Mycoremediation

Anchorage International Airport (AIA) Landspread Site

Pilot Study Report 2018

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Introduction

The goal of this pilot study is to test the effectiveness of Fungi to efficiently lower diesel range (DRO) hydrocarbons in Alaskan soils below the action level. Here *effectively* stands for higher rate (decreasing level per time) as what is observed in the currently used land spreading method. The secondary goal is to test different strains of fungi and to develop best practices methods (BPMs) to accommodate regulatory and client needs for remediation. Examples of such aspects would be:

- Simple,
- Low maintenance,
- Cost-effective application,
- Containment of contaminants on remediation site,
- Prevention/avoidance of invasive spread of fungi.

The pilot study was implemented on an exclusively used landspread site leased to AFSC from the Ted Stevens Anchorage International Airport (AIA). The site holds only jet fuel contaminated soil from previous spills.

Principles of Mycoremediation

Mushroom
Fruiting Body

Mycelium
Root Network

Enzyme
Work force

Fungi use their enzymes, usually used to mobilize nutrients in the soil, to degrade contaminants.

Principles of Mycoremedia

Versatile peroxidase
(P eryngii)

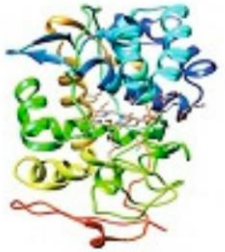
Two phases can be distinguished in Mycoremediation

Phase 1: Biomass extension

- Microbes are using primary enzymes to establish a nutrient platform.
- Enzymes are outside the mycelium in the soil
- Primary enzymes make nutrients available to fungi and other plants

Enzymes are a biological catalyst that "speed up" chemical reactions without being consumed. They are proteins folded into complex shapes that allow smaller molecules to fit into them. They function like a key and lock, a molecule (key) fits exactly the enzymatic receptor (lock) to be broken down.

Steps of Mycoremediation



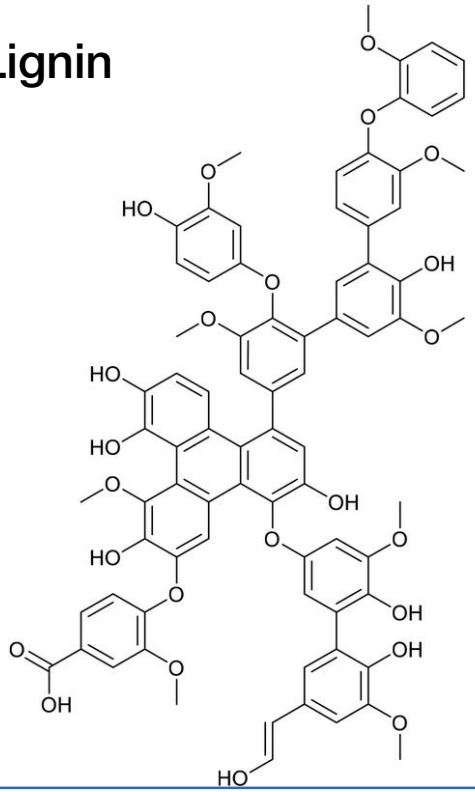
Versatile peroxidase
(P eryngii)

Phase 2: Mycelium Trains itself

- Once fungi run out of habitat they switch to produce secondary enzymes that are adapted to the environment
- In our case they are able to break down Petroleum Hydrocarbons and use them as food compounds

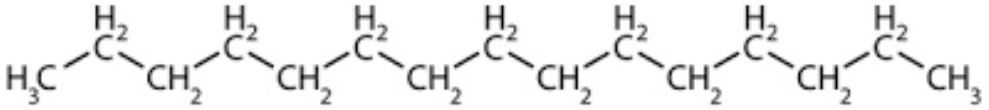
Enzymes change their primary shape (folding pattern) to fit the specific molecular structure of the contaminant to use it as a nutrient source.

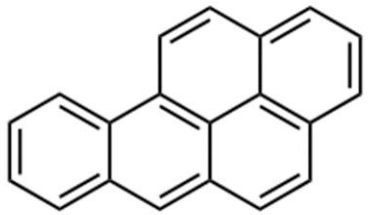
Lignin



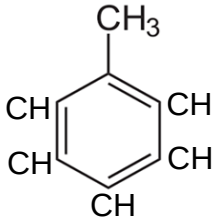
Petroleum Hydrocarbons

Pentadecane





Benz[a]pyrene



Toluene

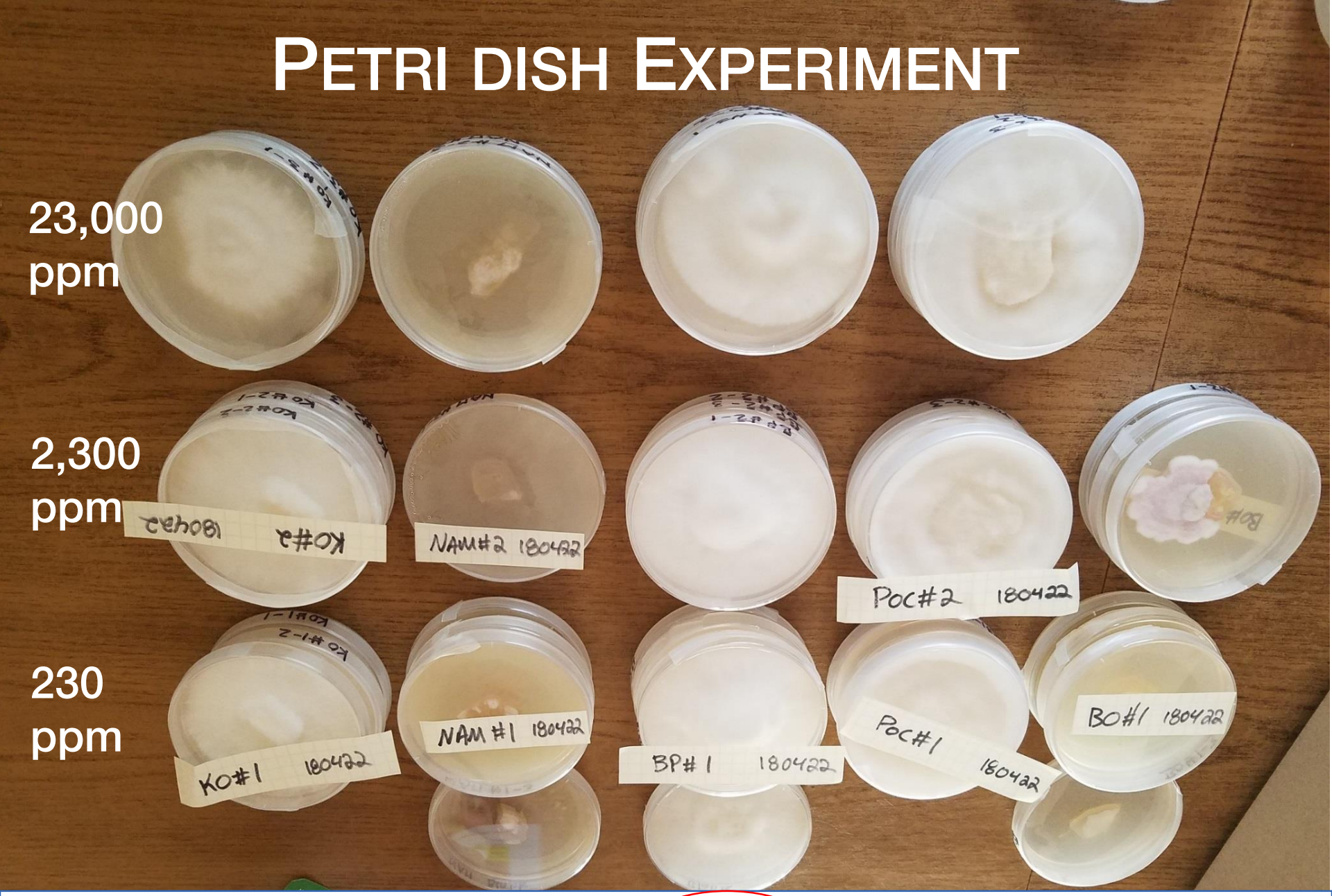
WOOD DEGRADING MUSHROOMS like Oyster Mushrooms are commonly used in petroleum hydrocarbon remediation as they are used to degrade and convert Lignin, which is a major compound in wood and of similar molecular structure than petro-hydrocarbons, to food. Picture to the left shows mycelium expanding into wood.

