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Utility of lipid biomarkers in support of bioremediation efforts at army sites

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Abstract

Lipid biomarker analysis has proven valuable in testing the hypothesis that attributes of the extant microbiota can directly reflect the occurrence of contaminant biodegradation. Two past research efforts have demonstrated this utility and are described here.

A 4.5 m vertical core was obtained from a diesel fuel oil contamination plume. Core material was assayed for total petroleum hydrocarbons (TPH) and bacterial membrane phospholipids (PLFA) via a single solvent extraction. Microbial viable biomass and the relative abundance of Gram-negative bacterial PLFA biomarkers were found to be significantly correlated with TPH concentration. The core TPH profile also revealed two distinct areas where the average TPH level of 3000 $\mu g\ g^{-1}$ fell to near detection limits. Both areas were characterized by a three-fold decrease in the hexadecane/pristane ratio, indicating alkane biodegradation, and a distinct PLFA profile that showed a close similarity to the uncontaminated surface soil.

Low-order, incomplete detonations can deposit hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) into training range surface soils. Since surface soils are exposed to temporal and diurnal moisture cycles, we investigated the effect two very different soil moisture tensions had on the in situ microbiota and RDX biodegradation. Saturated soils were characterized by rapid RDX biodegradation, 4 day half-life, a decrease in number of species detected and increase in PLFA biomarkers for Gram-negative proteobacteria (n16:1ω7c, n18:1ω9c, and n18:1ω7c) and Gram-positive firmicutes (i15:0 and a15:0). Terminal restriction fragment length polymorphism (T-RFLP) profiles of endpoint microbial communities indicated a shift from 18 to 36% firmicutes, the loss of gamma-proteobacteria and the emergence of alpha-proteobacteria.

These two past research efforts demonstrated the utility of the lipid biomarker analysis in identifying microbial community characteristics that were associated with two very different soil contaminants. Lipid biomarkers defined areas of TPH biodegradation and identified community shifts as a result of soil conditions that affected explosives fate. Information like this can be used to enhance the predictive power of ecological models such as the Army Training and Testing Area Carrying Capacity for munitions model [ATTACC].
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Keywords: Lipid biomarkers; PLFA; TPH; RDX; Contaminated soil

1. Introduction

> "The microbial community contains a record of the sum microbial response to the environment, which is written from changes in the biochemistry of the extant organisms" (David C. White).

Contaminant effects on the in-situ microbiota are generally continuous and the changes induced in community structure and activity can become integrated over long periods of time (Smith et al., 1986). For example, a contaminant can trigger the loss or emergence of a particular genera or species of microorganism or trigger a specific catabolic capacity within a single species (Ringelberg et al., 2001). Consequently, it is hypothesized that the in-situ microbiota contains a signature of past and present contaminant exposure and utilization. A comprehensive measure of the in-situ microbial ecology of a site will yield the identity of such a signature.

Currently, there is no universally accepted protocol for monitoring site microbial ecology. Culture and isolation techniques can provide valuable information regarding organism presence and potential activity, but it is now known that organisms in culture poorly reflect in-situ diversity and activity levels (American Academy of Microbiology, 2002). Molecular

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based assays are now recognized as a non-biased and accurate means of determining in-situ microbial community structure, diversity and activity. With a combination of molecular assays, it is now possible to obtain a comprehensive and quantitative definition of the in-situ microbial ecology of a site.

Here, we report findings from two studies where the in-situ microbiology surrounding total petroleum hydrocarbons (TPH) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) biodegradation was described using "whole-sample" characterization techniques or the direct assay of microbes in situ. The area selected for the TPH investigation was used as a fuel dispensing facility from late 1940 through early 1990. The subsurface had a mean hydraulic conductivity of 1.98×10^{-2} cm/s and this level of permeability raised concern over the transport of the contamination plume into underlying groundwater (approximately 5 m below the surface). Since nearby drinking water supplies were in close proximity, environmental health issues were paramount and, as a result, remedial actions were initiated. As part of the remedial actions, this study focused on examining the subsurface microbial ecology and the relationships that existed between the extant microbiota and the contamination. The intent of the study was to identify community attributes that were associated with the *in situ* biodegradation of the petroleum hydrocarbons.

In the other study, we monitored shifts in microbial community composition resulting from two different soil water potentials in the presence of added RDX. Our previous work had demonstrated that RDX biodegradation proceeded at a rate seven times faster under water saturated versus an unsaturated condition. Here we show that the increase in the rate of RDX biodegradation coincided with a big shift in microbial community composition. The working hypothesis is that soil moisture content imparts a major influence on soil microbial community composition that, in turn, can directly or indirectly affect RDX biodegradation. PLFA and TRFLP analyses were used to identify the nature of the community shift.

