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1 Alteration of microbial community structure affects diesel
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20 **Running head:** Community structure affects Arctic soil diesel biodegradation

Abstract

A wide range of microbial taxa are active in hydrocarbon-contaminated Arctic soils, and many are capable of hydrocarbon metabolism. The most effective hydrocarbon degraders may not naturally dominate following contamination events, so shifts in microbial abundance could potentially increase hydrocarbon biodegradation. In this study, we contaminated an Arctic soil with diesel, and used gentamicin and vancomycin to inhibit distinct portions of the microbial community. We measured diesel loss using gas chromatography, bacterial and fungal abundance with qPCR, and assessed bacterial diversity and community composition through Ion Torrent sequencing of 16S rRNA gene amplicons. The combined addition of both antibiotics increased diesel biodegradation significantly relative to the no antibiotic treatment, despite reduced bacterial and fungal abundance; however, this effect was not observed when nutrients were also added. All treatments produced unique bacterial communities, and both *Xanthomonadaceae* and *Micrococcineae* were dominant in the dual antibiotic treatment. The bacterial communities resulting from dual gentamicin and vancomycin addition were similar both with and without nutrients, although nutrient addition produced a much larger fungal population, which may partly explain the differences in biodegradation between these two treatments. These results suggest that the most efficient hydrocarbon-degrading community may not always be promoted naturally in contaminated soils.

Introduction

Hydrocarbon-degrading microorganisms inhabit many ecosystems (Greer *et al.*, 2010) and can metabolize and degrade petroleum contaminants for use as energy and carbon sources. Harnessing this activity for the purpose of contaminant removal is referred to as bioremediation. Several *in situ* treatments are currently used to enhance hydrocarbon bioremediation in contaminated soils, including supplementation with nutrients and/or oxygen to stimulate the growth of the existing microbial community (Powell *et al.*, 2006; Delille & Coulon, 2008; Yergeau *et al.*, 2009), or the addition of cultured hydrocarbon-degrading microorganisms (Thomassin-Lacroix *et al.*, 2002; Thompson *et al.*, 2005). Effective treatments are especially important in hydrocarbon-contaminated Arctic soils, as substantial hydrocarbon biodegradation may only occur when temperatures exceed 0°C (Walworth *et al.*, 2001), which equates to roughly two months each year in the high Arctic (Bell *et al.*, 2011). The effectiveness of bioremediation treatments, specifically nutrient amendment, has varied across hydrocarbon-contaminated soils (Powell *et al.*, 2006; Delille & Coulon, 2008; Yergeau *et al.*, 2009; Bell *et al.*, In press), suggesting that more targeted approaches may be required.

The role of cooperative interactions between microbial species in hydrocarbon-contaminated soils has been discussed by several authors (Brennerova *et al.*, 2009; Ciric *et al.*, 2010; Vilchez-Vargas *et al.*, 2010), but little attention has been paid to the potentially negative effects of interspecies competition. The growth and activity of some organisms can be reduced in the presence of competing species (Case & Gilpin,

1974; Mille-Lindblom *et al.*, 2006; Meidute *et al.*, 2008; Violle *et al.*, 2011), so the inhibition of taxa that are less efficient at hydrocarbon biodegradation may in fact increase the bioremediation capacity of a mixed microbial community. A small number of microbial species likely govern the majority of energy flux in hydrocarbon-contaminated soils (Dejonghe *et al.*, 2001), and while specialized microorganisms are required to transform substances such as uranium (Gihring *et al.*, 2011) and chlorinated solvents (Tas *et al.*, 2010), the ubiquity of natural hydrocarbons means that many co-existing taxa have evolved pathways that can be used to metabolize petroleum contaminants. In addition, species that are not directly involved in hydrocarbon biodegradation may obtain carbon and energy through cross-feeding (DeRito *et al.*, 2005), while still using important resources such as nutrients, and colonizing strategic spaces in soil such as the surfaces of carbon substrates or plant roots. Despite extreme climatic conditions, microbial diversity in Arctic soils is similar to what is found at lower latitudes (Neufeld & Mohn, 2005; Chu *et al.*, 2010), and hydrocarbon-contaminated Arctic soils have also been shown to maintain a variety of active taxa (Greer, 2009; Bell *et al.*, 2011).

A positive relationship between microbial richness/diversity and productivity has sometimes been observed, supporting the biodiversity and ecosystem productivity hypothesis (Bell *et al.*, 2005; Gravel *et al.*, 2011). Nevertheless, this relationship is function-specific (Griffiths *et al.*, 2000; Peter *et al.*, 2011), and can depend more on the presence of specific taxa than diversity *per se* (Salles *et al.*, 2009; Peter *et al.*, 2011). Reduced microbial diversity has even been linked to increased productivity under some

circumstances. For example, following the reduction of soil diversity through chloroform fumigation, some investigators found that added straw and ryegrass decomposition increased, while others noted increased thymidine and leucine incorporation (Degens, 1998; Griffiths *et al.*, 2000). Increased diversity has also negatively affected decomposition in cultured microbial assemblages when species that did not contribute substantially to this process were able to dominate (Jiang, 2007). This reverse relationship has also been observed in the context of bioremediation, as the chloroform fumigation of soil containing the pesticide 2,4-dichlorophenoxyacetic acid prior to inoculation with *Alcaligenes xylosoxidans* led to more than a 10-fold increase in contaminant reduction (Gunalan & Fournier, 1993). This means that the loss of specific inhibitory groups could actually reduce limitations on productivity. This mirrors well-studied relationships among macroorganisms, whereby the loss of key species that maintain ecological equilibrium can lead to the rapid depletion of resources (Beschta & Ripple, 2009), which is precisely the goal in bioremediation.