LABORATORY TESTING


Clean lab @ *FarNorth Fungi*

- The first step in the study was to test mushroom strains to their ability to withstand diesel range organics.
- Petri dishes were prepared with growth media mixed with different concentrations of Diesel: 23,000 ppm, 2,300 ppm and 230 ppm. Controls without diesel were also prepared.
- Petri dishes were inoculated with mycelium from *Brown Oyster (BO), King Oyster (KO), Pleurotus Ostreatus Columbinus* (Blue Oyster) (POC), *Namecoi Pholiot (NAM) and Black Poplar (BP).*

Petri dish Experiment



King Oyster	Nameco i Phpliot	Black Poplar	Blue Oyster	Brown Oyster

Production of Spawn

- We selected the Blue Oyster for this experiment. Its growth in the petri dish was faster than the Black Poplar.
- The next step is to grow enough spawn to be able to inoculate the study area.
- Bags of grain are inoculated with mycelium from Blue Oyster mushrooms. This is the spawn (like seeds) that will be used to inoculate the soil.

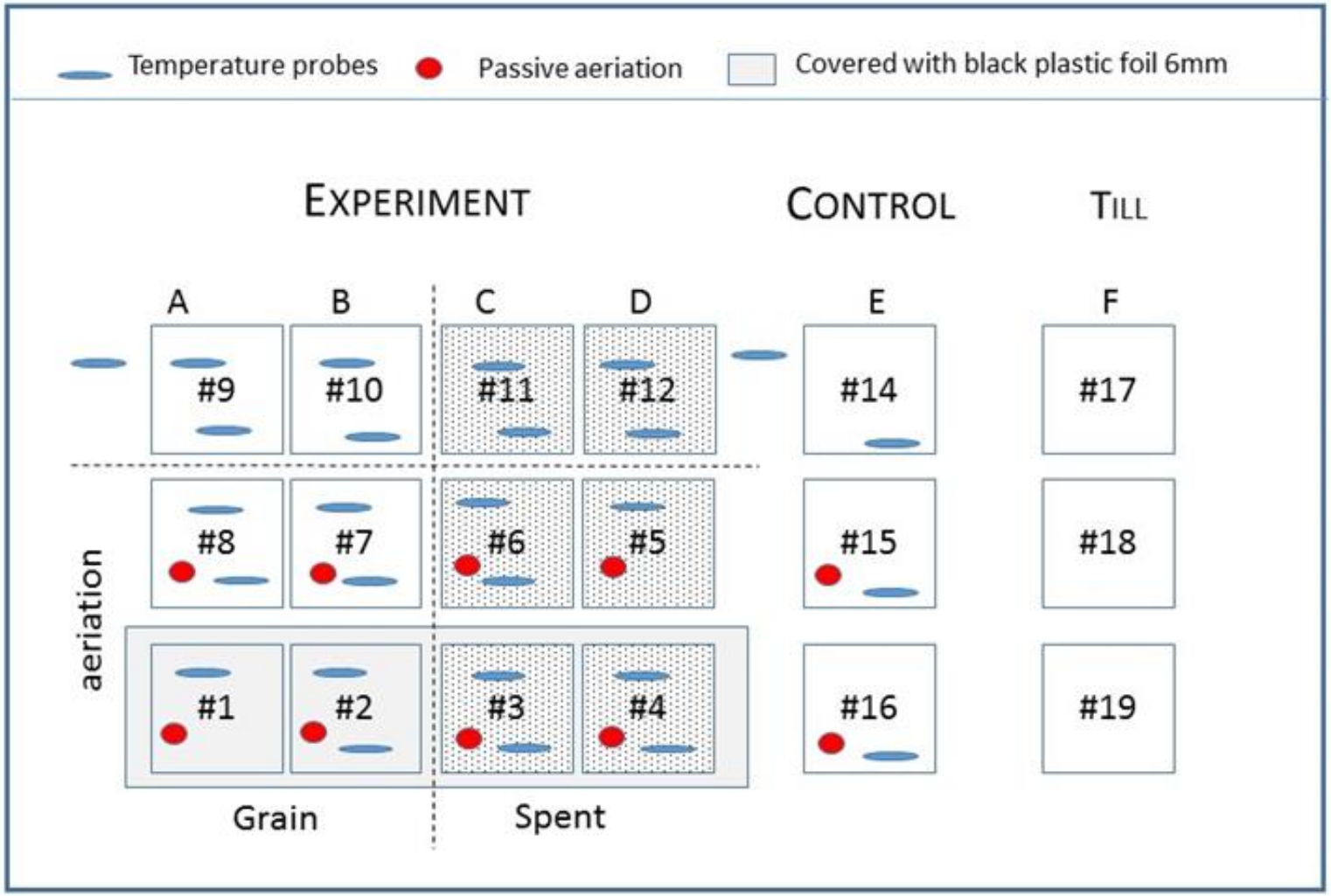
Production of Spawn

- Small sections of mycelium from petri dishes are mixed with grain in plastic bags (left). Here the grain provides the food for the mycelium to grow. Once enough mycelium is produced this grain-mycelium mixture is used as spent to inoculate the contaminated soil.

Agar Agar + Mycelium

Grain + Mycelium + Enzymes = Spent

EXPERIMENTAL SETUP



Experimental site description

- Plots row A and B are inoculated with spent
  - Plots 1 and 2 have passive aeration, weed guard and plastic sheeting
  - Plots 7 and 8 have passive aeration and weed guard
  - Plots 9 and 10 have weed guard
- Plots row C and D are inoculated with Spawn
  - Plots 3 and 4 have passive aeration, weed guard and plastic sheeting
  - Plots 5 and 6 have passive aeration and weed guard
  - Plots 11 and 12 have weed guard
- Control E: no inoculation
  - Plots 16 and 15 have passive aeration and weed guard
  - Plot 14 has weed guard
- Control F: no inoculation, tilling
  - Plots 17 to 19 have no aeration, no weed guard, tilling only
- Each plot is 1.5 feet by 1.5 feet.

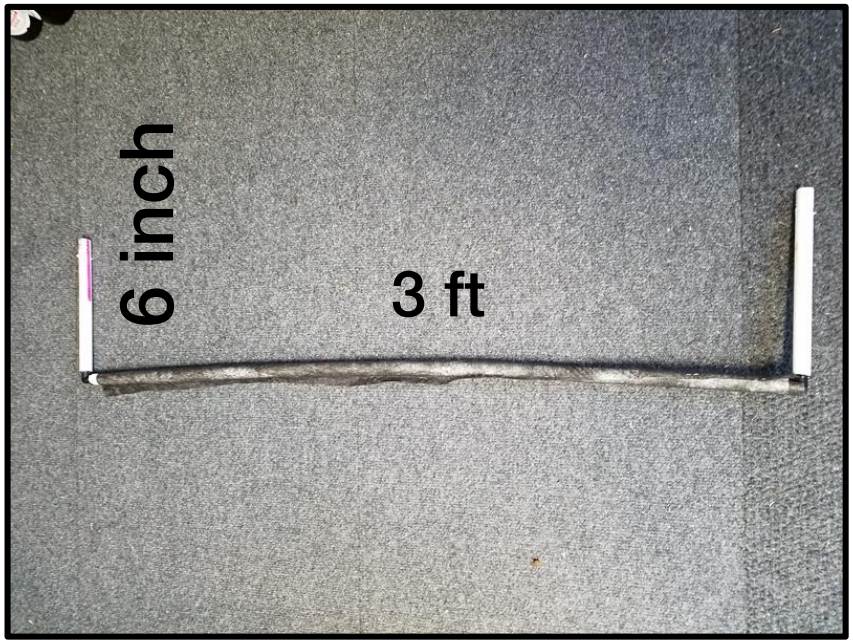
Site Preparation

Step 1: Preparing the ground by turning the soil over and raking.

