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Supplement of

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 TPH measurements from each plot. For each plot three subsections were measured, data presented as average and standard error. Soil TPH measurements are presented, and statistics were calculated across three time points: after one month of phytoremediation treatment (1995), one year of treatment (1996) and 16 years of treatment (2011). Overall percent loss represents the difference in TPH measured between 1995 and 2011. Data reaggregated from (Reynolds et al., 1997b, a; Leewis et al., 2013).Different letters indicate significant differences in TPH concentrations between treatments within a time point (P < 0.05 pair-wise Wilcox test).

Contaminant	Original Treatment	TPH (ppm) 1995			TPH (ppm) 1996			TPH (ppm) 2011			Overall Loss (%)
CrudeOil	c1	5313.95	± 138.03	a	3948.04	± 688.59	b	656.00	± 21.67	c	88%
	c2	5562.93	± 625.89	a	4409.48	± 44.41	b	744.67	± 53.04	c	87%
	f	4772.65	± 220.63	a	4017.23	± 372.17	b	631.67	± 11.46	c	87%
	p1	5897.50	± 648.80	a	3879.16	± 169.99	b	664.67	± 38.00	c	89%
	p1f	4806.89	± 209.04	a	3374.28	± 545.00	b	638.33	± 37.29	c	87%
	p2	6216.97	± 1369.67	a	3849.75	± 152.26	b	759.00	± 18.25	c	88%
	p2f	4067.25	± 893.86	a	3191.01	± 269.57	a	737.67	± 22.41	c	82%
Diesel	c1	5652.96	± 181.57	a	2904.77	± 386.75	b	316.00	± 15.56	c	94%
	c2	4919.41	± 1165.74	a	2105.60	± 262.69	b	339.33	± 7.43	c	89%
	f	3538.46	± 1057.88	a	1313.56	± 108.73	b	403.33	± 11.86	b	93%
	p1	3280.68	± 820.33	a	2951.23	± 510.57	a	340.67	± 16.69	b	90%
	p1f	3103.13	± 2203.77	a	1192.71	± 247.06	b	430.33	± 18.59	c	86%
	p2	4267.83	± 140.19	a	2746.77	± 158.34	b	392.33	± 15.83	c	91%
	p2f	1998.09	± 386.71	a	1071.39	± 380.47	b	395.67	± 9.86	b	80%

Table S3. 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)*. Different letters indicate significant differences in soil chemistry measurement between treatments by soil type(P < 0.05 pair-wise Wilcox test).*