In this study, we used antibiotics targeting different portions of the microbial community to determine whether shifts in the relative abundance of taxa would affect the biodegradation of added diesel in a high Arctic soil. Although these antibiotics are not 100% effective in the inhibition of entire phylogenetic groups, antibiotic addition has been shown to predictably shift the bacterial composition of complex communities (Antonopoulos *et al.*, 2009; Robinson & Young, 2010), as opposed to methods such as serial dilution and chloroform fumigation which are less targeted. In this study, we used gentamicin and vancomycin to target mainly Gram-negative and Gram-positive bacteria,

respectively, as most of the identified hydrocarbon degraders in contaminated Arctic soils are members of the phyla *Proteobacteria* (Gram-negative) and *Actinobacteria* (Gram-positive) (Greer *et al.*, 2010; Bell *et al.*, 2011). We have previously observed that the composition of bacterial communities in Arctic soils following moderate diesel contamination is driven by soil parameters such as organic matter, as opposed to hydrocarbon-degrading efficiency (Bell *et al.*, In press). In the current study, we aimed to determine whether modifying the bacterial community within a single Arctic soil could affect rates of diesel biodegradation.

Materials and Methods

Soil collection and microcosm setup

The top 15 cm of an uncontaminated soil was collected from CFS-Alert, Nunavut. Details of the CFS-Alert site are provided in Bell *et al.* (2011). The soil had an average pH of 7.88, an organic matter content of $4.90 \pm 0.16\%$, and a starting water content of $12.20 \pm 0.35\%$. Bulk soil was contaminated to a final concentration of ~ 5500 mg/kg of ultra low sulphur diesel. This soil was then incubated for one week at 1°C to allow weathering of the most volatile components of the diesel fuel, as short-term evaporative weathering can occur within one week of contamination (Neff *et al.*, 2000). Homogenized soil was split in half, with one half receiving 250 mg/kg of monoammonium phosphate (MAP), which was identified previously as the optimal biostimulation treatment for hydrocarbon-contaminated soils at CFS-Alert (Greer, 2009). Four antibiotic treatments were then applied to each half of the treated soil, using

gentamicin and vancomycin (Table 1). Concentrations were determined through pilot experiments in which we determined the lowest dose required to produce sustained changes in community structure over 6 weeks (data not shown).

Vancomycin and gentamicin are bactericidal antibiotics (Shelburne *et al.*, 2004). Vancomycin is a glycopeptide antibiotic that inhibits the synthesis of peptidoglycan for bacterial cell walls, and primarily targets Gram-positive bacteria (Reynolds, 1989), while gentamicin is an aminoglycoside that acts mostly through protein synthesis inhibition, and is particularly effective against many Gram-negative bacteria (Jana & Deb, 2006). Both antibiotics are known to have some activity against both Gram-negative and Gram-positive bacteria, but we are unaware of any direct sensitivity of fungi to these compounds. Three 30 g replicates of each treatment were incubated at 10°C for 6 weeks in loosely-capped Falcon tubes. To ensure that soil water content did not limit microbial activity, 2.5 ml of sterile water was added equally to treatments after the first 3 weeks of incubation. The final average water content was $18.7 \pm 0.25\%$. At the end of the incubation, soil was used for DNA extraction and hydrocarbon analysis.

Quantification of hydrocarbons

Diesel was quantified from each sample replicate at the end of the incubation, as well as from three replicate baseline samples, which were taken from soil directly prior to the 10°C incubation. Procedures for analysis of diesel concentrations were adapted from the Canada-Wide Standard for Petroleum Hydrocarbons in Soil, as used previously for the determination of hydrocarbon concentrations in sub-Arctic soils (Chang *et al.*,

2011). Two grams of wet soil were extracted for each sample replicate using a 20 ml mixture of acetone:hexane (1:1 v/v). To determine extraction efficiency, 20 ppm of stock octacosane (C28) was added. Sonication was performed in a bath for 30 min, and soil particles then settled overnight. To remove remaining water, 5 ml of clear supernatant was mixed with 1 g of anhydrous Na₂SO₄. The supernatant was again removed and shaken with 0.1 g of grade 62 activated silica gel to purify the sample. Prior to GC analysis, 20 ppm of stock triacontane (C30) was added as an internal standard to each sample. Analysis of F2 (C10-C16) and F3 (C16-C34) hydrocarbon fractions was performed on a Hewlett Packard 6890 gas chromatograph connected to a flame ionization detector. An automatic sampler injected 1 µl of sample to a DB-1 capillary column (15m x 530 µm x 0.15 µm) from Agilent technologies (Santa Clara, CA, USA). Oven temperature was held at 35°C for 2 min, and was raised by 30°C min⁻¹ to 300°C, and then held for 5 min, with helium as the carrier gas. The injector was maintained at 35°C for 0.1 min, and was increased to 350°C at a rate of 500°C min⁻¹. The detector was maintained at 350°C during quantification. Quantification of F2 and F3 fractions was performed using a calibration curve prepared with decane (C10), hexadecane (C16), and tetratriacontane (C34). Percent F2+F3 degradation was calculated separately for each replicate as: 100- [(final F2+F3)/(baseline average F2+F3)*100]. The average baseline diesel concentration was determined to be 5545 ± 292 (standard error) mg/kg soil.

