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Long-term Effects of Nutrient Addition and Phytoremediation on Diesel and Crude Oil Contaminated Soils in subarctic Alaska

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Abstract

Phytoremediation is a potentially inexpensive method of detoxifying contaminated soils using plants and associated soil microorganisms. The remote locations and cold climate of Alaska provide unique challenges associated with phytoremediation such as finding effective plant species that can achieve successful site clean-up despite the extreme environmental conditions and with minimal site management. A long-term assessment of phytoremediation was performed which capitalized on a study established in Fairbanks in 1995. The original study sought to determine how the introduction of plants (*Festuca rubra*, *Lolium multiflorum*), nutrients (fertilizer), or their combination would affect degradation of petroleum hydrocarbon (TPH) contaminated soils (crude oil or diesel) over time. Within the year following initial treatments, the plots subjected to both planting and/or fertilization showed greater overall decreases in TPH concentrations in both the diesel and crude oil contaminated soils relative to untreated plots. We re-examined this field site after 15 years with no active site management to assess the long-term effects of phytoremediation on colonization by native and non-native plants, their rhizosphere microbial communities and on petroleum removal from soil. Native and non-native vegetation had extensively colonized the site, with more abundant vegetation found on the diesel contaminated soils than the more nutrient-poor, more coarse, and acidic crude oil contaminated soils. TPH concentrations achieved regulatory clean up levels in all treatment groups, with lower TPH concentrations correlating with higher amounts of woody vegetation (trees & shrubs). In addition, original treatment type has affected vegetation recruitment to each plot with woody vegetation and more native plants in unfertilized plots. Bacterial community structure also varies according to the originally applied treatments. This study suggests that initial treatment with native tree species in combination with grasses could be an effective means for phytoremediating petroleum contaminated soils and promoting ecological recovery in cold regions.

Keywords

Remediation; Re-vegetation; Soil petroleum hydrocarbon; Microbial Degradation

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Introduction

At high latitudes, petroleum products are often the primary source of fuel in both urban and rural environments. Petroleum hydrocarbons (PHCs) can be introduced into the environment through natural seepage and surface spills such as leaking pipelines and storage tanks. There are more than 500 formerly used defense sites owned by the Department of Defense in Alaska with approximately 1000 individual areas of soil contamination, many contaminated with petroleum (Reynolds and Koenen, 1997; Yergeau et al., 2012). Although the need to remediate contaminated sites is not unique to high latitudes, remediation in these regions is often confounded by a lack of infrastructure, reduced degradation rates, and high expense associated with traditional remediation approaches. In Alaska, the human health risk of contaminated sites is also exacerbated by the fact that many rural communities are located off road systems and near contaminated sites, and these communities rely heavily upon subsistence food harvesting, which can increase the exposure of inhabitants to contaminants. Phytoremediation may offer one solution to feasibly reduce contaminant levels and human health risks in these communities.

There are currently two main remediation strategies for PHC contaminated sites; physical-chemical remediation by contaminant removal and bioremediation through the use of microorganisms and plants (Uhlik et al., 2009). Physical-chemical remediation methods are the conventional approach to remediation of contaminated soils; however, such methods can be extremely costly, involving removal and incineration or transfer to hazardous waste landfills. In remote regions, such remediation methods are often not available or are prohibitively expensive due to the lack of roads and other infrastructure (Slater et al., 2011). In addition to the expense, removal of contaminated soils by physical means can also leave much of the contaminant behind in the form of loose soil. Alternative methods of contaminant abatement can include landfarming, biopiles and other forms of bioremediation such as phytoremediation (Palmroth et al., 2002; Paudyn et al., 2008).

Landfarming is common in cold regions. In landfarms, contaminated soils are spread over large areas and can be amended with nutrients, water, and surfactants in an effort to reduce the limitations associated with microbial degradative processes (Filler et al., 2006; Sanscartier et al., 2009). Periodic tilling may be performed to aerate soils and promote volatilization of contaminants. In biopile systems, contaminated soils are amended with nutrients and water then mounded over an aeration piping system. Soils can also be amended with organic material as a bulking agent to increase soil porosity and supply carbon. Aeration systems are varied and can be active or passive, and also could incorporate a heating system to increase soil temperature and therefore extend degradation treatment season (Sanscartier et al., 2009).

