.182, P=0.029), but not significantly different between soil treatment or the interaction between the factors (**Table 5**). However, within the vermiculite treatment there was a significant difference between the natural and restored habitats (**Figure 9**). In Saif mining site (restored in 2015), richness was insignificant across habitat, soil treatment, and the interaction between the factors (**Table 5**). However, within the vermiculite treatment there was a significant difference between the natural and restored habitats (**Figure 9**).

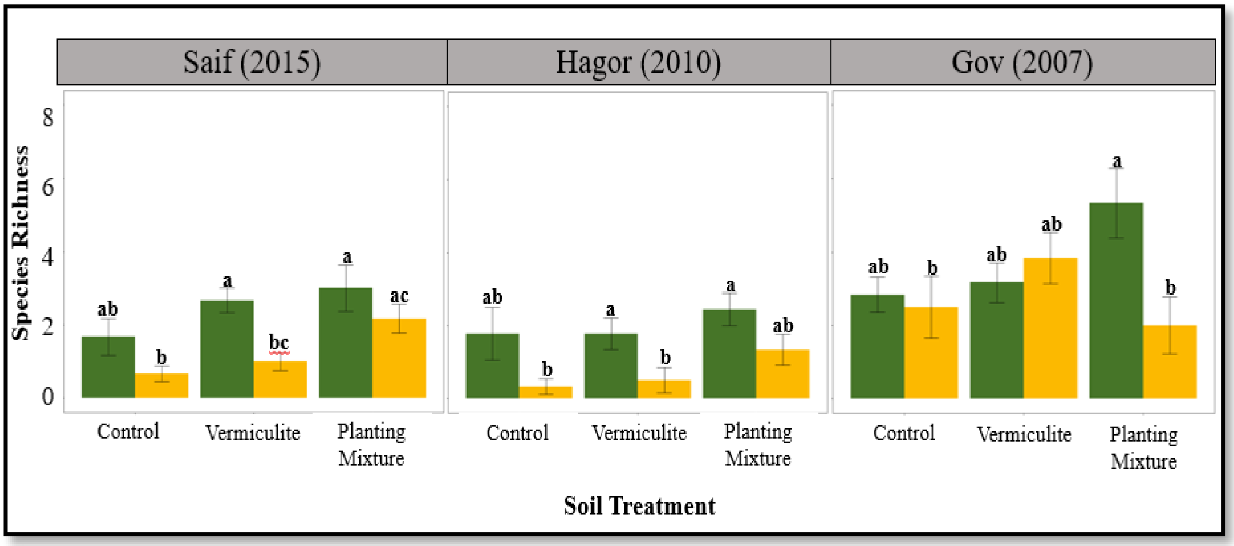

Figure 9: Species richness of germinated plants as a function of treatment and habitat in the different mining sites – Saif, Hagor, and Gov. Habitat is represented by different colors: natural (green) and restored (yellow). Different letters indicate Dunn's significant differences for each site individually.

Table 5: Summary of generalized linear mixed model (GLMM) testing the effect of habitat and treatment on plant richness for the different mining sites (Gov, Hagor and Saif). Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

	Richness			
	Estimates	**SE**	**z value**	**P value**
Gov (restored 2007)				
Habitat				
Restored	-0.179	0.484	-0.369	0.712
Treatment				
Vermiculite	0.111	0.332	0.335	0.738
Planting mixture	0.633	0.299	2.117	0.034*
Habitat×Treatment				
Restored×Vermiculite	0.316	0.469	0.675	0.500
Restored×Planting mixture	-0.856	0.488	-1.755	0.079
Constant term	1.017	0.334	3.041	0.002
Plot				
Variance	0.106	0.326		
Hagor (restored 2010)				
Habitat				
Restored	-1.671	0.766	-2.182	0.029*
Treatment				
Vermiculite	0.000	0.353	0.000	1.000
Planting mixture	0.319	0.328	0.971	0.332
Habitat×Treatment				
Restored×Vermiculite	0.406	0.978	0.415	0.678
Restored×Planting mixture	1.068	0.855	1.249	0.212
Constant term	0.559	0.271	2.064	0.039
Plot				
Variance	0.030	0.174		
Saif (restored 2015)				
Habitat				
Restored	-0.916	0.592	-1.549	0.121
Treatment				
Vermiculite	0.470	0.403	1.166	0.244
Planting mixture	0.588	0.394	1.490	0.136
Habitat×Treatment				
Restored×Vermiculite	-0.065	0.761	-0.085	0.932
Restored×Planting mixture	0.591	0.695	0.851	0.395
Constant term	0.511	0.316	1.615	0.106
Plot				
Variance	0.000	0.000		

Comparing species composition in Gov mining site, ‘Gov Restored 2’ plot clustered separately from ‘Gov Restored 1’ plot and the two Gov natural plots (**Figure 10**). Species composition was insignificant between habitat, soil treatment, or the interaction between them (**Table 6**). However, there was a significant difference between the plots of the same habitat (PERMANOVA: Psuedo-F=3.805; P(perm)<0.001; **Table 6**), more specifically between the natural plots (PERMANOVA pairwise comparison: Psuedo-t=2.243; P(MC)=0.039; **Table 7**). The trend in Hagor mining site was similar in that species composition was not significant between habitat, soil treatment, or the interaction between them, yet was significant between plots of the same habitat (Psuedo-F=3.343; P(perm)=0.008; **Table 6**). Specifically, between ‘Hagor Natural 1’ and ‘Hagor Natural 3’ (Psuedo-t=2.709; P(MC)=0.03; **Table 7**) and marginally significant between ‘Hagor Natural 2’ and ‘Hagor Natural 3’ (Psuedo-t=2.176; P(MC)=0.051; **Table 7**). Most of the natural habitat clustered together, yet not all. Also, the restored habitat in Hagor mining site clustered in two distinct groups, with one sample clustering separately (**Figure 10**). In Saif mining site, natural habitat and restored habitat clearly clustered in two distinct groups, with an exception of one restored sample with planting mixture that clustered with samples from the natural habitat (**Figure 10**). This was represented by a significant difference between the habitats (Psuedo-F=2.827; P(MC)=0.016; **Table 6**). However, species composition was insignificant across soil treatment, plots, or the interaction between habitat and soil treatment (**Table 6; Table 7**).