Contam.	Original Treatment	pH (ppm)		NO^{-}_{3}-N (ppm)		P (ppm)		K (ppm)		CEC (meq/100)		%C		Sand (%)		Silt (%)		Clay (%)	
Crude Oil	c1	6.87 ± 0.04	ac	30.00 ± 1.67	a	2.67 ± 0.19	a	50.67 ± 1.39	ac	2.56 ± 0.06	a	1.29 ± 0.09	ac	75.73 ± 1.68	a	15.80 ± 1.67	a	8.47 ± 0.19	a
	c2	7.14 ± 0.01	a	4.67 ± 0.84	a	1.00 ± 0.00	a	35.33 ± 1.35	c	1.99 ± 0.02	b	0.92 ± 0.02	bc	78.93 ± 0.51	a	14.27 ± 0.38	a	6.80 ± 0.33	a
	f	6.80 ± 0.05	c	17.67 ± 2.17	a	134.00 ± 13.53	a	58.00 ± 2.96	a	2.43 ± 0.06	a	1.15 ± 0.03	ac	80.53 ± 0.34	a	12.00 ± 0.18	a	7.47 ± 0.19	a
	p1	7.26 ± 0.03	b	2.67 ± 0.96	a	1.67 ± 0.19	a	32.33 ± 0.84	bc	1.88 ± 0.10	b	0.87 ± 0.03	bc	80.40 ± 0.69	a	13.47 ± 0.50	a	6.13 ± 0.19	a
	p1f	6.57 ± 0.02	b	17.33 ± 2.69	a	89.67 ± 8.26	a	57.33 ± 0.84	a	2.49 ± 0.02	a	1.50 ± 0.02	a	79.00 ± 0.94	a	15.03 ± 0.85	a	5.97 ± 0.10	a
	p2	7.09 ± 0.03	c	3.33 ± 0.69	a	1.33 ± 0.19	a	26.33 ± 0.69	bc	1.66 ± 0.04	b	0.94 ± 0.02	b	77.60 ± 0.00	a	17.27 ± 0.19	a	5.13 ± 0.19	a
	p2f	6.69 ± 0.04	c	2.67 ± 0.51	a	158.67 ± 17.28	a	55.33 ± 3.02	a	2.08 ± 0.02	b	1.28 ± 0.00	a	79.60 ± 0.67	a	14.60 ± 0.67	a	5.80 ± 0.00	a
Diesel	c1	5.96 ± 0.15	acd	32.33 ± 2.14	a	4.00 ± 0.58	a	59.67 ± 2.52	a	4.68 ± 0.15	a	2.75 ± 0.08	a	77.53 ± 0.62	ac	19.33 ± 0.50	a	3.13 ± 0.19	ac
	c2	5.51 ± 0.01	acd	13.67 ± 0.69	a	2.00 ± 0.00	a	52.67 ± 3.10	a	4.93 ± 0.19	a	3.23 ± 0.17	a	80.60 ± 0.88	abc	16.33 ± 0.91	a	3.07 ± 0.08	ac
	f	5.14 ± 0.04	bcd	39.67 ± 2.80	a	91.33 ± 6.68	a	62.33 ± 3.75	a	5.59 ± 0.28	a	3.51 ± 0.12	a	78.13 ± 0.08	ab	18.40 ± 0.24	a	3.47 ± 0.19	ac
	p1	5.40 ± 0.03	bcd	18.33 ± 2.17	a	2.00 ± 0.00	a	46.33 ± 1.64	a	5.01 ± 0.08	a	3.48 ± 0.07	a	82.03 ± 0.25	bc	15.40 ± 0.46	a	2.57 ± 0.21	a
	p1f	4.72 ± 0.01	b	81.00 ± 9.45	a	96.00 ± 2.08	a	79.33 ± 5.64	a	5.31 ± 0.15	a	3.17 ± 0.16	a	81.07 ± 0.40	abc	14.47 ± 0.34	a	4.47 ± 0.08	c
	p2	5.36 ± 0.04	bcd	40.67 ± 15.49	a	2.67 ± 0.38	a	51.33 ± 4.25	a	4.88 ± 0.24	a	3.08 ± 0.07	a	81.67 ± 0.17	b	14.07 ± 0.27	a	4.27 ± 0.19	c
	p2f	5.11 ± 0.05	bcd	17.67 ± 3.83	a	161.00 ± 17.21	a	115.33 ± 9.25	a	6.05 ± 0.22	a	3.02 ± 0.10	a	82.93 ± 0.51	c	13.13 ± 0.51	a	3.93 ± 0.19	ac

Table S4. 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).

Please see excel file.