Soil DNA extraction, 16S rRNA amplification, and Ion Torrent sequencing

Total soil DNA was extracted from 10 g of soil from each of three replicates per treatment using the MoBio UltraClean® Mega Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). Partial 16S rRNA amplicons were produced from each extract with the universal bacterial primers E786 (5'-GATTAGATACCCTGGTAG-3') and U926 (5'-CCGTCAATTCCTTTTRAGTTT-3') (Baker *et al.*, 2003), and were each labeled with adaptor sequences and unique multiplex identifier codes (MID) for Ion Torrent sequencing. PCR reactions were performed using 12 µl of KAPA2G Robust DNA Polymerase mix (KapaBiosystems Inc., Boston, MA, USA), 12 µl of nuclease-free deionized water, and 40 pmol of each of the appropriate forward and reverse primers. PCR cycling consisted of an initial 5 min denaturing step at 95°C, 30 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, and a final elongation step of 7 min at 72°C. Gel purification of amplicons was performed using the illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Piscataway, NJ, USA), and DNA in the purified eluate was quantified using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Burlington, ON, Canada), pooled in an equimolar ratio, and diluted to a concentration of 5×10^7 molecules for sequencing. Sequencing was performed on an Ion Torrent Personal Genome Machine™ using the Ion Xpress™ Template Kit and the Ion 314™ chip following manufacturer's protocols. Ion Torrent sequencing produces microbial community profiles that are very similar to those produced by Roche 454 sequencing (Yergeau *et al.*, 2012).

qPCR

Quantitative real-time PCR (qPCR) was performed to determine relative 16S rRNA and fungal ITS/5.8s gene abundance. All qPCR reactions were performed in 20- μ l volumes using the SYBR green QuantiTect PCR mix (Qiagen, Mississauga, Ontario, Canada) in a Rotor-Gene 3000 apparatus (Corbett Life Science, Sydney, New South Wales, Australia). We used the primer sets described in Fierer *et al.* (2005) for quantifying total bacteria (Eub338/Eub518) and fungal (5.8s/ITS1f) populations. Conditions were as described in Yergeau *et al.* (2010). Standards were made from 10-fold dilutions of linearized plasmids containing the gene fragment of interest that was cloned from amplified pure culture DNA (16S rRNA, $R^2 = 0.999$, efficiency=97%; fungal ITS/5.8s, $R^2 = 0.990$, efficiency=82%). Tests with Lambda phage DNA as in Yergeau *et al.* (2009) showed little to no qPCR inhibition, so values were not adjusted (data not shown).

Sequence and statistical analysis

Sequences were binned by multiplex identifier (MID) code, and MID codes were trimmed from each sequence. Filtering of sequences was performed using a moving average Q15 cutoff; if the average of 5 bases along the sequence fell below Q15, the sequence was trimmed at that point. Reads of less than 100 bp were removed from analysis. Taxonomic identities were assigned to sequences using the RDP Pipeline Classifier (<http://pyro.cme.msu.edu/>). The relative abundance of bacterial families within each treatment was determined, and these were ranked across all treatments, and the thirty taxa with the highest abundance values were selected. The abundance of each family was normalized to the maximum abundance for that group across samples,

and these were visualized using a heat map created in R with the 'gclus' and 'vegan' packages.

The relationship between samples was determined by mapping sequences against the Green Genes core dataset using Fast UniFrac (Hamady *et al.*, 2010), and the resulting distance matrix was visualized using PCoA analysis in R. Processing of OTUs was performed mostly in MOTHUR (Schloss *et al.*, 2009) after the number of sequences was standardized between samples by eliminating sequences with random numbers as in Fortunato *et al.* (2012). Sequences were aligned to the Green Genes core set with 'align.seqs', and using the following parameters: ksize=9, align=needleman, gapopen=-1. Sequences were filtered using 'filter.seqs', and putatively noisy sequences were removed using the 'pre.cluster' algorithm that is based on the procedure by Huse *et al.* (2010), and potential chimeras were removed with 'chimera.uchime' and 'remove.seqs'. A distance matrix was created using 'dist.seqs', and average neighbor clustering was done using 'cluster.split' and setting method=average. Shannon Diversity values for each replicate were obtained using 'summary.single'.

One-way ANOVA was used to statistically test for differences between treatment means in hydrocarbon biodegradation, qPCR results, and the relative abundance of major taxonomic groups. Differences between means were then assessed using Tukey's HSD test with a significance cutoff of $P=0.05$. Bacterial 16S rRNA and fungal ITS/5.8s gene abundance, as well as bacterial:fungal gene quantification ratios were log transformed prior to ANOVA, since values spanned several orders of magnitude. All ANOVAs were performed in JMP 8.0 (SAS Institute, Cary, NC).

Sequences were deposited...