> "A definition of natural attenuation in terms of a site's microbial ecology is defensible, whereas a definition in terms of contaminant chemistry and/or site geochemistry or hydrology is only circumstantial" (David C. White).

2. Materials and methods

2.1. TPH Study

2.1.1. Sample material

For the TPH study, subsurface material was collected from Fort Drum, NY, USA in three 5 ft × 3 in. ID split-barrel samplers fitted with a butyrate liner and driven with a 140 lb hammer dropped from 30 in (Talley et al., 2004). Sample material was mostly homogenous and comprised primarily of medium sands with some fines. All sampling materials were steam sterilized prior to use. Recovered cores were labeled, capped, sealed in wax and transported to laboratory at 5 °C in a refrigerated truck. One gram samples of material were collected over the entire length of the core.

2.1.2. Analyses

Sample total petroleum hydrocarbons (TPH) and PLFA were co-recovered by extracting 1 g of material in 3.5 ml of methylene chloride, methanol and aqueous phosphate buffer. The soil solvent mixture was subjected to 2 min. of sonication and extracted for a period of 3 h. at room temperature. Additional aliquots of methylene chloride and water (1 ml each) were then added to the mixture, shaken vigorously, and allowed to separate overnight. The organic phase was recovered and passed through a silica gel column (0.5 g). The column was eluted sequentially with 5 ml methylene chloride, 5 ml acetone and 5 ml methanol, collecting each eluent.

One µl of the methylene chloride eluent was injected on a HP5890 gas chromatograph equipped with a SPB-5 capillary column (60 m, .32 mm ID, .25 µm film) programmed from 50 °C (2 min) to 310 °C (3 min) at 4 °C/min for total TPH quantification. A splitless injection of 1 min was used at a purge of 80 ml/min. The injector was maintained at 250 °C and the flame ionization detector at 320 °C. Equal response was assumed for all peaks eluting between the retention times of 10 and 50 min. An internal standard calculation was applied to peak areas with nonadecanoic acid methyl ester at 50 pmole μl^{-1} serving as the internal standard.

PLFA were then recovered from the methanol eluent as described in White and Ringelberg (1998). PLFA were first trans-esterified in mild-alkaline methanol to obtain fatty acid methyl esters and then separated and quantified via capillary gas chromatography — mass spectrometry. Mass spectra were collected at 70 eV using positive electron impact ionization. An internal standard calculation was applied to peak areas with nonadecanoic acid methyl ester at 50 pmole μl^{-1} serving as the internal standard. Each PLFA profile represented a fingerprint of the extant microbial community with the sum total of all detected PLFA representing the total in situ viable microbial biomass (Balkwill et al., 1988).

2.2. RDX study

2.2.1. Sample material

For the RDX study, surface soil was collected by hand from Ft. Greely, Alaska and shipped to the laboratory at ambient temperature. The material was sieved (4 mm) and air dried for storage. Dry soils (5 g each) were pre-incubated in 40-mL vials in 1 ml H_2O (Milli-Q grade) at room temperature (21 °C ± 2 °C) for 48 h to acclimate the system prior to RDX addition. Individual microcosms were then augmented with 2 or 25 ml aqueous RDX to a final concentration of 10 mg RDX L^{-1} (unsaturated and saturated, respectively). Control microcosms were established by autoclaving (120 °C for 20 min) replicate microcosms three consecutive times, with an 8-hour resting period between sterilizations. The saturated microcosms also received 100 µL of a 0.1% resazurin solution as a redox indicator. Following the RDX addition, microcosms were incubated for a total of 840 h in the dark without agitation at room temperature (21 °C ± 2 °C). Three replicate vials were sacrificed at 0, 48, 168, 504, and 840 h to obtain microbiological and contaminant chemistry data.

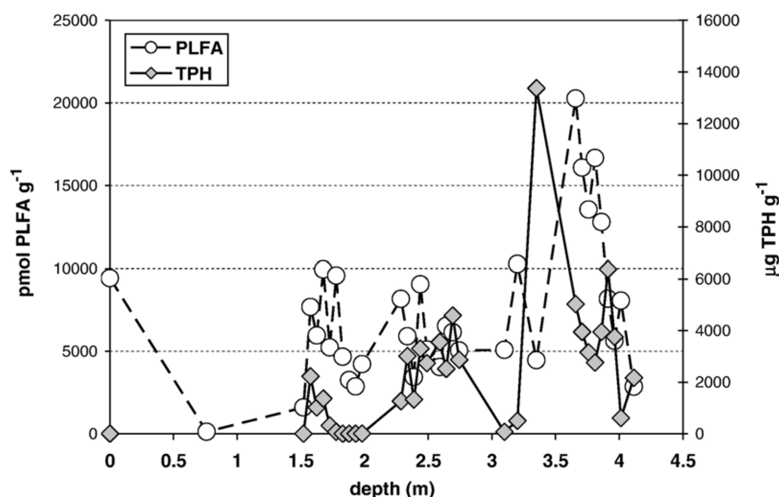

Fig. 1. TPH concentration and PLFA abundance are plotted against depth for the Fort Drum subsurface core materials. Microbial biomass (PLFA) correlated with the hydrocarbon (TPH) contamination.

