Intrinsyx Environmental

Intrinsyx Environmental Case Study

Endophyte-Enhanced Phytoremediation - Petroleum Hydrocarbons

Former Diesel & Gasoline Transfer Station, Dos Palos, CA

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1. Summary

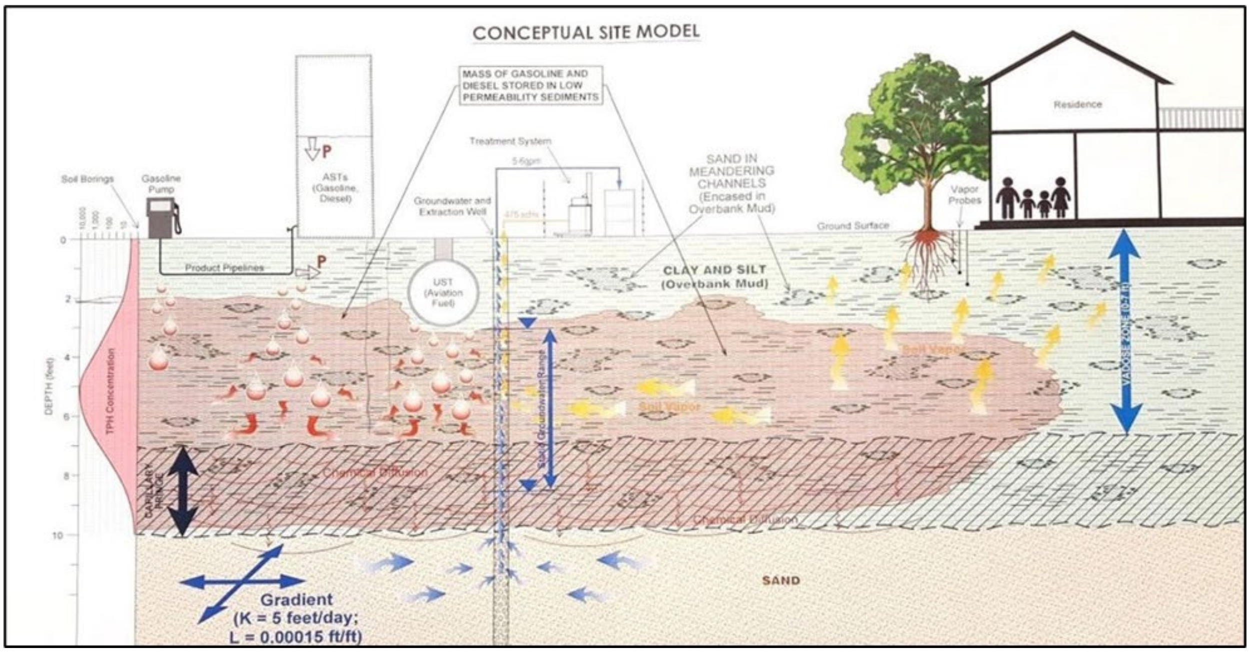
- Environmental Setting: Former diesel gas transfer station in the Central Valley of California with brackish groundwater (EC 3.0 dS/m ; TDS > 2,000 mg/L) and a high salinity sodic soil (EC 4.4 dS/m ; pH 7.8).
- Scale of Installation: over 1,000 trees, planted in 3 phases across 3.5 acres.
- Contaminants of Concern: Light non-aqueous phase liquid (LNAPL), dissolved phase diesel range organics, gasoline range organics, polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPH), benzene, toluene, ethylbenzene, xylenes (BTEX).
- Brief Description of Remedy: Phase A: Rescue poplar trees from phytotoxic effects of petroleum hydrocarbons by inoculating trees with PD1 endophyte, Phase B: planting of 484 salt and boron tolerant RRR hybrid poplar and salt resistant willow trees inoculated with PD1 endophyte, Phase C: planting of 434 poplar and willow trees inoculated with PD1 endophyte.
- Key Successes: Saving the client >$1 million in O&M costs, provide thriving phytoremediation solution in soil and groundwater saturated with fuel, establish hydrological control, enhance natural source zone depletion rates.

Figure 1: Former gas station and fuel transfer terminal in the Central Valley, CA

2. Site Description

The Dos Palos site is a former petroleum transfer terminal located in the northwestern area of the San Joaquin Valley in Central CA. The facility was used to store and dispense gasoline and diesel fuel, using underground storage tanks (USTs) and above-ground storage tanks (ASTs). In 1988, two 5000-gallon aviation fuel USTs were removed from the premises and revealed petroleum hydrocarbon contamination of the soil and groundwater (California Water Board, 2006). The Merced County Environmental Health Department investigated the site in 1988 and found contamination due to leaky pipes and surface spills. The site was transferred to the California Water Board in 2003 after several rounds of investigations that demonstrated petroleum hydrocarbon impacts to the groundwater and soil beneath the site (California Water Board, 2006).