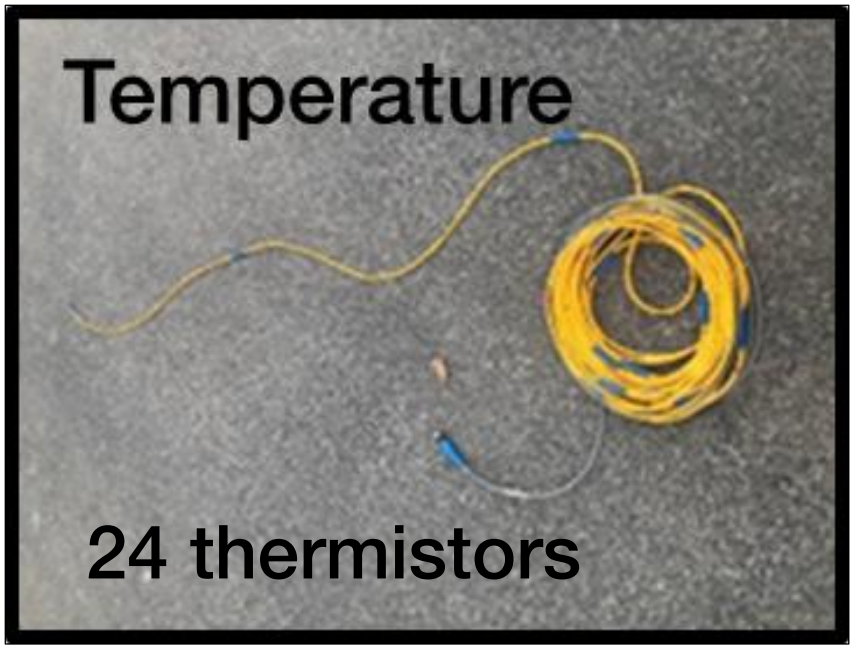
Step 2: Adding sawdust and wood shavings as initial food source.

Step 3: Inoculating with spawn and spent.

VENTILATION TUBES AND TEMPERATURE SENSORS



Customized ventilation tubes that were installed at 3-inch depth



Beaded Stream temperature sensors with datalogger were provided by PND, Torston Mayerbergern

Completed Installation

After inoculation, plots were covered with 6 millimeter black plastic foil or weed guard which allows air and moisture to penetrate.

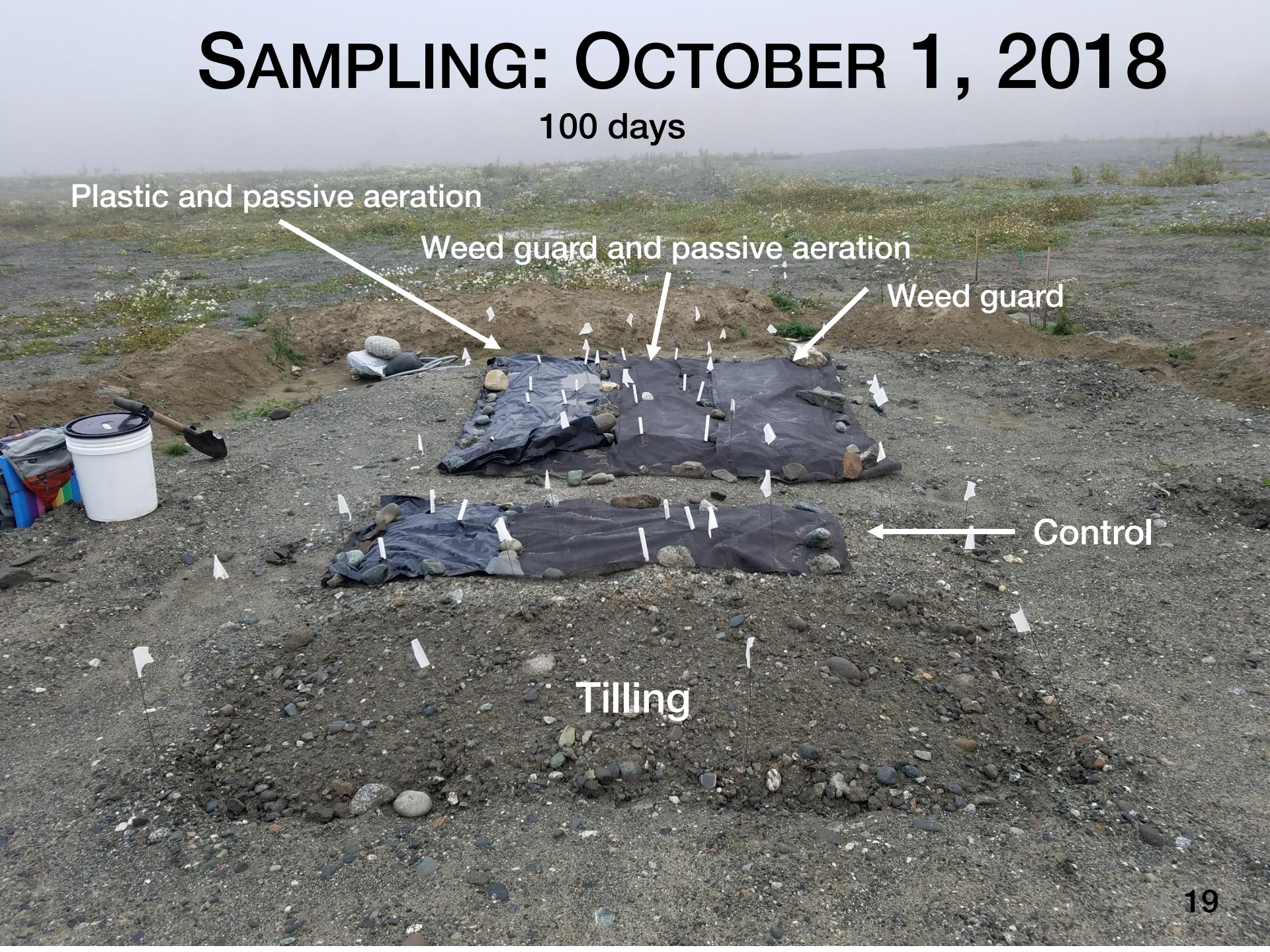
June 22, 2018

Site visit August 8, 2018

47 days later: large fruiting bodies of Oyster mushrooms visible.