2.2.2. Analyses

Total extractable RDX was recovered as described in Ringelberg et al. (2003). Briefly, solids were extracted in 10 ml acetonitrile (ACN) with sonication for 18 h at 20 °C. Liquids were assayed directly following centrifugation. ACN extracts were diluted with water and water extracts diluted with ACN, then both were filtered (0.45 µm Milipore Millex-FH, Bedford, MA) prior to analysis by HPLC. RDX was quantified on a 15-cm by 3.9-cm (4-µm) Nova Pak C8 (Waters Millipore) column by elution in 1.5 ml min^{-1} of 15:85 isopropanol:H_2O (v:v). Absorbance at 254 nm was recorded.

Microbial populations were fingerprinted via PLFA and by the terminal restriction fragment length polymorphism (T-RFLP) assay (Gruntzig et al., 2002). For PLFA, approximately 2 g of microcosm solids were extracted in 7.6 ml of chlorofrom:methanol:phosphate buffer (1:2:0.8, v:v:v) and processed as described in White and Ringelberg (1998).

For the T-RFLP assay, approximately 1 g of microcosm solid was extracted for total genomic DNA using the MoBio UltraClean DNA isolation kit (MoBio Laboratories, Carlsbad, CA). A portion of the 16S gene was then amplified via PCR using primers 8F and 926R. Following purification, the amplicon was

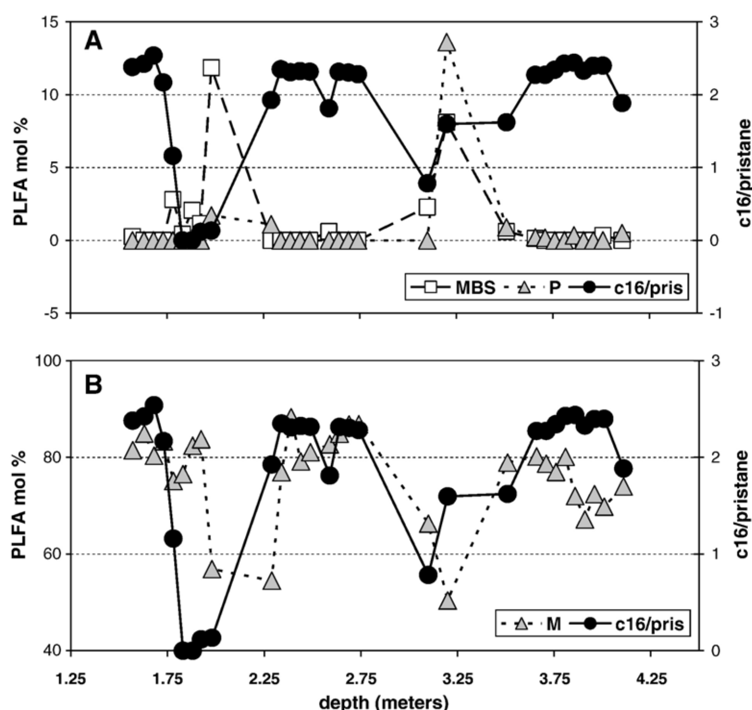

Fig. 2. The TPH biodegradation ratio, hexadecane (C16) to pristane, is plotted against depth for the Fort Drum subsurface core materials. Co-plotted in panel A are the mole percentages of PLFA biomarkers for actinomycetes, mid-chain methyl branched saturates (MBS) and for micro-eukaryotes, polyunsaturates (P), and in panel B the PLFA biomarkers for Gram-negative bacteria, monounsaturates (MONO).

digested using the Hha I, Msp I and Rsa I endonucleases (20 U each at 37 °C for 3 h.). The digested fragments were then separated and sized via capillary electrophoresis. Fragment sizes were compared to those contained in the RDP-II database (Ribosomal Database Project, Michigan State University) using the FragSorter program (Ohio State University). The goal was to taxonomically assign the individual fragments (and associated abundance).

3. Results and discussion

3.1. In situ characterization of TPH biodegradation

3.1.1. Contaminant chemistry

TPH concentration vs. depth is plotted for each point sampled along the continuous core (Fig. 1). The highest TPH concentration, 13,000 μg g^{-1}, was detected at a depth of 3 m. One gram of soil was found to contain sufficient amounts of contaminant and microbial cells for analysis. TPH recovery by the method described above was found comparable to EPA methods 3550 and 8015. In addition, A few 10 g core samples were extracted to assure that the 1 g extractions were not influenced by spatial heterogeneity. Results from the 1 and 10 g extractions were within 1 standard deviation of each other.