Figure 2: Conceptual site model showing the depth and movement of contaminants in the soil and aquifer

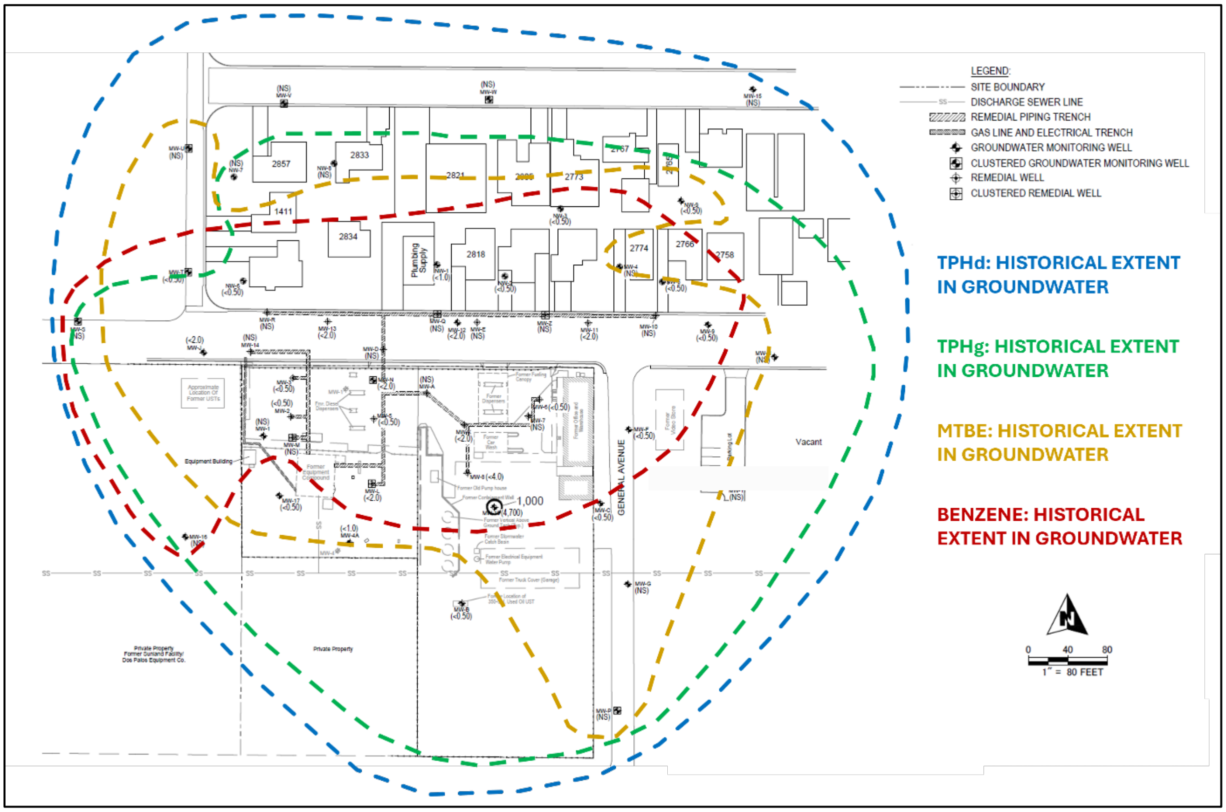


During the 1988 UST removal, strong petroleum odors were present in the UST excavation. During the subsequent investigations, separate-phase hydrocarbons were found floating on the relatively shallow water table (4-5 ft). The plume spread out along the water table in all directions from the original contamination site. Residences were located to the north of the fueling station and were affected by the plume migrating in that direction. Some groundwater monitoring was done during the initial investigation, finding high levels of benzene, toluene, xylenes, TEL, TPHd, and TPHg. Additional groundwater monitoring was performed in 1994, but there were no sampling or monitoring activities from 1998-2002. In 2002, sampling and monitoring resumed, again showing petroleum hydrocarbon contamination of the water table with a maximum layer thickness of 5.24 feet on top of the water table. In 2003, regulatory oversight was transferred from Merced County to the California Water Board due to the impacts on the groundwater in the area (California Water Board, 2006).