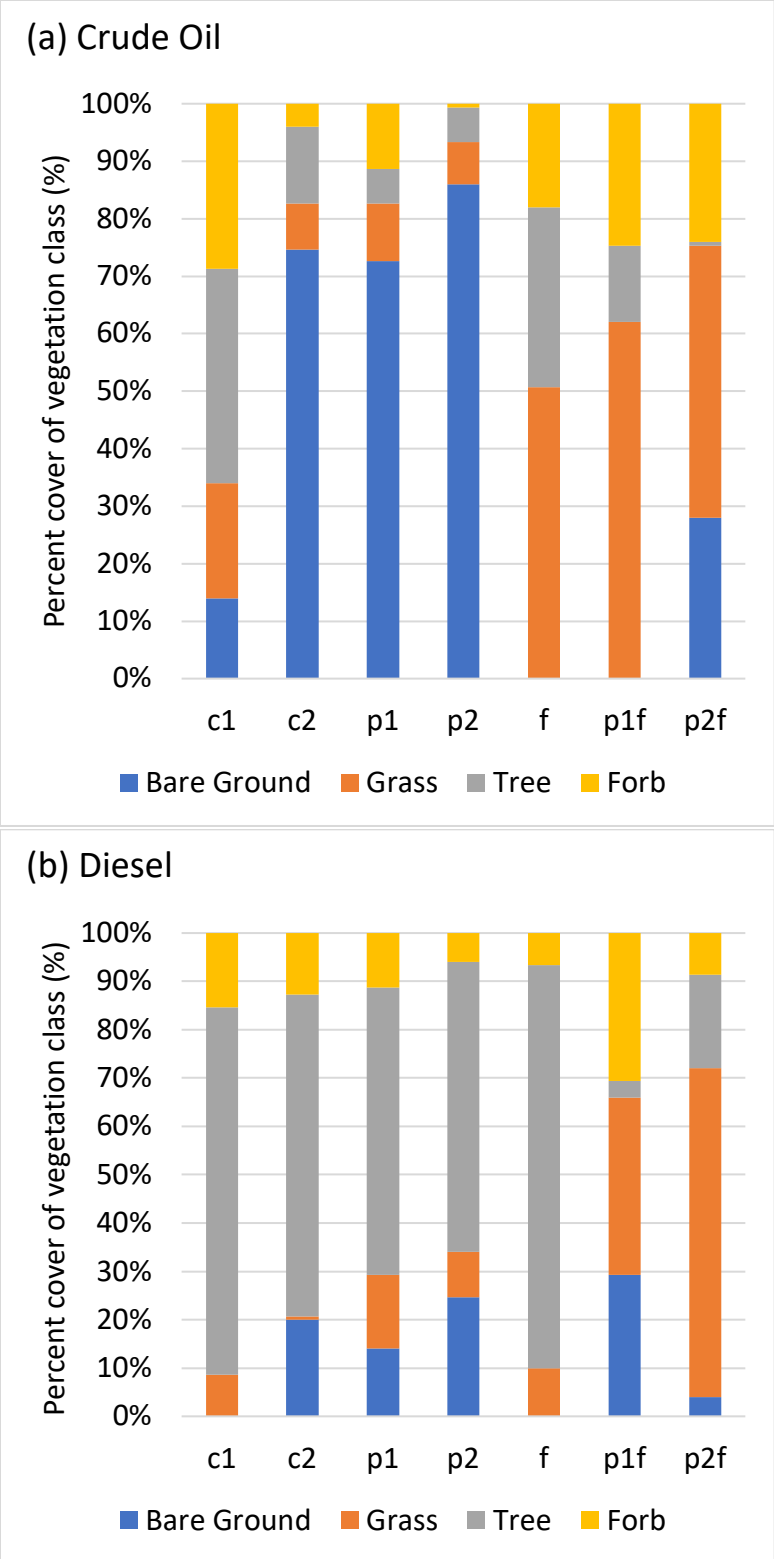

Figure S1. Estimated percent cover of vegetation groups in either (a) Crude Oil or (b) Diesel plots. Measurements were based on visual estimates in each of the six sub-plots. For accompanying statistics, see Figure 2.

