Aerobic biofilms grown from Athabasca watershed sediments are inhibited by increasing concentrations of bituminous compounds

Yergeau, Étienne; Lawrence, John R.; Sanschagrin, Sylvie; Roy, Julie L.; Swerhone, George D. W.; Korber, Darren R.; Greer, Charles W.

Publisher's version / Version de l'éditeur:
https://doi.org/10.1128/AEM.02216-13
Applied and Environmental Microbiology, 79, 23, pp. 7398-7412, 2013-09-20

AEM Accepts, published online ahead of print on 20 September 2013
Appl. Environ. Microbiol. doi:10.1128/AEM.02216-13

Title: Aerobic biofilms grown from Athabasca watershed sediments are inhibited by increasing bituminous compounds concentrations

Authors: Etienne Yergeau[1]#, John R. Lawrence[2] Sylvie Sanschagrin[1], Julie L. Roy[2], George D.W. Swerhone[2], Darren R. Korber[3], and Charles W. Greer[1]

Affiliations: [1]National Research Council Canada, Montreal, Canada
[2]Environment Canada, Saskatoon, Canada
[3]University of Saskatchewan, Saskatoon, Canada

Keywords: Athabasca River / Biofilms / Next-generation sequencing / Oil sands / Sediments

Running title: Athabasca River bitumen effects on biofilms

Date: September 4, 2013

#Correspondence to: Etienne Yergeau, National Research Council Canada, 6100 Royalmount, Montreal, Qc, H4P 2R2, Canada. Phone: 514-496-6152. Fax: 514-496-6265.
Etienne.Yergeau@cnrc-nrc.gc.ca

Abstract

Sediments from the Athabasca River and its tributaries naturally contain bitumen at various concentrations, but impacts of this variation on the ecology of the river are unknown. Here, we used controlled rotating biofilm reactors in which we re-circulated diluted sediments containing various concentrations of bituminous compounds taken from the Athabasca River and three tributaries. Biofilms exposed to sediments having low and high concentrations of bituminous compounds were compared. The latter were 29% thinner, had a different extracellular polysaccharide composition, 67% less bacterial biomass per μm^2, 68% less cyanobacterial biomass per μm^2, 64% less algal biomass per μm^2, 13% fewer protozoa per cm^2, were 21% less productive, had a 33% reduced content in chlorophyll a per mm^2 and a 20% reduction in the expression of photosynthetic genes, but had a 23% increase in the expression of aromatic hydrocarbon degradation genes. Within the Bacteria, differences in community composition were also observed, with relatively more *Alphaproteobacteria* and *Betaproteobacteria* and less *Cyanobacteria*, *Bacteroidetes* and *Firmicutes* in biofilms exposed to high concentrations of bituminous compounds. Altogether, our results suggest that biofilms that develop in the presence of higher concentrations of bituminous compounds are less productive and have lower biomass, linked to a decrease in the activities and abundance of photosynthetic organisms, likely due to inhibitory effects. However, within this general inhibition, some specific microbial taxa and functional genes are stimulated because they are less sensitive to the inhibitory effects of bituminous compounds or can degrade and utilize some bitumen associated compounds.

42 **Introduction**

43 Oil sands are unconventional petroleum deposits where bitumen, a dense and viscous form of
44 petroleum, is found in combination with sand, clay and water. One of the largest bitumen
45 reservoirs, the Athabasca oil sands, is located in Northeastern Alberta, Canada, along the
46 Athabasca River. Here, as the Athabasca River and its tributaries (e.g., Ells, Steepbank and
47 Firebag) cut through the Athabasca oil sands formation, oil sands are eroded, making the water
48 and sediments naturally-enriched in various bituminous compounds. These compounds are
49 mainly asphaltenes, but also various aromatic hydrocarbons (typically high molecular weight,
50 partially saturated polycyclic aromatic hydrocarbons similar to hopanoids), naphthenic acids
51 and alkanes (49, 50). The concentrations of these compounds vary naturally due to geological
52 and hydrological factors, but also because of aerial deposition from oil upgrading activities (24-
53 26). There are also some indications of a potential hydraulic connectivity between the oil sands
54 tailings ponds (which are often above grade, setting up a hydraulic head) and the at-grade or
55 subsurface natural water bodies (22). Many microorganisms, often belonging to the
56 *Proteobacteria,* can degrade and utilize hydrocarbon compounds that are found in bitumen (12,
57 52, 53). The *Betaproteobacteria* were previously shown to be positively correlated to various
58 hydrocarbon indicators in a field survey of sediments from the Athabasca River, suggesting that
59 they might be able to use some of the bituminous compounds as carbon sources (57). However,
60 the Athabasca River is generally considered to be nitrogen and phosphorus limited (8), which
61 probably strongly limits the utilization of bituminous compounds by microorganisms, as even
62 with abundant carbon sources, microbial growth cannot occur without N and P.

Although some bituminous compounds like asphaltenes and alkanes are generally not toxic to microbial activity, other compounds like polycyclic aromatic hydrocarbons and naphthenic acids can inhibit microbial growth. Although some bacteria can metabolize them (5, 6, 23), naphthenic acids can have negative effects on bacteria (15, 21), algae (20, 31) and cyanobacteria (42). Aromatic hydrocarbons are also known to be toxic to a variety of microbial processes (47, 51) and to bacteria (14, 34). Photosynthetic organisms were reported as being more strongly inhibited by contaminants than other organisms (17, 57), but also as being stimulated indirectly by oil addition through decreased grazing pressure (9). In river ecosystems, biofilms and flocs containing photosynthetic organisms are in large part responsible for ecosystem productivity and are used as a nutrient source by higher trophic levels. Any changes in microbial community productivity and nutrient cycling activities will have a direct influence on other trophic levels.

In freshwater ecosystems, the majority of microbial activity is associated with surfaces. Biofilms form at a specific location and respond to local environmental conditions such as current velocity, nutrients (27) and pollutants (56, 58), which makes them ideal resident communities for environmental monitoring. They also regulate the physical and chemical microhabitat and contribute to ecosystem processes as a whole (2). In addition, there is recruitment of populations to form the biofilm that represents the selection pressure for those conditions and locations (4). Therefore the biofilm community is more representative of what would be happening within a region of the river and in/on the sediment in particular than the planktonic community. The response of river biofilms to contaminant presence could therefore impact biodiversity, ecosystem health and functioning. The presence of various contaminants

was shown previously to influence biofilm aquatic microbial communities, even at concentrations that were generally considered to be sub-inhibitory (56, 58).

Our objective was to observe, under controlled *in vitro* conditions, the type of biofilm communities that would develop from sediments taken from various parts of the Athabasca watershed, which would be indicative of the impact of the variation in bituminous compounds on microbial communities. To do this, sediments from various locations were diluted in Athabasca River water taken from a reference location and re-circulated in rotating annular reactors and the biofilm architecture, community composition and activities were measured after 8 weeks. A large array of techniques was used to describe the functional and biological differences in microbial communities. We hypothesized that the hydrocarbons contained in bitumen would be inhibitory for microbial growth, but that dilution in the reactor would decrease this inhibition and provide a supplementary carbon source for adapted microbial communities.

Material and Methods

Sediment and water samples

Sediment and water for reactor set-up (see below) were sampled on September 23, 2010. A single large 10 kg sediment sample (top 15 cm of the sediment) was taken from each of the sites (12 samples) using a stainless steel shovel in less than 1 m of water. See Yergeau et al. (57) for a map of the sampling sites. Three sampling sites were chosen for the Athabasca River: a reference site near Fort McMurray (AR; 56.72°N, 111.40°W; 19.41 km from the nearest tailings pond), one site upstream of the oil sands mining activities (US; 56.87°N, 111.44°W; 4.69 km) and one site directly downstream of Suncor mining activities (DS; 57.06°N, 111.52°W; 6.90 km). Nine other sampling sites were chosen in three of the Athabasca tributaries: upper (EU; 57.23°N, 111.89°W; 22.69 km), mid (EM; 57.24°N, 111.77°W; 19.41 km) and lower (EL; 57.26°N, 111.72°W; 21.12 km) Ell's River (just outside the mining area); upper (FU; 57.34°N, 110.48°W; 73.15 km), mid (FM; 57.44°N, 110.89°W; 59.67 km) and lower (FL; 57.52°N, 111.11°W; 57.75 km) Firebag River (outside the mining area); upper (SU; 56.86°N, 111.13°W; 16.16 km), mid (SM; 56.99°N, 111.34°W; 10.69 km), and lower (SL; 57.02°N, 111.47°W; 10.80 km) Steepbank River (directly in the mining area). Sediment samples were collected, transferred to plastic bags, transported in coolers to the laboratory within 48 h and kept at 4°C in the dark until used in reactor set-up (14 days). Water for the experiment was taken at the reference site near Fort McMurray (AR). The chemical characteristics of the water were as follow: dissolved organic carbon (DOC), 4.5 mg/L; dissolved inorganic carbon, 28.2 mg/L; SO_4, 34.6 mg/L; Ca, 35.7 mg/L; Mg, 9.87 mg/L; K, 1.15 mg/L; Cl, 3.22 mg/L; conductivity, 0.298 µS; pH, 8.61; O_2, 9.05 mg/L; NH_3, 0 mg/L. Examination of an extensive database on reference sites in the Athabasca indicates that

120 median concentrations were 5, 13 and 26 µg L^{-1} total phosphorus and 2, 2 and 8 µg L^{-1} total
121 dissolved phosphorus for the upper, mid and lower Athabasca River, respectively. Total
122 nitrogen concentrations at reference sites were 200 and 361 µg L^{-1} for the mid and lower
123 Athabasca River, respectively (8). The reference site is considered to be both nitrogen and
124 phosphorus limited based on periphyton assessments (7). The water used for dilution (AR site)
125 contained negligible or undetectable levels of the various hydrocarbon indicators.
126
127 *Experimental design and sampling*
128 Rotating annular reactors containing 450 ml were set up as previously described (28, 29).
129 Sediment slurry (500 ml, >60% solid) was added to a 10 L container and then filled with
130 Athabasca River Reference water (from the AR site) (this represented a 1/20 dilution). The
131 overlying water plus sediment was then continuously recirculated through 3 replicate reactors
132 for each treatment (each replicate reactor was supplied independently via a peristaltic pump
133 but from the same reservoir and was randomly placed on the reactor bench) for about 50 days
134 at a rate of 500 ml per day at 22±2°C, giving 36 bioreactors. The reactors were run with
135 continuous illumination (2.1 µE s^{-1} m^{-2}) as described in Neu et al. (37). Each replicate reactor
136 contained twelve identical coupons, made in-house out of polycarbonate as previously
137 described (29). Each analysis was done on subsamples of three randomly selected replicate
138 biofilm coupons. Confocal laser scanning microscopic imaging was performed at 5 random
139 locations on transects across a 1 cm^2 subsample piece of the biofilm coupons. Subsampling for
140 other analyses (protozoan counts, thymidine incorporation, carbon utilization analyses,
141 molecular/genomic) was also carried out using randomly-selected subsamples from among the

12 identical coupons in each replicate reactor. For DNA-RNA work, biofilm was harvested by scraping off one coupon from each replicate reactor and flash freezing the biomass in liquid nitrogen. Since all replicate reactors were inoculated with the same dilution for each sediment source and concentrations were not measured directly in the reactors, statistical significance cannot be tested. For correlation analyses (see below), we used the same concentrations for the three replicate reactors.