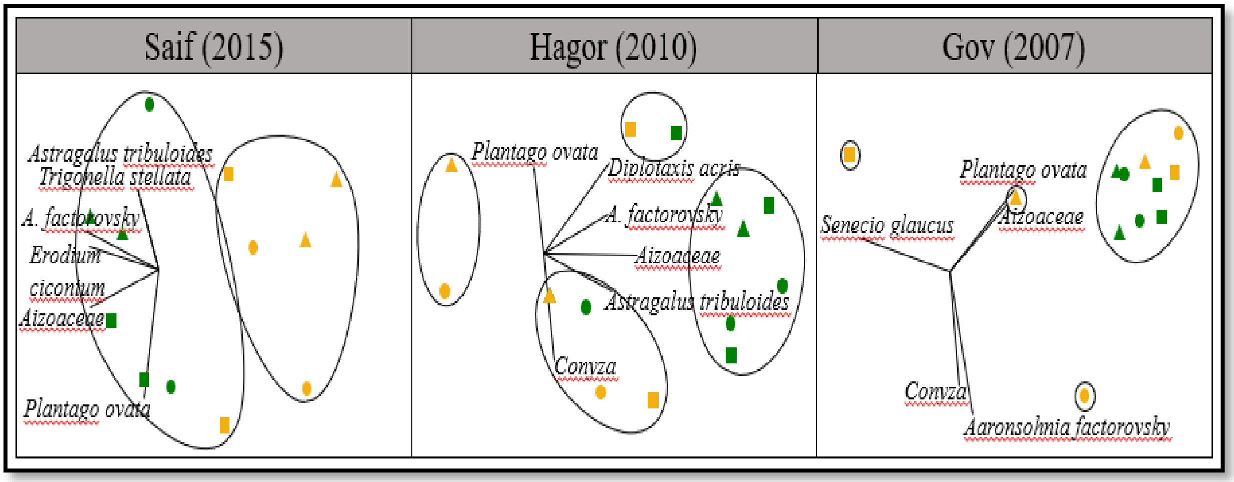

Figure 10: NMDS ordination based on Bray-Curtis similarities (calculates for square-root transformed abundances), presenting the plant community composition of samples from sites Saif, Hagor, and Gov. Different colors represent natural (green) or restored (yellow) habitats, and different shapes represent control (triangle), vermiculite (circle), and planting mixture (square). Stress values are 0.18, 0.12, and 0.03 for Saif, Hagor, and Gov respectively. The overlaid circles denote 60% similarity between clusters. Vectors denote plant species with a Pearson correlation >0.5.

Table 6: Results of permutational multivariate analysis of variance (PERMANOVA), testing for the effect of habitat and treatment on the composition of the plant community for the different mining sites (Gov, Hagor and Saif). Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
Gov (restored 2007)							
Habitat	1	2595.60	2595.60	0.746	1.000	3	0.574
Treatment	2	2291.80	1145.90	1.253	0.263	92836	0.337
Plot (Habitat)	2	6958.30	3479.20	3.805	0.000***	90785	0.018
Habitat×Treatment	2	2008.70	1004.30	1.098	0.375	92970	0.409
Res	4	3657.50	914.38				
Total	11	17512.00					
Hagor (restored 2010)							
Habitat	1	4016.50	4016.50	1.624	0.299	10	0.240
Treatment	2	2958.90	1479.40	1.999	0.098	94895	0.114
Plot (Habitat)	3	7421.70	2473.90	3.343	0.008***	94385	0.012
Habitat×Treatment	2	1458.00	728.98	0.985	0.458	94771	0.456
Res	6	4440.30	740.05				
Total	14	20145.00					
Saif (restored 2015)							
Habitat	1	3302.90	3302.90	7.989	0.331	3	0.016*
Treatment	2	2178.60	1089.30	1.784	0.158	95070	0.179
Plot (Habitat)	2	826.83	413.42	0.677	0.705	92506	0.680
Habitat×Treatment	2	348.07	174.04	0.285	0.951	94890	0.938
Res	4	2442.10	610.52				
Total	11	9098.40					

Table 7: Results of pairwise tests of PERMANOVA for factors habitat, treatment, and plot on the composition of the plant community for the different mining sites (Gov, Hagor and Saif). Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