From 1996 to 2005, remediation methods included LPH bailouts, dual-phase extraction, and removal of liquid-phase hydrocarbons using pneumatic skimmer pumps. A SVE system was added in 2005 but was constantly being fouled by the diesel and gas derivatives on-site, making it problematic and costly to maintain (California Water Board, 2006).

In March and April of 2017, ETIC planted 274 hybrid poplar trees to study the feasibility of using hybrid poplar trees to supplement and enhance groundwater recovery beneath the site. One year after planting, 70% of the trees were sick or dying due to the phytotoxic conditions. Intrinsyx Environmental performed a tree health assessment in 2018 and proposed inoculation with PD1 endophyte—a microbe native to the Pacific Northwest that lives inside poplar trees and provides tolerance to hydrocarbon Phytotoxicity (Khan, et al., 2014).

Figure 3: Plume map showing the extent of the TPHd, TPHg, MTBE, and benzene contamination



3. Remedial Objectives

The shallow depth of the water table, volatility of contaminants, and proximity to residential properties poses many risks to environmental and human health on this site. The Dos Palos site is located in the Delta-Mendota subbasin which is a long, narrow groundwater basin that covers portions of Sacramento, San Joaquin, Stanislaus, Merced, Madera, and Fresno counties. Beneficial water uses include irrigation for agriculture and urban usage (Northern and Central Delta-Mendota Region GSP, 2019). Diesel and gasoline derivatives are volatile and move readily through different mediums. The initial rounds of investigation revealed contamination of soil and groundwater below the USTs, but further investigation revealed that contaminants such as TPHd, TPHg, benzene, toluene, MTBE, and xylenes had spread in all directions on the water table.

In 1996, floating product was reported in 21 monitoring wells: 12 on-site and 9 off-site wells (California Water Board, 2006). The nearby residences to the north of the initial contamination site became increasingly exposed to contaminants in drinking water and soil vapor as the plume migrated in all directions. These contaminants volatize from the dissolved phase to the vapor phase and can potentially migrate indoors through basements and other pathways. Diesel and gasoline vapors are carcinogenic, irritating to the lungs, can have effects on the nervous system, cause dizziness and headaches (Agency for Toxic Substances and Disease Registry, 1995). During soil vapor sampling events in 2005, TEL and benzene concentrations were found to be above the California Human Health Screening Level at residences on the Northern perimeter of the plume (California Water Board, 2006).

3.1 Remedial Site Goals

1. Maintain, operate, and modify the remediation systems to capture, cleanup the pollutants, and prevent the further migration of pollutants from the site, and to provide continuous capture of vapors from the contaminant plume.
2. Continue operation of the remediation system until the Water Board approves, in writing, the cessation of operation.

3.2 Phytoremediation Goals

1. Establish healthy poplar trees
2. Rescue existing distressed trees from the phytotoxic effects of site contaminants
3. Create a cone of depression in local groundwater to prevent contamination plume from migrating offsite
4. Protect the trees from contaminant phytotoxicity with PD1 endophyte
5. Enhance rhizosphere breakdown of source zone contaminants with tree roots and microbial amendments
6. Decrease the rate of off-gassing VOC

4. Remedial Approach

Final selected remedy: Endophyte-assisted phytoremediation using PD1 endophyte and salt and boron tolerant RRR hybrid poplar and willow trees.

4.1 Remedy Description

Intrinsyx Environmental was originally consulted to save an existing phytoremediation pilot site via root drench inoculation with PD1 bacterial tree endophyte. After increasing the tree survival rate from 30% to >95%, Intrinsyx Environmental was contracted for scientific consultation, biological direction, and system design for Phase B and C of the phytoremediation plan using salt and boron tolerant RRR hybrid poplars, mycorrhizal amendments, and white-rot fungi supplied by The Remediators, Inc.

4.2 Approach Benefits

The effectiveness of the PD1 strain was demonstrated in a wide screening of many endophytes. PD1 outcompeted other strains in the breakdown of PAHs and tolerance to salt and temperature stress. PD1 also provided tolerance to plants exposed to otherwise lethal concentrations of phenanthrene and increased willow trees' ability to transpire normally while removing phenanthrene from the soil. The use of the PD1 endophyte, coupled with salt/boron hybrid poplars and mycorrhizal amendments to help with the uptake of water and minerals is what made phytoremediation possible on this challenging site (Khan, et al., 2014).

