Supplementary Materials

Long-term legacy of phytoremediation on plant succession and soil microbial communities in petroleum-contaminated sub-Arctic soils

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Table S1: Assignments of phospholipid fatty acid (PLFA) biomarkers to biological group

Classification	PLFA Biomarkers
Gram-positive bacteria	i15:0; a15:0; i16:0; i17:0; a17:0
Gram-negative bacteria	cy17:0; 18:1ω7c
Fungi	18:3ω3,6,9; 18:2ω6,9; 18:1ω9
Actinomycetes	10Me 16:0; 10Me 17:0; 10Me 18:0
AM Fungi	16:1ω5
Protozoa	20:4ω6c
Unspecific biomarkers	15:0

Table S2. Soil chemistry measurements and textural characteristics from each plot. For each plot three subsections were measured, data presented as average and standard error. Data reaggregated from (Leewis et al., 2013)

Contaminant	Original Treatment	pH (ppm)			NO$_3^-$-N (ppm)			P (ppm)			K (ppm)			CEC (meq/100)			%C		
CrudeOil	c1	6.87	±	0.04	30.00	±	1.67	2.67	±	0.19	50.67	±	1.39	2.56	±	0.06	1.29	±	0.09
	c2	7.14	±	0.01	4.67	±	0.84	1.00	±	0.00	35.33	±	1.35	1.99	±	0.02	0.92	±	0.02
	f	6.80	±	0.05	17.67	±	2.17	134.00	±	13.53	58.00	±	2.96	2.43	±	0.06	1.15	±	0.03
	p1	7.26	±	0.03	2.67	±	0.96	1.67	±	0.19	32.33	±	0.84	1.88	±	0.10	0.87	±	0.03
	p1f	6.57	±	0.02	17.33	±	2.69	89.67	±	8.26	57.33	±	0.84	2.49	±	0.02	1.50	±	0.02
	p2	7.09	±	0.03	3.33	±	0.69	1.33	±	0.19	26.33	±	0.69	1.66	±	0.04	0.94	±	0.02
	p2f	6.69	±	0.04	2.67	±	0.51	158.67	±	17.28	55.33	±	3.02	2.08	±	0.02	1.28	±	0.00
Diesel	c1	5.96	±	0.15	32.33	±	2.14	4.00	±	0.58	59.67	±	2.52	4.68	±	0.15	2.75	±	0.08
	c2	5.51	±	0.01	13.67	±	0.69	2.00	±	0.00	52.67	±	3.10	4.93	±	0.19	3.23	±	0.17
	f	5.14	±	0.04	39.67	±	2.80	91.33	±	6.68	62.33	±	3.75	5.59	±	0.28	3.51	±	0.12
	p1	5.40	±	0.03	18.33	±	2.17	2.00	±	0.00	46.33	±	1.64	5.01	±	0.08	3.48	±	0.07
	p1f	4.72	±	0.01	81.00	±	9.45	96.00	±	2.08	79.33	±	5.64	5.31	±	0.15	3.17	±	0.16
	p2	5.36	±	0.04	40.67	±	15.49	2.67	±	0.38	51.33	±	4.25	4.88	±	0.24	3.08	±	0.07
	p2f	5.11	±	0.05	17.67	±	3.83	161.00	±	17.21	115.33	±	9.25	6.05	±	0.22	3.02	±	0.10

Table S3: Count data of the plant species present in each treatment cell (average and standard deviation of the 6 pseudo-replicated cells). Top of table is plants and seedlings (< 20 cm tall), bottom of table is woody shrubs and trees with height > 20 cm. Treatments are defined as follows: no treatment (c1, c2), planted with annual ryegrass (p), a mix of annual ryegrass and Arctared fescue (p2), treated with fertilizer (f), and/or no added nutrients (no "f" indicated).

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Table S4: Average and standard deviation of PLFAs (nmol per gram soil) for total microbial biomass, total bacterial biomass, and subgroups as identified in table S1.

