Effects of Crude Oil, Dispersant, and Oil-Dispersant Mixtures on Human Fecal Microbiota in an *In Vitro* Culture System

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ABSTRACT The Deepwater Horizon oil spill of 2010 raised concerns that dispersant and dispersed oil, as well as crude oil itself, could contaminate shellfish and seafood habitats with hazardous residues that had potential implications for human health and the ecosystem. However, little is known about the effects of crude oil and dispersant on the human fecal microbiota. The aim of this research was to evaluate the potential effects of Deepwater Horizon crude oil, Corexit 9500 dispersant, and their combination on human fecal microbial communities, using an *in vitro* culture test system. Fecal specimens from healthy adult volunteers were made into suspensions, which were then treated with oil, dispersant, or oil-dispersant mixtures under anaerobic conditions in an *in vitro* culture test system. Perturbations of the microbial community, compared to untreated control cultures, were assessed using denaturing gradient gel electrophoresis (DGGE), real-time PCR, and pyrosequencing methods. DGGE and pyrosequencing analysis showed that oil-dispersant mixtures reduced the diversity of fecal microbiota from all individuals. Real-time PCR results indicated that the copy numbers of 16S rRNA genes in cultures treated with dispersed oil or oil alone were significantly lower than those in control incubations. The abundance of the *Bacteroidetes* decreased in crude oil-treated and dispersed-oil-treated cultures, while the *Proteobacteria* increased in cultures treated with dispersed oil. In conclusion, the human fecal microbiota was affected differently by oil and dispersed oil, and the influence of dispersed oil was significantly greater than that of either oil or dispersant alone compared to control cultures.

IMPORTANCE There have been concerns whether human health is adversely affected by exposure to spilled crude oil, which contains regulated carcinogens, such as polycyclic aromatic hydrocarbons. In this study, we determined the effect of BP Deepwater Horizon crude oil and oil dispersant on the human intestinal microbiota, since there is the potential that low-level residues of petrochemicals could contaminate seafood. The results of this study will increase our understanding of the ecophysiological changes in the microbial communities of the human gastrointestinal tract with respect to crude oil exposure.

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The Deepwater Horizon oil spill event resulted in an estimated 4.9 million barrels of crude oil being spilled into the northern Gulf of Mexico between 20 April 2010 and 15 July 2010, spread over 600 miles of coast from Florida to Texas (1, 2). To mitigate the effect of the oil spill, accelerate natural dispersion, and enhance biodegradation, approximately 1.5 million gallons of a chemical dispersant, Corexit 9500, were sprayed onto the surface of the Gulf of Mexico and also applied at the underwater pipe source of the leak (3). Affected areas used for commercial and recreational fishing were closed, due to concerns for seafood safety, in May 2010, and were completely reopened to fisheries in April 2011 (4). Despite the oil being highly dispersible and readily biodegradable (1), concerns have been raised about the short- and long-term impacts of residual oil components from the Deepwater Horizon oil spill on the environment and exposed vulnerable populations (5).

Crude oil consists of a complex mixture of organic compounds that includes volatile and nonvolatile components, such as saturated hydrocarbons, cycloalkanes, aromatic compounds, polycyclic aromatic hydrocarbons (PAHs), polar compounds, and resins/asphaltene compounds. Because some PAHs are considered potential human carcinogens that accumulate in seafood products, several studies have used total petroleum hydrocarbon (TPH) concentrations and PAH levels as indicators of potential oil contamination in seafood at oil spill sites (6–9). The toxicity of oil and oil dispersants to aquatic organisms has been widely evaluated (10–13). Several groups have studied the toxicity of dispersants and dispersed oil, the effects of oil and dispersants on soil and near-shore marine microbial communities, and the impacts of oil on mangrove bacterial populations (3, 14–16).

