The impact of biostimulation on the indigenous microbial communities in oil-contaminated desert soils in southern Israel

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Introduction

The Evrona nature reserve, located in southern Israel, experienced two oil spills that occurred in 1975 and 2014 (Tran et al., 2018 Fig.1). The two contaminated areas are situated near each other, with a few hundred meters distance between them (Nothers et al., 2017). The oil pollution-induced severe soil hydrophobicity (Gordon et al., 2018; Li et al., 2021) decreases the density of young trees, inhibits seed germination and growth (Tran et al., 2018), and reduces the bacterial species (Girsowicz et al., 2018). Therefore, this site's remediation of hydrocarbon contaminations is essential to this fragile desert ecosystem. We suggested that the contaminated soil microbial community can degrade the polluting oil and restore it at least partially if stimulated. Therefore, the objectives of our study were to investigate (1) the microbial communities in response to the biostimulation of water, nutrients, and biosurfactant and (2) the relationship between hydrocarbon metabolism genes and the physicochemical properties of the soil.

Figure 1. Aerial photographs of the December 2014 petroleum oil spill in the Evrona nature reserve, southern Israel. Photographed were taken on 4th Dec 2014

Material and Methods

The soil samples used in this study were obtained from a previous experiment (Li et al., 2021). Briefly, soils were collected from oil-contaminated spill sites in 1975 and 2014 and incubated for one year and a half. The soil samples were incubated at water contents of 20 and 20, and 50% (w/w) and amended with a Nutrient solution of 150 mg/l ammonium sulfate and 150 mg/l potassium phosphate in tap water. Surfactant solution addition contains 200 mg/l Rhamnolipids. Incubation was at 25 °C and soil was mixed every two or three days to keep the oxygen levels in the sealer container. DNA was extracted at the beginning and end of the experiment, and the *alkB, nahAc,* and *phe* genes were quantified by qPCR. The V3-V4 region of the 16S rRNA gene of bacteria and archaea was sequenced. The sequence data were analyzed with mothur and R packages.

Results and Discussion

The microbial community structures were evaluated in the 1975 and 2014 amended contaminated soils. As shown in Figure 2A, the most significant proportion of unique operational taxonomic units (OTU) was observed in 75 controls in 1975 samples. 75W20 and 75W20Nut presented 29.1% OTUs in common for 1975 soil treatments. The proportion of unique bacterial OTUs was most remarkable in 14 control (3.4%), followed by 14W50 (2.6%) and then other soils for 2014 soil treatments (Figure 2B). The proportions of shared archaeal OTU were more than 90% for all the 1975 and 2014 soils, more significant than that of shared bacterial OTU.

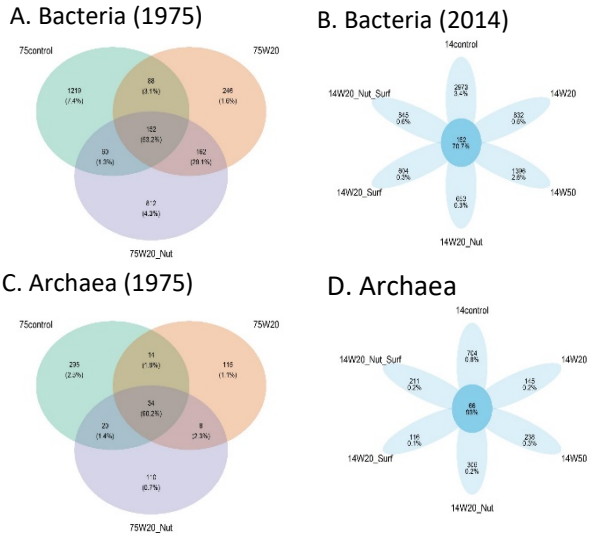

Figure 2. Venn diagram showing the number and percentage of OTUs of bacterial populations for (A) 1975 soil samples and (B) 2014 soil samples, and of archaeal populations for (C) 1975 soil samples and (D) 2014 soil samples at the end of incubation.