Contaminant	Original Treatment	Total Microbial Biomass			Total Bacterial Biomass			G+			G-			Actinobacteria			Fungi			Protozoa			Unclassified		
Crude Oil	c1	20.33	±	9.23	8.99	±	3.4752	3.3	±	1.3	5.7	±	2.3	1.46	±	0.53	7.63	±	4.2	0.6	±	0.3	0.2	±	0.1
	c2	6.104	±	0.68	3.62	±	0.4229	1.9	±	0.2	1.7	±	0.2	0.87	±	0.1	1.56	±	0.4	0.1	±	0	0.1	±	0
	f	19.85	±	3.45	9.43	±	1.7092	3.8	±	0.8	5.6	±	1	1.15	±	0.51	7.18	±	1.9	0.3	±	0.2	0.2	±	0
	p1	6.557	±	4.79	3.17	±	0.7301	1.6	±	0.5	1.6	±	0.3	0.91	±	0.26	0.73	±	0.3	0.1	±	0.1	0	±	0
	p1f	22.89	±	3.32	11	±	1.2089	4.1	±	0.7	6.9	±	0.8	1.49	±	0.65	7.43	±	1.8	0.5	±	0.1	0.2	±	0
	p2	9.073	±	0.28	5.8	±	0.0682	2.7	±	0.1	3.1	±	0.2	1.36	±	0.04	1.66	±	0.2	0.2	±	0.1	0.1	±	0
	p2f	18.75	±	3.47	10.1	±	0.873	4.4	±	0.3	5.7	±	0.7	1.63	±	0.47	4.55	±	1.4	0.5	±	0.1	0.2	±	0
Diesel	c1	10.95	±	2.11	4.53	±	0.6023	1.8	±	0.3	2.7	±	0.3	0.86	±	0.16	3.97	±	1.5	0.2	±	0.1	0.1	±	0
	c2	11.52	±	4.28	4.87	±	1.5338	2.4	±	1	2.5	±	0.7	0.92	±	0.2	4.21	±	1.9	0.2	±	0.1	0.1	±	0.1
	f	23.96	±	3.61	7.03	±	0.7067	2.7	±	0.4	4.3	±	0.4	1.07	±	0.14	11.6	±	2.4	0.6	±	0.1	0.3	±	0.1
	p1	9.941	±	1.13	4.28	±	0.3316	1.9	±	0.2	2.4	±	0.2	0.9	±	0.08	3.55	±	0.7	0.2	±	0	0.1	±	0
	p1f	19.51	±	5.84	7.09	±	2.3147	3	±	1.2	4.1	±	1.2	1.19	±	0.43	8.23	±	3.4	0.9	±	0.4	0.1	±	0.1
	p2	12.99	±	5.05	4.97	±	1.0348	2.1	±	0.5	2.9	±	0.5	0.92	±	0.16	5.1	±	3.2	0.4	±	0.1	0.1	±	0.1
	p2f	10.53	±	4.68	5.42	±	2.1746	2.5	±	1.1	2.9	±	1.1	1.14	±	0.34	2.59	±	1.4	0.6	±	0.6	0.1	±	0

Table S5: Average and standard deviation of number of OTUs (Num. OTU) and Shannon diversity index for the 16S rRNA gene sequence data.