Results

Diesel biodegradation

The amount of diesel degraded during the 6-week incubation varied between treatments (One-way ANOVA; $P=0.0083$), and was significantly higher ($P<0.05$) in the GVu treatment than in the NAm and VAm treatments (Figure 1). Diesel loss in GVu was almost significantly higher than what was observed in the GEm ($P=0.071$), VAm ($P=0.075$), and NAm ($P=0.080$) treatments. Excluding GVu and GEm, MAP addition led to increased diesel biodegradation overall, although this difference was not quite significant (paired Student's t-test; $P=0.086$).

qPCR

Bacterial 16S rRNA gene abundance varied significantly between treatments (One-way ANOVA; $P < 0.0001$). The addition of gentamicin led to copy numbers that were approximately two orders of magnitude lower than were quantified in other treatments, and there were no obvious differences between soils with and without MAP added (Figure 2a). Fungal ITS/5.8s gene abundance also varied between treatments (One-way ANOVA; $P < 0.0001$). Similar to 16S rRNA quantification, gene copies were lowest in the GEm and GVu treatments, although MAP addition did increase fungal populations in the GEm and GEm treatments. MAP addition reliably increased fungal copy numbers regardless of antibiotic treatment, and gene copies were highest in VAm

(Figure 2b). The ratio of 16S rRNA to fungal ITS/5.8s copies differed between treatments (One-way ANOVA; $P < 0.0001$), and was lowest in the presence of both antibiotics and MAP, irrespective of antibiotic treatment (Figure 2c).

16S rRNA diversity and composition

After filtering, we obtained a total of 138 968 usable reads, with an average of 5147 reads per replicate. A one-way ANOVA of Shannon Diversity values was significant ($P=0.046$), although no pairwise differences were observed using Tukey's HSD test. Comparison of all the soils that were not treated with MAP did show a significant difference between VAu and GEu ($P<0.05$). Nevertheless, there was a general decline in species diversity in all treatments relative to the baseline soil with the exception of the VAu treatment (Table 2). Mean OTU diversity was not significantly correlated with total hydrocarbon biodegradation, although the general trend showed increased biodegradation with decreased diversity (linear regression; $R = -0.478$, $P=0.23$). Fast UniFrac analysis showed that OTU composition separated primarily by whether or not gentamicin was present (Figure 3). Samples separated on the secondary axis based on the specific antibiotic treatment (with the exception of the baseline group which clustered with VAu), and then by the presence or absence of MAP. Interestingly, VAu grouped very closely with BAu, which is in line with the high diversity that was observed in this treatment.

Comparison of the thirty most abundant groups at the family level showed differences between all treatments (Figure 3). The grouping of treatments based on the

relative abundance of these families matched the pattern of the Fast UniFrac analysis, with the exception of the baseline group which formed an outgroup in this analysis. Following diesel contamination, many groups within the *Bacteroidetes* and *Firmicutes* declined. The most striking shift within the contaminated soils was that *Dietziaceae* and *Nocardiaceae*, two of the most abundant groups in the no antibiotic treatments, were undetectable when antibiotics were added. Sequences related to the *Micrococcineae* suborder of *Actinobacteria* were highly represented in all soils treated with gentamicin. Unclassified *Actinomycetales* were also prevalent in these soils, and over 75% of these sequences had a best match of *Micrococcineae* that fell below the 0.50 classification cutoff. All soils receiving gentamicin were also characterized by a higher relative abundance of most of the major families of *Alphaproteobacteria*, with the exception of the *Caulobacteraceae* which were more abundant in the vancomycin-treated and no antibiotic soils. *Xanthomonadaceae* were more abundant in VAu, VAm, GVu and GVm than in the other treatments.

The ratio of Gram-negative to Gram-positive sequences varied by treatment ($p=0.0067$). Compared with the no antibiotic treatment, there was no significant difference in the ratio of Gram-negative to Gram-positive sequences when vancomycin was added, while the addition of gentamicin alone led to a significant decline in the proportion of Gram-negative sequences (Figure 4a). The addition of MAP led to a decreased Gram-negative to Gram-positive ratio in the no antibiotic treatment, but increases in both the vancomycin and gentamicin treatments, although these differences were not significant.

The most abundant *Gammaproteobacteria* and *Actinobacteria* classifications were compared at the family level, and on average constituted over 50% of the sequences in all soils treated with gentamicin, as well as in the NAM treatment (Figure 4b). As mentioned earlier, the *Nocardiaceae* and *Dietziaceae* were two of the most abundant families following contamination in the absence of antibiotics, particularly following the addition of MAP, but were undetectable when antibiotics were present. ANOVA comparisons of the contaminated samples showed a significant difference between treatments in the abundance of *Xanthomonadaceae* ($p = 0.0366$), but treatments could not be separated by a Tukey's HSD test. There were also differences in the abundance of all unclassified *Micrococceae* and *Actinomycetales* sequences ($p < 0.001$), with a significant difference between soils receiving gentamicin and all other treatments.

Discussion

In this study, we used diesel to contaminate a low organic matter soil from the Canadian high Arctic, and observed that all antibiotic treatments altered soil microbial composition. In one treatment, the extent of diesel biodegradation was significantly higher than in the no antibiotic control following a 6-week incubation. Interestingly, total degradation was not tied to estimates of fungal and bacterial biomass, suggesting that many microorganisms may not contribute substantially to hydrocarbon metabolism under standard bioremediation conditions. While inhibition via antibiotics did not increase bioremediation in all cases, these results indicate that microbial community

composition likely does affect the efficiency of diesel biodegradation, and that the community that forms naturally in a given soil following diesel contamination may be suboptimal for bioremediation purposes.