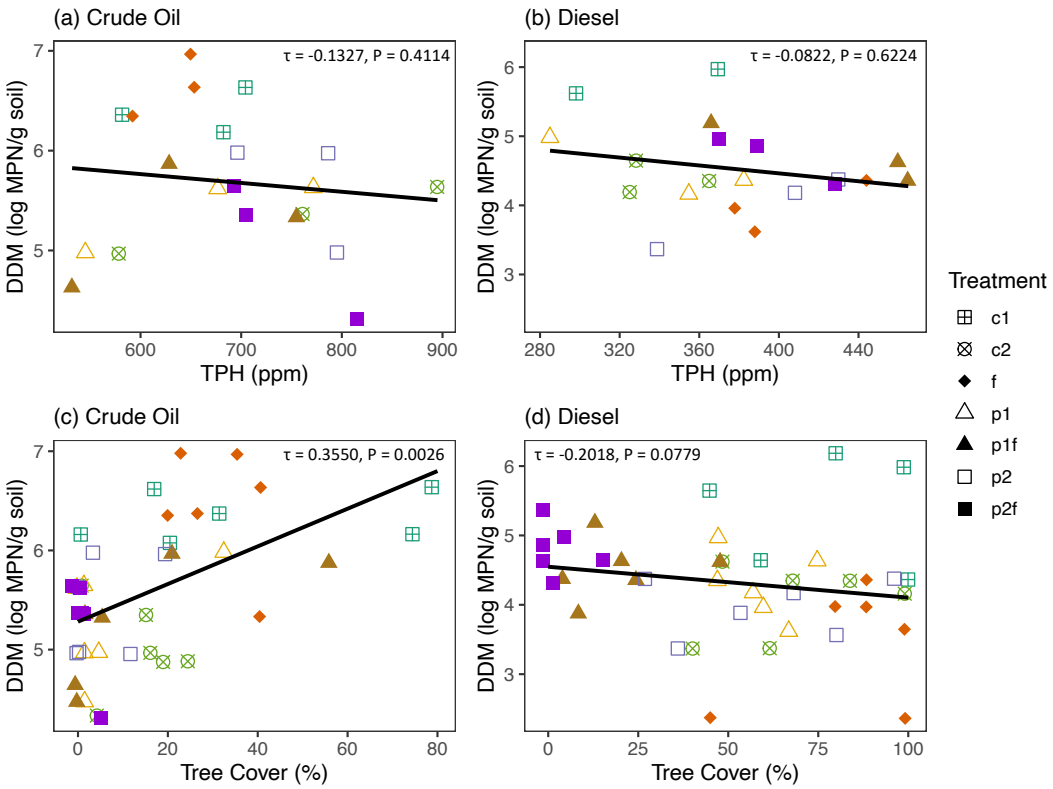

Figure S2. Relationship between the number of culturable diesel-degrading microorganisms (DDM) and total petroleum hydrocarbon concentrations (TPH; a & b) or percent cover of trees (c & d) in soils contaminated with crude oil (a &c) or diesel (b & d). Solid symbols indicate treatments originally fertilized. Treatments indicated are as follows: no treatment (c1, c2), planted with annual ryegrass (p1), a mix of annual ryegrass and Arctared fescue (p2), treated with fertilizer (f), and/or no added nutrients (no "f" indicated). The statistical relationship between variables is displayed on each graph.