Both landfarming and biopiles are relatively low-cost and simple techniques and their use is wide spread across northern latitudes. However, these *ex-situ* strategies for contaminant abatement require the input of expensive elements such as heavy equipment for tillage, maintenance of aeration systems or reapplication of fertilizer multiple times a year. In remote regions with little or no road access, these strategies quickly become unreasonably expensive due to the cost of materials and travel to the site. Depending on the specific site management methods applied, these techniques may also leave contaminated soils exposed in stockpiles located at or near the community landfill. In addition, both landfarming and biopiles can disturb fragile ecosystems and can cause unnecessary destruction of the natural landscape.

Plant assisted bioremediation, or phytoremediation, is potentially an inexpensive and effective alternative method for cleanup of soil contaminants (Singer et al., 2003a; Wenzel,

2008). Phytoremediation is an attractive approach to bioremediation because it does not necessarily involve large soil disruptions and can result in intact, biologically active soils with minimal financial input (Aken et al., 2010). However, phytoremediation can take longer to achieve remedial targets than conventional cleanup methods (Palmroth et al., 2002). Phytoremediation relies on plant root systems to foster the growth and activity of microorganisms through release of metabolic products, improved aeration and/or other mechanisms (Singer et al., 2003a). Increased microbial activity in plant-associated soils can lead to biodegradation of pollutants through either induction of microbial degradation pathways or co-metabolism. Some of the aromatic compounds released by plant roots share chemical similarities to pollutants, such as PHCs, or an intermediary in the metabolism of pollutants, such as salicylic acid. Plant aromatic compounds have been shown to induce the microbial degradation of petroleum (McCutcheon and Schnoor, 2003; Siciliano et al., 2003; Singer et al., 2003b). Co-metabolism of pollutants by soil microbial communities can occur when secondary compounds that are structurally analogous to contaminants are available to the community (Donnelly et al., 1994; Gilbert and Crowley, 1997; Miya and Firestone, 2001). At high latitudes, such as in Alaska, many plant species have unusually high concentrations of secondary compounds, which are thought to have evolved as a defense against damage, such as from UV radiation or herbivory, and are therefore particularly attractive for investigations into microbial degradation of aromatic compounds (Bryant et al., 1991, 1994; Hadacek, 2002; Stark et al., 2008). With increased concentrations of secondary compounds at higher latitudes, increased rates of contaminant disappearance may be achievable, which would be advantageous for promoting contaminant biodegradation in the rhizosphere. Another important advantage of using plants native to higher latitudes is their adaptation to the extreme environmental conditions, such as low temperature and nutrient limitation.

In this study we investigated the long-term effects of phytoremediation on contaminant disappearance at a site in Fairbanks, Alaska, where a phytoremediation experiment on soils contaminated with crude oil or diesel fuel was previously conducted (Reynolds and Koenen, 1997; Reynolds et al., 1997b; Reynolds et al., 1999). Experimental field plots were established and subjected to different fertilization and planting regimes, while petroleum disappearance was monitored for two years. The site then underwent colonization by local plants and natural attenuation for 15 years. In this study, we re-examine this field site 15 years later to assess the long-term effects of phytoremediation on colonization by native plants, their rhizosphere microbial communities and petroleum removal from soil. The data provide a novel, long-term assessment of the feasibility of rhizoremediation with native plants and soil microbial communities for achieving regulatory cleanup guidelines in cold regions.

Background and Site Description

A phytoremediation field study was initiated in 1995 and 1996 at the Farmers Loop Permafrost Research Facility field site of the Army Corps of Engineers Cold Regions Research and Engineering Laboratory (ACE CRREL) (Reynolds and Koenen, 1997; Reynolds et al., 1997a; Reynolds et al., 1997b; Reynolds et al., 1999). The study sought to compare the effects of nutrients and vegetation on rhizosphere-enhanced bioremediation of PHC contaminated soils. Crude oil and diesel contaminated soils were used for the experiment: crude oil contaminated soils were collected from a gravel pad at a pump station on the Trans-Alaska pipeline, and diesel contaminated soils were collected during the removal of an underground storage tank. Soils were transported to the Farmers Loop Facility and placed, separately, in adjacent lined and bermed areas approximately 21 × 3 m, and 60 cm deep (Figure 1 and 2). Each of the soil piles was subdivided by wooden beams into seven treatment plots. For each soil the treatments included: three levels of rhizosphere