Confocal laser scanning microscopy (CLSM)

Examination of all stained and control materials was carried out with an MRC 1024 confocal laser scanning microscope (formerly Bio-Rad, now Zeiss, Jena, Germany) attached to a Microphot SA microscope (Nikon, Tokyo, Japan) equipped with a Nikon, 10X NA 0.3, water immersible lens. Coupons from each of the replicate reactors were cut into 1 cm^2 pieces and mounted in small Petri dishes using Dow Corning #3140 acid-free silicone (WPI, Inc., Sarasota, FL.) and then stained and analyzed according to Lawrence et al (28). In brief: bacteria were stained with the fluorescent nucleic acid stain (SYTO 9, Green); a lectin probe (*Triticum vulgaris*-TRITC [tetramethyl rhodamine isothiocyanate], Red) was used to visualize exopolymer, while autofluorescence was used to detect algal and cyanobacterial cells (Blue) as described previously in detail (39). The lectins *Arachis hypogaea, Canavalia ensiformis, Glycine max, Triticum vulgaris* and *Ulex europaeus* conjugated to fluorescein isothiocyanate (FITC) were applied individually for *in situ* analyses of polymer composition, as described by Neu et al. (38). Digital image analysis of the CLSM optical thin sections was performed by using NIH Image version 1.61 (http://rsb.info.nih.gov/nih-image/) with macros written for semi-automated

quantification as described in Manz et al. (33). This allowed determination of biofilm thickness, bacterial cell area (biomass), exopolymer biomass, cyanobacterial biomass, and total photosynthetic biomass at various depths. Three-color red–green–blue projections (bacterial cells = green, polymer = red, and algal autofluorescence = blue) of the biofilms were computed. Based on controls, there was no background detectable that could be interpreted as hydrocarbon and the threshold setting for algal+cyano autofluorescence limits and detection of weaker signals.

Protozoan and micrometazoan enumeration, carbon utilization pattern and thymidine incorporation

Protozoa and micrometazoa were enumerated as follows: Samples were removed from the reactors on a weekly basis and the numbers of protozoa and micrometazoa manually counted on replicate 2 cm^2 subsamples using phase contrast microscopy. The abundance values presented are the cumulative abundance over the course of the 8 weeks of the experiment. Carbon utilization patterns were determined for biofilm samples using commercial Eco-plates (Biolog, Hayward, CA), as described previously (30). Thymidine incorporation was carried out using tritiated thymidine following the standard protocol of Robarts and Wicks (44). All negative controls were killed with formaldehyde at 0.4% final concentration.

Nucleic acid extraction and rRNA subtraction

Frozen biofilm strips were thawed in 2 volumes (approximately 5 ml) of RNAlater (Ambion, Life Technologies, Burlington, Ontario, Canada) and centrifuged at 8,000x g for 12 minutes. The

DNA and RNA from resulting cell pellets were simultaneously extracted using a homemade bead-beating protocol with subsequent phenol-chloroform purification, as previously described (54, 55). Extracts were separated in two parts, one was treated with DNAse to produce RNA and the second one was treated with RNAse to produce DNA. Total RNA was amplified using the Message Amp kit (Ambion) and rRNA was subtracted following the protocol described by Stewart et al. (48), with the exception that the T7 promoter was coupled to the forward primer instead of the reverse primer because the MessageAmp procedure produces antisense RNA.

Ion Torrent 16S rRNA gene sequencing

Partial 16S rRNA gene amplicons were produced using the universal primers E786 (5'-GATTAGATACCCTGGTAG-3') and U926 (5'-CCGTCAATTCCTTTRAGTTT-3') (1) containing the 10-bp multiplex identifiers (MID) and adaptor sequences for Ion Torrent sequencing described previously (3, 57). Reactions were performed in 25-µl volumes containing 1 µl of template DNA, 0.3 µM each primer, 0.4 mg/ml of BSA, 0.2mM of dNTPs and 0.05 U/µl of rTaq DNA polymerase (GE Healthcare, Baie d'Urfé, Canada). Cycling conditions involved an initial 5 min denaturing step at 95°C, followed by 25 cycles of 30 s at 95°C, 30 s at 55°C, and 45 s at 72°C, and a final elongation step of 3 min at 72°C. All PCR products were purified on agarose gels using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and quantified using the PicoGreen dsDNA quantitation assay (Invitrogen, Carlsbad, CA). All the 36 amplification products from the different samples were pooled in an equimolar ratio and sequenced together. A total of $3.50x10^7$ molecules were used in an emulsion PCR using the Ion OneTouch™ 200 Template Kit (Life Technologies) and the OneTouch™ instruments (Life Technologies) according to the

manufacturer's protocol. The sequencing of the pooled library was done using the Personal Genome Machine™ (PGM) system and a 314 chip with the Ion Sequencing 200 kit according to the manufacturer's protocol. Sequences were binned by MID, after which MIDs were trimmed from each sequence. Sequences having an average Phred quality score below 20, or being less than 100 bp, were then filtered out of the dataset. Taxonomic identities were assigned to sequences using the "multiclassifier", which is the local multi-sample version of the RDP Pipeline Classifier (http://pyro.cme.msu.edu/). Weighted-normalized Unifrac distances between each sample pair were calculated using the FastUnifrac website (18) based on the GreenGene core dataset. For OTU calculation, sequence data were randomly normalized to 1964 sequences and were then de-noised using the procedure of Quince et al (43). These sequence data have been submitted to the NCBI SRA database under accession No. SRP026549 (NCBI BioProject PRJNA210445).

RNA sequencing

Total rRNA-subtracted RNA was reverse-transcribed using the SuperScript III kit (Invitrogen). Illumina libraries were prepared following the protocol of Meyer and Kircher (36), with indices 7 to 42 pooled together and sent for three lanes of Illumina HiSeq 2000 paired-end 2x101 bp sequencing at The Center for Applied Genomics of the Hospital for Sick Children, Toronto, Canada. Resulting data were split into 216 files (36 samples x 2 reads x 3 lanes). These sequence data have been submitted to the NCBI SRA database under accession No. SRP026549 (NCBI BioProject PRJNA210445). Data from the different lanes were pooled together and the resulting 72 files were filtered using a custom-made Perl script. Sequences were trimmed to the first

occurrence of an undefined base (N) or of a low quality base (below phred-like score of 20) and sequences shorter than 75 bp were filtered out. The resulting high-quality sequences were submitted to MG-RAST 3.0 (35) for automated annotation. No attempt was made to assemble the paired reads as overlaps were not observed. Annotation results from the two paired files were summed after preliminary analyses that revealed no significant differences between the paired datasets. We also looked more specifically at key genes related to aerobic hydrocarbon utilization: alkane hydroxylases and ring-opening dioxygenases. For alkane hydroxylase, we used the MG-RAST "all annotations" functionality with the GenBank database and summed the abundance of reads matching either "alkane monooxygenase" or "alkane hydroxylase". For the ring-opening dioxygenase, we used the MG-RAST "hierarchical classification" functionality with the M5NR database and summed the abundance of reads for three categories of ring-opening dioxygenases. The intradiol ring-opening dioxygenase represents a sum of MG-RAST functions "Catechol 1,2-dioxygenase (EC 1.13.11.1)", "Catechol 1,2-dioxygenase 1 (EC 1.13.11.1)", "Intradiol ring-cleavage dioxygenase (EC 1.13.11.1)", "Protocatechuate 3,4-dioxygenase alpha chain (EC 1.13.11.3)", and "Protocatechuate 3,4-dioxygenase beta chain (EC 1.13.11.3)". The extradiol ring-opening dioxygenase represents a sum of MG-RAST functions "catalytic subunit of meta cleavage enzyme", "Catechol 2,3-dioxygenase (EC 1.13.11.2)", "extradiol dioxygenase large subunit", "Protocatechuate 4,5-dioxygenase alpha chain (EC 1.13.11.8)", "Protocatechuate 4,5-dioxygenase beta chain (EC 1.13.11.8)", "Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39)", and "1,2-dihydroxynaphthalene dioxygenase". The gentisate/homogentisate ring-opening dioxygenase represents a sum of MG-RAST functions

"Gentisate 1,2-dioxygenase (EC 1.13.11.4)" and "Homogentisate 1,2-dioxygenase (EC 1.13.11.5)".

Data analysis

All statistical analyses were carried out in R (v 2.13.2, The R Foundation for Statistical Computing). Normal distribution and variance homogeneity of the data was tested using the "shapiro.test" and "bartlett.test" functions, respectively. If the data was not normally distributed or did not show homogeneous variance, it was log transformed before ANOVA analyses. ANOVA were carried out using the "aov" function, with the post-hoc Tukey Honestly Significant Difference tests being performed with the "TukeyHSD" function. Spearman rank-order correlations were carried out using the "cor" and "cor.test" functions. Similarity matrices were calculated using the "vegdist" function of the "vegan" package using "Bray" for Biolog substrate utilization patterns and for mRNA functional classification. These matrices and the Unifrac matrix were then used for principal coordinate analyses (PCoA) that were carried out using the "pcoa" function of the "ape" package. Vectors of summed relative abundance at the phyla/class level, substrate or SEED functional hierarchy were overlaid on the ordinations.

Results

Sediments chemical analyses

A subset of the sediments analyzed in Yergeau et al. (57) was diluted in Athabasca River water (1:20) and used to inoculate rotating annular reactors. Since the same sediment was recirculated in triplicate reactors and hydrocarbon concentrations were not measured directly in the re-circulated water, chemical data cannot be tested for significance. The sediments had very different concentrations of bituminous compounds, with Ell's River lower, Firebag River lower and Steepbank River mid and lower sediment samples generally having values one or more orders of magnitude higher than other samples. In these samples with high levels of bituminous compounds, up to 12.76% of the total organic matter was due to the presence of total petroleum hydrocarbons (TPH) (Table 1). In contrast, the samples showing the lowest values for bituminous compounds generally had less than 1% of their organic matter being related to hydrocarbons (Table 1).

Biofilm architecture

Biofilms were 29.0% thinner in the samples exposed to the sediments containing the highest concentration of hydrocarbon (black bars) when compared to the other treatments (white and grey bars), and many of the pairwise differences were significant (Fig. 1). Consequently, biofilm thickness was significantly and negatively correlated to most of the hydrocarbon indicators, with stronger correlations (up to r_s=-0.77, $P<0.001$) with EPA-PAH, alkylated PAHs and aromatic compounds (Table 2). The composition of the extracellular polysaccharide (EPS) matrix varied considerably between the different treatments based on lectin binding assays (Fig. 1). $Glc(NAc)_2$

(N-acetylglucosamine), NeuNAc (N-Acetylneuraminic acid) was 35.9% less abundant in the samples exposed to the sediments containing the highest concentration of hydrocarbon (black bars) when compared to the other treatments (white and grey bars) (Fig. 1), resulting in significant ($P<0.05$) negative correlations with concentrations of naphthenic acids, EPA-PAH, alkylated PAHs and aromatic compounds (Table 2). In contrast, terminal α or β galNAc (terminal α- or β-linked N-acetylgalactosamine) was 207.6 % more abundant in the samples exposed to the sediments containing the highest concentration of hydrocarbon (black bars) when compared to the other treatments (white and grey bars) (Fig. 1), resulting in significant ($P<0.01$) positive correlation with TPH, TSH and TAH (Table 2).