Groups	Pseudo-t	P(perm)	Unique perms	P(MC)
Gov (restored 2007)				
Habitat				
Natural, Restored	0.864	1.000	3	0.575
Treatment				
Control, Vermiculite	1.251	0.258	2518	0.296
Control, Planting mixture	1.351	0.161	2513	0.248
Vermiculite, Planting mixture	0.875	0.684	2519	0.544
Plot (Site)				
Gov Natural 1, Gov Natural 2	2.243	0.016	60	0.039*
Gov Restored 1, Gov Restored 2	1.923	0.050	60	0.087
Hagor (restored 2010)				
Habitat				
Natural, Restored	1.274	0.297	10	0.240
Treatment				
Control, Vermiculite	1.424	0.161	53952	0.185
Control, Planting mixture	1.421	0.144	52706	0.172
Vermiculite, Planting mixture	1.398	0.127	70624	0.171
Plot (Site)				
Hagor Natural 1, Hagor Natural 2	1.833	0.134	60	0.136
Hagor Natural 1, Hagor Natural 3	2.709	0.169	60	0.030*
Hagor Natural 2, Hagor Natural 3	2.176	0.034	60	0.051
Hagor Restored 1, Hagor Restored 2	1.495	0.168	54	0.193
Saif (restored 2015)				
Habitat				
Natural, Restored	2.827	0.335	3	0.016*
Treatment				
Control, Vermiculite	1.078	0.414	2517	0.402
Control, Planting mixture	2.263	0.042*	2517	0.050
Vermiculite, Planting mixture	0.833	0.657	2518	0.599
Plot (Site)				
Saif Natural 1, Saif Natural 2	1.531	0.181	60	0.189
Saif Restored 1, Saif Restored 2	0.324	0.884	60	0.911

Evaluation of active seeding in the restored mines

Greenhouse experiment 2 included soil of control and enriched samples from the restored plots and seeds from 10 native desert species that were artificially introduced into the soil samples. A total of 6072 seedlings from 17 species emerged (the 10 artificially added species and seven naturally occurring species). Of the seven naturally occurring species, one was identified to the family level, one was identified to the genus level, and 5 were identified to the species level. Two species that were artificially introduced (*A. factorovsky* and *P. ovata*) showed a higher abundance than the number of planted seeds in 'Gov Restored 1' plot.

Abundance was higher in the oldest restoration site (Gov) than in the other two sites (GLMM: z=-5.241, P<0.001; **Table 8**; **Figure 11**). In all sites, abundance was larger in samples with an actively added seed bank than control samples (**Figure 11**). More specifically, abundance differed significantly between the control without actively added seeds and the control (GLMM: z=8.106, P<0.001), vermiculite (z=8.051, P<0.001), and planting mixture (z=10.561, P<0.001) that included the actively added seed bank (**Table 8**). However, there was no significant difference across the soil treatments with the actively added seed bank (**Figure 11**).

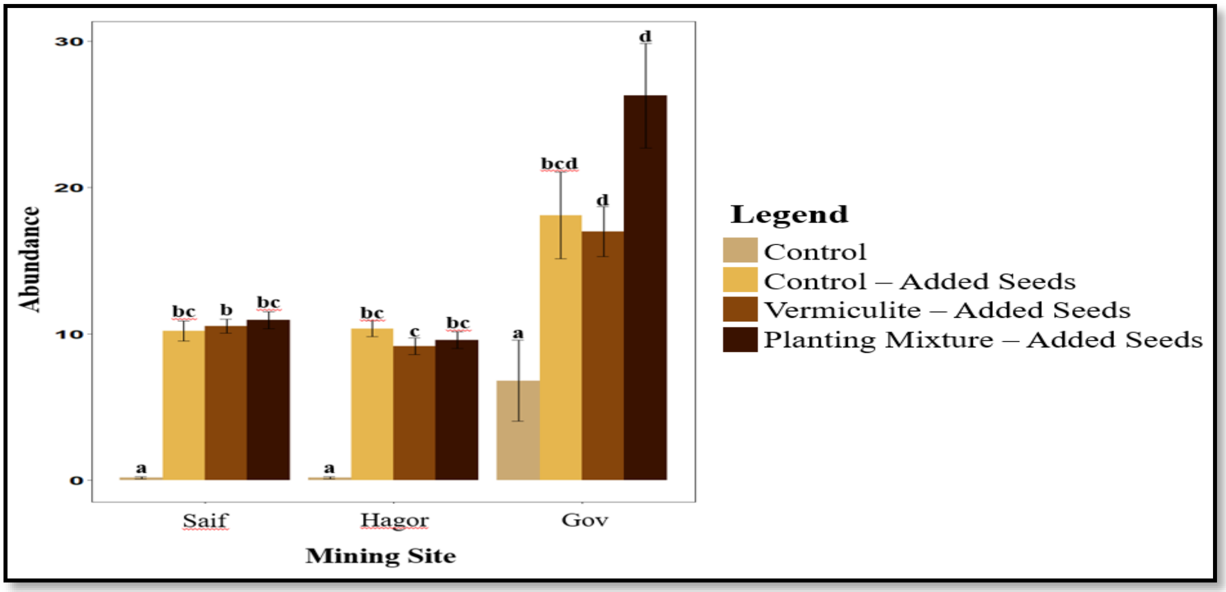

Figure 11: Abundance of germinated plants as a function of treatment in the restored habitat in the different mining sites Saif (restored 2015), Hagor (restored 2010), and Gov (restored 2007). Soil treatments are represented by different colors. Different letters indicate Dunn's significant differences.