Work coming out of the Exxon Valdez oil spill in Prudhoe Bay, Alaska indicated that chemical signatures could be used to identify the occurrence of microbial biodegradation of crude oil (Prichard and Cosca, 1991). Other researchers have also found that a decrease in the ratio of n-alkanes over branched acyclic isoprenoids coincides with biodegradation activity (McIntyre et al., 2007). Two areas along the continuous core showed decreases in the ratio of hexadecane to pristane, 1.7 to 2.2 m and

Table 1
Contaminant chemistry, microbial biomass, community composition and K-means assignments from Fort Drum subsurface core material taken at each depth sampled

Depth (m)	Contaminant chemistry		Microbial biomass	Microbial community composition					K-means association[a]		
	TPH (μg g^{-1})	Hexadecane/pristane	PLFA (pmole g^{-1})	Trans/cis[b]	TBS[c]	MONO[d]	MBS[e]	POLY[f]	(Group #)		
				(Mole percentage)					#1	#2	#3
0	4	–	9425	0.08	12.6	53.1	12.9	2.9			X
0.76	0	–	128	0.00	3.1	39.5	3.2	13.6			X
1.52	3	–	1597	0.00	18.2	55.0	6.1	2.0			X
1.57	2229	2.4	7676	0.24	9.1	81.5	0.3	0.0		X	
1.63	1022	2.4	5955	0.24	5.9	84.9	0.0	0.0		X	
1.68	1367	2.5	9938	0.21	10.1	80.3	0.0	0.0		X	
1.73	326	2.2	5233	0.19	8.4	83.3	0.0	0.0		X	
1.78	79	1.2	9575	0.09	13.4	75.1	2.8	0.0			X
1.83	1	0.0	4662	0.05	15.0	76.5	0.4	0.0			X
1.88	1	–	3254	0.04	7.7	82.4	2.1	0.0			X
1.93	7	0.1	2872	0.23	6.1	83.7	1.1	0.0			X
1.98	13	0.1	4213	0.09	9.2	56.9	11.9	1.7			X
2.29	1268	1.9	8171	0.34	13.0	54.5	0.0	1.1		X	
2.34	3010	2.3	5903	0.25	7.6	76.9	0.0	0.0		X	
2.39	1332	2.3	3432	0.23	4.5	88.3	0.0	0.0		X	
2.44	3303	2.3	9053	0.34	9.5	79.2	0.0	0.0		X	
2.49	2738	2.3	5098	0.23	8.1	81.1	0.0	0.0		X	
2.59	3556	1.8	4039	0.33	6.3	82.7	0.6	0.0		X	
2.64	2520	2.3	6530	0.28	5.3	84.9	0.0	0.0		X	
2.69	4584	2.3	6148	0.32	4.0	86.8	0.0	0.0		X	
2.74	2868	2.3	5040	0.36	4.1	86.8	0.0	0.0		X	
3.10	76	0.8	5083	0.11	14.1	66.3	2.3	0.0			X
3.20	507	1.6	10,259	0.13	8.1	50.4	8.1	13.6			X
3.35	13,369	1.6	4457	0.26	4.2	78.9	0.6	0.9	X		
3.66	5022	2.3	20,272	0.31	4.1	80.1	0.1	0.2	X		
3.71	3947	2.3	16,087	0.22	4.6	78.7	0.0	0.2	X		
3.76	3155	2.3	13,547	0.15	2.1	77.0	0.0	0.0	X		
3.81	2762	2.4	16,670	0.16	1.2	80.1	0.0	0.0	X		
3.86	3950	2.4	12,792	0.16	2.9	72.0	0.0	0.3	X		
3.91	6379	2.3	8179	0.17	7.0	67.1	0.0	0.0	X		
3.96	3759	2.4	5633	0.17	3.5	72.4	0.0	0.0	X		
4.01	613	2.4	8062	0.19	7.3	69.8	0.3	0.0	X		
4.11	2174	1.9	2850	0.22	5.2	74.0	0.0	0.5	X		

[a] K-means cluster groups from arcsin transformed PLFA mole percentages, whole profiles.
[b] Trans/cis — sum of two ratios: 16:1ω7t/16:1ω7c and 18:1ω7t/18:1ω7c.
[c] Sum of all terminally branched saturated PLFA.
[d] Sum of all monounsaturated PLFA.
[e] Sum of all mid-chain methyl branched saturated PLFA.
[f] Sum of all polyunsaturated PLFA.