enhancement and two nutrient levels. The three levels of rhizosphere enhancement included unplanted (-P), annual ryegrass (*Lolium multiflorum*, 1P), or a mixture of annual ryegrass and arctared fescue (*Festuca rubra*, 2P). Grasses were chosen for their cold hardiness, rapid growth, and tolerance to low-fertility soils. The two nutrient levels were either control (no fertilizer, -F) or added nutrients (+F). Commercially available fertilizer (granular 20-20-10) was surface applied at approximately 620 g/m² of N, P, and K. The annual ryegrass and arctared fescue mixture was 1:1 by weight, and seeding was done by hand at approximately 10.8 g/m².

Triplicate soil samples were taken from each of the plots to sample for TPH at day 0 and after 54 days and 238 days of treatment (described below). After 238 days of treatment, TPH concentrations in both the plant + fertilizer treatment and soils without any amendments had decreased relative to the initial TPH concentrations (measured at day 0). The planted and fertilized treatment had significantly lower TPH concentrations than the untreated soils (Figure 3).

After completion of the initial phytoremediation project in 1996, the site was not actively managed for approximately 15 years. No nutrient or seed amendments were made during this time. In 2010–2011 the site was re-examined and our follow up study was conducted.

Materials and Methods

Site Preparation and Sampling

A follow up study of the initial phytoremediation experiment conducted at the Farmers Loop Permafrost Research Facility field site was conducted in June 2011. The original soils and treatment plots remained intact and were used with minor modification to the sampling design. Each of the seven treatment plots per contaminant was subdivided into six 1 × 1.5 m² sections using twine. Two soil samples were collected from each of the sub-sections into both a glass jar with Teflon lined lids for TPH analysis or two separate sterile plastic zip-top bags for microbial analyses. Soil samples were collected from four random points within each sub-section at a depth of 10 cm and homogenized in the sampling container. Samples were sieved through a sterile 2.5 mm sieve and stored at -80°C until analysis for molecular and TPH analysis or at 4°C for culture-based microbial assays.

Vegetation Characterization

All vegetation within each sub-section was counted and identified to the species level (Johnson et al., 1995). Percent cover of dominant vegetation, bare ground, and mosses were visually assessed. Tree height, basal diameter, and canopy cover were also measured for each tree at the site. Trees were defined as such when main-stem height was greater than 20 cm, and trees shorter than 20 cm were counted as “seedlings”.

Analysis of TPH in Soils

In the initial study (1995), TPH was extracted by sonication with methylene chloride (EPA Method 3550B). Anhydrous Na₂SO₄ was added to the soils during extraction as a drying agent and extracts were then analyzed by GC-FID.

For the follow up study, total petroleum hydrocarbons (TPH) were analyzed by Alpha Analytical (320 Forbes Boulevard, Mansfield, MA). Soil samples were extracted and analyzed using a gas chromatograph equipped with a flame ionization detector (GC/FID). The temperature program and associated experimental conditions were optimized to obtain maximum resolution in an 80 min chromatographic run representative of hydrocarbons in the n-octane (C8) to n-tetracontane (C40) range. Qualitative evaluation of the sample was

conducted by reviewing the sample chromatogram in conjunction with a chromatogram of an alkane series generated with the same chromatographic conditions (EPA Method 8015M). Quantitative determination of the sample hydrocarbon concentration was performed in accordance with EPA Method 8015M.

Analysis of Nutrients in Soils

Soil physical and chemical analyses were conducted at the University of Alaska Fairbanks, Palmer Center for Sustainable Living. Properties assessed were: pH, % loss on ignition (C), CEC, NO_3^- , NH_4^+ , P, K, and soil particle size (Jackson, 1958; Day, 1965; Holmgren et al., 1977; Bremner, 1982; Peech, 1982; Michaelson et al., 1987).

Microbial Community Analyses

Total soil DNA was extracted using the FastDNA SPIN kit for soil (MP Biomedicals, Ohio, USA) following the manufacturer's instructions. DNA was eluted into 50 μL of water and stored at -20°C until analysis. DNA concentrations were evaluated by measuring absorbance at 260 and 280 nm using a NanoDrop ND-1000 Spectrophotometer (Thermo Fischer Scientific, USA).