Table 8: Summary of generalized linear mixed models (GLMM) testing the effect of site and treatment on plant abundance for the restored areas with an induced seed bank. Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

	Abundance			
	Estimates	**SE**	**z value**	**P value**
Site				
Hagor	-3.295	0.629	-5.241	0.000***
Saif	-3.295	0.629	-5.241	0.000***
Treatment				
Control – Added Seeds	1.187	0.146	8.106	0.000***
Vermiculite – Added Seeds	1.188	0.148	8.051	0.000***
Planting mixture – Added Seeds	1.528	0.145	10.561	0.000***
Site×Treatment				
Hagor×Control – Added Seeds	2.941	0.487	6.044	0.000***
Saif×Control – Added Seeds	2.928	0.487	6.016	0.000***
Hagor×Vermiculte – Added Seeds	2.818	0.487	5.783	0.000***
Saif×Vermiculite – Added Seeds	2.959	0.487	6.076	0.000***
Hagor×Planting mixture – Added Seeds	2.521	0.486	5.185	0.000***
Saif×Planting mixture – Added Seeds	2.658	0.486	5.470	0.000***
Constant term	1.501	0.318	4.723	0.000
Plot				
Variance	0.172	0.415		

Species composition was clustered into three distinct groups: control soil samples without an added seed bank, the soil samples of ‘Gov Restored 1’ plot, and all the other soil samples with an actively added seed bank (**Figure 12**). The only exception is the control soil sample without an added seed bank for ‘Gov Restored 1’ plot, which is standalone between the control group and ‘Gov Restored 1’ group (**Figure 12**). Species compositison differed significantly across soil treatment (PERMANOVA: Psuedo-F=15.874, P(perm)<0.001), and was not significant between site, plot, and interaction between site and treatment (**Table 9**). More specifically, species composition differed significantly between the control without actively added seeds and the control (PERMANOVA pairwise comparison: Psuedo-t=3.916, P(perm)=0.002), vermiculite (Psuedo-t=4.195, P(perm)=0.001), and planting mixture (Psuedo-t=3.963, P(perm)=0.001) that included an actively added seed bank (**Table 10**). Also, there was a significant difference between the control soil samples and the planting mixture soil samples with an actively added seed bank (Psuedo-t=3.307, P(perm)=0.006; **Table 10**). Lastly, there was a significant difference between the two Gov restored plots (Psuedo-t=1.858, P(perm)=0.001; **Table 10**).

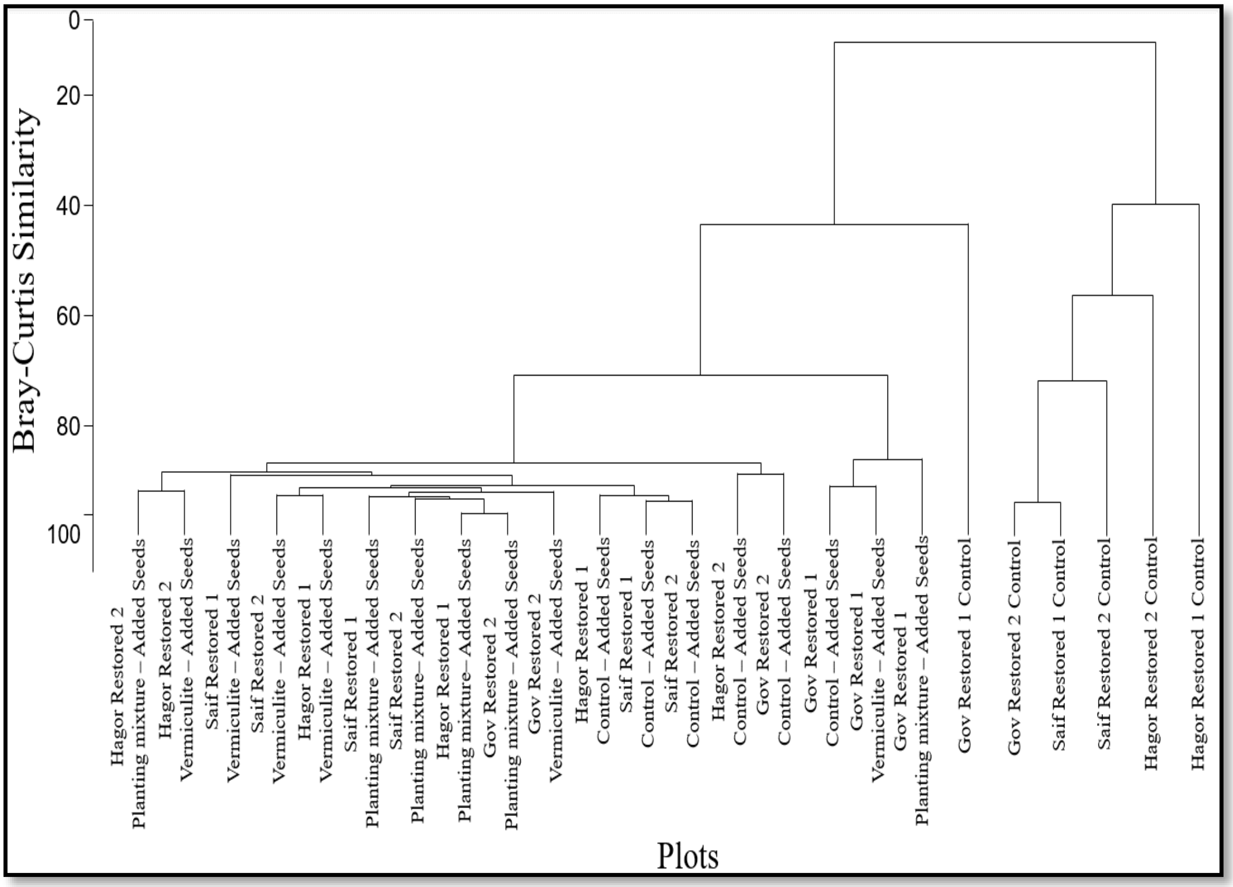

Figure 12: Relative similarity (Bray-Curtis index, calculated for square-root transformed abundances) dendrogram of species composition in the study plots. The legend signifies the site (Gov – restored in 2007, Hagor – restored in 2010, or Saif – restored in 2015), the habitat (natural or restored), the plot number, the soil treatment (control, vermiculite, or planting mixture), and if seeds were artificially added.