2.7 to 3.5 m (Fig. 2). These two areas were identified as zones where *in situ* biodegradation of petroleum hydrocarbons had occurred or was an active process at the time of sampling. Both proposed biodegradation zones were bordered on either side by samples containing 500–5000 μg g^{-1} TPH.

3.1.2. Microbial community analysis

The intent of this study was to identify community attributes that were associated with the *in situ* biodegradation of the petroleum hydrocarbons. Microbial biomass (pmole PLFA g^{-1} soil) was found to run parallel to contaminant concentrations and a significant positive correlation, Spearman $r=0.56$, $p=.0005$, was identified between the two measures (Fig. 1). A conversion of the PLFA values to cell numbers, assuming that 1 pmole PLFA is equivalent to 2.5×10^4 bacterial cells (Balkwill et al., 1988), indicated the surface soil to contain approximately 4×10^8 cells g^{-1}. This biomass level dropped by an order of magnitude by the 0.8 m depth, which is a typical phenomenon for microbial distribution in soils (for example — Federle et al., 1986). However, microbial biomass rebounded to greater than surface cell numbers between 1.5 m and 4.5 m (Fig. 1). The relationship between biomass and TPH concentration suggests that a significant interaction existed between the indigenous microbiota and the contamination. Margesin et al. (2007) came to a similar conclusion in the evaluation of a different diesel-oil contaminated soil.

Specific microbial PLFA biomarkers were then examined in relationship to the alkane/acyclic isoprenoid ratio (Fig. 2). Two structural classes of PLFA, the mid-chain branched saturates and the polyunsaturates, showed an inverse relationship to the hexadecane/pristane ratio (Fig. 2A). Mid-chain branched saturates, in particular the 10-methyl branched moieties, are indicative of a number of actinomycetes whereas the polyunsaturates are indicative of micro-eukaryotes (Lechevalier and Lechevalier, 1988; Pinkart et al., 2002). Species of both fungi and actinomycetes have been shown to degrade petroleum hydrocarbons (Mancera-Lopez et al., 2007; Baek et al., 2004). However, another possibility, put forth by Margesin et al. (2003), is that, although present, the fungi and actinomycetes may not have been actively involved in the TPH biodegradation. A number of researchers have seen the enrichment of Gram-negative bacteria as a result of TPH contamination (see Balba et al., 1998 for a brief review). Being r-strategist organisms, certain Gram-negative bacteria proliferate when an excess of added carbon, in this case TPH, is present. K-strategists, such as Gram-positive bacteria, actinomycetes, and fungi that are likely present in the soil prior to the contamination event, are more efficient organisms and therefore more successful in resource limited situations. The biodegradation of petroleum hydrocarbons by r-strategists could have allowed the k-strategists to resume their natural position within the soil community. In this study, we also observed a high percentage of Gram-negative bacterial PLFA biomarkers in association with high TPH concentrations (Table 1 and Fig. 2B). Monounsaturated PLFA are indicative of Gram-negative bacteria and were by far the most prominent PLFA detected in these samples.

Exploratory statistics were then used to determine if a whole community attribute could be related to the observed changes in