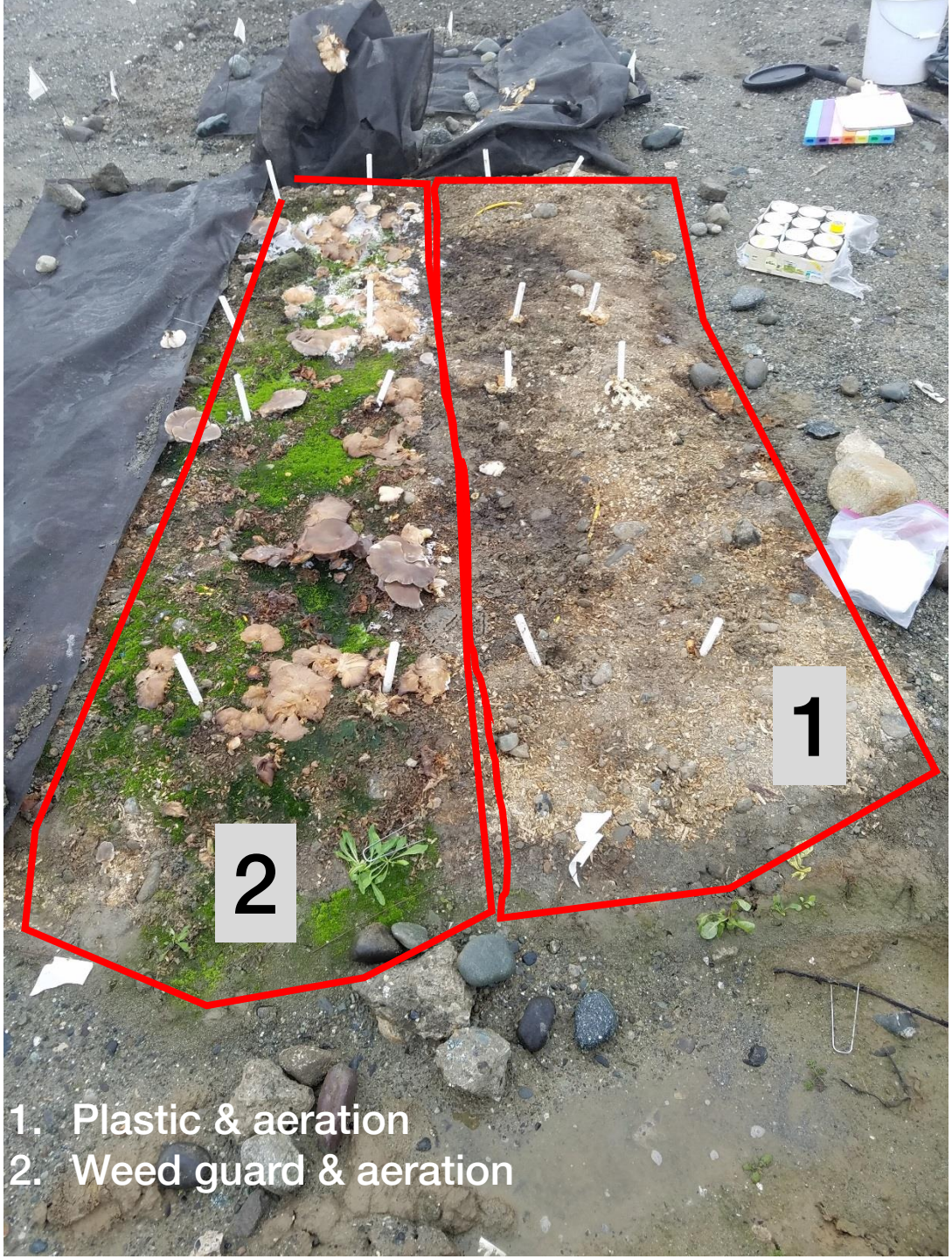
Site visit August 8, 2018

Sampling: October 1, 2018



Uncovering the plastic covered and aeriated side.

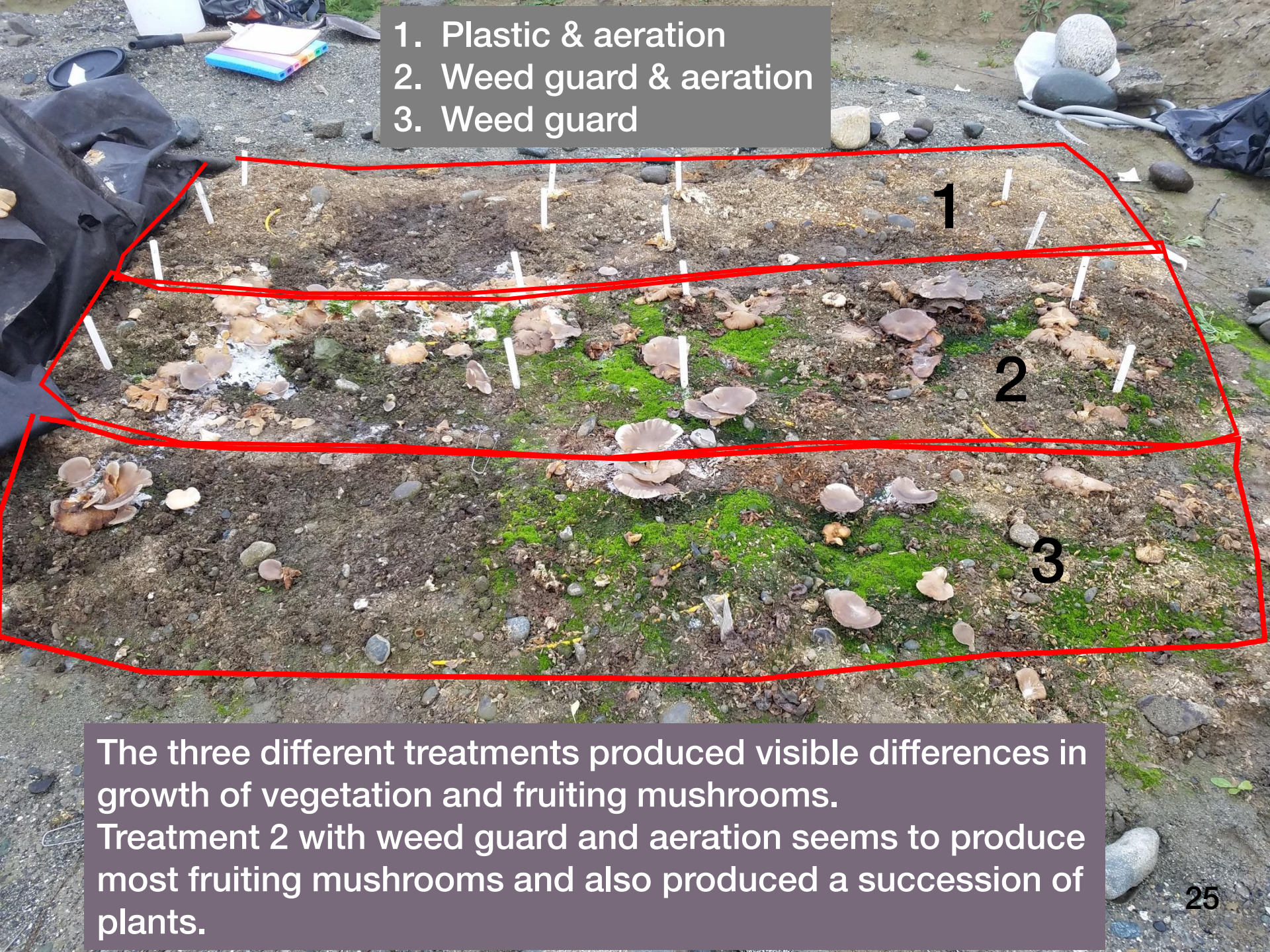
Mushroom growth was mainly around aeration tubes and along the border of the plastic cover