Bacterial community profiling using T-RFLP was performed on extracted total community DNA. PCR reactions were carried out using a 5' 6-carboxyfluorescein-labelled 27F and unlabeled 1392R primers with a total reaction volume of 25 μL . The PCR reaction contained final concentrations of 1 $\times$ PCR buffer, 3 mM MgCl_2 , 200 μM dNTPs and 0.1 μM BSA (New England Biolabs, Beverly, MA, USA), 200 nM each primer, 0.5 U Taq DNA polymerase and 1 μL template DNA. Thermal cycler conditions were as follows: denaturation at 94°C for 5 min; 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min 40 s; and a final extension step of 72°C for 10 min. PCR products were purified using the Qiaquick PCR purification kit (Qiagen, Valencia, CA, USA) and quantified using the Nanodrop ND-1000 spectrophotometer. Then 40 ng of the PCR product was digested for 3 h at 37°C using HhaI restriction enzyme, with *Pseudomonas stutzeri* DNA run as a control to verify complete digestion. After digestion, fragments were precipitated overnight using 1.5 μL of sodium acetate (0.75M), 6 μg of glycogen per reaction (molecular grade), and 47 μL ethanol and centrifuged at 14 000 r.p.m. for 35 min. Pellets were resuspended in 1.0 μL H_2O , 0.5 μL of ROX-labelled MapMarker 1000 ladder (BioVentures, Murfreesboro, TN, USA) and 9.5 μL deionized formamide, and then run on an ABI 3100 Genetic Analyzer. T-RFLPs were analyzed using GeneMapper software version 3.7 (Applied Biosystems, Foster City, CA, USA). Terminal restriction fragments less than 50 bp and above 1000 bp in size were excluded from analyses.

Statistical Analyses

Data were tested for normality by testing the residuals of each data set, and were found to be non-normally distributed. Vegetation and TPH data were analyzed individually to detect trends using appropriate non-parametric tests (NMDS, Kruskal-Wallis test). Relationships between vegetation and TPH were analyzed using regression analyses. Vegetation communities were tested using ANOVA. Microbial community data was analyzed using detrended correspondence analysis (DCA). All statistical analyses were conducted in PAST (Version 2.15, April 2012, Hammer and Harper 2001), significance was accepted at $p=0.05$ (95% confidence level) for all statistical analyses.

Results and Discussion

Soil TPH concentrations were significantly lower in 2011 than in samples quantified in 1996, when contaminant levels were last quantified and published for the site (Figure 3). In

both the diesel and crude oil contaminated soils in 2011, contaminant levels in all treatment plots had dropped by 80–95% of levels last measured in 1996, well below the ADEC cleanup level for diesel range organics (1000 mg/kg) (ADEC, 2012). This suggests that natural attenuation (no amendments), land farming or phytoremediation are all capable of achieving cleanup limits for hydrocarbon contaminated soils with initial concentrations of greater than 8300 mg/kg within ~15 years. The concentration of residual hydrocarbons remained higher in the crude oil contaminated soils than in the diesel contaminated soils, which could be because recalcitrant compounds constitute a greater portion of the crude oil than diesel fuels (Reynolds et al., 1997a), as well as the fact that the crude oil contaminated soil was more nutrient poor, more coarse, and more acidic. For most PHCs, reduction rates are often initially rapid, followed by a period of much slower losses, similar to the observed effects here. These data demonstrate that, despite slow disappearance rates, TPH disappearance continues to occur and, for soils contaminated with up to 3422 mg/kg diesel, can reach clean up levels in less than two decades and that for near-surface contaminants, phytoremediation, land farming or natural attenuation may provide an effective low-cost treatment.