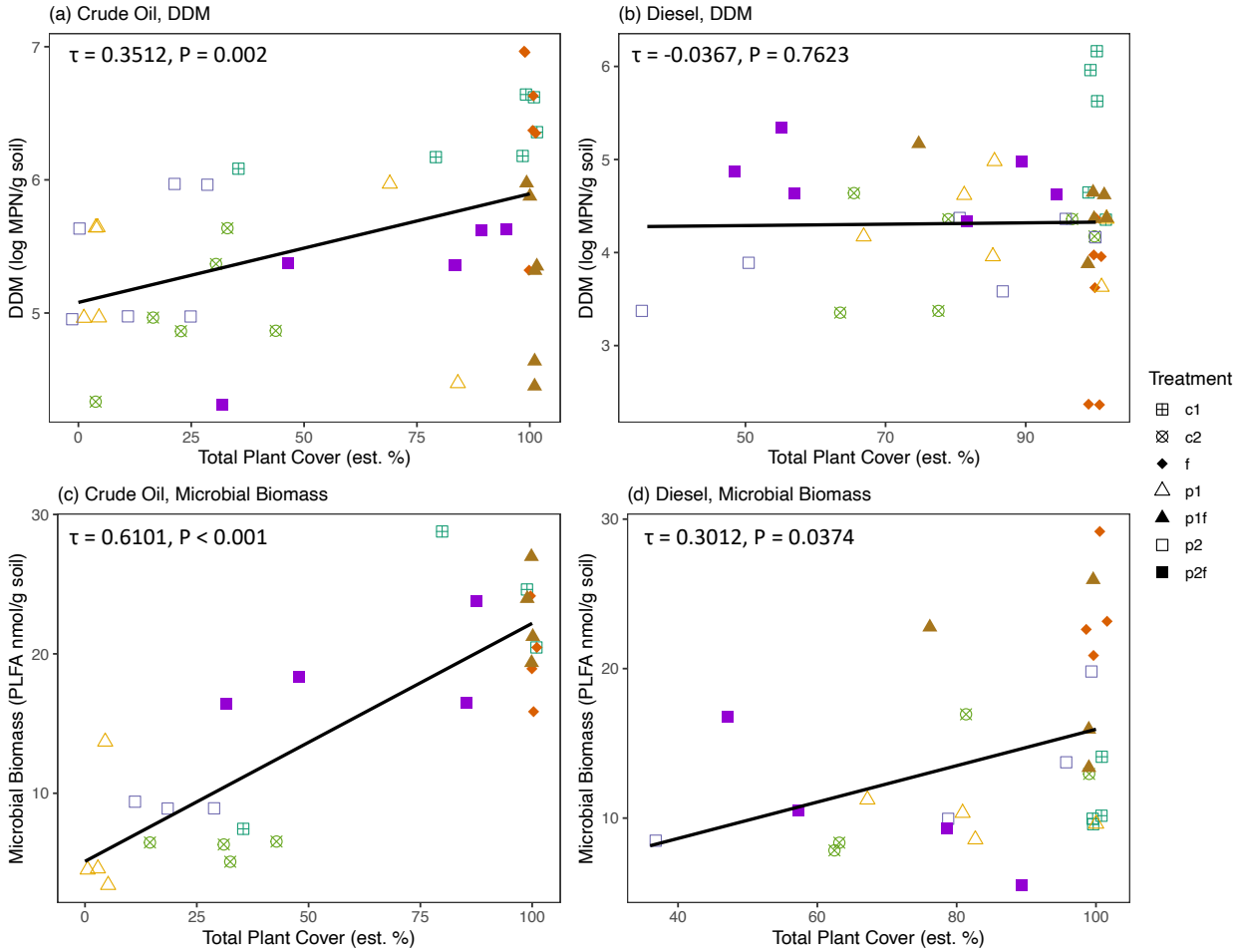

Figure S3. Relationship between the number of culturable diesel-degrading microorganisms (DDM (a & b) or total microbial biomass as measured by PLFAs (c & d) and total estimated plant cover in crude oil (a &cC) or diesel (b & d) contaminated soils. Solid symbols indicate treatments originally fertilized. Treatments indicated are as follows: no treatment (c1, c2), planted with annual ryegrass (p1), a mix of annual ryegrass and Arctared fescue (p2), treated with fertilizer (f), and/or no added nutrients (no "f" indicated). The statistical relationship between variables is displayed on each graph.