The abundance of *Firmicutes* and *Gemmatimonadetes* decreased in the above treatments compared to the control soil at the end of incubation. No apparent changes were observed between the samples from the start and at the end of incubation for 2014 control and 2014 clean soils. For the bacterial communities in 1975 soils, the abundance of *Chloroflexi*, *Verrucomicrobia*, and *Deinococcus-Thermus* increased in the biostimulation treatments. In addition, the two dominant archaeal phyla were *Euryarchaeota* and *Thaumarchaeota* for 1975 and 2014 soil treatments. The correlations between soil hydrophobicity, identified by water drop penetration time (WDPT) and molarity of ethanol drop (MED), total petroleum hydrocarbon (TPH), and the abundance of genes were tested. Gene *alkB*, encoding the alkane monooxygenase, was negatively correlated with WDPT, MED, and TPH in 1975 contaminated soils, while the correlation was positive in 2014 contaminated soils, even though not significant. The abundance of gene *nahAc* (encoding naphthalene dioxygenase) was positively correlated ($p < 0.05$) with WDPT, MED, and TPH in 2014 contaminated soils (Figure 6A). Additionally, a negative correlation was observed between the abundance of *phe* (encoding phenol monooxygenase) and WDPT, MED, and TPH for 2014 soils.

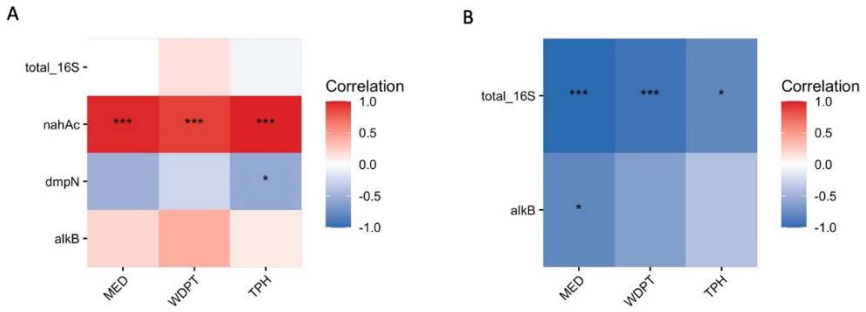

Figure 3. Correlations between the abundance of genes (copies/dry weight soil) and soil properties (WDPT, MED, and TPH) for the bacterial populations in (A) 2014 contaminated and (B) 1975 contaminated samples. "*" indicates $p < 0.05$, "**" indicates $p < 0.01$ and "***" indicates $p < 0.001$.

We conclude that oil pollution remarkably affects microbial compositions and promotes the growth of hydrocarbon degraders in desert soils. Biostimulation of water saturation, nutrients, and biosurfactants further shaped the indigenous communities along the 1.5-year incubation. *Proteobacteria* and *Actinobacteria* comprise most microbial populations in oil-polluted and clean soils. The microbial richness and diversity were higher in the untreated oil-polluted soils compared to the biostimulation treatments. While the hydrocarbon removal may not depend on the overall communities or richness but on the species that potentially contribute to the degradation. *Firmicutes* were considered significant hydrocarbon degraders in the current oil-polluted soils. The abundance of *Firmicutes* increased in response to the high levels of hydrocarbons under supportive environmental factors. Then it decreased along with the long hydrocarbon levels and competition with other species (e.g., *Chloroflexi*). Enriching *Firmicutes* and hydrocarbon-degrading genes in the 14control and 14W50 at the end of incubation also supported this. The correlation between the *nahAc* gene and WDPT, MED, and TPH may indicate that aromatic hydrocarbons are significant factors of the soil hydrophobicity at the end of incubation. Therefore, the bioremediation of aromatic hydrocarbons may need further exploration.

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