5. Monitoring and Results

5.1 Phase A

The application of PD1 endophytes to trees in Phase A resulted in the successful rescue of the initial phytoremediation site and increased the overall health of the trees in the long term. All the stressed trees, except for the 30 reserved as a control group, were inoculated using 160 mL of PD1 endophyte per tree as a root drench. One year after the root drench, the inoculated trees showed significantly more growth than the uninoculated trees. Trees treated with our PD1 endophytes exhibited 100% greater growth and demonstrated extensive new shoot growth This was also echoed by the SPAD Chlorophyll values, with inoculated trees having an average SPAD chlorophyll value of 49.8 and uninoculated having an average SPAD chlorophyll value of 33.8. The tree survival rate after PD1 endophyte treatment was >95%.

Figure 4: Tree mortality rate during Phase A of phytoremediation

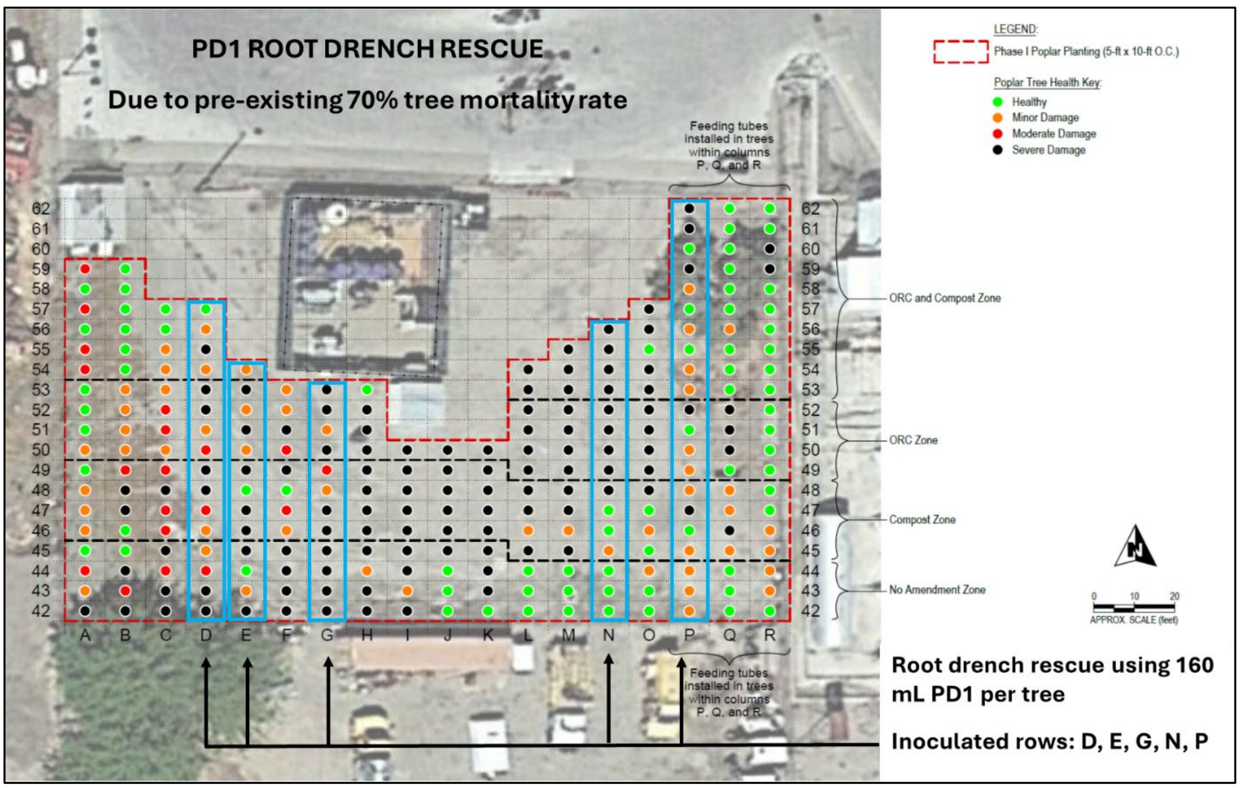


Figure 5: Picture showing growth differences of PD1 inoculated vs uninoculated hybrid poplars on the site