Biofilm community composition

The biofilm community composition was analyzed by Ion Torrent 16S rRNA gene sequencing, direct counts and confocal laser scanning microscopy. The microbial communities exposed to the sediments having the highest concentrations of hydrocarbons (Ell's lower, Firebag lower and Steepbank mid and lower) were visually very different from the other biofilms when observed by confocal laser scanning microscopy (Fig. 2). Similar differences were found upon analysis of each biofilm for relative abundance of algae, *Cyanobacteria* and Bacteria using confocal laser scanning microscopy (Fig. 3). *Cyanobacteria*, algae and Bacteria were 67.7%, 63.6% and 67.2% less abundant, respectively, in the samples exposed to the sediments containing the highest concentration of hydrocarbon (black bars) when compared to the other treatments (white and grey bars) (Fig. 3). Significant negative correlations were observed between algae, *Cyanobacteria* and Bacteria and various hydrocarbon indicators ($P<0.001$,

313 P<0.05 and P<0.01, respectively), with the strongest correlations being with EPA-PAH, alkylated
314 PAHs and aromatic compounds (Table 2). The cumulative number of protozoa observed over
315 the course of the 8 week experiment (with observation every week) was also 13.4% lower in
316 the samples exposed to the sediments containing the highest concentration of hydrocarbon
317 (black bars) when compared to the other treatments (white and grey bars) (Fig. 3). Protozoa
318 counts were also negatively correlated to most of the hydrocarbon indicators measured
319 (P<0.01), with total aromatic hydrocarbon, total petroleum hydrocarbon and naphthenic acid
320 concentrations having the strongest correlations (Table 2).
321 The bacterial community, as measured by Ion Torrent 16S rRNA gene sequencing, was
322 dominated by *Proteobacteria*, with the *Alphaproteobacteria* and *Betaproteobacteria* classes
323 being highly abundant in most treatments (19.6-36.0% and 14.7-43.8% of total community,
324 respectively) (Fig. 4a and Table S1). Most of the phyla/classes were significantly (P<0.05)
325 affected by the experimental treatments, with the exception of the *Cyanobacteria* (Table S1).
326 The *Alphaproteobacteria* and the *Betaproteobacteria* were often significantly more abundant in
327 the biofilms exposed to sediments containing the highest hydrocarbon concentrations (Alpha:
328 29.0% (high) vs. 23.3% (medium-low); Beta: 32.4% vs. 28.9%) (Fig. 4a and Table S1), and were
329 positively correlated to several hydrocarbon indicators (P<0.01 and P<0.001, respectively), with
330 the highest coefficient being for EPA-PAH, alkylated PAHs and aromatic compounds (Table 3).
331 The *Gammaproteobacteria* class was significantly more abundant in two treatments,
332 downstream of Suncor and Steepbank River upper (29.0% and 27.2% of total community,
333 respectively) (Fig. 4a and Table S1), but only showed a significant positive correlation (P<0.01)
334 with naphthenic acid concentration (Table 3). The *Bacteroidetes* were significantly more

abundant in the biofilms exposed to sediments from the Firebag River (15.7-23.2% of total community) (Fig. 4a and Table S1), which resulted in significant negative correlations ($P<0.001$) with EPA-PAH, alkylated PAHs and aromatic compounds (Table 3). The *Actinobacteria* were significantly more abundant in the biofilms that developed from Firebag River lower and upper, Ell's river mid and Steepbank River upper sediments (6.8-9.3%) (Table S1), which resulted in a significant positive correlation ($P<0.01$) with naphthenic acids and negative correlations ($P<0.001$) with EPA-PAH, alkylated PAHs and aromatic compounds (Table 3).

The relationships between phyla/classes and samples mentioned above were also visible in the principal coordinate analyses of the Unifrac distance onto which the phylum/class data were projected. The ordination also revealed that the biofilms exposed to sediments from the Firebag River were harbouring similar bacterial communities, with relatively more *Actinobacteria* (average Firebag River: 5.04% vs. average other samples: 4.29%), *Deltaproteobacteria* (2.42% vs. 1.79%) and *Bacteroidetes* (12.59% vs. 8.70%) than other treatments (Fig. 5a). These samples were also relatively richer in *Acidobacteria* (1.58% vs. 1.39%) and *Cyanobacteria* (0.06% vs. 0.02%) and poorer in *Betaproteobacteria* (29.02% vs. 33.22%) and *Alphaproteobacteria* (25.73% vs. 25.96%) (Fig. 5a). In contrast, most of the biofilms exposed to the lower Ell's River and the Steepbank River sediments were richer in *Beta-* (average lower Ell's River and the Steepbank River: 34.27% vs. average other samples 27.97%), *Alpha-*(27.70% vs. 24.00%) and *Gammaproteobacteria* (17.76% vs. 17.28%) (Fig. 5a). The remaining biofilms were relatively enriched in *Planctomycetes* (average other samples: 1.54% vs. average lower Ell's River and the Steepbank River: 0.90%), *Firmicutes* (2.32% vs. 1.39%) and *Cyanobacteria* (0.07% vs. 0.005%).

357 We compared the microbial communities of the biofilms with the ones detected in a
358 previous microbial field survey of the sediments used in this study (57). When looking at each
359 phylum/class individually, no significant Spearman correlations were found between the biofilm
360 and sediments, suggesting that, across the different samples, the relative abundance of
361 particular taxa in the sediment was not predictive of its relative abundance in the biofilm. When
362 comparing the relative abundance of all phyla/classes across pairs of samples, significant
363 Spearman correlations were found between sediments and their associated biofilm, but also
364 between almost all biofilm-sediment pairs, indicating that the relative community composition
365 at the phylum/class level was not more similar when comparing a sediment sample with its
366 associated biofilm than a random biofilm-sediment pair. Similarly, when comparing Unifrac
367 distances, the biofilm bacterial communities were not more similar to their associated sediment
368 bacterial communities, than to other sediment bacterial communities. Altogether, these results
369 indicate that biofilm microbial communities could not be predicted from the sediment
370 microbial communities and that some selection of microorganisms had occurred because not all
371 microorganisms present in the sediment can live in a biofilm.
372 The number of OTUs detected in the microbial communities was highly variable among
373 replicate samples (Fig. S1). This variability precluded the identification of any significant
374 differences between the treatments. The number of OTUs was generally negatively correlated
375 with the different hydrocarbon indicators, but again, none of these trends were significant at
376 $P<0.05$.
377
378 *Biofilm activities*

379 Biofilm activities were measured by bacterial thymidine incorporation, chlorophyll a
380 measurement, Biolog assays and Illumina mRNA sequencing. Bacterial production, as measured
381 by thymidine incorporation rates, was 206.6% higher in the biofilms exposed to sediments from
382 the Firebag River (mid and lower) and the Ell's river (mid) (Fig.6), which were not among the
383 sediments having the lowest hydrocarbon concentrations. However, significant ($P<0.05$)
384 negative correlations were observed between bacterial production and different hydrocarbon
385 compounds, with the strongest negative correlations being with the concentration of total
386 aromatic compounds, EPA-PAHs and alkylated PAHs (Table 2).
387 The active community composition, based on the taxonomic affiliation of the sequenced
388 mRNA in MG-RAST, was dominated by *Proteobacteria*, mainly the *Alpha-*, *Beta-*, and
389 *Gammaproteobacteria* classes (12.9-20.6%, 11.7-19.2% and 17.1-22.8% of total activity,
390 respectively) (Fig. 4b and Table S2). The *Firmicutes* and *Cyanobacteria* were much more active
391 than expected based on their relative abundance in 16S rRNA gene libraries (10.4-17.2% vs. 0.9-
392 4.8% and 3.6-13.4% vs. 0.0-0.4%, respectively)(Fig. 4a vs. 4b). The *Cyanobacteria* were
393 significantly less active (by 56.4%) when exposed to the sediments from the Reference Site, the
394 lower Ell's River, and the mid and Lower Steepbank River (Fig. 4b and Table S2), and showed
395 significant negative correlations ($P<0.001$) with EPA-PAH, alkylated PAHs and aromatic
396 compounds concentrations (Table 3). The activities of *Firmicutes* were also significantly ($P<0.05$)
397 and negatively correlated with TPH, TAH, EPA-PAH, alkylated PAHs and aromatic compounds
398 concentrations, even though most of the differences between treatments were not significant
399 (Table 3). *Alphaproteobacteria* and *Betaproteobacteria* were 7.6% and 21.3% more active,
400 respectively, in biofilms exposed to sediments containing higher concentrations of

401 hydrocarbons (black lines) when compared to the other treatments (white and grey lines) (Fig
402 4b and Table S2), which resulted in significant positive correlations (P<0.001) between the
403 activities of these two groups and EPA-PAH, alkylated PAHs and aromatic compound
404 concentrations (Table 3). The relative activity of *Actinobacteria* was significantly (P<0.05) and
405 positively correlated with the concentration of naphthenic acids (Table 3).

406 Chlorophyll a and photosynthesis-related transcripts were 33.3% and 19.5% less
407 abundant, respectively, in the samples exposed to the sediments containing the highest
408 concentration of hydrocarbons (black bars) when compared to the other treatments (white and
409 grey bars) (Fig.6). Chlorophyll a was significantly (P<0.001) and negatively correlated with the
410 concentration of total aromatic compounds, EPA-PAHs and alkylated PAHs (Table 2). Similarly,
411 the expression of photosynthesis-related genes was significantly (P<0.01) and negatively
412 correlated with the concentration of total aromatic compounds, EPA-PAHs and alkylated PAHs
413 (Table 2). A large majority of the *Cyanobacteria* families were negatively correlated to the
414 concentrations of TPH and total aromatic compounds, while almost as many families were
415 positively and negatively correlated to naphthenic acids and n-alkanes (Fig. S2). Very few
416 phyla/classes showed a clear majority of families positively correlated to TPH, n-alkanes and
417 aromatic compounds, with only *Verrucomicrobia* and *Actinobacteria* having a clear majority of
418 families showing positive correlations (Fig. S2). In contrast, for naphthenic acids, most of the
419 phylum/classes had a majority of their families positively correlated with NA concentration (Fig.
420 S2).

421 The biofilms that developed in the presence of the Firebag River lower sediments
422 degraded a higher percentage of the substrates in Biolog assays and clustered away from most

other treatments (Fig. 5b). Within the other treatments, there was clustering of the Steepbank and Ell's River lower samples observed in the lower left quadrant of the PCoA ordination plot (Fig. 5b). The relative abundance of the different functions detected following mRNA sequencing was also used to create an ordination of the samples (Fig. 5c). The biofilms exposed to the Firebag River sediment were clearly distinct from the other samples, and this was partly due to a higher relative abundance of transcripts related to photosynthesis and lower relative abundance of transcripts related to phosphorus, fatty acids, aromatics, secondary metabolism and stress (Fig. 5c). The biofilms that developed in the presence of sediments from the Steepbank River, lower and upper Ell's River and Athabasca River Reference site and downstream of Suncor, clustered together on the left side of the first axis, having relatively higher expression of genes related to fatty acids, aromatics, potassium, secondary metabolism, virulence, iron and amino acids and relatively lower expression of genes related to photosynthesis, respiration and sulfur (Fig. 5c). The biofilms exposed to sediments from the upper Steepbank River, the mid Ell's River and upstream of Suncor clustered together, with relatively more transcripts associated with sulphur metabolism, photosynthesis, respiration and carbohydrates and fewer related to potassium, virulence, iron, amino acids and mobile elements (Fig. 5c). For the most part, photosynthesis-related functions were negatively correlated to different hydrocarbon indicators (Fig. S3), while the relative abundance of functions related to potassium were mostly negatively correlated to TPH and naphthenic acids and mostly positively correlated to n-alkanes (Fig. S3). For the other functional groups (carbohydrates, N, P, Fe and S metabolism), a majority of functions were positively correlated to TPH, naphthenic acids, n-alkanes and aromatic compounds (Fig. S3).