Table 9: Results of permutational multivariate analysis of variance (PERMANOVA), testing for the effect of site and treatment on the composition of the plant community for the restored plots with an actively added seed bank. Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
Site	2	2036.80	1018.40	0.906	0.669	15	0.510
Treatment	3	25183.00	8394.30	15.874	0.000***	95059	0.000
Plot (Site)	3	3370.70	1123.60	2.125	0.097	94870	0.109
Site×Treatment	6	2073.50	345.58	0.654	0.767	94413	0.759
Res	9	4759.20	528.81				
Total	23	37423.00					

Table 10: Results of pairwise tests of the factors site (Gov – restored 2007, Hagor – restored 2010, Saif – restored 2015), soil treatment, and plot for the restored areas with an actively added seed bank. Significant p-values are labeled with * < 0.05, ** < 0.01, and *** < 0.001.

Groups	Pseudo-t	P(perm)	Unique perms	P(MC)
Site				
Gov, Hagor	0.968	0.670	3	0.464
Gov, Saif	0.761	1.000	3	0.593
Hagor, Saif	1.473	0.335	3	0.235
Treatment				
Control, Control – Added Seeds	3.916	0.002**	94073	0.003
Control, Vermiculite – Added Seeds	4.195	0.001**	94265	0.002
Control, Planting mixture – Added Seeds	3.963	0.001**	94112	0.002
Control – Added Seeds, Vermiculite – Added Seeds	1.680	0.112	94844	0.115
Control – Added Seeds, Planting mixture – Added Seeds	3.307	0.006**	94604	0.010
Vermiculite – Added Seeds, Planting mixture – Added Seeds	1.892	0.079	95030	0.092
Plot (Site)				
Gov Restored 1, Gov Restored 2	1.858	0.001**	839	0.092
Hagor Restored 1, Hagor Restored 2	0.779	0.551	839	0.551
Saif Restored 1, Saif Restored 2	1.573	0.430	824	0.202

Discussion

In this research, I sought to explore vegetation patterns in the field and germination potential under greenhouse conditions of the Zin valley plant community after a severe disturbance due to open-pit phosphate mining. I compared communities in restored (post-mining) plots with adjacent natural plots not affected by mining (i.e. reference), using control and enriched soil samples. I also evaluated the differences between plant communities from restored mining fields of various restoration years. Additionally, I actively added native seeds to restored control and enriched soil samples to test germination potential. I hypothesized that changes in the plant communities will be apparent along both spatial and temporal scales, and that enriched soil samples will have higher community measures (abundance, species richness, and community composition). I also hypothesized that adding seeds will increase plant community measures, and that these measures will be even higher in enriched soil samples.

The results of the vegetation survey indicated that the prevalence of plant species in the restored habitat of the young and intermediate mining sites (i.e. Saif and Hagor, respectively) are distinctly different from the oldest restored mining site (i.e. Gov) and all the natural habitat in the various sites (**Figure 6**). In the intermediate site, only eight perennial species were recorded, and no annual species at all (personal observations). In the youngest site, no plant species were recorded. This suggests that given time, the plant community can recover with no active assistance from a drastic disturbance, albeit slowly. This is in accordance with other passive restoration studies (Bradshaw 1997; Zahawi *et al.* 2014; Miao *et al.* 2016). However, attempting to link these results in a successional manner and not for each site specifically may lead to false conclusions about the quality of the restoration process.

Furthermore, while abundance was not measured, I observed two noteworthy findings. First, two restored plots of the oldest restoration site (Gov) differed significantly from each other. ‘Gov Restored 1’ is situated in a depression that allows for many plants to flourish, while ‘Gov Restored 2’ is located atop a hill with significantly less vegetation. This finding implies that while time is certainly a factor in the restoration process, other factors (such as topography and geomorphology) may also play a significant role. Second, within the natural habitat, Gov mining site exhibited a much higher abundance than the other two sites, even while prevalence was relatively similar. This indicates that even within the natural habitat there is high variation and endeavoring to compose broad conclusions about the entire mining area without considering the distinction between sites would be inaccurate.

On a spatial scale, I evaluated germination potential of restored mines and reference areas within mining sites (i.e. restored habitat against adjacent natural habitat in each mining site) and between various soil treatments. This was done to determine whether any difference between the two habitats resulted from either seed bank reduction or soil deficiency. The results of the abundance data showed that the young and intermediate sites differed significantly between their natural and restored habitat while the oldest site did not (**Figure 8**). Across the natural habitat, the enriched (which were assumed to be improved) soil samples did not exhibit significantly higher abundances. A similar trend occurred across the restored habitat. This indicates that a lack of seed bank in the soil accounts for the differences between the natural and restored plots. Additionally, a noteworthy trend is that Gov mining site exhibited a much higher abundance than the other two sites.

The conclusions above are strengthened when evaluating the abundance of the active seeding experiment in the restored mining sites (**Figure 11**). In the control samples, the seed bank was clearly lacking. However, once additional seeds were introduced, no differences were found between the various soil treatments within each site. This clearly indicates that the soil itself does not hinder germination. Still, while not statistically significant, I found much higher abundances in Gov than the other two sites, suggesting seed bank establishment in that site. Also, while not statistically significant, there is an increase in the abundance of germinated plants within the planting mixture treatment. This indicates that while the control soil doesn’t hinder germination, the supplementary nutrients that the planting mixture provides results in a higher number of seeds are able to germinate.