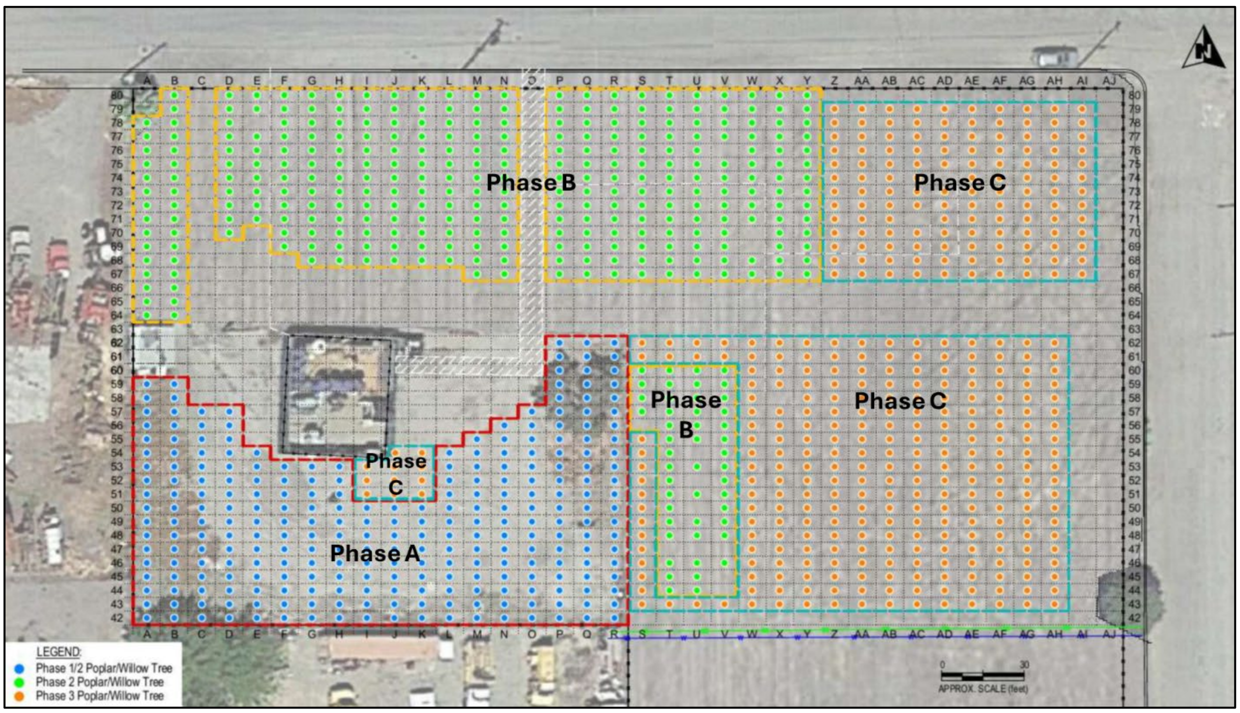
Table 1: Table showing percent recovery after Intrinsyx Environmental's involvement on the site (calculated by tree health metrics such as height, chlorophyll content, diameter at breast height, and number of shoots). Asterisks indicate rows of trees that were severely affected by the growing conditions on-site and in very poor health.

Tree row	Percent recovery	Inoculation status
A	11.1%	
B	11.10%	
C	12.50%	
D*	37.50%	inoculated
E*	30.80%	inoculated
F*	8.30%	
G*	NQ	inoculated
H*	8.30%	
I*	11.10%	
J*	11.10%	
K*	22.20%	
L*	15.40%	
M*	NQ	
N*	6.70%	inoculated
O*	12.00%	
P*	19.00%	inoculated
Q	4.80%	
R	14.30%	

5.2 Phase B and C

After the successful recovery of the trees in Phase A, hybrid poplars and willows were planted across the entire site and inoculated with PD1 endophytes in Phase B and C bringing the total to 1,400 trees (see Figure 6). The endophytes provided enhanced establishment for trees planted in phases B and C, resulting in a 98% survival rate. The rate of treatment by endophyte-assisted hybrid poplars exceeded the rate of treatment by the groundwater pump-and-treat and the soil vapor extraction systems, eventually leading to the decision by the Central Valley Regional Water Quality Control Board to discontinue the previous systems and allow phytoremediation to be the only type of remediation on the site. Between May and September 2019, the client installed three temperature and oxidation-reduction potential (ORP) sensor probes; installed two pressure transducers/piezometers; and collected carbon dioxide flux samples from twenty-five sampling points, current planting density of 870 trees per acre.