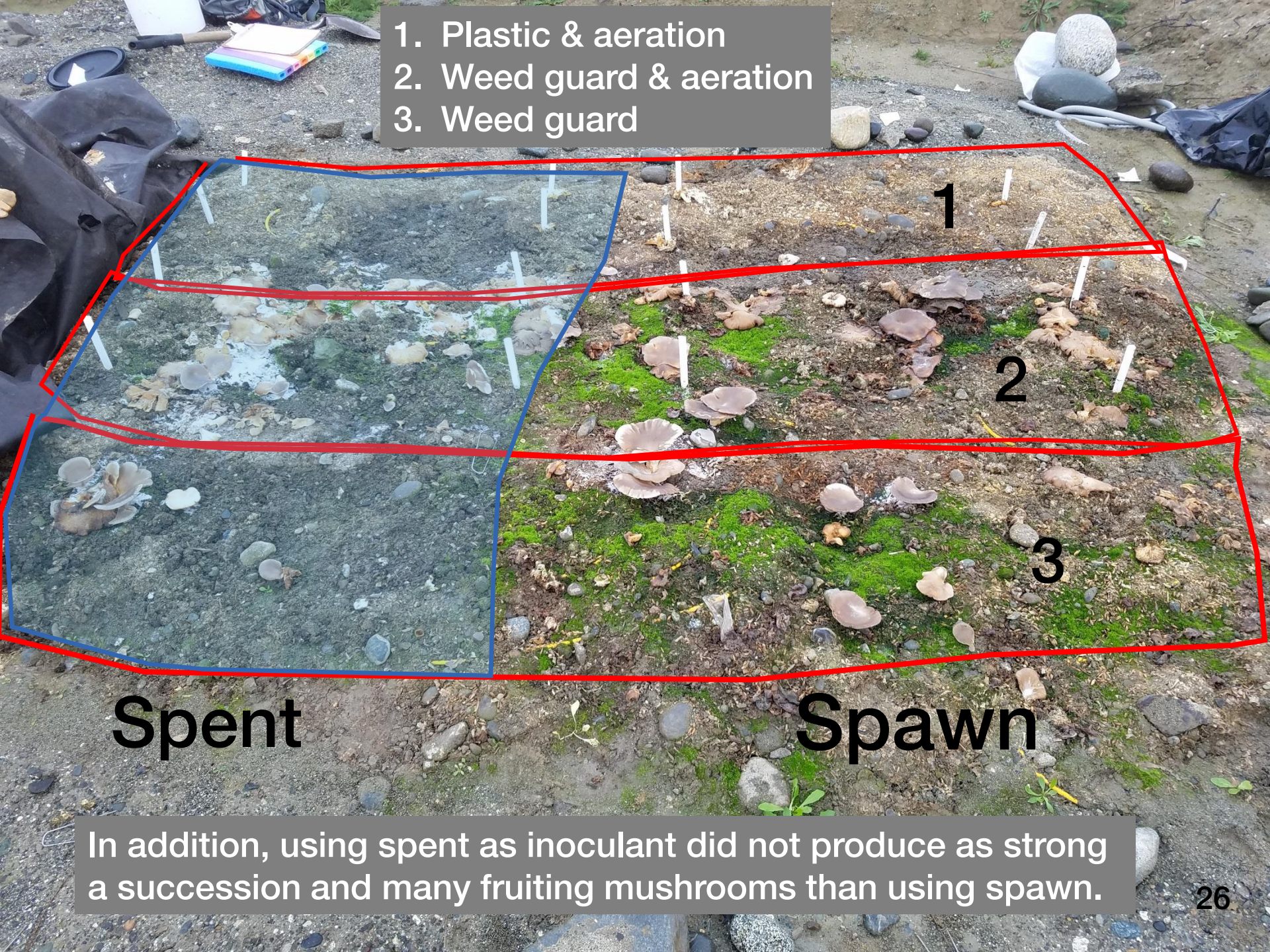
Compared to the plastic cover (1), weed guard and aeration (2) promoted growth of fruiting mushrooms. Even in October the growth of new mushrooms was visible. In addition, vegetation, mostly moss and plaintain, was abundant.


Mycelium

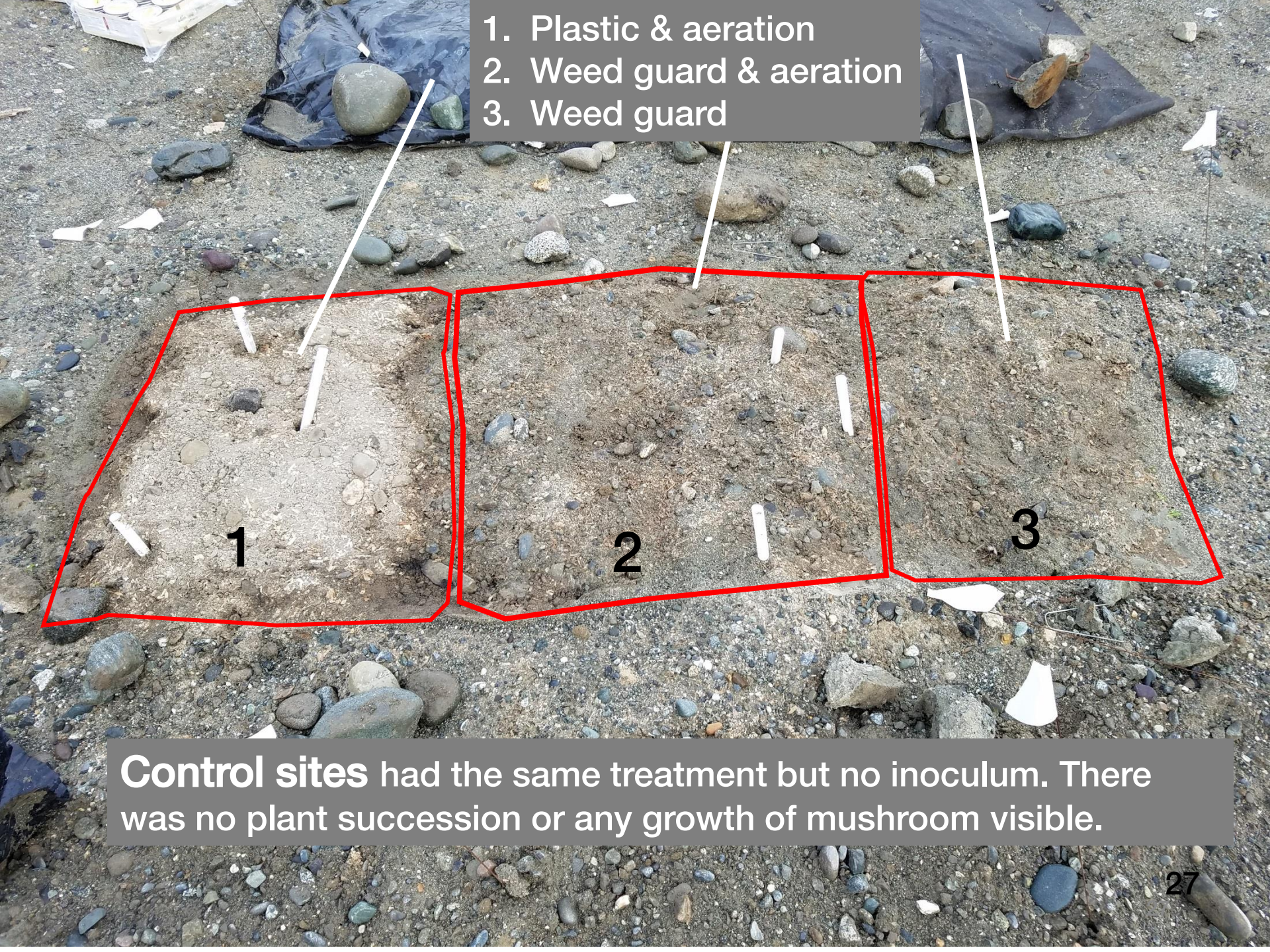

1
2
3
24



The three different treatments produced visible differences in growth of vegetation and fruiting mushrooms.
Treatment 2 with weed guard and aeration seems to produce most fruiting mushrooms and also produced a succession of plants.



In addition, using spent as inoculant did not produce as strong a succession and many fruiting mushrooms than using spawn.



Control sites had the same treatment but no inoculum. There was no plant succession or any growth of mushroom visible.