445 No significant pairwise differences among the treatments were observed for the relative
446 expression of alkane monooxygenase genes (Fig. 7). Similarly, very few significant pairwise
447 differences were observed between treatments for the various classes of aromatic ring-cleaving
448 dioxygenases (Fig. 7). For the extradiol type dioxygenases, the only significant difference was
449 between the lower Ell's River treatment and the Firebag River treatments (Fig. 7). For the
450 intradiol type dioxygenases, the Suncor Upstream treatment induced significantly less
451 expression than the Reference site, the lower Ell's River and the lower Steepbank River
452 treatments (Fig. 7). For the gentisate/homogentisate type dioxygenases, the lower Steepbank
453 River treatment induced significantly more expression than the Suncor Upstream treatment
454 (Fig. 7). The relative expression of alkane monooxygenase genes was not correlated to any of
455 the chemical parameters measured, while the expression of cytochrome P450 alkane
456 hydroxylase (CYP153) was significantly ($P<0.05$) and negatively correlated to the concentration
457 of n-alkanes (Table 2). The expression of aromatic ring-cleaving dioxygenase genes was
458 significantly ($P<0.05$) and positively correlated to the concentration of a variety of chemical
459 compounds, with the strongest correlations often being observed with EPA-PAH, alkylated
460 PAHs and aromatic compounds (Table 2).
461

462 **Discussion**

463 In the present study, biofilms were grown in rotating annular reactors to provide a
464 representation of the periphyton of the Athabasca River and its tributaries. Biofilms form at a
465 specific location in response to local environmental conditions; as such they make an ideal
466 resident community for environmental monitoring. They also regulate the physical and
467 chemical microhabitat and contribute to ecosystem processes as a whole (2). In addition there
468 is recruitment of populations to form the biofilm that represents the selection pressure for
469 those conditions and location (4). Therefore the biofilm community is more representative of
470 what would occur within a region of the river and in/on the sediment in particular than the
471 planktonic community. Thus we focused our attention on these communities. Our results
472 suggest that variation in bituminous compound content of the river sediments leads to major
473 differences in biofilm microbial communities. In agreement with our initial hypothesis, two
474 mechanisms, namely inhibitory and increased energy and carbon effects, appear to be at play
475 during the exposure of biofilm microbial communities to bitumen-containing river sediments.
476 Firstly, inhibitory effects were observed as the river sediments having the highest
477 concentrations of bituminous compounds generally led to the development of less productive
478 biofilms, where photosynthetic organisms were particularly repressed as compared to biofilms
479 that developed from river sediments having lower bituminous compound concentrations.
480 Secondly, within the microbial communities, some taxonomic groups (mainly *Proteobacteria*)
481 and functional genes (ring-opening dioxygenases) increased their relative abundance and
482 activity with increasing concentrations of hydrocarbon compounds. So, for general inhibition of
483 the microbial communities, some microorganisms appeared to be stimulated by higher

484 hydrocarbon concentrations, either because they could take advantage of this carbon source or
485 because they were more tolerant to the inhibitory effect of some bituminous compounds.
486
487 *Inhibitory effects*
488 Constant exposure of sediments to higher concentration of bituminous compounds resulted in
489 a decrease in all the productivity indicators (thymidine incorporation, chlorophyll a, and
490 photosynthesis gene expression) and reduced biofilm thickness. These results suggest that the
491 hydrocarbons found in the Athabasca watershed negatively affect the indigenous biofilm-
492 forming microbial communities. Several compounds present in bitumen are inhibitory to
493 organisms. Naphthenic acids (NA) are generally thought to be the most toxic components (10,
494 19), and were shown to not only negatively influence plants, birds, fish, toads, frogs and rats
495 (11, 16, 32, 41, 45) but also to negatively influence bacteria (15, 21), algae (20, 31),
496 cyanobacteria (42) and protozoa (46). However, in the present study, the strongest negative
497 correlations were often observed with aromatic compounds, while very few significant
498 correlations were found with NA and alkanes, suggesting that the aromatic fraction of bitumen
499 might be the most inhibitory or the most available for microorganisms. Aromatic hydrocarbons
500 are recognized to be toxic to a variety of microbial processes. For instance, methanogenesis in
501 sludge was 50% inhibited by a variety of aromatic compounds at concentrations ranging from
502 3.4 to 57.3 mM (5mM≈500 ppm) (47). For nitrification in soils, the no-observed-effect
503 concentrations (NOECs) ranged from 22 to 1,100 mg kg^{-1} (51). However, when directly
504 confronted with aromatic compounds, bacteria appeared to be more sensitive with half
505 maximal effective concentrations (EC50) of luminescent bacterial assays for different PAHs

506 ranging from 0.53 to 24.39 ppm (µg mL^{-1}) (14, 34). In contrast, the EC50 of naphthenic acids in
507 luminescent bacterial assays was reported to range from 41.9 to 64.9 ppm (mg L^{-1}) (15) and
508 even the more toxic aromatic alkanoic naphthenic acids and their degradation products had
509 EC50 ranging from 9.4 to 69.2 ppm (mg L^{-1}) (23). In the current study, NAs were measured as a
510 class using LC-MS-MS and there is no speciation data available, but the concentrations
511 estimated in the reactors (≤0.2ppm, assuming a complete dissolution in the re-circulated water)
512 are considerably lower than those reported in the above toxicological tests. In contrast, for
513 aromatic compounds, the concentrations estimated in the reactors (e.g. EPA-PAH≈1ppm,
514 assuming a complete dissolution in the re-circulated water) are within the range reported as
515 inhibitory in laboratory assays. This might explain the greater number and the higher
516 correlation coefficients of negative correlations between our microbiological indicators to
517 aromatic compounds compared to other hydrocarbon compounds. However, although
518 estimated concentrations of PAH and NA were mostly lower than the levels reported as
519 inhibitory in toxicology tests, a long term cumulative exposure to a persistent suite of
520 contaminants in the environment might affect microbial communities regardless of relatively
521 low concentration.

522 The activities of photosynthetic organisms were strongly repressed when exposed to
523 sediments having the highest content of bituminous compounds. A previous field survey of the
524 bacterial and archaeal communities in sediments from the Athabasca River, its tributaries and
525 oil sand tailings ponds revealed a significant decrease in *Cyanobacteria* relative abundance with
526 increasing sediment hydrocarbon content (57). *Cyanobacteria* appear to be particularly
527 sensitive to environmental disturbance as exposure of reactor grown biofilms to some

528 pharmaceutical products also resulted in a reduction of the relative abundance of
529 *Cyanobacteria* and a reduction of the expression of photosynthetic genes (58). *Cyanobacteria*
530 could thus potentially be used as a bio-indicator to follow the impact of oil sands mining
531 operations. This might be, however, confounded by the apparent insensitivity of some algae to
532 PAHs (13). In aquatic ecosystems, *Cyanobacteria* are involved in major processes (C and N
533 fixation) that are at the base of ecosystem productivity. The lowered photosynthetic activity
534 observed in most biofilms exposed to sediments having higher bituminous compound content
535 could cascade effects on the entire microbial community as well as on higher trophic levels.
536 This is consistent with the generally lower productivity and biomass in these biofilm samples.
537 One indication of this cascade effect on higher trophic levels is the lower abundance of
538 protozoa in reactors inoculated with sediments having high bituminous compound
539 concentrations. However, a reduction in protozoa also results in diminished grazing pressure on
540 the microbial communities (40), which would lead to thicker biofilms if nothing else was
541 influencing the microbial community.
542
543 *Increased carbon and energy*
544 The microbial communities present in the Athabasca watershed are constantly exposed to
545 various components of bitumen through erosion of the oil sands by the river flow and
546 atmospheric deposition through the activity of bitumen upgrading (24-26). One of our
547 hypotheses was that these microbial communities were adapted to this constant flow of
548 organic compounds and were taking advantage of this increased carbon and energy input. The
549 concentration of TPH observed could represent an important carbon and energy source for the

550 microbial community as, in the sediments containing the highest concentrations of TPH, a large
551 portion of the organic matter was due to the presence of bituminous compounds (up to
552 12.76%, Table 1). However, in the case of an increased carbon and energy effect, we would
553 have expected the biofilms developed from sediments containing higher concentrations of
554 bituminous compounds to produce more biomass, which was not the case. In fact, increased
555 bituminous concentrations had mainly inhibitory effects on biofilms, with lowered thickness,
556 lower bacterial, cyanobacterial and algal biomass and lower bacterial productivity. The
557 decrease in biomass/productivity indicators could also be related to an increased selection
558 pressure for the specialist subset of the microbial community that can use bituminous
559 compounds as carbon sources, which might be less productive than other members of the
560 community. In fact, the relative expression of functional genes related to extradiol ring-opening
561 dioxygenases and the relative abundance and activities of *Alphaproteobacteria* and
562 *Betaproteobacteria* was positively correlated with hydrocarbon concentration. Microorganisms
563 of the Athabasca watershed are known to be able to degrade bituminous compounds, many of
564 which belong to the *Proteobacteria* (12, 52, 53). The *Betaproteobacteria* class and the
565 *Proteobacteria* phylum were previously shown to be positively correlated to various
566 hydrocarbon indicators in a field survey of sediments from the Athabasca River, its tributaries
567 and oil sands tailings ponds (57). Alternatively, an increased proportion of bituminous
568 compounds among carbon sources could reduce biofilm productivity as many bituminous
569 compounds are difficult/recalcitrant substrates.

570 The Athabasca river near the oil sands mining operations is considered to be both
571 nitrogen and phosphorus limited based on periphyton assessments (7), and an increased

carbon and energy source might not have an impact if microbial communities are otherwise limited. The carbon and energy effect and the inhibitory effect due to bituminous compounds simultaneously influence Athabasca watershed microorganisms, and inhibitory effects are probably more visible when growth is limited by available nutrients and the input of carbon compounds from bitumen cannot be fully exploited. It appears that in the first 8 weeks of biofilm formation, the inhibitory effects of bituminous compounds override any potential increases in carbon and energy, as all productivity indicators were negatively correlated to bituminous compound concentration. However, in the longer term, the relative increases observed in some bacterial taxa (e.g. *Alpha*- and *Betaproteobacteria*) and in the expression of hydrocarbon degrading genes with increasing bituminous compound concentrations might lead to an adapted community and potentially to biofilms of similar thickness and productivity than those exposed to lower bituminous compound concentrations.

Inhibitory effects and increased carbon and energy effects of bituminous compounds were not the only mechanisms influencing biofilm microbial communities, as the correlation coefficients observed with hydrocarbon concentrations were not perfect and some of the biofilms exposed to sediments did not behave exactly as other biofilms exposed to similarly contaminated sediments. For instance, cyanobacteria, algae and bacteria were lower in the upper Steepbank River, Suncor downstream and Athabasca Reference site where TPH and NA concentrations were also low, while bacterial production was higher in the lower Firebag River where hydrocarbons were higher. Other mechanisms that were not explored here are differences in N and P concentration of the sediments, highly tolerant microorganisms in the original sediment microbial population, sediment granulometry or interactions with other

biota. This is illustrated by the clustering of samples coming from the Firebag River in some of the ordinations shown in Fig. 5. The incomplete solubility and organic matter binding of the bituminous compounds, especially PAHs, could also explain some of the discrepancies observed, as the concentrations in the sediments do not perfectly reflect the concentrations available to the biofilms in the reactors. Even with all these confounding factors, several highly significant correlations, with correlation coefficients below -0.7 and P-values well below 0.001, were found between biological indicators and the concentration of various bituminous compounds. Since most of these correlations were negative, this clearly indicates that biofilm forming organisms in the Athabasca watershed are strongly inhibited by increasing concentrations of bituminous compounds.

In conclusion, the results presented here showed that after 8 weeks of incubation, increased exposure to bituminous compounds reduced biofilm productivity and biomass while stimulating particular microbial taxa and some aerobic hydrocarbon degrading genes. Natural and anthropogenic increases in bituminous substance release in the Athabasca watershed will, in the short term, strongly reduce microbial productivity, but, in the longer term, could lead to the stimulation of already occurring microbes that can utilize the carbon present in bituminous compounds for growth and partly restore productivity, if other nutrients are not limiting.