Although many toxicological studies have been conducted, little is known about the effects of exposure to crude oil and dispersants on the human intestinal microbiota. The bioaccumulation of dangerous petrochemical compounds like PAHs in aquatic or-
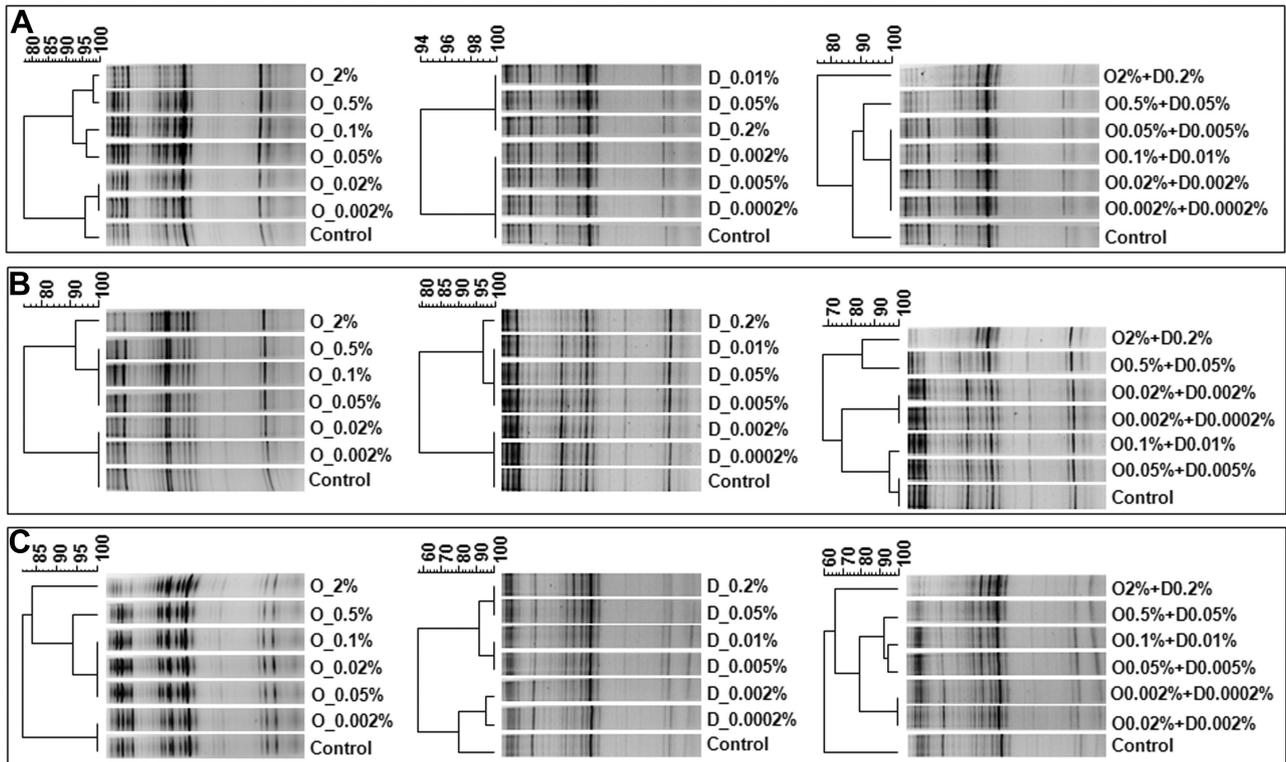
FIG 1 Cluster analyses of denaturing gradient gel electrophoresis (DGGE) profiles from fecal microbiota cultures treated with crude oil (O_proportion of treated oil), dispersant (D_proportion of dispersant), or oil-dispersant mixture (O_proportion of oil plus D_proportion of the dispersant) compared with control incubations (not treated). Fecal samples from three different individuals (individuals A, B, and C) were compared to each other.

ganisms and the potential chance to contaminate seafood products via food chain dynamics may cause unintended consequences to the intestinal microbiota of the consumer and pose a public health risk. Potential consequences include a shift in the diversity of the microbial community, which may alter the ability of the indigenous microbiota to prevent colonization of the gastrointestinal tract by potential enteropathogens or affect the host physiology and immune system (17, 18). Although there may be a low potential for crude oil residues to bioaccumulate in seafood and be ingested, to our knowledge, there are few data available on the impact of crude oil and dispersed oil on the composition of the human intestinal microbiota.