Microbial population size and biodegradation

Diesel degradation in GVu was significantly higher than that observed in NAu, and nearly significantly higher than NAM, even though the number of 16S rRNA gene copies in GVu was roughly two orders of magnitude lower. This result was surprising, but could indicate that a large percentage of the microbial population is not actively involved in biodegradation, as has been observed in a diesel-degrading mixed culture (Hesselsoe *et al.*, 2008). Previous studies conducted at CFS-Alert showed that heterotrophic colony-forming units varied by more than an order of magnitude in hydrocarbon-contaminated biopiles, with no observed difference in ¹⁴C-hexadecane mineralization (Greer, 2009). An increased carbon mineralization to biomass ratio in metal contaminated soils has been assumed to be related to increased energy expenditure associated with heavy metal tolerance (Nakatsu *et al.*, 2005), and tolerance to antibiotic stress may have similarly affected the antibiotic-treated soils, as antibiotic resistance is known to exist in the Arctic (Allen *et al.*, 2010; D'Costa *et al.*, 2011). Even cultured isolates that are grown without added stressors can vary substantially in terms of the amount of carbon that they divert to CO₂, biomass, and metabolites during hydrocarbon biodegradation (Bouchez *et al.*, 1996), so changes to the community composition alone could be responsible for these trends.

It was also interesting to note that fungal abundance declined in gentamicin-treated soils. This may have been due to a direct toxic effect of gentamicin on fungi, or to a decline in bacterial groups that promote fungal growth.

Fungal response to MAP

Little is known about how fungi respond to biostimulation in polar regions (Hughes & Bridge, 2009). In temperate soils, however, addition of mixed nutrient compost and NPK fertilizer to hydrocarbon-contaminated soils led to increases in fungal CFU counts and PLFA quantification, respectively (Margesin *et al.*, 2007; Li *et al.*, 2012). We observed that MAP addition significantly increased fungal abundance under all antibiotic treatments and in the absence of antibiotics, despite having only minimal effects on bacterial abundance. Although not statistically significant, we observed increases in hydrocarbon biodegradation in MAP-treated soils that were concurrent with fungal population increases (excluding GVu and GVm), but this does not necessarily indicate that fungi are efficient hydrocarbon degraders in these soils. For example, selective inhibition of soil bacteria and fungi using streptomycin and cycloheximide showed that bacteria were responsible for 82% of hexadecane mineralization, while fungi were responsible for only 13%, while both groups contributed equally to consumption of added glucose (Song *et al.*, 1986). Substantial and direct involvement of Arctic fungi in the bioremediation of hydrocarbon-contaminated soils remains to be demonstrated, as carbon from soil organic matter and bacterial biomass and metabolites may also supplement fungal metabolism. Nevertheless, fungi are known to

indirectly assist hydrocarbon biodegradation in some cases, as bacterial dispersal in unsaturated soil matrices can be greatly facilitated by the presence of fungal mycelia (Furuno *et al.*, 2010).

Bacterial diversity

Shifts in bacterial diversity were not significant due to high variation within some treatments, although diversity generally declined across treatments relative to the baseline soil, with the exception of the VAu treatment. Vancomycin actually appeared to promote diversity relative to the contaminated soils in the absence of antibiotics, and VAu also grouped very closely with the initial baseline soil in the UniFrac analysis. This may be due to vancomycin limiting fast-growing groups following the addition of diesel and nutrient substrates. Gram-positive *Actinobacteria* are specifically known to respond quickly to nutrient inputs in a variety of soils (Ramirez *et al.*, 2012), and both the *Dietziaceae* and *Nocardiaceae* were promoted in the NAu and NAm treatments, but inhibited by all antibiotic treatments. Diversity was lowest in soils treated with gentamicin, likely due to higher toxicity at the concentration added, or a wider range of bacterial targets. There was a negative but insignificant correlation between diversity and hydrocarbon biodegradation, suggesting that altering the abundance and relationships of specific microbial taxa may be more important than non-specifically modifying diversity.

Distinct bacterial community composition across treatments

Bacterial community composition varied between all treatments, although addition of antibiotics appeared to be more important than addition of MAP. Other studies of contaminated soils at CFS-Alert have identified *Pseudomonas* and *Rhodococcus* as dominant, and potentially important, hydrocarbon-degrading genera (Whyte *et al.*, 2002; Yergeau *et al.*, 2012). In this study, we chose to compare the relative abundance of bacterial families rather than genera, since the number of sequences that classified to the genus level varied widely between taxonomic groups. Nevertheless, we found that *Actinobacteria* such as *Nocardiaceae*, the family to which *Rhodococcus* belongs, and the closely related *Dietziaceae*, dominated NAm and NAm soils, as did the *Gammaproteobacteria* family *Xanthomonadaceae*, which is closely related to the *Pseudomonadaceae*. Although the *Nocardiaceae* and *Dietziaceae* are Gram-positive, the fact that they were suppressed under all antibiotic treatments is not surprising. *Dietzia* are highly sensitive to a variety of antimicrobials (Pilares *et al.*, 2010), as are *Rhodococcus* and other closely related genera, particularly to gentamicin and vancomycin (Soriano *et al.*, 1995). In the absence of these groups, diesel biodegradation was equal if not greater, demonstrating that a variety of effective diesel degraders were present in this soil. Both *Pseudomonas* and *Xanthomonas* have been shown to possess some gentamicin resistance (Heuer *et al.*, 2002), yet *Xanthomonadaceae* declined in the GEu and GEm treatments. This group remained dominant in the GVu and GVm treatments, however, possibly due to inhibition of competing taxa by vancomycin that were resistant to gentamicin alone.