Acknowledgments

This Project was undertaken with the financial support of Environment Canada's Strategic Applications of Genomics in the Environment (STAGE) Program.

References

1. **Baker, G. C., J. J. Smith, and D. A. Cowan.** 2003. Review and re-analysis of domain-specific 16S primers. J Microbiol Meth **55:**541–555.
2. **Battin, T. J., L. A. Kaplan, J. Denis Newbold, and C. M. E. Hansen.** 2003. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. Nature **426:**439-442.
3. **Bell, T. H., E. Yergeau, C. Maynard, D. Juck, L. G. Whyte, and C. W. Greer.** 2013. Predictable bacterial composition and hydrocarbon degradation in Arctic soils following diesel and nutrient disturbance. ISME J **7:**1200-1210.
4. **Besemer, K., H. Peter, J. B. Logue, S. Langenheder, E. S. Lindstrom, L. J. Tranvik, and T. J. Battin.** 2012. Unraveling assembly of stream biofilm communities. ISME J. **6:**1459-1468.
5. **Blakley, E. R.** 1978. The microbial degradation of cyclohexanecarboxylic acid by a β-oxidation pathway with simultaneous induction to the utilization of benzoate. Can. J. Microbiol. **24:**847-855.
6. **Blakley, E. R.** 1974. The microbial degradation of cyclohexanecarboxylic acid: a pathway involving aromatization to form p-hydroxybenzoic acid. Can. J. Microbiol. **20:**1297-1306.
7. **Chambers, P., A. Dale, G. Scrimgeour, and M. Bothwell.** 2000. Nutrient enrichment of northern rivers in response to pulp mill and municipal discharges. J. Aquat. Ecosyst. Stress Recovery **8:**53-66.
8. **Chambers, P. A., J. M. Culp, N. E. Glozier, K. J. Cash, F. J. Wrona, and L. Noton.** 2006. Northern rivers ecosystem initiative: Nutrients and dissolved oxygen - issues and impacts. Environ. Monit. Assess. **113:**117-141.
9. **Chronopoulou, P.-M., A. Fahy, F. Coulon, S. Païssé, M. Goñi-Urriza, L. Peperzak, L. Acuña Alvarez, B. A. McKew, T. Lawson, K. N. Timmis, R. Duran, G. J. C. Underwood, and T. J. McGenity.** 2013. Impact of a simulated oil spill on benthic phototrophs and nitrogen-fixing bacteria in mudflat mesocosms. Environ. Microbiol. **15:**242-252.
10. **Clemente, J. S., and P. M. Fedorak.** 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere **60:**585-600.
11. **Crowe, A. U., A. L. Plant, and A. R. Kermode.** 2002. Effects of an industrial effluent on plant colonization and on the germination and post-germinative growth of seeds of terrestrial and aquatic plant species. Environ. Pollut. **117:**179-189.
12. **Del Rio, L. F., A. K. M. Hadwin, L. J. Pinto, M. D. MacKinnon, and M. M. Moore.** 2006. Degradation of naphthenic acids by sediment micro-organisms. J. Appl. Microbiol. **101:**1049-1061.
13. **Djomo, J. E., A. Dauta, V. Ferrier, J. F. Narbonne, A. Monkiedje, T. Njine, and P. Garrigues.** 2004. Toxic effects of some major polyaromatic hydrocarbons found in crude oil and aquatic sediments on *Scenedesmus subspicatus*. Water Res. **38:**1817-1821.
14. **El-Alawi, Y. S., B. J. McConkey, D. G. Dixon, and B. M. Greenberg.** 2002. Measurement of Short- and Long-Term Toxicity of Polycyclic Aromatic Hydrocarbons Using Luminescent Bacteria. Ecotoxicol. Environ. Saf. **51:**12-21.
15. **Frank, R. A., R. Kavanagh, B. Kent Burnison, G. Arsenault, J. V. Headley, K. M. Peru, G. Van Der Kraak, and K. R. Solomon.** 2008. Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere **72:**1309-1314.
16. **Gentes, M.-L., C. Waldner, Z. Papp, and J. E. G. Smits.** 2006. Effects of oil sands tailings compounds and harsh weather on mortality rates, growth and detoxification efforts in nestling tree swallows (*Tachycineta bicolor*). Environ. Pollut. **142:**24-33.

17. **Gilde, K., and J. L. Pinckney.** 2012. Sublethal Effects of Crude Oil on the Community Structure of Estuarine Phytoplankton. Estuaries Coasts **35:**853-861.
18. **Hamady, M., C. Lozupone, and R. Knight.** 2010. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. ISME J **4:**17-27.
19. **Han, X., M. D. MacKinnon, and J. W. Martin.** 2009. Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS. Chemosphere **76:**63-70.
20. **Headley, J. V., and D. W. McMartin.** 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. J. Environ. Sci. Health., Part A **39:**1989-2010.
21. **Herman, D. C., P. M. Fedorak, M. D. MacKinnon, and J. W. Costerton.** 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Can. J. Microbiol. **40:**467-477.
22. **Holden, A. A., R. B. Donahue, and A. C. Ulrich.** 2011. Geochemical interactions between process-affected water from oil sands tailings ponds and North Alberta surficial sediments. J. Contam. Hydrol. **119:**55-68.
23. **Johnson, R. J., B. E. Smith, P. A. Sutton, T. J. McGenity, S. J. Rowland, and C. Whitby.** 2011. Microbial biodegradation of aromatic alkanoic naphthenic acids is affected by the degree of alkyl side chain branching. ISME J **5:**486-496.
24. **Kelly, E. N., D. W. Schindler, P. V. Hodson, J. W. Short, R. Radmanovich, and C. C. Nielsen.** 2010. Oil sands development contributes elements toxic at low concentrations to the Athabasca River and its tributaries. Proc. Natl. Acad. Sci. USA **107:**16178-16183.
25. **Kelly, E. N., J. W. Short, D. W. Schindler, P. V. Hodson, M. Ma, A. K. Kwan, and B. L. Fortin.** 2009. Oil sands development contributes polycyclic aromatic compounds to the Athabasca River and its tributaries. Proc. Natl. Acad. Sci. USA **107:**16178-16183.
26. **Kurek, J., J. L. Kirk, D. C. G. Muir, X. Wang, M. S. Evans, and J. P. Smol.** 2013. Legacy of a half century of Athabasca oil sands development recorded by lake ecosystems. Proc. Natl. Acad. Sci. USA **110:**1761-1766.
27. **Larson, C. A., and S. I. Passy.** 2013. Rates of species accumulation and taxonomic diversification during phototrophic biofilm development are controlled by both nutrient supply and current velocity. Appl. Environ. Microbiol. **79:**2054-2060.
28. **Lawrence, J. R., M. R. Chenier, R. Roy, D. Beaumier, N. Fortin, G. D. W. Swerhone, T. R. Neu, and C. W. Greer.** 2004. Microscale and molecular assessment of impacts of nickel, nutrients, and oxygen level on structure and function of river biofilm communities. Appl. Environ. Microbiol. **70:**4326–4339.
29. **Lawrence, J. R., G. D. W. Swerhone, and T. R. Neu.** 2000. A simple rotating annular reactor for replicated biofilm studies. J. Microbiol. Methods **42:**215–224.
30. **Lawrence, J. R., B. Zhu, G. D. W. Swerhone, J. Roy, L. I. Wassenaar, E. Topp, and D. R. Korber.** 2009. Comparative microscale analysis of the effects of triclosan and triclocarban on the structure and function of river biofilm communities. Sci. Total Environ. **407:**3307-3316.
31. **Leung, S. S., M. D. MacKinnon, and R. E. H. Smith.** 2003. The ecological effects of naphthenic acids and salts on phytoplankton from the Athabasca oil sands region. Aquat. Toxicol. **62:**11-26.
32. **Lister, A., V. Nero, A. Farwell, D. G. Dixon, and G. Van Der Kraak.** 2008. Reproductive and stress hormone levels in goldfish (*Carassius auratus*) exposed to oil sands process-affected water. Aquat. Toxicol. **87:**170-177.
33. **Manz, W., K. Wendt-Potthoff, T. R. Neu, U. Szewzyk, and J. R. Lawrence.** 1999. Phylogenetic Composition, Spatial Structure, and Dynamics of Lotic Bacterial Biofilms Investigated by Fluorescent in Situ Hybridization and Confocal Laser Scanning Microscopy. Microb. Ecol. **37:**225-237.

34. **McConkey, B. J., C. L. Duxbury, D. G. Dixon, and B. M. Greenberg.** 1997. Toxicity of a pah photooxidation product to the bacteria *Photobacterium phosphoreum* and the duckweed *Lemna gibba*: Effects of phenanthrene and its primary photoproduct, phenanthrenequinone. Environ. Toxicol. Chem. **16:**892-899.
35. **Meyer, F., D. Paarmann, M. D'Souza, R. Olson, E. M. Glass, M. Kubal, T. Paczian, A. Rodriguez, R. Stevens, A. Wilke, J. Wilkening, and R. A. Edwards.** 2008. The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics **9:**386.
36. **Meyer, M., and M. Kircher.** 2010. Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing. Cold Spring Harb Protoc **doi:10.1101/pdb.prot5448**.
37. **Neu, T. R., G. D. W. Swerhone, U. Bockelmann, and J. R. Lawrence.** 2005. Effect of CNP on composition and structure of lotic biofilms as detected with lectin-specific glycoconjugates. Aquat. Microb. Ecol. **38:**283–294.
38. **Neu, T. R., G. D. W. Swerhone, and J. R. Lawrence.** 2001. Assessment of lectin-binding analysis for in situ detection of glycoconjugates in biofilm systems. Microbiology **147:**299-313.
39. **Neu, T. R., S. Woelfl, and J. R. Lawrence.** 2004. Three-dimensional differentiation of photo-autotrophic biofilm constituents by multi-channel laser scanning microscopy (single-photon and two-photon excitation). J. Microbiol. Meth. **56:**161-172.
40. **Parry, J. D.** 2004. Protozoan Grazing of Freshwater Biofilms. Adv. Appl. Microbiol. **54:**167-196.
41. **Pollet, I., and L. I. Bendell-Young.** 2000. Amphibians as indicators of wetland quality in wetlands formed from oil sands effluent. Environ. Toxicol. Chem. **19:**2589-2597.
42. **Quagraine, E. K., H. G. Peterson, and J. V. Headley.** 2005. In situ bioremediation of naphthenic acids contaminated tailing pond waters in the Athabasca oil sands region - Demonstrated field studies and plausible options: A review. J. Environ. Sci. Health. Part A Toxic/Hazard. Subst. Environ. Eng. **40:**685-722.
43. **Quince, C., A. Lanzen, R. J. Davenport, and P. J. Turnbaugh.** 2011. Removing noise from pyrosequenced amplicons. BMC Bioinformatics **12:**38.
44. **Robarts, R. D., and R. J. Wicks.** 1989. [Methyl-3H] thymidine macromolecular incorporation and lipid labeling: their significance to DNA labelling during measurements of aquatic bacterial growth rate. Limnol. Oceanogr. **34:**213-222.
45. **Rogers, V. V., M. Wickstrom, K. Liber, and M. D. MacKinnon.** 2002. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. **66:**347-355.
46. **Scarlett, A. G., C. E. West, D. Jones, T. S. Galloway, and S. J. Rowland.** 2012. Predicted toxicity of naphthenic acids present in oil sands process-affected waters to a range of environmental and human endpoints. Sci. Total Environ. **425:**119-127.
47. **Sierra-Alvarez, R., and G. Lettinga.** 1991. The effect of aromatic structure on the inhibition of acetoclastic methanogenesis in granular sludge. Appl. Microbiol. Biotechnol. **34:**544-550.
48. **Stewart, F. J., E. A. Ottesen, and E. F. DeLong.** 2010. Development and quantitative analyses of a universal rRNA-subtraction protocol for microbial metatranscriptomics. ISME J. **4:**896-907.
49. **Strausz, O. P., E. M. Lown, A. Morales-Izquierdo, N. Kazmi, D. S. Montgomery, J. D. Payzant, and J. Murgich.** 2011. Chemical Composition of Athabasca Bitumen: The Distillable Aromatic Fraction. Energy Fuels **25:**4552-4579.
50. **Strausz, O. P., A. Morales-Izquierdo, N. Kazmi, D. S. Montgomery, J. D. Payzant, I. Safarik, and J. Murgich.** 2010. Chemical Composition of Athabasca Bitumen: The Saturate Fraction. Energy Fuels **24:**5053-5072.