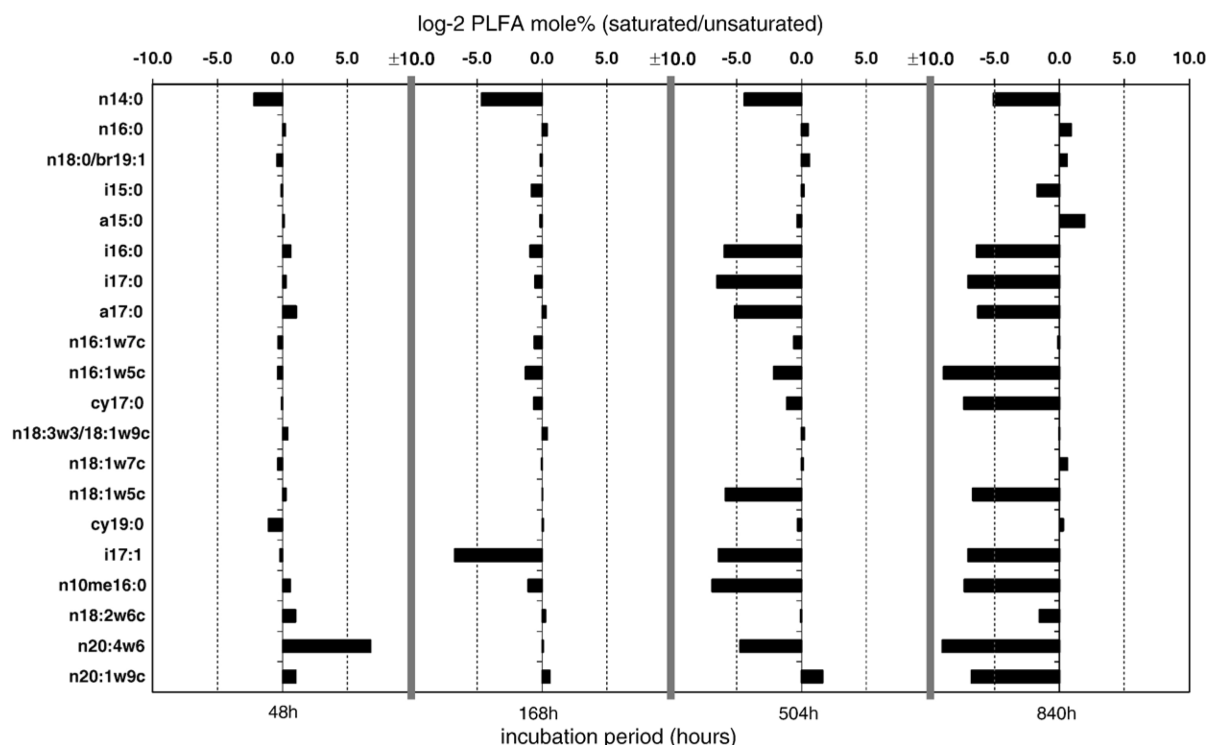

Fig. 3. Relative change in phospholipid fatty acids (PLFA) in saturated and unsaturated training range surface soils exposed to 10 mg L^{-1} of aqueous RDX. The data (mole %) are expressed on a log2 scale to highlight fold changes that occurred between the specified incubation periods. Relative to the unsaturated soil, only a few PLFA were enriched in the saturated soil.

TPH chemistry. By application of a K-means hierarchical cluster analyses, three community profiles of the greatest possible distinction were defined (Table 1). Community 3, characterized by significantly greater mole percentages of terminally and mid-chain branched saturated and polyunsaturated PLFA, was identified in samples exhibiting low TPH concentrations and a decreased hexadecane/pristine ratio. Community 3 samples included the surface and two near surface samples. Our interpretation is that this community represented a community of K-strategist microorganisms that persisted through the past TPH exposure and subsequent remediation. This is opposed to a community of actively biodegrading microorganisms, such as community 2, which is characterized by a high monounsaturated mole percentage and increased trans/cis ratio. These findings, monounsaturated % and high trans/cis ratios, are very similar to those observed by MacNaughton et al. (1999) when examining the microbiology surrounding crude oil biodegradation. The trans/cis ratio has been used as a descriptor of the physiological health of Gram-negative bacterial communities, with ratios less than 0.05 indicating a healthy community (White et al., 1998). The high trans/cis ratios observed here likely reflect the exposure of the Gram-negative populations to high TPH concentrations in a similar manner as when Gram-negative bacteria are exposed to solvents (Sikkema et al., 1995).

In summary, by examining the in situ microbial PLFA signatures, we were able to relate specific community attributes to a chemical measure of TPH biodegradation. TPH biodegradation could be characterized by either a whole community fingerprint (i.e. community 2) or by an individual PLFA attribute (i.e. tran/cis ratio). In addition, the successful attenuation of the diesel fuel spill could also be monitored. The identified community fingerprint, i.e. community 3, and individual PLFA attributes, i.e. actinomycete and micro-eukaryote