The bacterial communities in the soils treated with both gentamicin and vancomycin were quite similar to a mixed enrichment culture isolated from an oil-field in China that degraded more than 50% of 10 000 mg l⁻¹ crude oil in 7 days. The enrichment consisted of 7 genera including *Pseudomonas*, *Microbacterium* (*Micrococcineae*), and 3 genera of *Alphaproteobacteria*, two of which classify as *Rhizobiales* (Zhao *et al.*, 2011). All of these groups are closely related to those that increased in relative abundance in the gentamicin + vancomycin treatments, especially GVu, while many other taxa declined in abundance. Nevertheless, there appears to be an important difference in the diesel-degrading capacity of these two communities. Aside from a higher abundance of certain groups of *Alphaproteobacteria* in GVu, there was a much larger fungal population in GVm, which may have led to negative competitive interactions. Several bacterial isolates from polar regions have exhibited substantial antifungal activity (Shekh *et al.*, 2011), while mesophilic strains of *Xanthomonas*, *Pseudomonas*, and *Microbacterium* are also known to produce fungistatic compounds (Radtke *et al.*, 1994; Cavaglieri *et al.*, 2005; Weise *et al.*, 2012). While *Xanthomonadaceae* are known hydrocarbon degraders, they have also been shown to obtain carbon from co-occurring microorganisms (Lueders *et al.*, 2006), so a reduction in co-occurring taxa such as fungi may promote their use of hydrocarbon contaminants as a carbon source.

Conclusions

Most current approaches to the bioremediation of hydrocarbon-contaminated soils involve blanket treatments that aim to increase the biomass and activity of whole microbial communities. We have shown, however, that shifts in microbial composition can affect biodegradation within a single Arctic soil. This suggests that the most effective hydrocarbon-degrading communities are not always promoted naturally, or following treatments such as biostimulation. Future work should focus on determining the functional potential of the many microbial taxa that are active in hydrocarbon-contaminated Arctic soils, and on safe treatments that promote key groups that do not naturally dominate *in situ* communities.

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Table 1. Treatments and corresponding abbreviations for unamended (u) and monoammonium phosphate-treated (m) soils.

Antibiotic	Concentration (mg/g soil)	MAP added	Abbreviation
Baseline (untreated soil)	n/a	n/a	BAu
None	n/a	-	NAu
	n/a	+	NAm
Gentamicin	2.0	-	GEu

	2.0	+	GEm
Vancomycin	0.8	-	VAu
	0.8	+	VAm
Gentamicin + vancomycin	2.0 / 0.8	-	GVu
	2.0 / 0.8	+	GVm

Table 2. Average Shannon diversity values for each treatment with standard deviations.

Treatment	Shannon diversity (n=3)
Baseline	4.91 ± 0.02
No antibiotics	4.65 ± 0.53
No antibiotics (MAP)	4.63 ± 0.92
Gentamicin	4.07 ± 0.46
Gentamicin (MAP)	4.10 ± 0.39
Vancomycin	5.05 ± 0.02
Vancomycin (MAP)	4.80 ± 0.28
Gent + Vanc	4.27 ± 0.09
Gent + Vanc (MAP)	4.21 ± 0.09

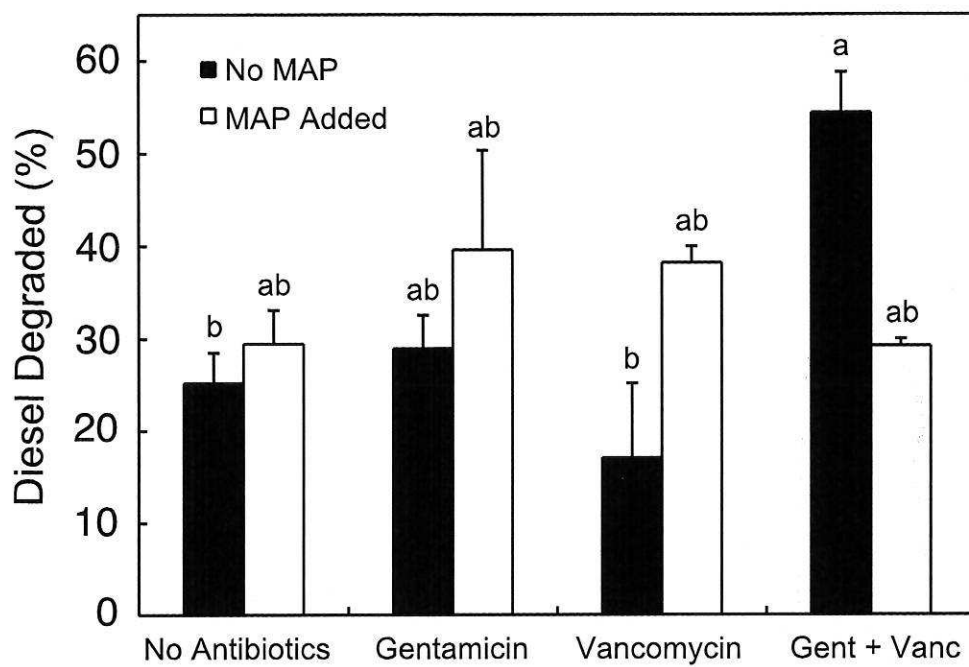
Figure 1. Diesel degraded (%) in soils over the 6-week incubation period following treatment with various antibiotic treatments, and with or without monoammonium phosphate (MAP) added. Error bars represent standard error (n=3). Different letters above columns indicate that means were significantly different as determined by Tukey's HSD test (P<0.05) following one-way ANOVA.