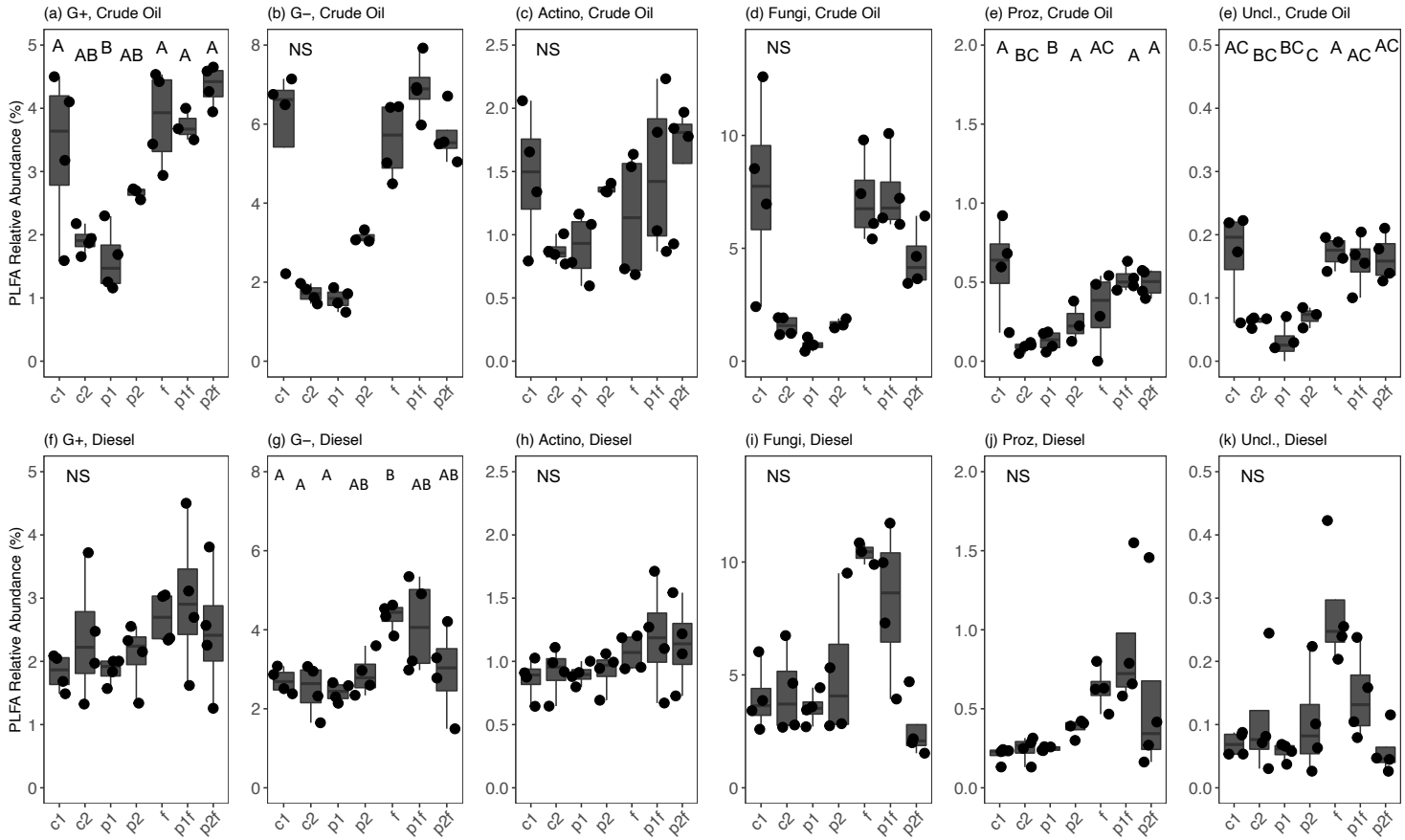

Figure S4. Relative abundance of PLFAs (nmol per gram soil) subgroups as identified in table S1 in crude oil (a-e) or diesel (f-k) contaminated soils. Abbreviations represent the following subgroups: G+ are gram positive bacteria, G- are gram negative bacteria, Actino. are Actinobacteria, and Unclass. are PLFAs unclassified. Letters indicate significant differences between treatments ($P < 0.05$). The letters "NS" indicates no significant differences found between treatments.