Species richness was low across the different mining sites and habitats (**Figure 9**). The low number of species is not surprising in an environment such as a hyper-arid desert. Yet, differences in species richness between the natural and restored plots are still apparent. In the oldest restored site, richness in the natural area increased and was significantly different than the restored habitat with the addition of planting mixture. This indicates that the soil seed bank of Gov mining site is relatively diverse and when given better conditions, more species can germinate. When considering species composition (**Figure 10**), only the youngest site showed a distinct community composition between natural and restored habitats. In the oldest site, the plot ‘Gov Restored 2’ clustered separately from the other restored plot and the natural plots (which clustered together). This indicates that while ‘Gov Restored 1’ reached a recovery point where species composition resembles the reference plots, the same cannot be said from ‘Gov Restored 2’. This implies that time since restoration might not be the most significant factor for restoration, and this correlates with the observation from the vegetation survey.

On a temporal scale, I evaluated germination potential of restored mines and reference areas between mining sites (i.e. habitat across the different mining sites). When considering the restored mining sites, the sites served as a proxy for time since restoration (Gov restored in 2007, Hagor in 2010, and Saif in 2015). Abundance and species richness alone did not reveal any significant differences. However, the species composition in the natural habitat of Gov was significantly different than the other two sites (**Figure 7**). These results go in line with results from the field survey and from the germination analysis within each site, showing that there is high variation between Gov and the other two sites, and that the natural habitat does not necessarily display similar plant communities. All this implies that the different study sites might not be comparable and that comparisons between them on a temporal scale might result in misleading ecological insights. Furthermore, the large variation between the two oldest restoration plots suggests that time since restoration was not the most significant factor that helped vegetation recover, but rather the topographical layout of that particular plot.

I postulated two non-mutually exclusive hypotheses regarding seed establishment: (1) The seed bank hypothesis – in the restored habitat, relative to the adjacent natural habitat, the seed bank (i.e. germination potential) is poorer and less abundant; (2) The soil composition hypothesis – post-mining soils’ characteristics prevent germination and limit vegetation growth. Altogether, my findings indicate that the lack of seed bank is the major limiting factor for the recovery and vegetation establishment in the restored plots. Clearly, seeds from the adjacent natural habitat are barely reaching the restored plots. Therefore, helping reestablish the seed bank upon completing restoration should be given precedence.

Additionally, it appears that the mining activity did not compromise the soil composition and germination is still able to take place once seeds are restored. Overall, four different soil enriching treatments were tested for their potential to enhance germination. Vermiculite and planting mixture were tested in my greenhouse experiments, and another experiment was carried out as part of undergraduate project testing sand and straw. None of these treatments caused a significant increase in germination potential, even when added to natural soils. It is worth noting that the straw was not sterilized and many of the sprouts were of non-native plants, giving further evidence that the topsoil is not compromised. An analysis of the topsoil of the oldest and intermediate sites did not reveal many significant differences between the natural and restored soils (T. Gabay, unpublished data; **Appendix table 5**). Perhaps this is because open-pit mining techniques cause less contamination than other forms of mining, such as tailing or fracking. Also, the phosphate is removed as a complete layer of rock, and therefore does not leave much less contaminated residue in the soil.

Finally, even though all the mining sites display similar abiotic conditions and were assumed to be homogenous, the vegetation differs among the sites. Vegetation, especially in harsh conditions of a hyper-arid desert, relies on microtopography and specific niches to survive (Kemp 1989; Gutterman 1993; Fenner & Thompson 2005). In this case, heterogeneity among the different sites, however small, lead to observed changes in vegetation patterns. Therefore, a more nuanced context of the landscape is required when restoring areas with a hyper-arid nature.

As stated before, the interface between vegetation research, mining, and arid lands in an ecological restoration context is lacking. Hyper-arid deserts are particularly ignored, neglected, and are often thought of as barren lands that can be destroyed without considering subsequent restoration practices. In such cases (as this study) where restoration does occur, most of the focus lies in esthetic rather than ecological considerations. This study is the first step towards providing both practical propositions for the ecological restoration of phosphate mines in Zin valley and increasing our overall understanding of restoration in hyper-arid environments after a severe disturbance.

Practically, this study highlights active steps that can be taken to improve restoration efforts of the area. First, during restoration planning of the landscape, site-specific ecological measures should be considered. Models that help prioritize parameters that restoration efforts should focus on need to be examined and implemented (Bielecka & Król-Korczak 2010; James & Carrick 2016; Carabassa *et al.* 2019). Second, the preservation of removed topsoil and plant material should be given higher priority. Studies show that proper management of topsoil is crucial for enhancing restoration efforts, especially of revegetation (Shackelford *et al.* 2018). However, research in this area is still ongoing, specifically on how to deal with large topsoil stockpiles and the long waiting periods until they are returned to the ground. Third, my findings highlight the fact that restoration, especially in extreme areas such as hyper-arid deserts, require assistance after such a severe disturbance such as mining. Many restoration efforts around the world immediately introduce active restoration measures, either through direct seeding or seedling plantings (Palma & Laurance 2015; Grant *et al.* 2016; Mattiske 2016; Larios *et al.* 2017). A study of revegetation projects done by Omar & Bhat (2008) in Kuwait (highly similar to Zin Valley in climatic conditions) showed very promising results. However, there is a serious lack of native plant research and seed storage and supplementation in Israel in general and of Negev species in particular. Fourth, an ongoing monitoring system should be considered to assess the restoration efforts after reclamation is complete. Long-term monitoring endeavors offer important insights for restoration projects (Brown 2005; Campbell *et al.* 2017; Shackelford *et al.* 2018) A monitoring system should be realized to insure the success of the restoration efforts of the company and increase our understanding for future reference. Future research directions from a practical prospective should focus on improving topsoil management, active restoration in the field, and monitoring efforts.