Results from vegetation surveys showed that the plant community had changed substantially from when the site was first established in 1995 (Figure 1 and 2). None of the originally planted grasses were found on the site, instead the plots have been colonized by both native and non-native Alaskan plants (Figure 4). The diesel-contaminated soils were more heavily colonized by plants than crude oil contaminated soils ($p=0.06$), and there were more and larger woody plants present in the diesel contaminated soil (Figure 4). This is likely due to the difference in soil type between the diesel and crude-oil contaminated soils. The two contaminated soils differed in soil texture: crude oil contaminated soils were gravel with a large grain size, while diesel contaminated soils were finer textured with more organic matter (Table 1). Coarser soils tend to have a lower cation exchange capacity (CEC) and less water and nutrient retention, which can mean more inhospitable conditions for plant and microbial growth (Bergamaschi et al., 1997; Fredlund et al., 1997; Lund et al., 1999). Fine-textured soils with higher soil organic matter can be a more favorable environment for plant colonization and microbial growth, conditions which could lead to increased contaminant degradation (Olk and Gregorich, 2006). Interestingly, the crude oil contaminated soils were more heavily colonized by non-native plants from the *Asteraceae* family, such as *Taraxacum officinale*, and from the *Fabaceae* family, such as *Vicia cracca* and *Trifolium hybridum*.

Current vegetation status also appears to be affected by the original treatment type: plots that were originally planted with grasses grouped together in ordination space (NMDS, Crude Oil: $R^2 = 0.7005$, Stress = 0.1927; Diesel: $R^2 = 0.6859$, Stress = 0.1622, Figure 5). NMDS also revealed that plots with no original treatment are associated with increased numbers of native plants (e.g. *Salix sp.*, *Oxytropis deflexa*) and that initially planted and fertilized plots were associated with increased numbers of non-native plants (e.g. *Agrimonia striatica*, *Taraxacum officinale*). Nutrient status and other soil parameters have different effects on vegetation depending on the contamination, and therefore soil, type (Table 1, Figure 5). Both nutrient levels and contaminant type have been found to affect vegetation survival and colonization (Chapin et al., 1986; Rohr et al., 2006). Fertilization may have had an effect on initial colonization and survivability of vegetation; however, this direct effect would only last until the fertilizer had either been depleted or dispersed through the site, typically less than a year. Although direct effects of the fertilizer would be fairly short-lived, it is possible that the fertilizer acted to prime plant succession or to allow for some seeds to colonize and establish in a particular treatment. Although the fertilizer may not remain in the soil over the long-term, turnover of nitrogen-rich plant biomass might also provide a long-term enhancement of available nitrogen (Ruess et al., 1996).

A critical question from the standpoint of bioremediation is how the initial vegetation and fertilization treatments affected contaminant degradation rates. It is important to note that one characteristic of petroleum biodegradation is the sequential loss or disappearance of petroleum components, in which more labile fractions are lost most rapidly while recalcitrant fractions remain and slowly degrade in weathered petroleum. As a result, biodegradation follows a somewhat asymptotic pattern rather than a linear progression. The initial 3-year study found increased rates of contaminant disappearance in plots that were both planted and treated with fertilizer within the first year of the field study (Reynolds and Koenen, 1997; Reynolds et al., 1997b; Reynolds, 2004). These rates were higher than in treatments that were only either planted or fertilized. Fifteen years later, significant differences in mean soil TPH concentrations between planting/fertilization treatments were not detected when mean TPH levels were compared at the plot level. Addition of vegetation and fertilizer appeared to accelerate TPH biodegradation relative to unplanted, unfertilized treatments initially, and over the long term all treatments arrived at a similar plateau.

Further statistical evaluation of the relationships between detailed vegetation data and TPH revealed that increased woody vegetation counts affected TPH disappearance over the long term. There was a negative relationship between number of woody plants present and soil TPH concentrations across all plots (Figure 6: crude oil contaminated soils; $R^2 = 0.315$, $p < 0.01$; Diesel contaminated soils; $R^2 = 0.452$, $p < 0.01$). In diesel contaminated soils, the current number of trees is significantly different between the original treatment types ($p < 0.001$) with significantly fewer trees in cells which were originally planted and fertilized ($p < 0.05$) compared to untreated and unplanted cells. Crude oil contaminated soils did not have differences between number of trees on each plot ($p = 0.06$). The data suggest that combined planting and fertilization accelerated initial diesel biodegradation rates, yet in the long term may slow remediation by limiting the establishment of trees in diesel-contaminated soil.