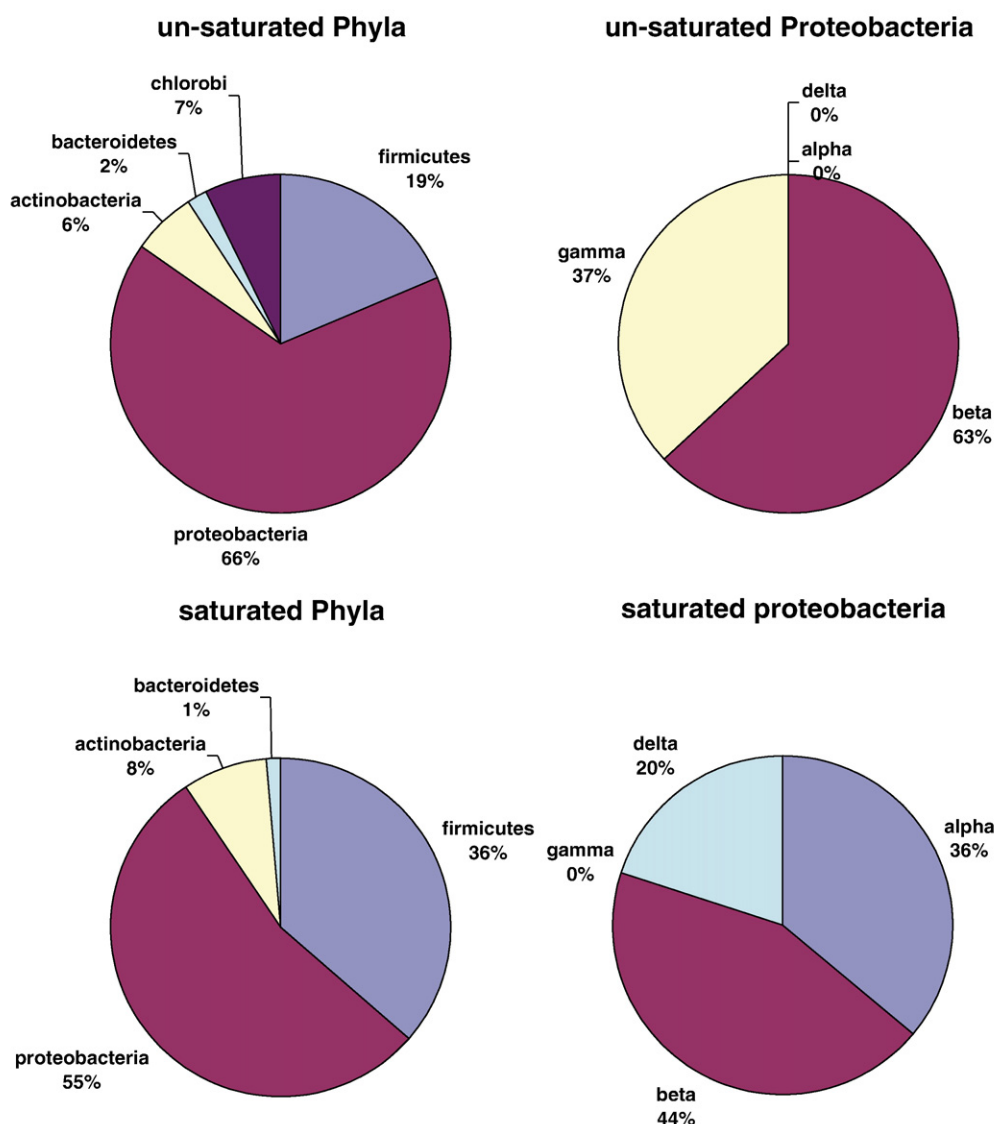

Fig. 4. The distribution of bacteria phyla and classes in saturated and unsaturated training range surface soils following 840 h of incubation are illustrated. Identifications were obtained by running the phylogenetic analysis tool (PAT) against the (RDB-II) ribosomal database on terminal restriction fragments. The analysis revealed an increase in the proportion of firmicutes and the emergence of alpha- and delta-proteobacteria in the saturated soil.

percentages, represented benchmarks or endpoints for identifying the successful bioremediation of the TPH contamination.

3.2. RDX biodegradation

The soil used in this study had a demonstrated capacity for the biological transformation of RDX (Ringelberg et al., 2003). Half-lives for the added RDX were 4 days saturated and 29 days unsaturated. These values were similar to those reported by Zhao et al. (2004) for aqueous RDX in marine sediment (at 10 °C) and Speitel et al. (2001) for RDX in a soil at a gravimetric moisture level of 8%. A number of reports have also shown that reduced conditions can be ideal for RDX biodegradation (McCormick et al., 1981; Boopathy et al., 1998; Guiot et al., 1999; Speitel et al., 2001). Since saturated soils can rapidly become anoxic, given sufficient biomass and bio-available carbon, soil moisture likely plays a pivotal role in RDX biodegradation.

The soils contained $\sim 10^8$ cells g^{-1} at the time RDX was added in aqueous form, which is a typical cell density for a surface soil. A plot of saturated to un-saturated PLFA (on a Log2 scale to enhance fold changes) showed a dramatic shift to occur in the saturated PLFA profile over the time course of the study (Fig. 3). Relative to the un-saturated soil, very few PLFA became enriched under the saturated condition. Instead there was a significant drop in the number of PLFA detected. The loss in PLFA diversity was also associated with a loss in total PLFA abundance, approximately 1/3 that of the un-saturated soil. The decreased biomass and fewer number of detectable PLFA was likely an indication of increased selective pressures on microbial growth, e.g. O_2 availability, as indicated by a color change in the added resazurin dye.

To further resolve the in situ descriptions of microbial community composition and to specifically address species numbers, the T-RFLP assay was applied. The number of bacterial species identified in the saturated soils was approximately 60% less than that observed in un-saturated soil (32 species un-saturated versus 13 species saturated). Microbial community development within the saturated soil was characterized by a shift toward bacterial species of the phylum firmicutes and alpha- and delta-classes of the phylum proteobacteria (Fig. 4). The increase in Gram-positive firmicutes from 19% (un-saturated) to 36% (saturated) corresponded with the PLFA biomarker analysis, i.e. increased % of a15:0. The observed shift in the proteobacteria from gamma, unsaturated, to alpha/delta, saturated, is likely reflected in the increased percentage of 18:1ω7c and cy19:0, both common in many proteobacteria (see Table 2 for a list of PLFA biomarkers detected in the saturated and unsaturated soils).