Conceptually, my findings strengthen results about removed seed banks in highly disturbed, hyper-arid areas, the importance of preserving topsoil, and site-specific restoration connotations. My findings advocate future research directions that will involve understanding dispersal mechanisms of native desert plants. This can help improve the topographic consideration in the restoration planning, which consequently might boost passive revegetation in the area. Another research direction could focus on the aboveground and belowground interactions of plants and their environment, including microbes and insects. This can help improve our understanding of the ecosystem functionality and thus allow for better planning and monitoring restoration practices. Finally, more attention should be placed on building a native seed pool and studying plant functional traits for restoration projects.

My results indicate a complex picture of vegetation reestablishment following the mining disturbance in Zin phosphate mines. The nature of soil seed banks and germination in general, and in arid environments specifically, is highly crucial for restoration potential after mining. This research is a first step in understanding plant communities and successional processes in post-mining areas of hyper-arid regions. Human development is not slowing down and is only expected to increase in years to come. Therefore, given how important ecosystem restoration will become in the coming decade (Aronson & Alexander 2013; Suding *et al.* 2015; UN General Assembly 2019), the novelty of this research offers exciting prospects and a chance to assist in the preservation of the unique ecosystem of the Zin valley desert.

Appendix

Appendix table 1: Vermiculite chemical composition (weighted %).

SiO_2	Silica	38-46
Al_2O_3	Aluminum	10-16
MgO	Magnesium	16-35
CaO	Calcium	1-5
K_2O	Potassium	1-6
Fe_2O_3	Iron	6-13
H_2O	Water	8-16
Other	-------	0.2-1.2

Appendix table 2: HR2 planting mixture chemical composition (weighted %).

Peat	43
Coconut	50
Quartz sand	7

Appendix table 3: List of natural plant species acquired from the Israeli Gene Bank.

Latin Name	**Common Name**
Chenopodiastrum murale	Nettle-leaved goosefoot
Astragalus tribuloides	------------------
Malva parviflora	Cheeseweed mallow
Aaronsohnia factorovsky	Aronsonia factorovsky
Plantago ovata	Desert indianwheat
Diplotaxis acris	Violet wall-rocket
Asteriscus graveolens	------------------
Centaurea pallescens	Pale star thistle
Stipa capensis	Common awn grass
Anabasis setifera	------------------

Appendix table 4: List of plant species identified in the study (field and greenhouse).

Latin Name	Common Name	Appearance in field	Appearance in greenhouse
Chenopodiastrum murale	Nettle-leaved goosefoot		X
Erodium crassifolium	Desert stork's-bill	X	X
Trigonella stellata	Star fenugreek	X	X
Astragalus tribuloides	--------------------	X	X
Malva parviflora	Cheeseweed mallow	X	X
Aaronsohnia factorovsky	Aronsonia factorovsky	X	X
Plantago ovata	Desert indianwheat	X	X
Diplotaxis acris	Violet wall-rocket	X	X
Ononis mollis	Restharrow	X	X
Asteriscus graveolens	--------------------	X	X
Filago pyramidata	Broadleaf cottonrose		X
Centaurea pallescens	Pale star thistle	X	X
Stipa capensis	Common awn grass	X	X
Stellaria pallida	Lesser chickweed		X
Senecio flavus	--------------------		X
Pteranthus dichotomus	--------------------	X	X
Cynodon dactylon	Bermudagrass		X
Kickxia acerbiana	Cancerworts	X	X
Senecio glaucus	Buck's horn groundsel	X	X
Fagonia mollis	Common fagonia	X	X
Conyza	Horseweed		X
Aizoaceae	--------------------		X
Aizoon hispanicum		X	
Mesembryanthemum nodiflorum		X	
Mesembryanthemum forskalii		X	
Erodium laciniatum	--------------------	X	
Anastatica hierochuntica	Rose of Jericho	X	
Reichardia tingitana	Poppy-leaved reichardia	X	
Pulicaria incisa	Undulate fleabane	X	
Matthiola livida	--------------------	X	
Zygophyllum dumosum	Bushy bean-caper	X	
Zilla spinosa	--------------------	X	
Salsola damascena	--------------------	X	
Androcymbium palaestinum	--------------------	X	
Hyoscyamus desertorum	--------------------	X	
Anabasis setifera	--------------------	X	
Ochradenus baccatus	--------------------	X	
Astragalus dactylocarpus	--------------------	X	
Pseuderucaria clavata	--------------------	X	
Moricandia nitens	Moricandia	X	
Helianthemum ventosum	--------------------	X	

Agathophora alopecuroides	--------------------	X	
Launaea angustifolia	--------------------	X	
Atriplex suberecta	--------------------	X	
Tamatix nilotica	--------------------	X	
Gymnocarpos decander	--------------------	X	
Rumex cyprius	--------------------	X	
Heliotropium arbainense	--------------------	X	
Arnebia decumbens	--------------------	X	
Caylusea hexagyna	--------------------	X	
Lasiopogon muscoides	--------------------	X	
Nitraria retusa	Salt tree	X	
Calendula arvensis	Field marigold	X	
Salvia aegyptiaca	--------------------	X	
Anvillea garcinii	Arabian oxeye	X	

Appendix table 5: Physicochemical soil properties. Values are means and standard errors of all topsoil samples from either restored or undisturbed plots in each sampling site. Adapted from Talia Gabay's unpublished data.