Therefore, the objective of this study was to investigate the potential effects of oil, dispersant, and dispersed oil on the human fecal microbiota as part of assessing the potential risk of exposure to petrochemical residues in seafood products. We report the effect of Deepwater Horizon source oil, dispersant Corexit 9500, and oil-dispersant mixtures on the fecal microbiota in *in vitro* cultures (19) by real-time PCR, denaturing gradient gel electrophoresis (DGGE), and 16S rRNA gene-based pyrosequencing to investigate alterations of the microbial community.

RESULTS

DGGE profiles of cultures treated with crude oil, dispersant, and oil-dispersant mixture. The DGGE profiles of bacterial 16S rRNA gene fragments, obtained from cultures of fecal suspensions treated with oil, dispersant, and oil-dispersant mixtures, showed the effect of exposure on the bacterial community compared to control cultures (Fig. 1). The differences between samples treated

with crude oil and samples treated with dispersant or dispersed oil were compared during the incubation period. Although the controls for samples treated with crude oil and dispersant or dispersed oil were different for the fecal suspensions from each individual, the DGGE profiles of triplicate samples were evaluated and found to be similar for each individual (Fig. 1). The DGGE profiles from cultures treated with the oil-dispersant mixture were changed more than the profiles from samples treated with either crude oil or dispersant alone. The similarities of gel profiles from cultures treated with dispersant alone (71.3% [mean value of three individual samples between control and the highest concentration exposure samples]) were higher than cultures treated with oil alone (70.8%) and with the oil-dispersant mixture (63.6%). The gel profiles showed more significant changes in cultures treated with oil-dispersant mixture than in cultures treated with crude oil alone, and the altered bands in cultures treated with crude oil alone were different with increasing concentration. DGGE profiles indicated that the fecal microbiota was perturbed by dispersed oil much more than by oil and dispersant alone compared to control cultures. Cultures treated with dispersant alone were excluded from further pyrosequencing analysis, since the dispersant alone did not alter the composition of the intestinal microbiota from any individual, as shown in the DGGE profiles (Fig. 1).

Pyrosequencing analysis of cultures treated with crude oil and oil-dispersant mixture. To refine and extend the initial DGGE results, pyrosequencing analysis was done on controls and crude oil- and dispersed-oil-treated culture samples from each individual. The pyrosequencing reads were obtained from 42

TABLE 1 Summary of pyrosequencing data obtained from fecal samples from three individuals incubated with crude oil alone and oil-dispersant mixtures[a]