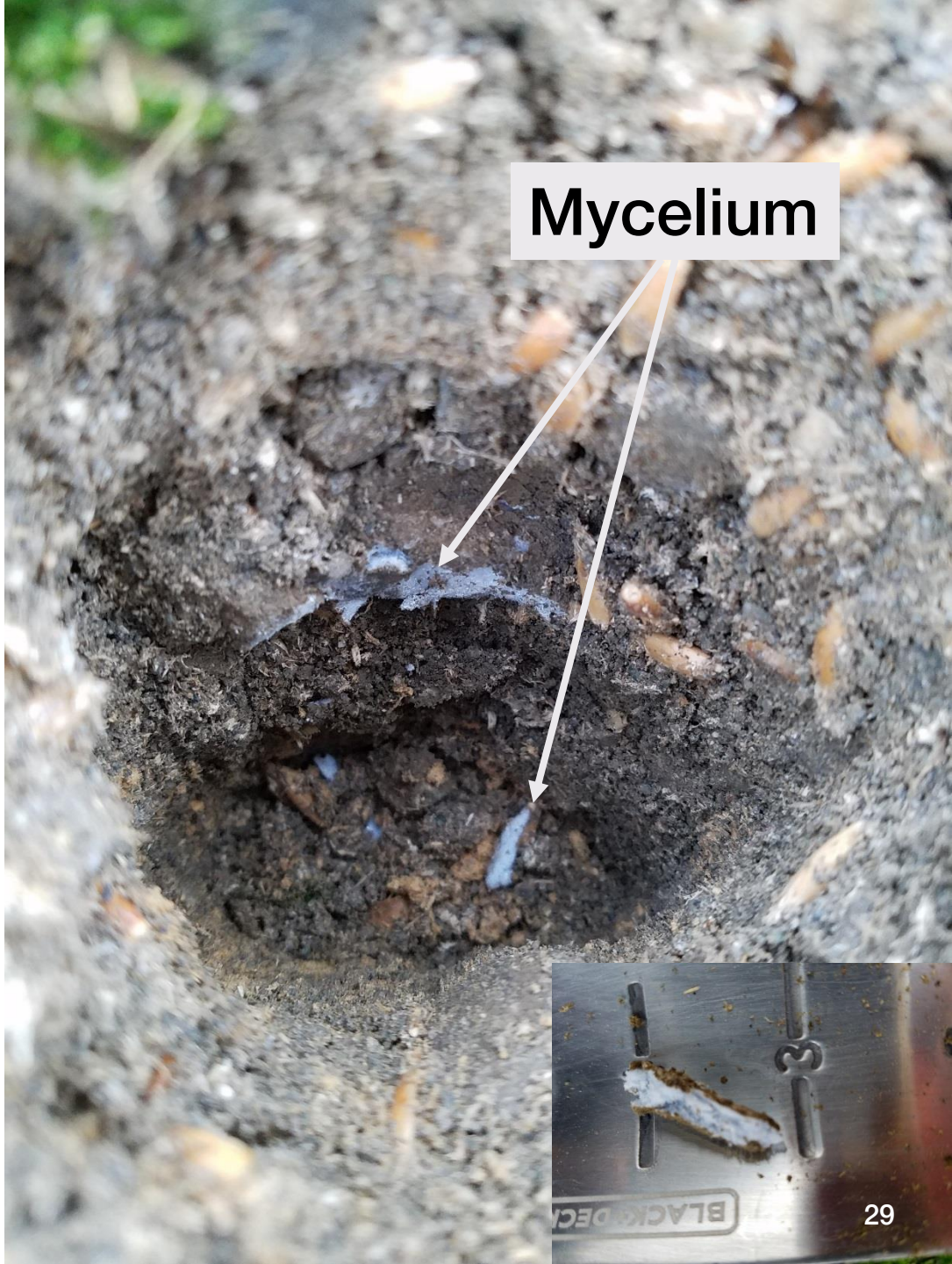
This picture shows a section of upper soil with black organic rich pockets, white mycelium, moss and Blue Oyster Mushroom.

SAMPLING

Samples were collected in three randomly selected locations in each plot. The collection was performed with a pointed small shovel integrating over 6-inch depth of the soil. A total of 100g of soil was collected in each plot. Sampling was performed on June 22nd after inoculation and October 1st.

The soil is a gravely sandy fill with some cobbles which makes taking representative homogeneous samples in small plots difficult.

The picture to the right shows that some mycelium grew down to 4-inch depth.



Soil Characteristics

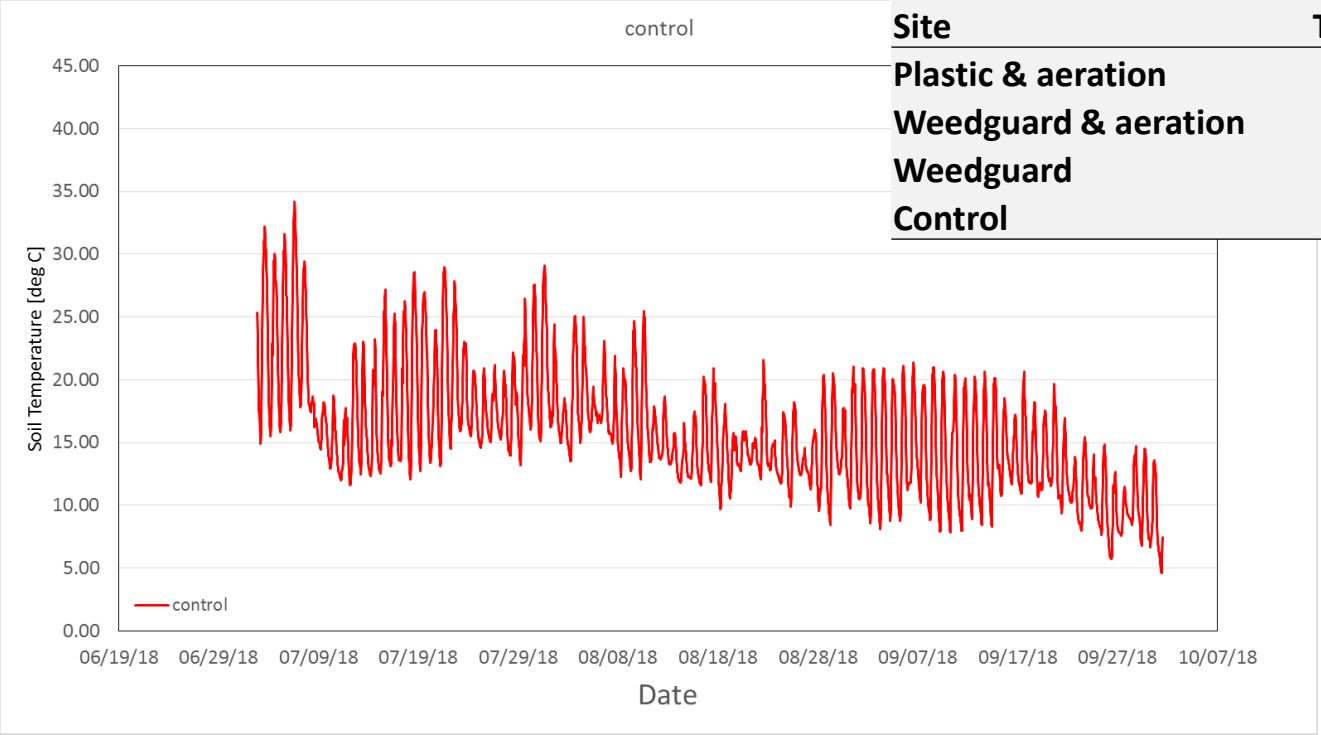
Parameter	Unit	Initial	Final
pH	/	7.6	7.6
Sp Conductivity	µS/cm	85.0	63.5
Moisture	%	6.2	8.2

Soil characteristics were analyzed on 5 grams of soil.
For specific conductivity (@ 25°C) and pH measurement samples were suspended in deionized water in a ratio 1:1 and pH and conductivity were analyzed using a *WTW multimerter probe 3800i*.
For moisture 5 grams of sample was dried at 50 C for 24 hours; moisture content was calculated based on the weight difference.
All parameters were very uniform for all plots between the beginning and end of the study period.