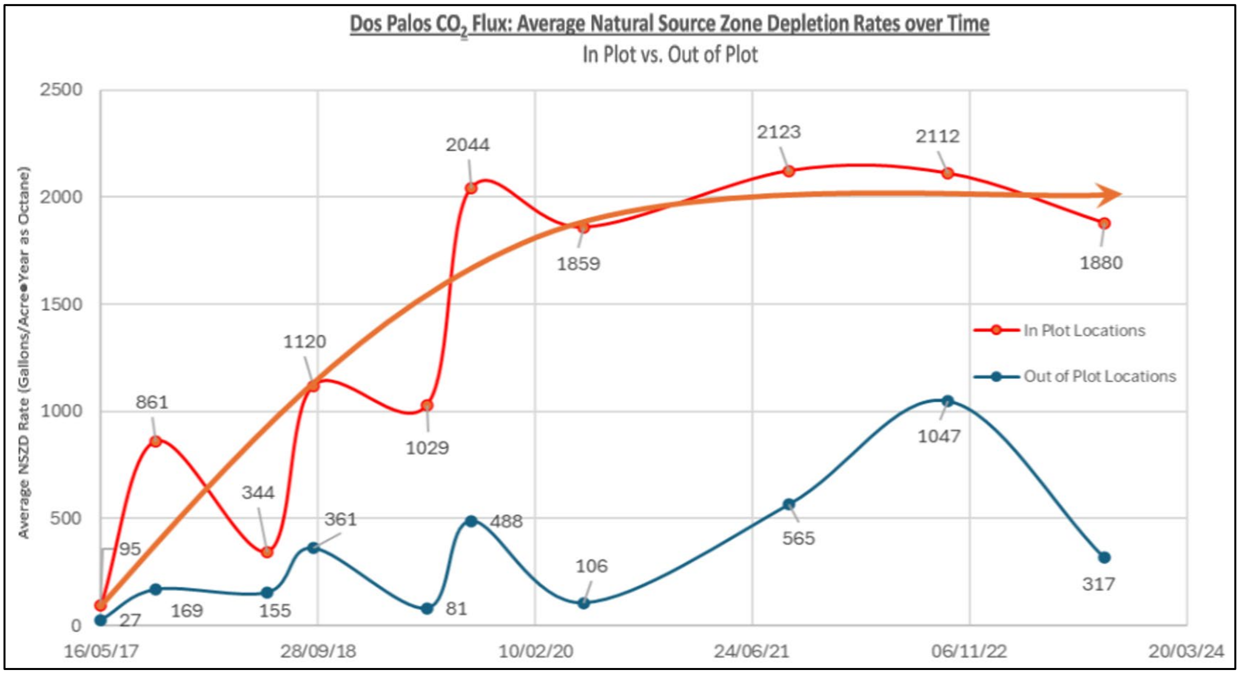
Figure 6: Map showing Phase B and Phase C expansions (relative to Phase A)



5.2.1 Natural Source Zone Depletion Rates

A year-over-year increase in in-plot natural source-zone depletion (NSZD) rates after planting inoculated trees shows that the trees accessed the source zone and used the contaminated water as part of their natural process (shown in Figure 7).

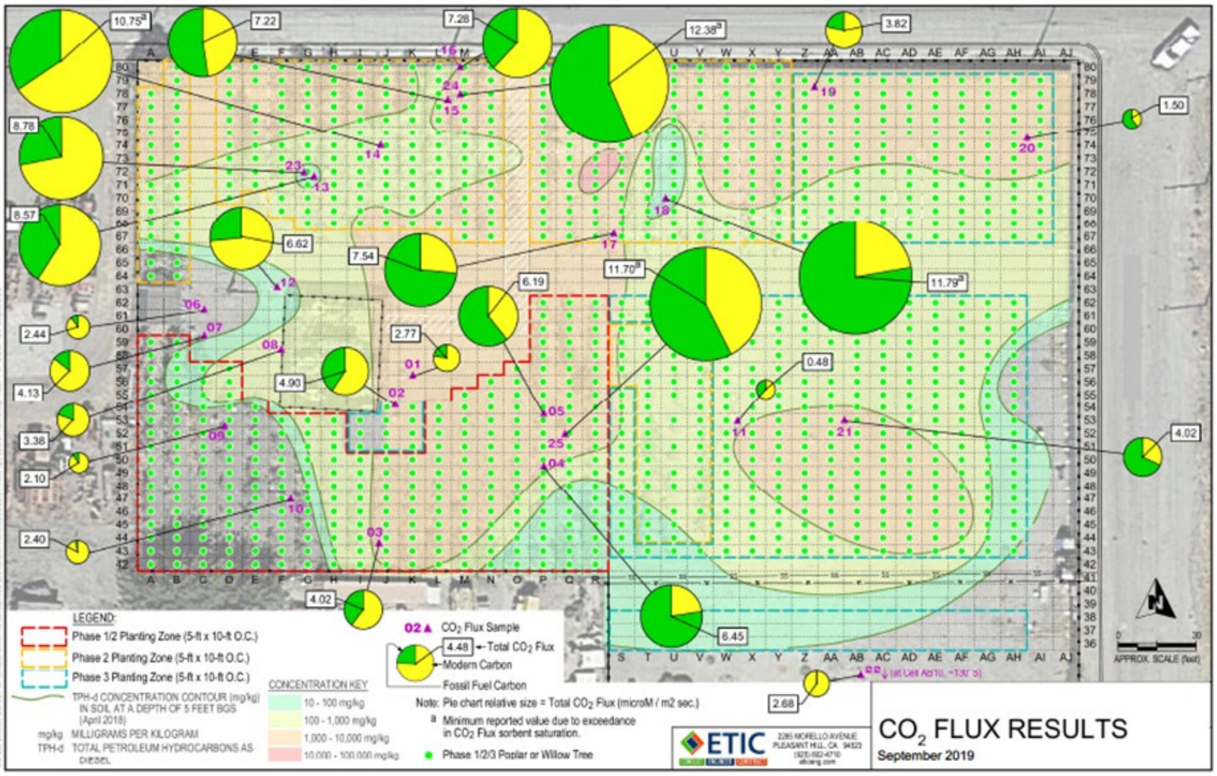
Figure 7: Average Natural Source Zone Depletion rates over time with the red line showing in-plot CO_2 flux locations and the blue line showing out-of-plot CO_2 flux locations