51. **Sverdrup, L. E., F. Ekelund, P. H. Krogh, T. Nielsen, and K. Johnsen.** 2002. Soil microbial toxicity of eight polycyclic aromatic compounds: Effects on nitrification, the genetic diversity of bacteria, and the total number of protozoans. Env. Toxicol. Chem. **21:**1644-1650.

52. **Wyndham, R. C., and J. W. Costerton.** 1981. Heterotrophic potentials and hydrocarbon biodegradation potentials of sediment microorganisms within the Athabasca oil sands deposit. Appl. Environ. Microbiol. **41:**783-790.

53. **Wyndham, R. C., and J. W. Costerton.** 1981. In vitro microbial degradation of bituminous hydrocarbons and in situ colonization of bitumen surfaces within the Athabasca oil sands deposit. Appl. Environ. Microbiol. **41:**791-800.

54. **Yergeau, E., S. Bokhorst, A. H. L. Huiskes, H. T. S. Boschker, R. Aerts, and G. A. Kowalchuk.** 2007. Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. FEMS Microbiol. Ecol. **59:**436–451.

55. **Yergeau, E., and G. A. Kowalchuk.** 2008. Responses of Antarctic soil microbial communities and associated functions to temperature and freeze-thaw cycle frequency. Environ. Microbiol. **10:**2223–2235.

56. **Yergeau, E., J. R. Lawrence, D. R. Korber, M. J. Waiser, and C. W. Greer.** 2010. Meta-transcriptomic analysis of the response of river biofilms to pharmaceutical products using anonymous DNA microarrays. Appl. Environ. Microbiol. **76:**5432-5439.

57. **Yergeau, E., J. R. Lawrence, S. Sanschagrin, M. J. Waiser, D. R. Korber, and C. W. Greer.** 2012. Next-generation sequencing of microbial communities in the Athabasca River and its tributaries in relation to oil sands mining activities. Appl. Environ. Microbiol. **78:**7626-7637.

58. **Yergeau, E., S. Sanschagrin, M. J. Waiser, J. R. Lawrence, and C. W. Greer.** 2012. Sub-inhibitory concentrations of different pharmaceutical products affect the meta-transcriptome of river biofilm communities cultivated in rotating annular reactors. Environ. Microbiol. Rep. **4:**350-359.

781 **Table 1**: Organic components concentration of the sediments used in the biofilm reactors

	Org. matter	TPH	TPH/ total C	TSH	TAH	NA	EPA PAH	Total alkylated PAHs	Total aromatic compounds	Total n-alkane
	% (w/w)	µg/g	%	µg/g	µg/g	mg/ml	ng/g	ng/g	ng/g	ng/g
Athabasca River										
Reference Site	2.10	251.3	1.20	104.0	147.3	ND	1176.3	1235.3	1406.3	5831.7
Suncor Upstream	2.20	104.9	0.48	52.2	52.7	1	188.6	188.2	248.6	4241.7
Suncor Downstream	1.35	68.5	0.51	32.6	35.9	ND	127.7	130.9	172.8	1014.3
Tributaries										
Ell's River Upper	3.18	66.3	0.21	35.1	31.2	1	218.7	197.7	299.5	2252.2
Ell's River Mid	1.06	139.6	1.32	69.5	70.1	3	433.6	677.1	711.9	660.1
Ell's River Lower	2.19	2792.4	12.76	1638.4	1154.0	4	9497.4	14661.6	15138.0	2111.3
Firebag River Upper	2.49	34.1	0.14	19.6	14.5	1	35.9	33.6	39.9	3576.2
Firebag River Mid	12.96	129.1	0.10	87.1	42.0	1	89.4	73.0	100.6	7815.9
Firebag River Lower	5.05	1995.4	3.96	977.9	1017.5	3	854.2	762.5	932.0	1703.5
Steepbank River Upper	5.53	121.2	0.22	52.7	68.5	4	82.3	105.4	125.7	4522.2
Steepbank River Mid	3.69	840.3	2.28	420.1	420.2	0	5854.3	8925.4	9525.8	1146.1
Steepbank River Lower	4.26	3879.8	9.12	1947.7	1932.1	3	9209.7	13348.1	13866.6	16570.7

782 All values except NA are per dry weight of sediments. TPH: total petroleum hydrocarbon; TSH: total straight chain hydrocarbon; TAH: total
783 aromatic hydrocarbon; NA: naphthenic acids; EPA-PAH: US Environmental Protection Agency 16 priority polycyclic aromatic hydrocarbon; PAH:
784 polycyclic aromatic hydrocarbon
785 ND: not determined. Data are modified from Yergeau et al. (57).

Table 2: Correlation coefficients between sediment chemical concentrations and biofilm biological data

	TPH	TSH	TAH	NA	EPA-PAH	Alkylated PAHs	Aromatic compounds	n-alkanes
Protozoa	**-0.71**	**-0.69**	**-0.74**	**-0.74**	*-0.51*	**-0.56**	**-0.56**	-0.01
Algae	**-0.60**	**-0.55**	**-0.66**	-0.24	**-0.72**	**-0.75**	**-0.75**	0.06
Cyanobacteria	-0.33	-0.32	-0.33	-0.02	-0.38	-0.40	-0.40	-0.06
Bacteria	*-0.50*	*-0.47*	*-0.54*	-0.22	**-0.63**	**-0.66**	**-0.66**	0.18
glc(NAc)$_2$, neuNAc	-0.22	-0.17	-0.28	-0.42	-0.39	-0.43	-0.43	0.25
Terminal β gal, galNAc	-0.08	-0.08	-0.09	0.05	-0.07	-0.09	-0.09	0.05
α man, α glc	0.18	0.17	0.21	0.26	0.38	0.37	0.37	-0.25
Terminal α or β galNAc	*0.50*	*0.52*	*0.45*	-0.01	0.29	0.27	0.27	0.11
α-L-fucose	0.35	0.36	0.28	-0.17	0.24	0.21	0.21	0.05
Thickness	*-0.54*	*-0.50*	**-0.59**	-0.36	**-0.74**	**-0.77**	**-0.77**	0.19
Bacterial productivity	-0.17	-0.15	-0.25	-0.15	-0.36	-0.41	-0.41	-0.23
Chlorophyll a	-0.28	-0.25	-0.33	-0.17	**-0.55**	**-0.58**	**-0.58**	0.15
mRNA								
Photosynthesis	-0.31	-0.30	-0.31	-0.03	*-0.47*	*-0.48*	*-0.48*	-0.14
Alkane monooxygenase	0.06	0.05	0.07	0.06	0.05	0.07	0.07	0.19
Cytochrome P450	-0.19	-0.25	-0.20	0.06	-0.19	-0.21	-0.21	-0.36
Extradiol	0.31	0.31	0.37	0.31	0.37	0.40	0.40	0.25
Intradiol	0.29	0.30	0.27	0.38	0.32	0.33	0.33	0.21
Gentisate/homogentisate	0.27	0.24	0.28	0.36	0.16	0.20	0.20	0.21
Sum aromatic deg. genes	0.41	0.39	*0.42*	*0.49*	0.38	0.41	0.41	0.23

Underline: P-value below 0.05; Italics: P-value below 0.01; Bold: P-value below 0.001

TPH: total petroleum hydrocarbon; TSH: total straight chain hydrocarbon; TAH: total aromatic hydrocarbon; NA: naphthenic acids; EPA-PAH: US Environmental Protection Agency 16 priority polycyclic aromatic hydrocarbon; PAH: polycyclic aromatic hydrocarbon

glc(NAc)$_2$: N-acetylglucosamine; neuNAc: N-Acetylneuraminic acid; terminal β gal: terminal β-galactose; galNAc: N-Acetylgalactosamine; α man: α mannose; α glc: α glucose; terminal α or β galNAc: terminal α- or β-linked N-acetylgalactosamine.

Intradiol represents a sum of MG-RAST functions "Catechol 1,2-dioxygenase (EC 1.13.11.1)", "Catechol 1,2-dioxygenase 1 (EC 1.13.11.1)", "Intradiol ring-cleavage dioxygenase (EC 1.13.11.1)", "Protocatechuate 3,4-dioxygenase alpha chain (EC 1.13.11.3)", and "Protocatechuate 3,4-dioxygenase beta chain (EC 1.13.11.3)"; Extradiol represents a sum of MG-RAST functions "catalytic subunit of meta cleavage enzyme", "Catechol 2,3-dioxygenase (EC 1.13.11.2)", "extradiol dioxygenase large subunit", "Protocatechuate 4,5-dioxygenase alpha chain (EC 1.13.11.8)", "Protocatechuate 4,5-dioxygenase beta chain (EC 1.13.11.8)", "Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39)", and "1,2-dihydroxynaphthalene dioxygenase"; Gentisate/homogentisate represents a sum of MG-RAST functions "Gentisate 1,2-dioxygenase (EC 1.13.11.4)" and "Homogentisate 1,2-dioxygenase (EC 1.13.11.5)".

Table 3: Spearman correlation coefficients between the most abundant phylum/class and sediment chemical concentrations.