SOIL CHARACTERISTICS

The soil is a Gravely Sandy Soil with some cobble size stones

TEMPERATURE



Site	T(ave) °C	Stdev
Plastic & aeration	15.77	4.03
Weedguard & aeration	15.76	4.28
Weedguard	15.93	4.53
Control	15.83	4.81

Temperature was measured hourly in each plot using *Beaded Stream* temperature string. Temperature probes were buried at 2-inch depth. No differences in temperature between each treatment and between treatments and control plot were seen (see table above). T ranged between 35 °C and 4.5 °C from July 2nd to October 1st.

Gas Chromatography

Moisture: About 20 grams of samples was weight to 0.01 gram accurate and dried at 50 °C for 24 hours. Difference in mass was used to calculate % moisture on dry weight basis.

Extraction: 10 grams of soil was extracted with 20 mL 1:1 Acetone:Hexane.

Mixture was shaken for 24 hours and filtered through 0.45 µm Teflon® filter.

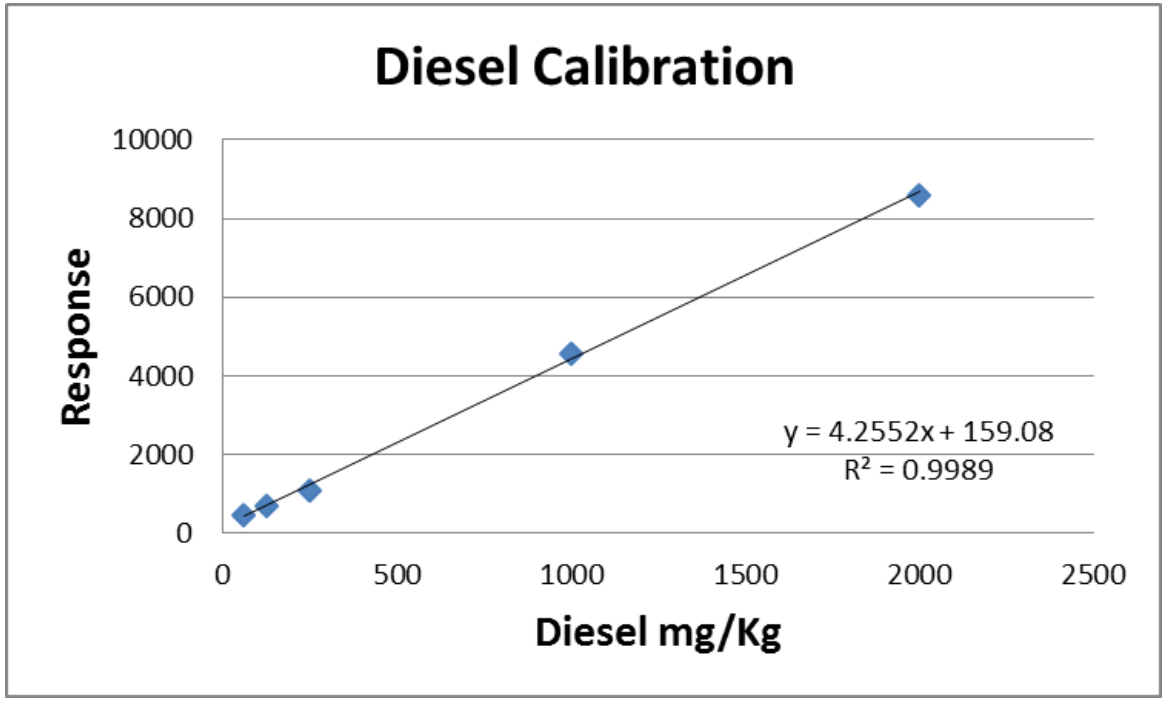
Analysis: 5 µl of the extract were injected into a SRI 8610C Gas Chromatograph.

- Column settings: keep at 50 °C for 3 minutes, ramp 10°C/minute to 250°C, keep at 250 °C for 5 minutes.
- Injector Temp: 250 °C
- FID Temp: 260 °C

Standard RESTEK 31214 DRO Mix with alkanes from C10 to C25.

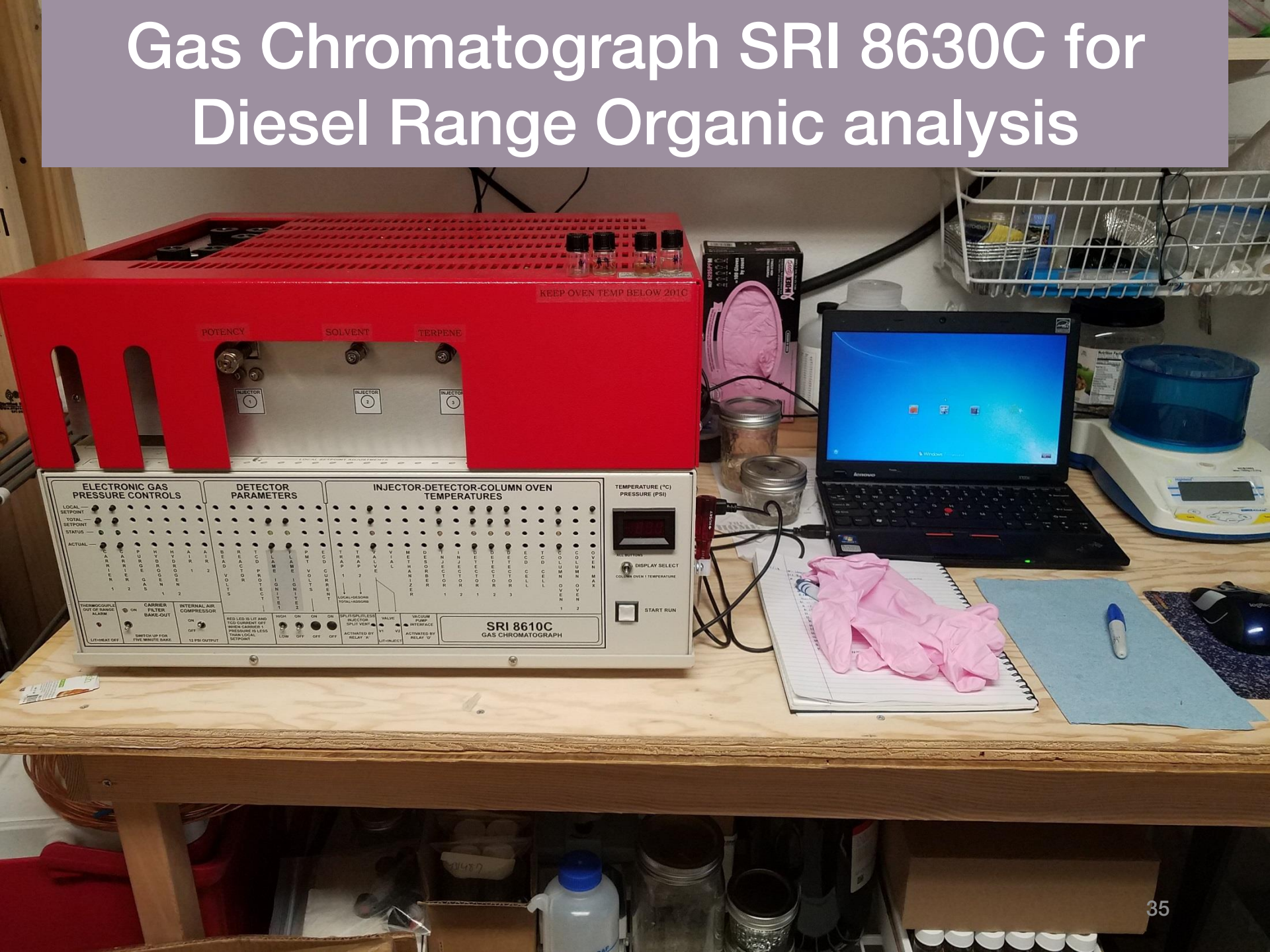
External Calibration was performed between 20 and 1000 ppm using commercial Diesel from Tesoro Station at corner of Debar and Airport Heights, Anchorage Alaska.