Comparison of the NSZD rates (gallons/year) inside and outside of the planted areas and within similar soil concentrations zones shows elevated hydrocarbon biodegradation rates within the phytoremediation test area. The magnitude of the increase in the NSZD rates varies relative to the hydrocarbon concentration in the vadose zone soil. For high-concentration zones (1,000-10,000 mg/kg diesel), the increases range from 2.7 to 4.5 times greater NSZD rates than out-of-plot zones with high concentrations. For medium concentration zones (100-1,000 mg/kg diesel), the increases range from 1.9 to 3.4 times greater NSZD rates than out-of-plot zones with medium concentrations. The enhancement of NSZD rates is due to several factors associated with planting trees at the site.

The CO_2 flux analysis was pivotal in proving that the endophyte-assisted poplars broke down hydrocarbons and fossil fuel-derived hydrocarbons at the fuel station site (shown in Figure 8). Figure 8 shows the CO_2 flux samples as a pie chart with yellow representing modern carbon and green representing fossil fuel-derived carbon. A typical by-product of root respiration and microbial activity is CO_2. Elevated concentrations of hydrocarbons provide a carbon source for both endophytes and soil microbes. The robust, endophyte-assisted trees uptake $C_xH_yO_z$ compounds and exude sugars and other nutrients, supporting increased rhizosphere activity and resulting in additional flux of respired CO_2 in the soil. Large portions of the CO_2 respired into the soil by the trees were fossil fuel-derived carbon, as proven by carbon dating, showing that the trees and tree-enhanced rhizosphere consumed and broke down oil and gas products. We can also see that off-plot, the pie chart is almost entirely yellow. Minimal fossil-fuel-derived CO_2 in off-plot samples demonstrates that the microbial community in that area could not metabolize fossil-fuel-derived CO_2. The CO_2 flux analysis shows that, despite the high levels of contamination in-plot, there is a thriving fungal and microbial flora because of the increase in respired, fossil-fuel-derived CO_2.