Figure 2. Gene quantification from qPCR assays of a) bacterial 16S rRNA, and b) fungal ITS/5.8s genes. Also shown is c) the ratio of 16S rRNA and fungal ITS/5.8s genes. Error bars represent standard error (n=3). Different letters above columns indicate that means

were significantly different as determined by Tukey's HSD test ($P < 0.05$) following one-way ANOVA.

Figure 3. Bacterial community composition as assessed by Ion Torrent sequencing of 16S rRNA amplicons. Samples were separated through principal component analysis using a distance matrix created in Fast UniFrac (upper panel). A heat map demonstrates variation in the relative abundance of the thirty most commonly identified taxa classified to the family level, with each taxa normalized to the maximum abundance for all treatments (lower panel). The majority of unclassified *Actinomycetales* had a best match of *Micrococcaceae*, so both of these groups are represented in bold.

Figure 4. The main effects of antibiotics on microbial community composition are shown by a) changes in the Gram-negative/Gram-positive sequence ratio, and b) the unnormalized abundance of the main groups of *Actinobacteria* and *Gammaproteobacteria* identified across soils. Error bars represent standard error ($n=3$). Different letters above columns indicate that means were significantly different as determined by Tukey's HSD test ($P < 0.05$) following one-way ANOVA.



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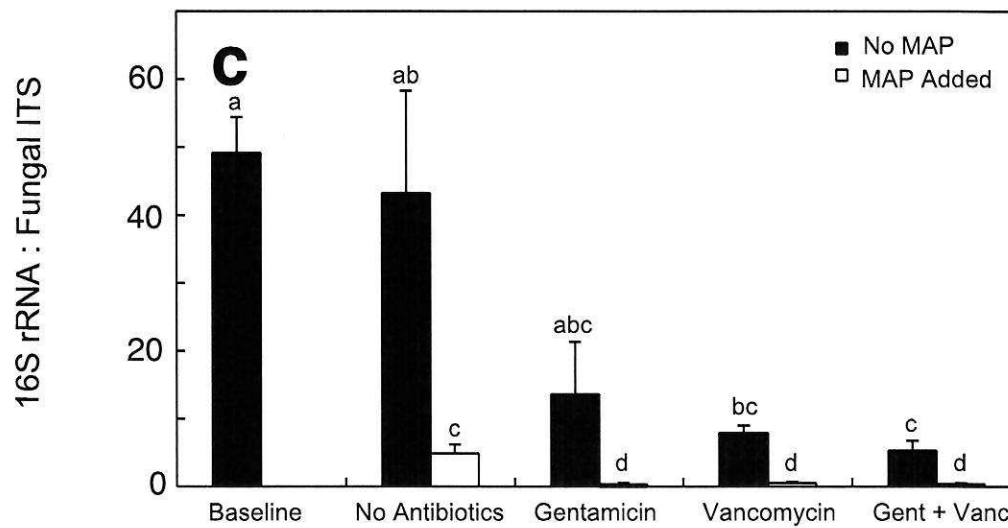
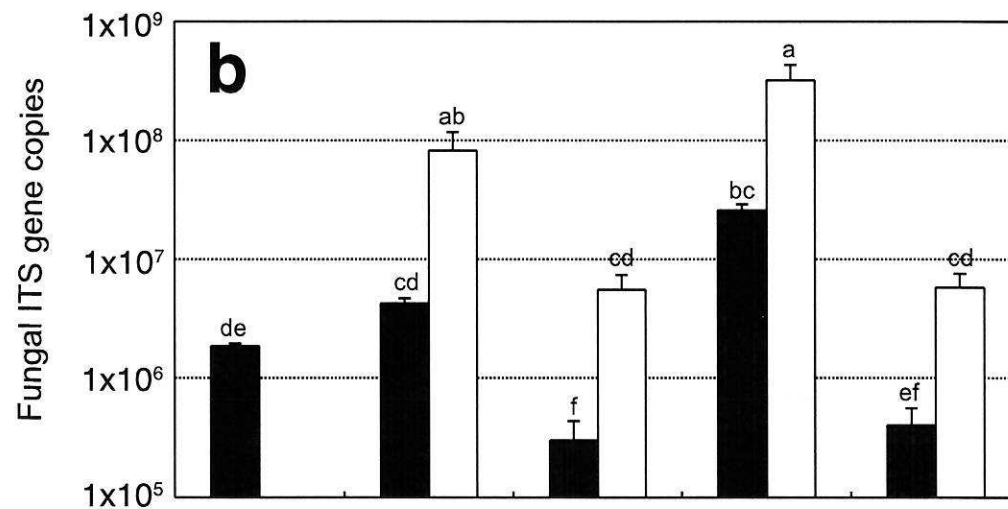
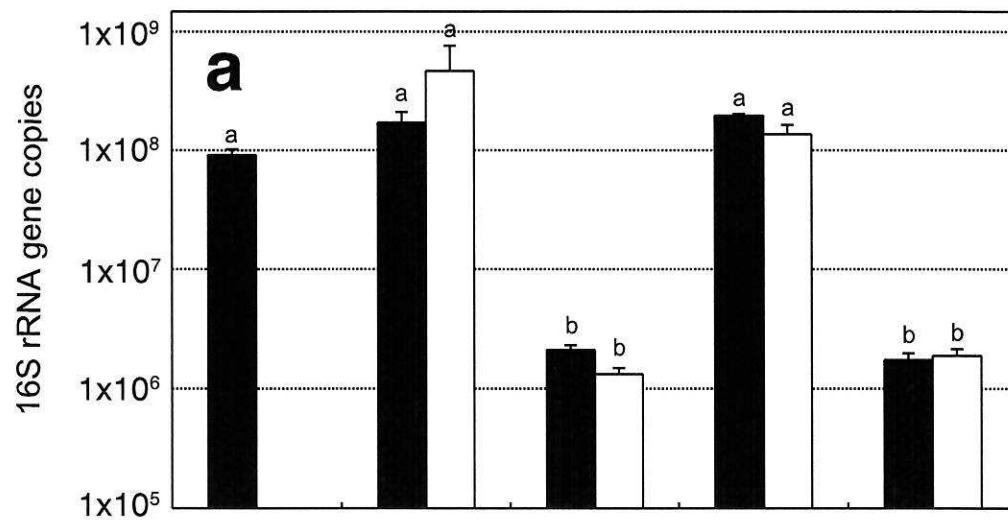
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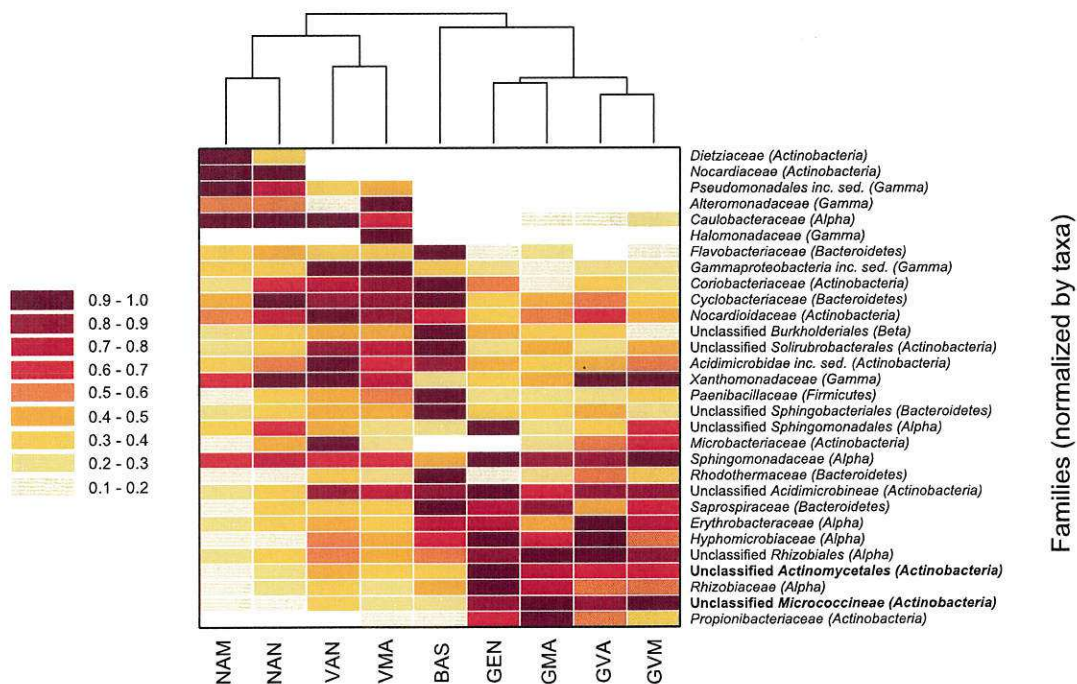
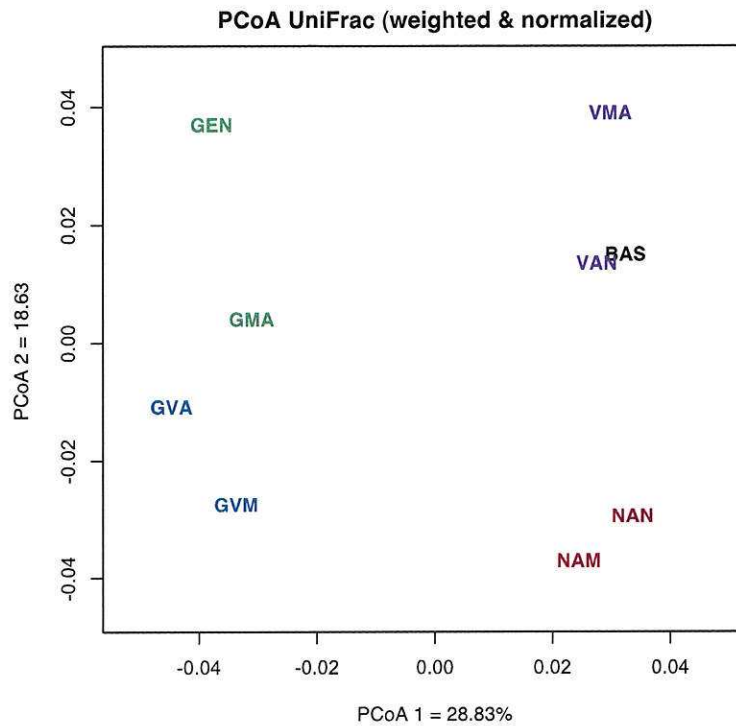
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