The dominant woody plant species present included willow (*Salix* spp.), Alaskan birch (*Betula neoalaskana*), white spruce (*Picea glauca*), and balsam poplar (*Populus balsamifera*) (Figure 4). Some northern tree populations (*Betula*) have been shown to have increased concentrations of phenolic secondary compounds with increasing latitude (Stark et al., 2008). Based on the secondary compound hypothesis (Robertson et al., 2010; Slater et al., 2011), this increasing secondary compound concentration could have resulted in increased degradation potential in the microbial community and a resulting decrease in soil TPH. Trees have more extensive root systems than some forbs and grasses, which may also contribute to rhizoremediation efficiency. *Salix* and *Populus* species are commonly used in remediation studies and have been found to lower TPH and reduce toxicity in TPH contaminated soils (Palmroth et al., 2002; Newman and Reynolds, 2004). Willows (*Salix* spp.) are very common throughout the Alaskan interior and Northern latitudes. Willows were found in each of the plots at the Farmers Loop Site, with willows being found up to 4 m tall in the cell with the lowest soil TPH concentration. In lab and greenhouse studies, a willow native to interior Alaska (*Salix alaxensis*) was found to accelerate microbial biodegradation of diesel range organics (McFarlin, 2010) and PCBs (Slater et al., 2011) in spiked soils.

Because microbial biodegradation is an important mechanism for TPH removal from soils, we investigated the structure of the bacterial community in an effort to determine the effect of TPH and vegetation on microbial community dynamics. Terminal restriction fragment length polymorphism (T-RFLP) data indicate that the current microbial community structure varies depending on the original treatments implemented 15 years ago (Figure 7). The original treatment type resulted in different microbial community structures, much as it did for vegetation dynamics (Figures 1, 2 and 4). This suggests that the original treatment

influenced vegetation structure and succession, which affected associated microbial communities by mechanisms such as rhizodeposition and the quantity and quality of aboveground and belowground litter. It has been shown that as trees age and the plant community matures, associated microbial populations change and may foster pollutant degrading microorganisms (Newman and Reynolds, 2004). It has also been found that with increasing quantities of vegetation there are increasing quantities of microorganisms on sites contaminated with PHCs (Reynolds et al., 1999; Margesin et al., 2007). The microbial data do not appear to indicate a significant relationship between contaminant concentration and bacterial community structure, nor a relationship between current nutrient status and bacterial community structure ($p>0.05$); however more studies should be conducted to further understand the relationship between soil parameters and the microbial community. Future studies should focus on the microorganisms involved in actual biodegradation of the contaminant; for example, the Most Probable Number analysis of petroleum degraders would be directly indicative of the degradation potential at the site and would be valuable for further site assessment and long-term monitoring. Determining the identity of petroleum-degrading microorganisms present would also be valuable, since detection of previously characterized petroleum degraders can reveal environmental preferences that may suggest effective ways to enhance biodegradation and to monitor biodegradative populations in the long term.

The results of this study indicate that trees and shrubs have a positive effect on TPH biodegradation by biostimulating microbial communities in the root zone. Yet, the use of grasses in the initial stages of this study were also clearly beneficial, as has been shown previously (Phillips et al., 2009). However, the grasses applied were annuals, requiring repeated seeding, which is labor-intensive and expensive over the long term. Instead of planting with exotic annual plants, another phytoremediation option would be the use of native perennial grasses or forbs, which are adapted to local conditions and have been shown to survive contamination and to induce degradation of petroleum hydrocarbons in previous studies located in Northern latitudes (Robson et al., 2003; Ferrera-Rodríguez et al., 2012). Initial cultivation of grasses appeared to inhibit natural recruitment of woody plants that were associated with increased long-term contaminant loss. Trees and shrubs can also be advantageous in that they offer increased rooting depth and long-term growth as compared to grasses and forbs. Our findings suggest that a combined approach of initial seeding with grasses and planting with trees or shrubs, such as with native willow cuttings, may lead to increased and sustained contaminant disappearance rates at a site. In addition to promoting biodegradation in surface soils, grasses would also help to provide a living cap on contaminated soils by stabilizing soils and minimizing contaminant escapement from the site via blowing dust. Trees or shrubs would act to increase long term biodegradation rates at deeper soil layers, provide soil stabilization, and may also provide added hydraulic control, preventing leaching of contaminants. More information regarding the interactions between native graminoids and woody plants planted together would be valuable for developing optimal long-term rhizoremediation strategies.