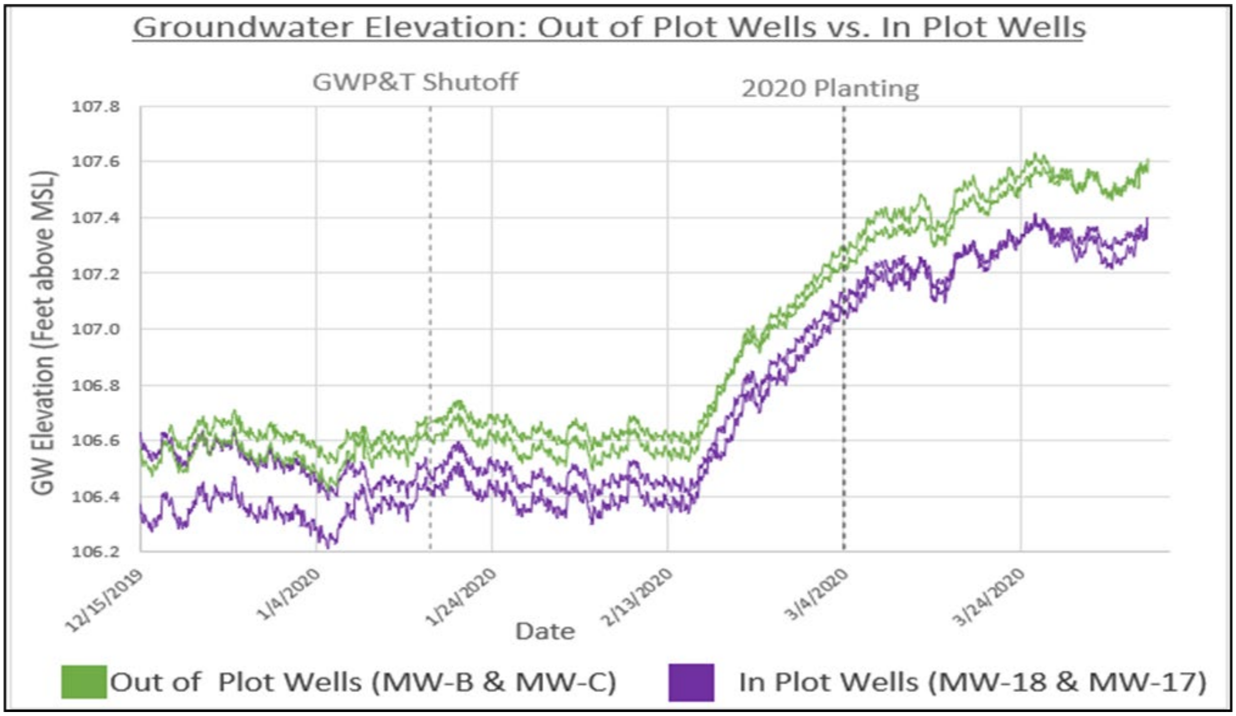
Figure 8: CO_2 flux sample analysis is depicted as pie charts with yellow representing modern carbon and green representing fossil fuel-derived carbon



5.2.2 Groundwater Uptake and Hydraulic Control

Decreases in the in-plot and off-plot groundwater elevation show that trees gained hydraulic control of the site and began mitigating the migration of contaminants off-site within one year of planting (shown in Figure 9). Before the groundwater pump and treat system was shut off, in-plot and off-plot groundwater monitoring wells had very similar groundwater elevation levels. In-plot groundwater levels were hovering around 106.6 feet above mean sea level (amsl), and off-plot groundwater levels were hovering around 106.5 feet amsl. After the groundwater P&T system was shut off in January 2020, the trees took control of the water table and created a zone of depression in-plot. The gap between the on-plot and off-plot groundwater has continued to widen over time, showing that the mature trees continue to create a zone of depression, and groundwater is flowing from off-plot to in-plot.

Figure 9: In-plot vs out-of-plot groundwater elevation (feet above MSL) with green representing an average of out-of-plot wells and purple representing an average of in-plot wells



6. Project Outcomes

6.1 RAOs/Project Objectives Achieved

PD1 endophytes rescued many trees from mortality and provided healthy growth of 95% of trees on site, despite phytotoxic concentrations, the daily groundwater consumption rate is about 17,400 to 43,500 gallons of water per acre planted. The average capture rate of the current GWP&T system is about 10,224 gallons per day. The yearly average groundwater consumption of the trees exceeded that of the GWP&T system during the 2020 growing season. The pressure transducer data collected confirms that the groundwater elevations in the tree plot areas are 0.1 to 0.2 feet lower than those outside the plot. In addition, the trees improve conditions for biodegradation near the root zone. The petroleum hydrocarbons in groundwater and soil are degraded in enhanced rhizosphere and drawn to the tree roots and then are rapidly degraded. In 2020, California Waterboard permitted the shutdown of the groundwater pump and treat system as well as the soil vapor extraction systems (Central Valley Regional Water Quality Control Board, 2020). In 2022, the California Waterboard authorized the deconstruction of both pump and treat and SVE systems. The groundwater and soil concentrations are steadily decreasing, and site closure is expected in approximately 5 years (from system decommissioning). By shutting down the pump and treat and SVE systems, the site owner saved over $1M/year in operation and maintenance costs.

Figure 10: 2021 pictures of trees on the western edge of the site

Figure 11: In 2021 Trees are beginning to resemble a forest, and local residents have requested removing fencing to be able to view the trees more clearly

7. References

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