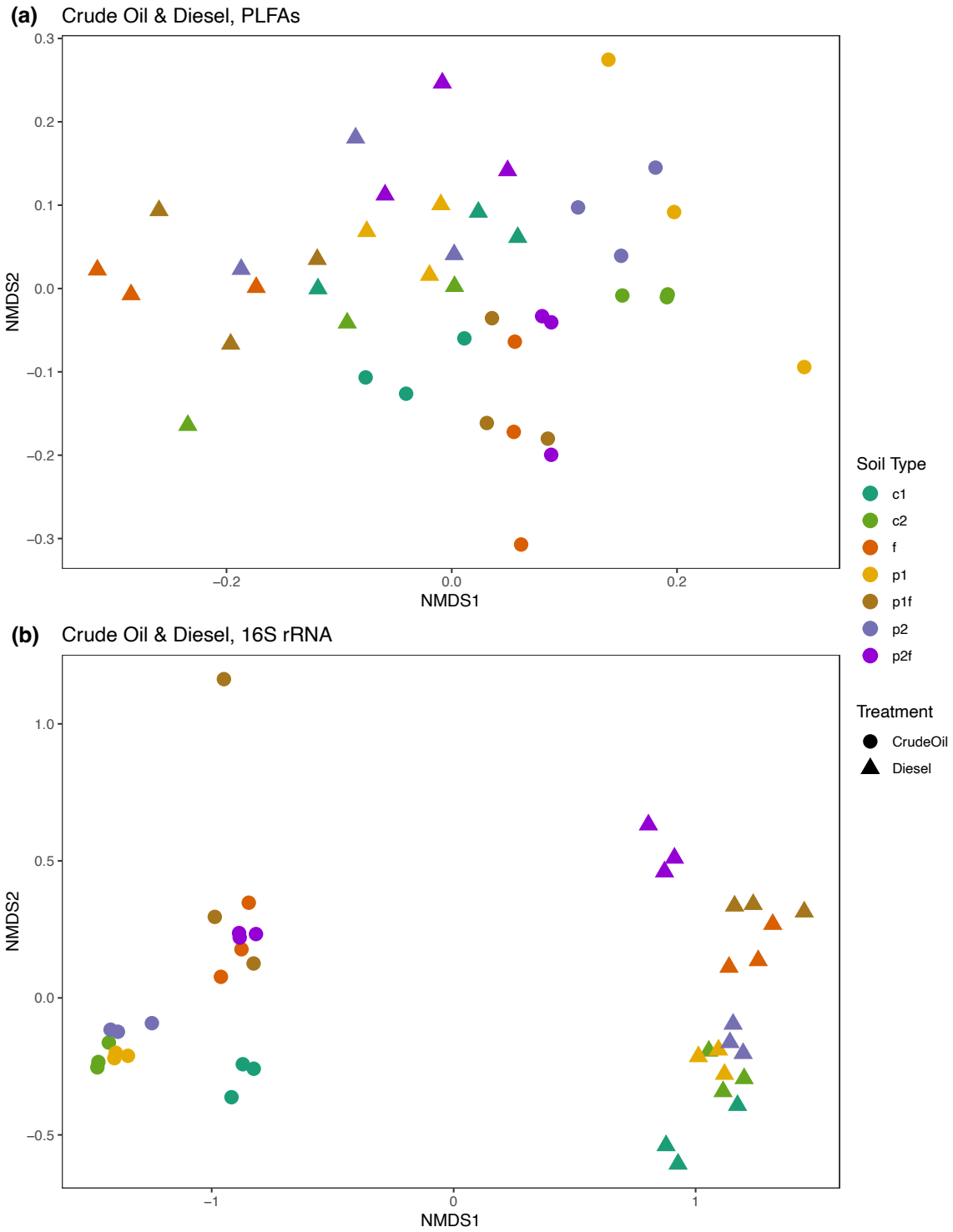

Figure S5. Non-metric multidimensional scaling ordination analysis (NMDS) of soil PLFAs (a) or 16S rRNA genes (b) including both crude oil (circle) and diesel (triangle) contaminated soils. Colours indicate original treatment.

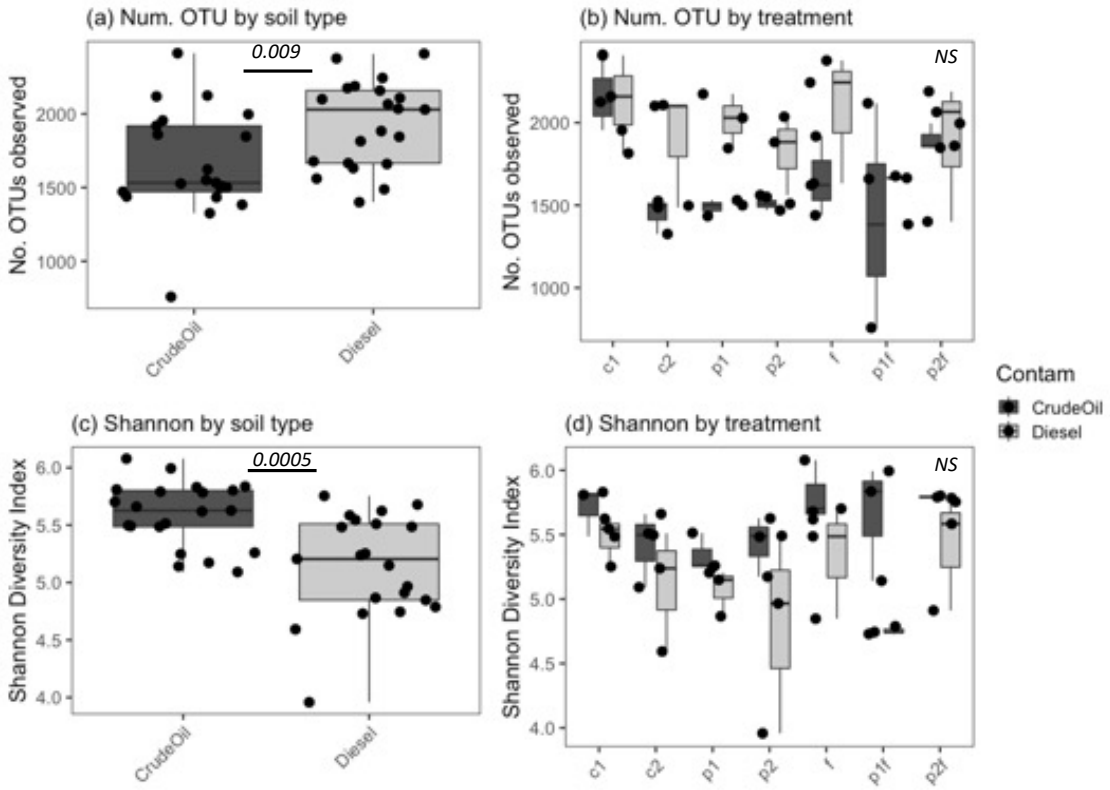

Figure S6. Measures of the number of observed OTUs (a & b) and Shannon alpha diversity index (c & d) for the 16S rRNA gene sequence data, displayed by soil type (a & c) or by treatment (b & d). The values shown are means with 95% confidence intervals (N = 6). Treatments indicated are as follows: no treatment (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). Significant differences are noted with the Kruskal-Wallace P-value displayed. The letters "NS" indicates no significant differences found between treatments.

Figure S7. 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).