	TPH	TSH	TAH	NA	EPA.PAH	Alkylated PAHs	Aromatic compounds	n-alkanes
16S rRNA gene								
Actinobacteria	-0.30	-0.30	-0.29	*0.46*	*-0.51*	*-0.47*	*-0.47*	-0.09
Bacteroidetes	-0.22	-0.22	-0.29	-0.05	**-0.56**	**-0.59**	**-0.59**	0.08
Cyanobacteria	-0.18	-0.17	-0.19	-0.05	-0.26	-0.27	-0.27	0.17
Firmicutes	0.00	0.07	-0.08	-0.41	0.10	0.04	0.04	0.40
Alpha-	**0.56**	*0.51*	**0.60**	0.29	**0.70**	**0.73**	**0.73**	*-0.48*
Beta-	0.21	0.24	0.25	-0.26	**0.55**	**0.56**	**0.56**	0.04
Gamma-	0.07	0.04	0.10	*0.52*	-0.15	-0.11	-0.11	0.14
Delta-	0.21	0.24	0.13	-0.08	0.05	0.01	0.01	-0.03
mRNA								
Actinobacteria	-0.04	-0.05	0.02	0.37	-0.28	-0.23	-0.23	0.17
Bacteroidetes	0.08	0.07	0.00	0.09	-0.12	-0.14	-0.14	0.36
Cyanobacteria	-0.27	-0.27	-0.28	0.03	**-0.56**	**-0.57**	**-0.57**	0.05
Firmicutes	-0.34	-0.32	-0.39	-0.05	*-0.52*	**-0.54**	**-0.54**	-0.16
Alpha-	0.30	0.29	0.30	-0.16	**0.60**	**0.60**	**0.60**	-0.12
Beta-	0.26	0.27	0.31	-0.21	**0.58**	**0.59**	**0.59**	0.06
Gamma-	0.25	0.21	0.26	0.24	0.26	0.29	0.29	0.01
Delta-	-0.10	-0.18	-0.09	-0.03	0.18	0.17	0.17	-0.39

Underline: P-value below 0.05; Italics: P-value below 0.01; Bold: P-value below 0.001

TPH: total petroleum hydrocarbon; TSH: total straight chain hydrocarbon; TAH: total aromatic hydrocarbon; NA: naphthenic acids; EPA-PAH: US Environmental Protection Agency 16 priority polycyclic aromatic hydrocarbon; PAH: polycyclic aromatic hydrocarbon

Figure legends

Figure 1: Biofilm thickness and composition of the extracellular polysaccharide (EPS) matrix based on lectin binding assays for biofilm grown in rotating reactors inoculated with sediments from the Athabasca River and its tributaries. Values are mean of triplicate measurements and different letters indicate significant differences in Tukey HSD post-hoc tests (at $P<0.05$). α man: α mannose; α glc: α glucose; $glc(NAc)_2$: N-acetylglucosamine; neuNAc (N-Acetylneuraminic acid); terminal α or β galNAc: terminal α- or β-linked N-acetylgalactosamine; terminal β gal: terminal β-galactose; galNAc: N-Acetylgalactosamine. Sediment [TPH]: White fill: [TPH]<125 µg/g; Grey fill: 125 µg/g<[TPH]<750 µg/g; Black fill: [TPH]>750 µg/g

Figure 2: Montage of confocal laser scanning microscopy images of biofilm grown in rotating reactors inoculated with sediments from the Athabasca River and its tributaries. Images were selected based on their similarity to the mean results obtained for algal, bacterial and cyanobacterial biomass for each treatment so they are the images that best represent the results obtained for any treatment via image analyses of the stack. (Bacteria = green, exopolymer = red, Cyanobacteria+Algae = blue).

Figure 3: Amount of bacterial, cyanobacterial and algal biofilm components based on confocal laser scanning microscopy imaging and numbers of protozoa based on direct counts over a period of 8 weeks for biofilm grown in rotating reactors inoculated with sediments from the Athabasca River and its tributaries. Values are mean of triplicate measurements and different letters indicate significant differences in Tukey HSD post-hoc tests (at $P<0.05$). Sediment [TPH]: White fill: [TPH]<125 µg/g; Grey fill: 125 µg/g<[TPH]<750 µg/g; Black fill: [TPH]>750 µg/g

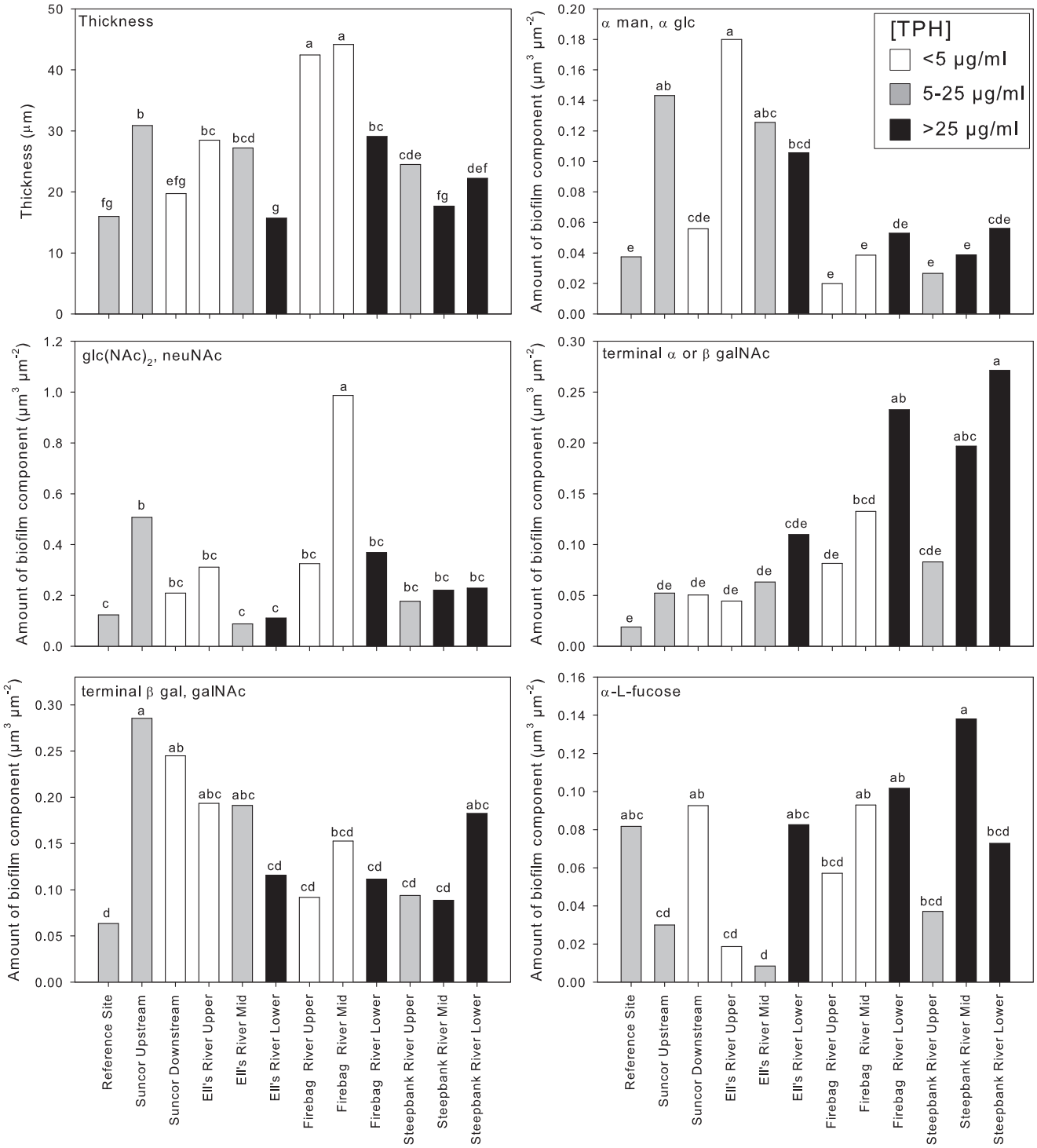
Figure 4: Bacterial community composition based on Ion Torrent 16S rRNA gene amplicon (~120 bp) sequencing (a) or on the taxonomic affiliation of the sequenced mRNA (b) for biofilm grown in rotating reactors inoculated with sediments from the Athabasca River and its tributaries. For each treatment, means of triplicates are presented. See Tables S1 and S2 for ANOVA and Tukey test results.

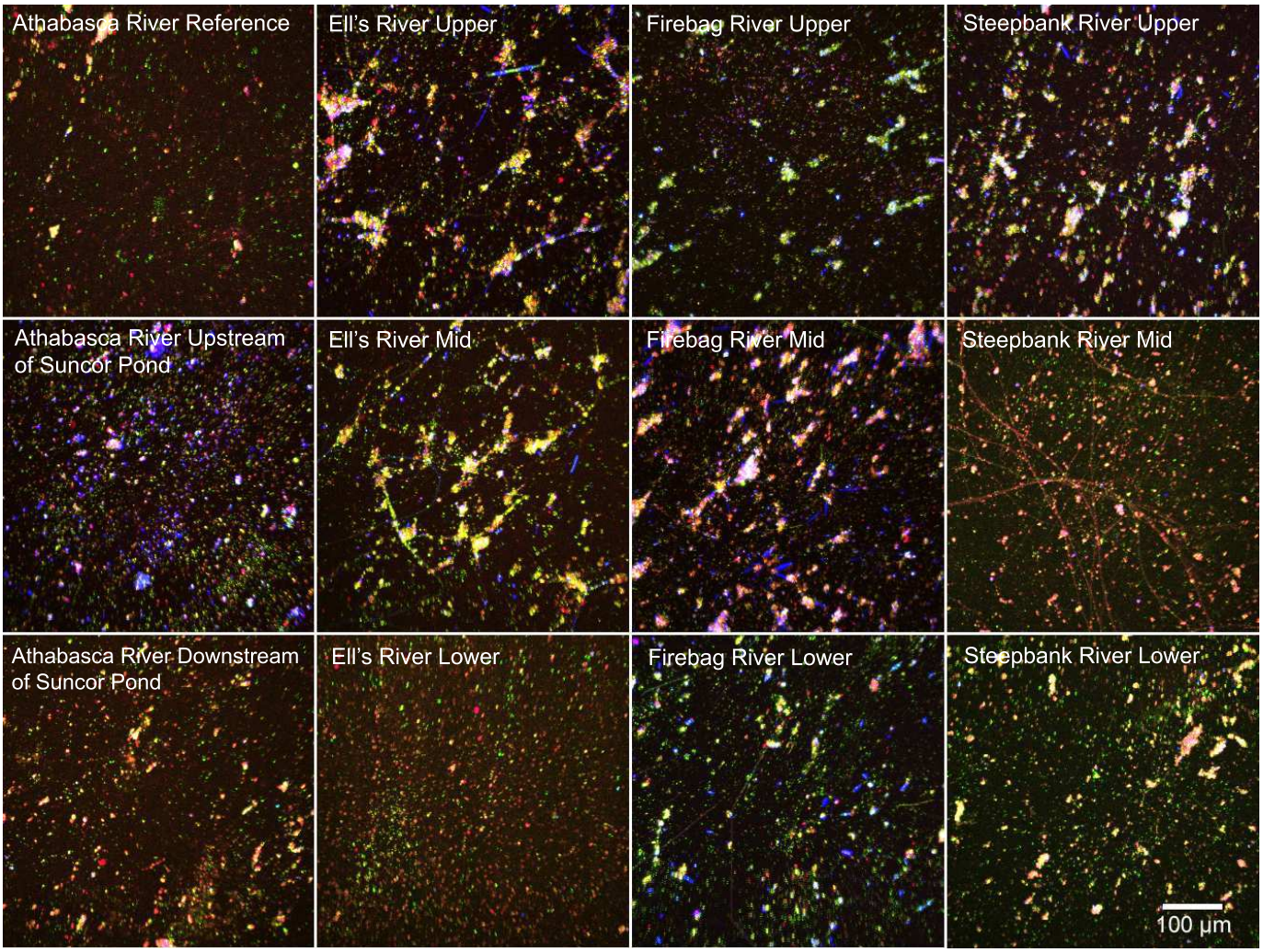
Figure 5: Principal coordinate analysis of the (A) community composition based on 16S rRNA gene amplicon sequencing (Unifrac values) (B) Biolog assay color intensity and (C) the functional composition based on mRNA sequencing for biofilm grown in rotating reactors inoculated with sediments from the Athabasca River and its tributaries. Arrows indicate samples having the highest values for that variable and were added as supplementary variables and are not involved in the calculation of the ordination. AR: Reference site, US: Upstream Suncor, DS: Downstream Suncor , EU: Ell's River Upper, EM: Ell's River Mid, EL: Ell's River Lower, FU: Firebag River Upper, FM: Firebag River Mid, FL: Firebag River Lower, SU: Steepbank River Upper, SM: Steepbank River Mid, SL: Steepbank River Lower. For (B), pol: polymer, sum of the color intensity for α-cyclodextrin, Tween 40 and Tween 80; cb: carbohydrate, sum of the color