Conclusions and Future Directions

This study investigated the long-term effects of phytoremediation on soils contaminated by crude oil and diesel fuel in interior Alaska. In the initial 3-year period post treatment, for both crude oil and diesel contaminated soils, rhizosphere enhancement with non-native grasses and nutrient amendments resulted in increased rates of TPH reduction relative to natural attenuation, nutrient additions alone, or plants alone (Reynolds et al., 1997a). Fifteen years later, we found that increased TPH disappearance appeared to be associated with increased numbers of trees and shrubs such as willow (e.g. *Salix bebbiana*, *Salix alexensis*, *Salix glauca*), birch (*Betula neolaskana*), white spruce (*Picea glauca*), and balsam poplar

(*Populus balsamifera*), all native to the region, which had colonized the site. The original field treatments appeared to affect the total vegetation present at the site, the plant successional trajectory, and the soil bacterial community structure. All plots achieved soil TPH levels that were below regulatory cleanup limits within 15 years. Phytoremediation and fertilization accelerated biodegradation initially as shown in the 3-year study using non-native annual grasses, and our long-term study revealed that colonization with trees and shrubs corresponded to increased extent of long-term petroleum removal. The original planting and fertilization strategy was effective for accelerating the reduction of contaminant levels and mitigating risk in a timely manner during the initial phase after contamination of a site. The increased TPH disappearance in plots with more trees and shrubs implies that initially planting with woody plants in addition to grasses may have the potential to achieve TPH reductions greater than those observed here. Native vegetation may be the best phytoremediation option for ensuring long term survival of plants and the associated increase in degradation rates with minimal labor and expense.

Phytoremediation appears to be an effective and potentially low-cost bioremediation strategy particularly applicable to remote sites that are inaccessible to heavy equipment, that have no infrastructure, or that are situated on fragile ecosystems. The use of local plants like willows, which are readily propagated from stem cuttings, can further reduce costs associated with phytoremediation activities. Phytoremediation represents an option for the many contaminated sites located in Northern communities where infrastructure and resources limit the feasibility of conventional remediation strategies.

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Highlights

- We examined effects of long-term phytoremediation treatment on petroleum contaminated soils
- After initial treatments were applied & with no active management, native plants colonized the site
- Current microbial community structure varies depending on original treatments
- Initial phytoremediation treatments continue to affect plant community structure
- Soil petroleum concentrations are below regulatory limits

Figure 1.
Photo overview of the study site in 1996 (top) and 2011 (bottom). Note tree establishment and colonization, and variety of vegetation colonizing the different plots.

Figure 2.

Aerial view of the design of the phytoremediation treatments at the site and the vegetation 15 years following establishment of the plots. Fourteen individual plots are present; half of the soils are contaminated with diesel fuel (top) and half with crude oil (bottom). Plots were planted with annual ryegrass (+1P) or a mix of annual ryegrass and arctared fescue (+2P), and treated with additional nutrients (+F) or no added nutrients (–F).

Figure 3.

Changes in TPH concentrations in crude oil (A) and diesel (B) contaminated soils. Error bars indicated 95% confidence interval. Plots were planted with annual ryegrass (+1P) or a mix of annual ryegrass and arctared fescue (+2P), and treated with additional nutrients (+F) or no added nutrients (–F (1) or –F (2)). Alaska Department of Environmental Conservation clean-up standard for this site is indicated at 1000 ppm.

Figure 4.

Total vegetation counts for crude oil (trees: top left, other vegetation: bottom left) and diesel (trees: top right, other vegetation: bottom right) contaminated soils. Plots were originally planted with annual ryegrass (+1P) or a mix of annual ryegrass and arctard fescue (+2P), and treated with additional nutrients (+F) or no added nutrients (–F (1) or –F (2)). Names designated with a (*) indicate native vegetation, names designated with a (+) indicate mixed native and non-native vegetation within a family, vegetation family names with no indicator are non-native.

Figure 5.

Nonmetric multidimensional scaling (NMDS) of vegetation data from crude oil (A) or diesel (B) contaminated soils. Blue points indicate plots planted and fertilized, green points are plots only planted, red points are plots only fertilized, and black points are plots with no added amendments. Vectors indicate environmental parameters.

Figure 6. Regression of TPH concentrations and counts of all woody trees and shrubs for crude oil (A) or diesel (B) contaminated soils.

Figure 7. Detrended correspondence analysis (DCA) of T-RFLP of 16S rRNA for all samples from the crude oil contaminated soils. The first two axes account for 30% of total variance

Table 1

Physical and chemical characteristics of soils from each plot.