GAS CHROMATOGRAPHY

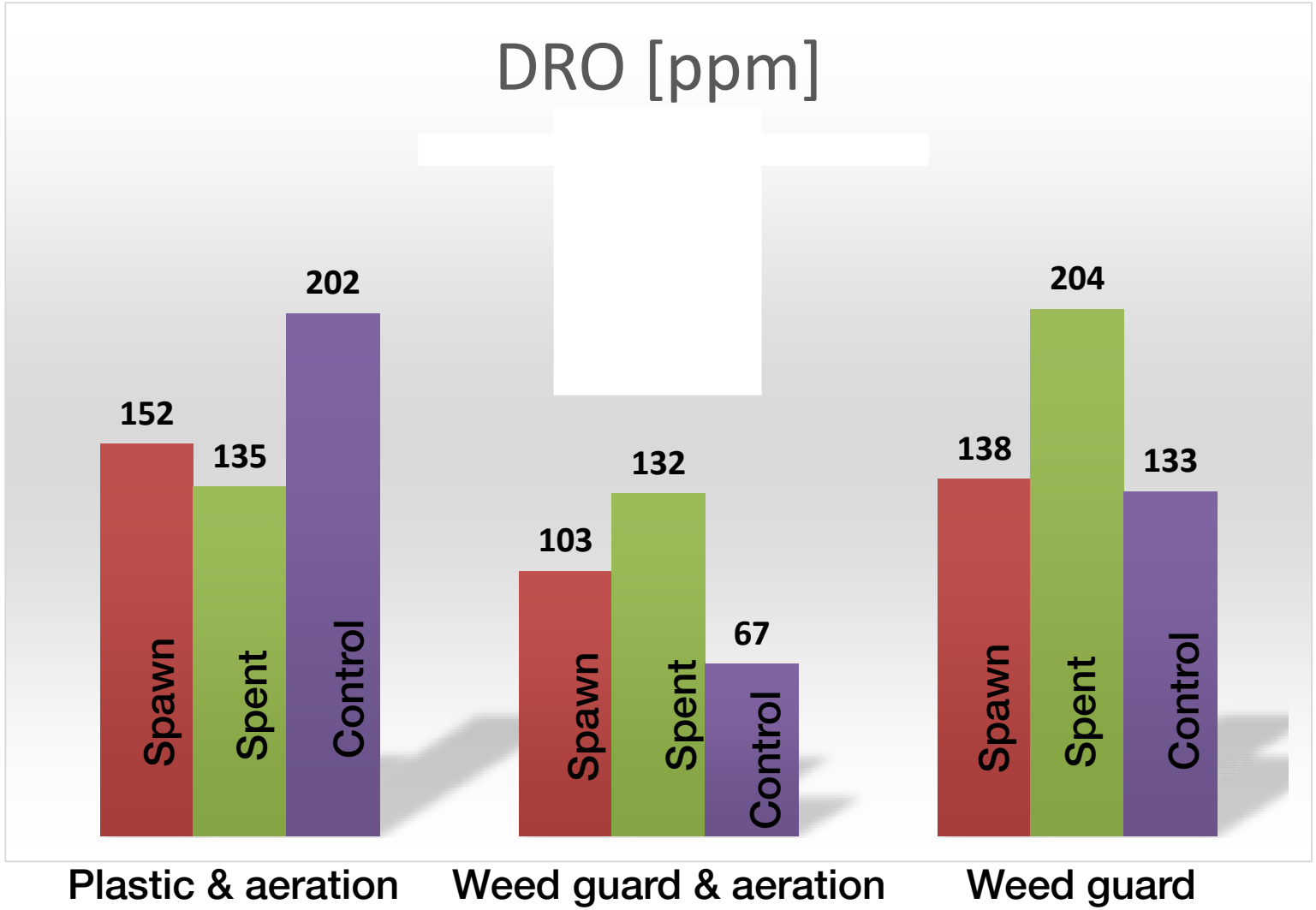


- DRO standard was used to determine retention time window of C11 to C25 . A Diesel sample was diluted using Hexane:Acetone in a ratio 1:1 as diluent to establish an external calibration (see above).
- Extraction efficiency was tested on two blank samples (sand) spiked with 200 ppm Diesel. Recovery was 92% and 84%.
- Calibration Verification standards were run every 10th sample.

Gas Chromatograph SRI 8630C for Diesel Range Organic analysis



RESULTS


DRO [ppm]
152
135
202
Spawn
Spent
Control
103
132
67
Spawn
Spent
Control
138
204
133
Spawn
Spent
Control
Plastic & aeration
Weed guard & aeration
Weed guard

Discussion

Site	DRO mg/kg	Treatment	RSD %
1	152	PLA	n/a
2	n/a	PLA	
3	90	PLA SP	67
4	181	PLA SP	
5	103	WGA SP	44
6	161	WGA SP	
7	103	WGA	1
8	102	WGA	
9	158	WG	29
10	118	WG	
11	246	WGSP	24
12	192	WGSP	
14	131	CRL WG	
15	66	CRL WGA	
16	202	CRL PLA	
Blank	<LOD		Recovery
MS #1	789		95%
MS #2	713		86%

PLA	Plastic and aeration
WGA	Weed guard and aeration
CRLA	Control and aeration
PL	Plastic
WG	Weed guard
CRT	Control
S	Spent is used instead of Spawn

The calibration, calibration continuation verification samples (CCV), blanks and matrix spikes (MS) indicate that the analytical method used here is robust. Initial concentration of DRO in the soil was analyzed by SGS on 6 samples for the 2018 land-spreaded soil and ranged between 220 mg/Kg and 3650 mg/Kg with an average of 1762 mg/Kg.

The results from samples collected on October 1st in our plots are shown in the figure above and table to the left. Diesel concentration is reported on a dry weight basis. The DRO concentrations of each treatment vary up to 67%; there is no clear difference in DRO concentration between control sites and inoculated sites. Part of the results may reflect the inhomogeneity of contaminant concentration in the soil which is also reflected in primary concentrations determined for the 2018 spreading area. In addition, sawdust and wood shavings added to the soil are causing a dilution effect which lowers the concentration artificially. To overcome the reduction of concentration due to dilution, we will determine the concentration of added organic material from wood shaving and sawdust by heating the sample to 440 °C to burn off the organics. This analysis will be carried out in January 2019.

Overall, we believe that during the first season the fungi established biomass and completed Phase 1 of the mycoremediation. Sampling and analysis of soils next year will give a better indication of how DRO degradation continues.

Conclusions

- Fungi established the biomass (Phase 1) by growing fruiting bodies and mycelium in the upper 3 inches of soil. Fungi enzymes unlocked nutrients and initiated plant succession towards establishing a soil ecosystem that will support degradation of DRO.

- Inhomogeneous soil and the addition of organic material makes it difficult to establish statistical significant results. Additional analysis to account for the dilution effect caused by sawdust and wood shavings will be performed in January.

Where to Go From Here?

The first summer was mainly used to establish the biomass needed for the mycelium to grow to depth. We expect summer 2019 results will show DRO degradation. We will sample at least 3 more times to document the progress of the degradation.

Acknowledgement

We thank Menzies Aviation for providing the opportunity for this Pilot Study. A special thank you to Amanda Tuttle and Laurie Butler and the Menzies crew for the excellent support they gave us in establishing the site and for providing us escorted access for site visits.
Thank you to PND and Torsten Mayrberger for lending the temperature probes.