Site	**Hagor**		**Gov**	
Plot Type	Undisturbed	Restored	Undisturbed	Restored
Saturation (%)	26.89±1.48	31.01±2.51	28.19±1.70	31.56±2.40
pH	7.98±0.20	7.61±0.24	8.01±0.25	7.95±0.10
EC (dS/m)	37.63±10.69	44.37±13.52	13.04±7.74	17.86±5.66
NH4 (mg/kg)	2.21±0.35	3.3±0.79	1.46±0.39	6.61±2.17
NO3 (mg/kg)	289.31±97.95	310.71±109.04	146.34±93.89	47.91±10.30
P (mg/kg)	8.18±1.04	17.2±3.24	9.41±1.53	10.16±2.50
K (mg/kg)	1.14±0.22	1.4±0.36	1.32±0.56	0.81±0.27
Sand (%)	60.5±4.26	69.41±3.70	68.18±5.25	70.26±2.17
Silt (%)	23.63±4.78	17.68±3.71	19.75±3.89	18.33±2.65
Clay (%)	15.86±2.95	12.9±2.27	12.06±2.51	11.4±2.35
SOM	1.47±0.44	0.39±0.08	1.24±0.14	0.72±0.18

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תוכן עניינים

הערכת נביטה של אתרים טבעיים ואתרים ששוקמו אקולוגית במכרות הפוספטים בבקעת צין, ישראל

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מערכות אקולוגיות נפגעות מאד בעקבות ההליך האנתרופוגני של כרייה. כריית פוספטים מתרחשת על פני 200 קמ"ר רבוע של מדבר הנגב בישראל. אולם, לא נבדקה ההשפעה של תהליכי השיקום המתמשכים של המכרות. לצמחים ובנק הזרעים שלהם יש תפקיד מרכזי בתהליכים שקורים בתוך מערכות אקולוגיות, ולכן יש להם חשיבות עצומה כאשר חוקרים שיקום אקולוגי. אני התמקדתי בשלושה אתרי כרייה ששוקמו בשנים שונות בתוך בקעת צין, והשוותי בין חברת הצומח והצלחת הנביטה בחלקות משוקמות ביחס לחלקות טבעיות סמוכות. אני שיערתי (1) שיש חוסר בבנק הזרעים בתוך החלקות המשוקמות; (2) שהרכב הקרקע המשוקם שונה מהקרקע הטבעית ומגביל נביטה. אני הקמתי שני ניסויי חממה בעזרת דגימות קרקע שנאספו מאתרי הכרייה השונים: (1) השוואה בין אזור טבעי מול משוקם, שטופלו באמצעות קרקע גננית או ורמקוליט; (2) הוספת זרעים מצמחייה טבעית ומקומית לקרקע משוקמת כדי לבחון את יכולת הנביטה שלהם. התוצאות הצביעו על כך שחוסר בבנק הזרעים הוא הגורם המגביל העיקרי בתהליך שיקום חברת הצומח ולא נראה שהרכב הקרקע מונע נביטה. שפע הפרטים היה נמוך משמעותית בחלקות משוקמות ביחס לחלקות טבעיות באתר הכרייה הצעיר ואתר הכרייה האמצעי. עושר המינים גם כן היה נמוך משמעותית, אולם רק בטיפול הורמקוליט. הרכב החברה גם היה שונה משמעותית. באתר הכרייה הישן ביותר, לא נמצאו הבדלים משמעותיים בשפע הפרטים או בהרכב החברה. עושר המינים נמצא משמעותית נמוך יותר בחלקות משוקמות ביחס לטבעיות רק כאשר נוסף טיפול של קרקע גננית. כאשר משווים חלקות משוקמות ששוקמו בשנים שונות, נגלה שהרכב החברה שונה משמעותית. אבל תוצאה זו הינה מטעה, מכיוון שנמצאו הבדלים משמעותיים בין שפע הפרטים והרכב החברה גם בין האזורים הטבעיים של אתרי הכרייה השונים. התוצאות שלי מראות תמונה מורכבת של התבססות מחודשת של הצמחייה בעקבות הפרעת הכרייה. עבודות שיקום נקודתיים צריכות להתמקד על שיפור הליך התכנון תרום-כרייה כדי להתאים מטרות ליעדים ספציפיים, שימור הקרקע העליונה (טופ-סויל), זריעה אקטיבית בחלקות המשוקמות כדי לזרז את התבססות הצמחייה, וניטור של כל התהליך והתוצאות הסופיות. בכללי, המחקר שלי הרחיב את הידע על שיקום צמחייה במדברים היפר-ארידים ובמכרות פוספטים. מחקר עתידי רצוי שהתמקד בדפוסי הפצה של צמחים מדבריים ובקשרים עליים/תחתיים. המחקר שלי שופך אור על המגבלות הצמחייה באזורים מופרעים היפר-ארידים והינו הבסיס לניסויי המשך שבודקים עבודות שיקום עתידיות של מכרות הפוספטים.

אוניברסיטת בן-גוריון בנגב

הפקולטה למדעי הטבע

המחלקה למדעי החיים

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שבט תש"פ פברואר 2020