Cell Number	Original Treatment	Contaminant	pH	NH ⁴⁺ (ppm)	NO ₃ ⁻ (ppm)	P (ppm)	K (ppm)	CEC (meq/100)	C (%)	Sand (%)	Silt (%)	Clay (%)
1	-P/-F1	Crude Oil	6.9 ± 0.1	<1	30.1 ± 5.02	2.68 ± 0.58	50.85 ± 4.2	2.6 ± 0.2	1.3 ± 0.3	75.7 ± 5	15.8 ± 5	8.5 ± 0.6
2	-P/+F	Crude Oil	6.8 ± 0.2	<1	17.7 ± 6.52	134.1 ± 40.2	58.18 ± 8.9	2.4 ± 0.2	1.2 ± 0.1	80.5 ± 1	12 ± 0.5	7.5 ± 0.6
3	-P/-F2	Crude Oil	7.1 ± 0	<1	4.68 ± 2.52	1 ± 0	35.44 ± 4.06	2 ± 0.1	0.9 ± 0.1	78.9 ± 1.5	14.3 ± 1.2	6.8 ± 1
4	+1P/-F	Crude Oil	7.3 ± 0.1	1	2.67 ± 2.9	1.67 ± 0.58	32.43 ± 2.53	1.9 ± 0.3	0.9 ± 0.1	80.4 ± 2.1	13.5 ± 1.5	6.1 ± 0.6
5	+1P/+F	Crude Oil	6.6 ± 0.1	1	17.4 ± 8.11	89.99 ± 24.9	57.54 ± 2.53	2.5 ± 0.1	1.5 ± 0.1	79 ± 2.8	15 ± 2.5	6 ± 0.3
6	+2P/-F	Crude Oil	7.1 ± 0.1	<1	4.51 ± 0.71	1.34 ± 0.58	26.4 ± 2.08	1.7 ± 0.1	1 ± 0.1	77.6 ± 0	17.3 ± 0.6	5.1 ± 0.6
7	+2P/+F	Crude Oil	6.7 ± 0.1	1	3.51 ± 0.71	158.8 ± 51.4	55.5 ± 9.1	2.1 ± 0.1	1.3 ± 0	79.6 ± 2	14.6 ± 2	5.8 ± 0
8	-P/-F1	Diesel	6 ± 0.5	<1	32.5 ± 6.46	4.02 ± 1.74	60 ± 7.63	4.7 ± 0.4	2.8 ± 0.3	77.5 ± 1.9	19.3 ± 1.5	3.1 ± 0.6
9	-P/+F	Diesel	5.1 ± 0.1	<1	40 ± 8.45	91.31 ± 19.7	62.11 ± 10.8	5.6 ± 0.8	3.5 ± 0.4	78.1 ± 0.2	18.4 ± 0.7	3.5 ± 0.6
10	-P/-F2	Diesel	5.5 ± 0	<1	13.8 ± 2.1	2.01 ± 0	53.02 ± 9.37	4.9 ± 0.6	3.2 ± 0.5	80.6 ± 2.7	16.3 ± 2.7	3.1 ± 0.2
11	-P/-F2	Diesel	5.4 ± 0.1	<1	18.5 ± 6.55	2.01 ± 0	46.66 ± 4.98	5 ± 0.3	3.5 ± 0.2	82 ± 0.8	15.4 ± 1.4	2.6 ± 0.7
12	+1P/+F	Diesel	4.7 ± 0	<1	80.9 ± 27.9	95.64 ± 6.34	79.53 ± 16.5	5.3 ± 0.5	3.2 ± 0.5	81.1 ± 1.2	14.5 ± 1	4.5 ± 0.2
13	+2P/-F	Diesel	5.4 ± 0.1	<1	40.6 ± 46.2	2.68 ± 1.16	51.68 ± 12.8	4.9 ± 0.7	3.1 ± 0.2	81.7 ± 0.5	14.1 ± 0.8	4.3 ± 0.6
14	+2P/+F	Diesel	5.1 ± 0.2	<1	17.8 ± 11.6	161.1 ± 52	115.1 ± 28	6.1 ± 0.7	3 ± 0.3	82.9 ± 1.5	13.1 ± 1.5	3.9 ± 0.6