Terminal restriction fragments for *Clostridia*, *Rhodococcus* and *Beijerinckia* species were identified in the saturated soil and not in the unsaturated soil. All three genera of bacteria contain species known to utilize RDX as a sole source of carbon and/or nitrogen (personal communications, Drs. Neal Adrian and Herb Fredrickson, U.S. Army-ERDC; Bhushan et al., 2003; Seth-Smith et al., 2002). The emergence of other species of alpha-and delta-proteobacteria, i.e. *Myxococcus*, *Methylosinus*, *Rhodobacter*, and *Sphingomonas* were also unique to the saturated soil. Although these organisms have not been directly tied to nitramine biodegradation, the latter three genera have all been tied to the biodegradation of aromatic compounds. Certain *Rhodobacter* and *Sphingomonas* species facilitate ring cleavage via the protocatchuate enzyme whereas certain *Methylosinus* species can facilitate ring cleavage via a monooxygenase as well as oxidize a variety of aliphatic compounds (Buchan et al., 2001; Wattian et al., 2001; Green and Dalton, 1989). Within the saturated soil, the majority of organisms identified showed traits related to contaminant biodegradation if not specifically nitramine biodegradation. In addition, two other related species, *Sporosarcina urea* and *Halobacillus halophilus*, were identified as the most abundant, i.e. greatest TRF areas, bacteria present in the saturated soil. Both species can have the ability to synthesize urease and are widely distributed in surface soils. The ability to release ammonium ions might also have played a role in RDX loss through base induced catabolism (Heilmann et al., 1996).

Even though no species were identified that are known to degrade RDX in the unsaturated soil, some species were identified that have been observed to degrade other environmental pollutants. *Burkholderia* spp. have been isolated that show the ability to biodegrade 2,4-dinitrotoluene. *Burkholderia* was one of the more abundant species detected in our tests. Strains of the beta-proteobacteria *Bordettella* and the gamma-proteobacteria *Pseudomonas stutzeri* are capable of degrading a variety of contaminants, including aromatic compounds (Eriksson et al., 2003; Spanggord et al., 1991). In short, the unsaturated soil fostered the development of a diverse community with biodegradative capabilities. However, an ideal soil moisture tension in terms of agricultural applications, i.e. 1/3 bar, is far less amenable to RDX biodegradation than is soil that is saturated.

In summary, we found that soil moisture tension had a significant effect on the development of indigenous microbes. Saturated soils fostered the development of a reduced environment that supported the complete biodegradation of the added RDX. The resulting, relatively simple, microbial community was enriched in firmicutes, and alpha- and delta proteobacteria. Specific bacterial genera known to degrade RDX were identified (i.e. *Clostridium* sp., *Rhodococcus* sp. and *Beijerinckia* sp.) as well as other genera with associated biodegradation capabilities

Table 2
PLFA biomarkers detected in Ft. Greely surface soils incubated under different soil moisture tensions

Organisms		PLFA biomarkers [a]
Eukaryotes	ω6 series	n18:2ω6c, n20:4ω6c
	ω3 series	n18:3ω3, n20:5ω3
Prokaryotes	Gram-positive	i14:0, a15:0, i16:0, i17:0, a17:0
	Gram-negative	
	Cyclopropyl	cy17:0, cy19:0
	ω7 series	n16:1ω7c, n18:1ω7c
	Other series	n16:1ω9c, n16:1ω5c, n17:1ω8c, n18:1ω9c n18:1ω5c, n19:1ω8c, n20:1ω9c
	Actinomycetes	n10me16:0, n10me17:0, n10me18:0, i17:1ω7c

[a] PLFA biomarkers are assigned to taxonomy according to Lechevalier and Lechevalier (1988), and Pinkart et al. (2002).

(i.e. denitrification and ring cleavage). In addition, alkaline hydrolysis of the added RDX may have occurred. The unsaturated soils exhibited a significantly different endpoint microbial community and succession that was characterized by a considerably lower rate and extent of RDX biotransformation. The aerobic nature of the soil fostered the development of a diverse bacterial community with a capacity for contaminant biodegradation, if not one ideally suited to RDX biotransformation.

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Dr. David C. White pioneered the development and use of membrane lipids in quantifying microbial communities in situ. This development has provided many researchers with a valuable tool for exploring microbes in a wide variety of environments. By applying this tool with care, insight into the movers and shakers of the microbial kingdom can be gained and this insight can and has been used to benefit us all. Thanks D.C.

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