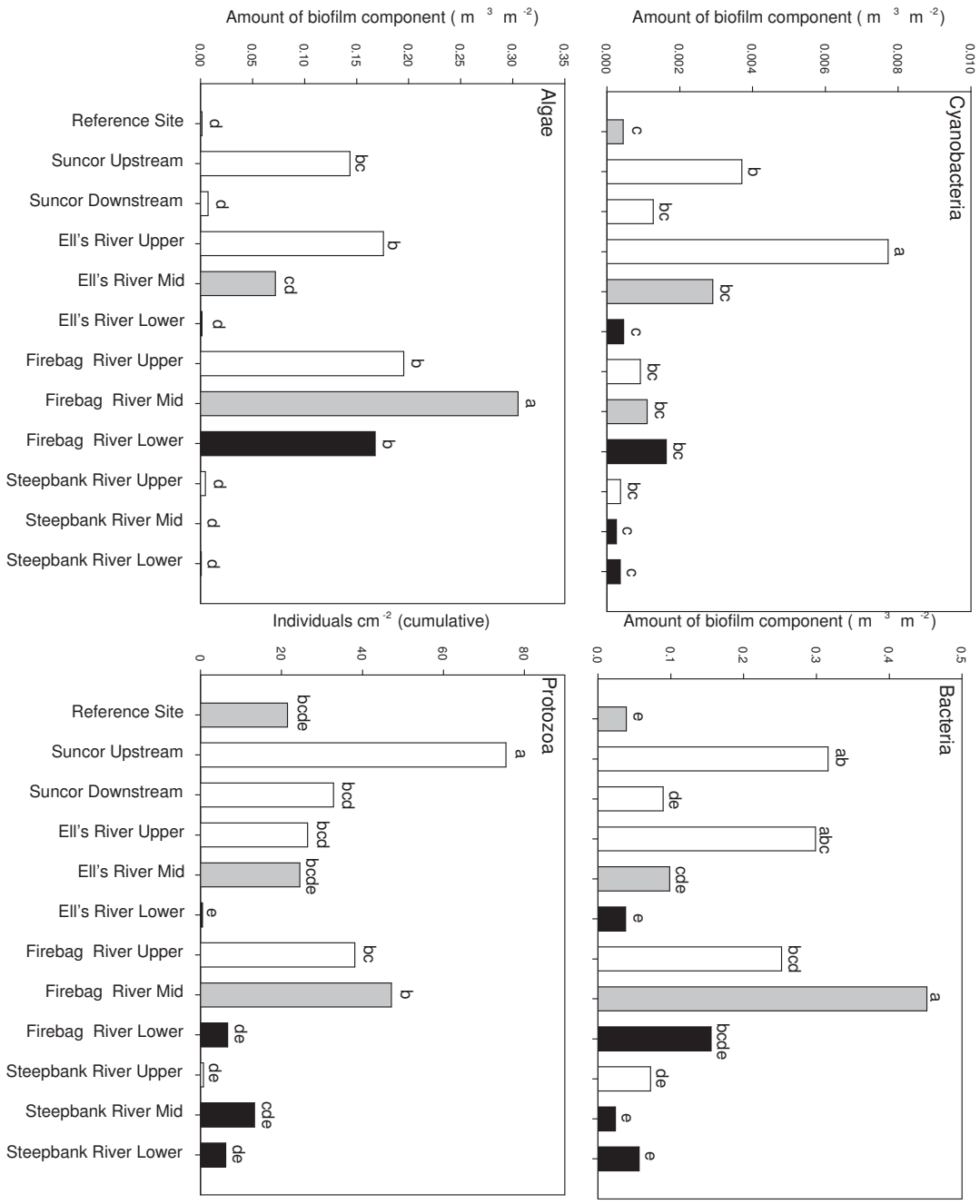
intensity for D-xylose, I-erythritol, glycogen, ß-methyl-D-glucoside, N-acetyl-D-glucosamine, D-cellobiose, α-D-lactose and D-mannitol; ca: carboxylic acid, sum of the color intensity for 2-hydroxy benzoic acid, α-keto butyric acid, itaconic acid, D-malic acid, D-galactonic acid γ-lactone, D-glucosaminic acid, 4-hydroxy benzoic acid, γ-hydroxybutyric acid and D-galacturonic acid; aa: amino acid, sum of the intensity for L-threonine, glycyl-L-glutamic acid, L-phenylalanine, L-serine, L-arginine and L-asparagine; am: amine, sum of color intensity for putrescine and phenylethylamine; ph: phosphorylated, sum of the color intensity for D,L-α-glycerol phosphate and glucose-1-phosphate; es: ester, color intensity for pyruvic acid methyl ester.

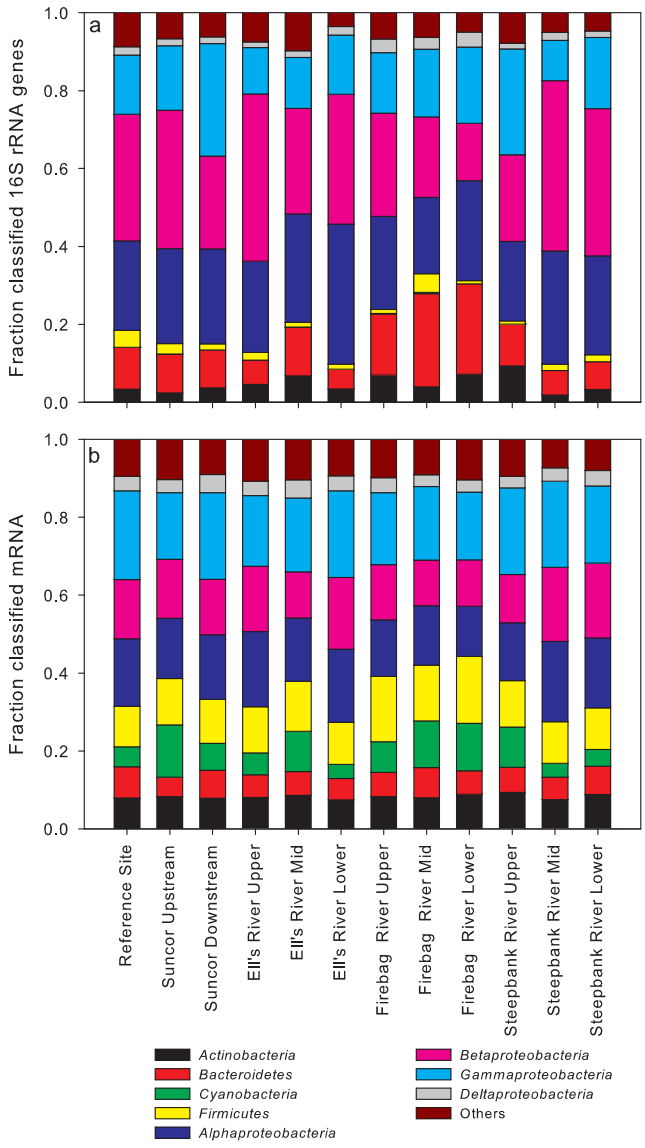
Figure 6: Bacterial productivity, chlorophyll a content and relative abundance of mRNA related to photosynthesis for biofilm grown in rotating reactors inoculated with sediments from the Athabasca River and its tributaries. Values are mean of triplicate measurements and different letters indicate significant differences in Tukey HSD post-hoc tests (at $P<0.05$). Sediment [TPH]: White fill: [TPH]<125 µg/g; Grey fill: 125 µg/g<[TPH]<750 µg/g; Black fill: [TPH]>750 µg/g

Figure 7: Relative abundance of mRNA related to alkane monooxygenase and extradiol, intradiol and gentisate/homogentisate type aromatic ring cleaving enzymes for biofilm grown in rotating reactors inoculated with sediments from the Athabasca River and its tributaries. Intradiol represents a sum of MG-RAST functions “Catechol 1,2-dioxygenase (EC 1.13.11.1)”, “Catechol 1,2-dioxygenase 1 (EC 1.13.11.1)”, “Intradiol ring-cleavage dioxygenase (EC 1.13.11.1)”, “Protocatechuate 3,4-dioxygenase alpha chain (EC 1.13.11.3)”, and “Protocatechuate 3,4-dioxygenase beta chain (EC 1.13.11.3)”; Extradiol represents a sum of MG-RAST functions “catalytic subunit of meta cleavage enzyme”, “Catechol 2,3-dioxygenase (EC 1.13.11.2)”, “extradiol dioxygenase large subunit”, “Protocatechuate 4,5-dioxygenase alpha chain (EC 1.13.11.8)”, “Protocatechuate 4,5-dioxygenase beta chain (EC 1.13.11.8)”, “Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39)”, and “1,2-dihydroxynaphthalene dioxygenase”; Gentisate/homogentisate represents a sum of MG-RAST functions “Gentisate 1,2-dioxygenase (EC 1.13.11.4)” and “Homogentisate 1,2-dioxygenase (EC 1.13.11.5)”. Values are mean of triplicate measurements and different letters indicate significant differences in Tukey HSD post-hoc tests (at $P<0.05$). Sediment [TPH]: White fill: [TPH]<125 µg/g; Grey fill: 125 µg/g<[TPH]<750 µg/g; Black fill: [TPH]>750 µg/g

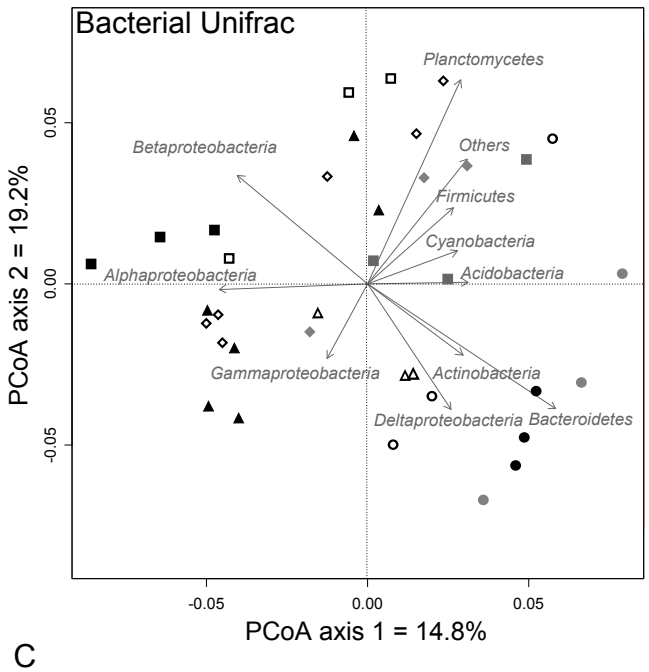




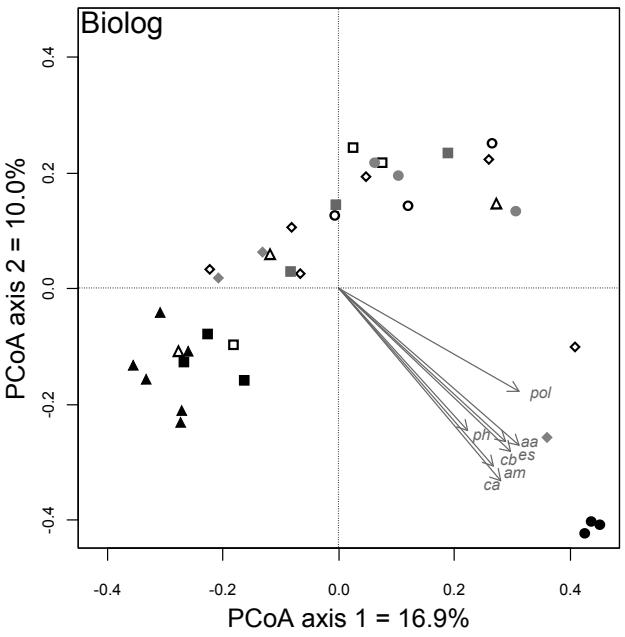




A



B



C