Contaminant	Original Treatment	Num. OTU			Shannon Index		
CrudeOil	c1	2164.00	±	230.98	5.71	±	0.19
	c2	1660.00	±	242.25	5.80	±	0.25
	f	1449.33	±	107.72	5.42	±	0.29
	p1	1488.67	±	49.97	5.34	±	0.15
	p1f	1420.00	±	680.71	5.66	±	0.45
	p2	1509.67	±	39.53	5.43	±	0.23
	p2f	1901.67	±	82.78	5.79	±	0.01
Diesel	c1	2127.33	±	297.77	5.47	±	0.20
	c2	2085.00	±	397.14	5.34	±	0.44
	f	1898.33	±	356.24	5.11	±	0.47
	p1	2016.67	±	165.40	5.07	±	0.18
	p1f	1667.00	±	8.19	4.75	±	0.03
	p2	1826.67	±	242.46	4.80	±	0.78
	p2f	1885.33	±	423.93	5.42	±	0.45
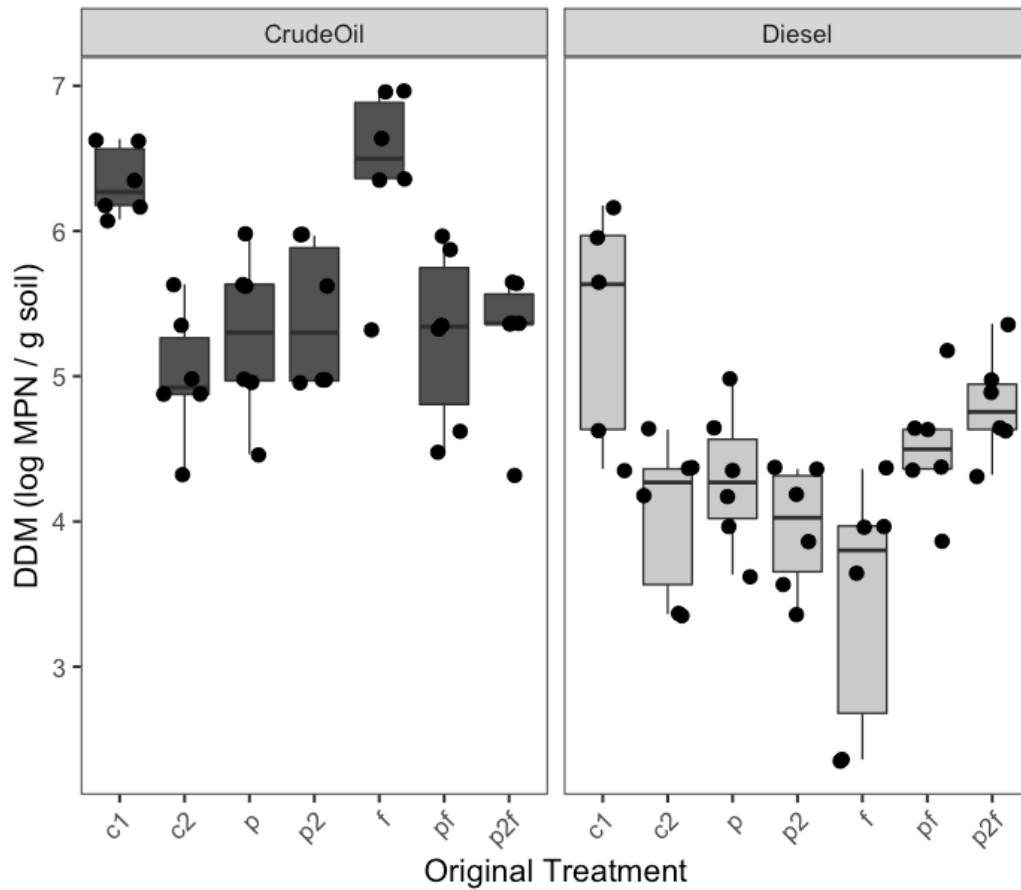
Figure S1: Most probable number (MPN) of diesel degrading microorganisms in either Crude Oil (dark grey) or Diesel (light grey) soils. Measurements were based on 10 triplicate 1 g soil samples. The values shown are means with 95% confidence intervals (N = 6). Treatments indicated are as follows: no treatment (c1, c2), planted with annual ryegrass (p), a mix of annual ryegrass and Arctared fescue (p2), treated with fertilizer (f), and/or no added nutrients (no "f" indicated).
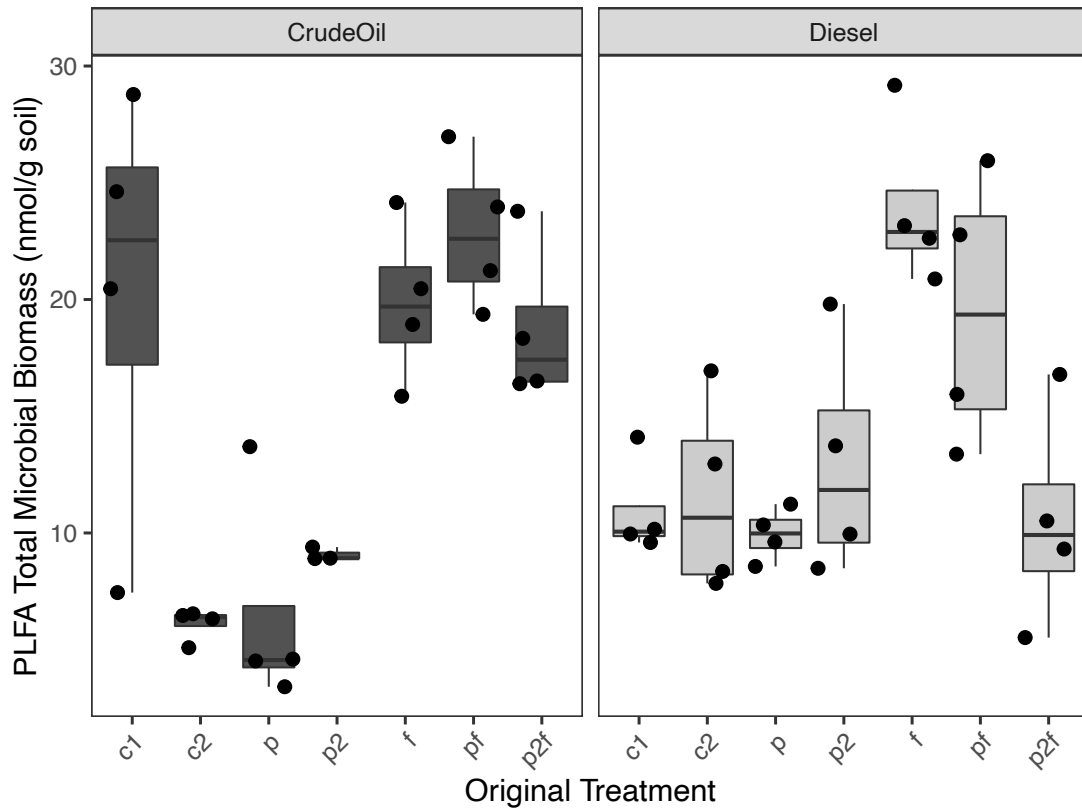
Figure S2: Total microbial biomass as measured by PLFA biomarkers.

Figure S3: heat map of the relative abundance of the families greater than 2% abundant in crude oil (left) or diesel (right) contaminated soils. The colours ranging from blue to red indicate the gradient in relative abundance of the family (R.A.% = Relative Abundance).