Treatment	Treatment concn (%)	Sample origin	No. of reads			Read length (bp)		Observed OTUs[b]	Chao1 estimation	Shannon index	Good's coverage
			Total	Filtered	Normalized	Mean	Maximum				
Crude oil alone	0	Individual A	2,965	2,744	2,500	466.3	519	620	1,696.45	5.04	0.84
	0.002		3,215	2,924	2,500	468.0	519	620	1,520.03	4.75	0.84
	0.02		2,964	2,730	2,500	466.5	514	578	1,491.50	4.77	0.85
	0.05		2,882	2,618	2,500	468.9	520	618	1,680.24	5.01	0.84
	0.1		3,009	2,796	2,500	468.3	518	493	1,146.61	4.88	0.88
	0.5		2,238	2,074	2,074	465.6	518	507	1,245.13	4.90	0.85
	2		2,503	2,245	2,245	469.5	512	468	1,072.59	4.61	0.87
	0	Individual B	3,195	2,929	2,500	467.2	515	676	1,659.46	5.29	0.82
	0.002		4,150	3,830	2,500	468.0	573	721	2,068.92	5.45	0.81
	0.02		3,465	3,197	2,500	468.1	515	724	1,742.60	5.47	0.81
	0.05		3,630	3,271	2,500	465.9	516	679	1,579.06	5.38	0.83
	0.1		3,541	3,282	2,500	466.6	521	697	1,723.29	5.38	0.82
	0.5		8,826	7,939	2,500	463.8	523	728	1,760.85	5.51	0.81
	2		14,203	12,579	2,500	461.1	553	721	1,681.56	5.48	0.82
	0	Individual C	5,815	4,971	2,500	461.4	519	634	1,389.49	5.10	0.84
	0.002		7,070	6,365	2,500	463.9	517	627	1,188.56	5.20	0.85
	0.02		5,277	4,695	2,500	462.5	516	679	1,566.17	5.32	0.83
	0.05		6,761	5,776	2,500	462.4	517	731	1,611.09	5.44	0.82
	0.1		6,232	5,510	2,500	416.5	537	807	1,637.56	5.76	0.80
	0.5		3,512	3,240	2,500	464.2	520	466	876.73	4.88	0.90
	2		6,607	5,396	2,500	460.6	517	852	2,192.13	5.50	0.77
Mixture of crude oil and dispersant	0	Individual A	6,671	6,090	2,500	474.7	607	316	593.47	3.48	0.93
	0.002		7,032	6,512	2,500	473.9	521	278	508.11	3.45	0.94
	0.02		9,008	8,272	2,500	473.0	567	345	634.60	3.61	0.92
	0.05		9,630	8,749	2,500	474.1	539	298	526.38	3.36	0.94
	0.1		8,296	7,627	2,500	474.5	548	279	473.26	3.03	0.94
	0.5		7,223	6,502	2,500	470.9	530	284	529.02	3.24	0.94
	2		8,082	7,163	2,500	470.7	514	214	448.19	2.23	0.95
	0	Individual B	3,379	3,267	2,500	473.5	514	250	385.30	4.08	0.96
	0.002		7,703	6,991	2,500	471.7	591	472	986.05	4.72	0.90
	0.02		5,250	4,860	2,500	475.1	527	447	810.90	4.84	0.91
	0.05		8,016	7,299	2,500	468.3	576	459	918.01	4.87	0.90
	0.1		2,675	2,496	2,496	471.1	517	300	489.02	4.47	0.95
	0.5		6,943	5,953	2,500	467.6	515	431	761.46	4.47	0.91
	2		2,659	2,471	2,471	469.7	510	169	252.18	3.03	0.98
	0	Individual C	1,717	1,620	1,620	472.5	513	244	514.93	4.00	0.92
	0.002		9,050	8,051	2,500	470.9	575	412	776.01	4.14	0.91
	0.02		2,275	2,134	2,134	474.7	517	221	346.16	4.16	0.96
	0.05		3,905	3,520	2,500	470.2	517	324	617.16	4.12	0.93
	0.1		5,046	4,498	2,500	473.7	519	344	535.34	4.17	0.94
	0.5		8,139	7,120	2,500	471.2	520	325	580.10	3.46	0.93
	2		5,677	4,869	2,500	471.9	514	208	409.28	2.81	0.95

[a] Estimated statistical values were obtained from the Mothur program.
[b] OTUs, operational taxonomic units.

samples of cultures treated with oil alone or with the oil-dispersant mixture. A total of 230,436 reads were obtained, and 207,175 sequences (89.9% of total obtained reads) were analyzed after filtering. Their average length and estimated statistical values, after normalization of read sizes, are shown in Table 1. The altered values for Shannon diversity index from cultures treated with the oil-dispersant mixture (1.16 [mean value for three individuals]) between the control cultures and cultures treated with 2% oil plus 0.2% dispersant were higher than those from the cultures treated with oil alone (0.24). This indicated that exposure to dispersed oil could influence the diversity of fecal microbiota.

To investigate the individual variability of fecal microbiota, the microbial compositions of *in vitro*-cultured fecal samples obtained from all three individuals were compared to each other at the phylum and genus levels (Fig. 2). Four phyla (*Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*) were dominant; the proportions were different in each individual (Fig. 2A). In the control cultures, the phylum *Firmicutes* was more abundant in individual B (48.5% of analyzed reads) than in individuals A (24.9%) and C (35.7%), whereas *Proteobacteria* was more abundant in individuals A and C (36.6% and 32.3%, respectively) than in individual B (7.9%). The abundance of *Bacteroidetes* was higher in individual B (33.9%) than in individuals A (31.3%) and C (25.1%). Seventeen genera were dominant in *Firmicutes*, and four genera were dominant in *Bacteroidetes* in all three individuals. *Faecalibacterium* was the dominant genus in *Firmicutes* in individual B, and *Bacteroides* was the dominant genus in *Bacteroidetes* in all three individuals (Fig. 2B and C).
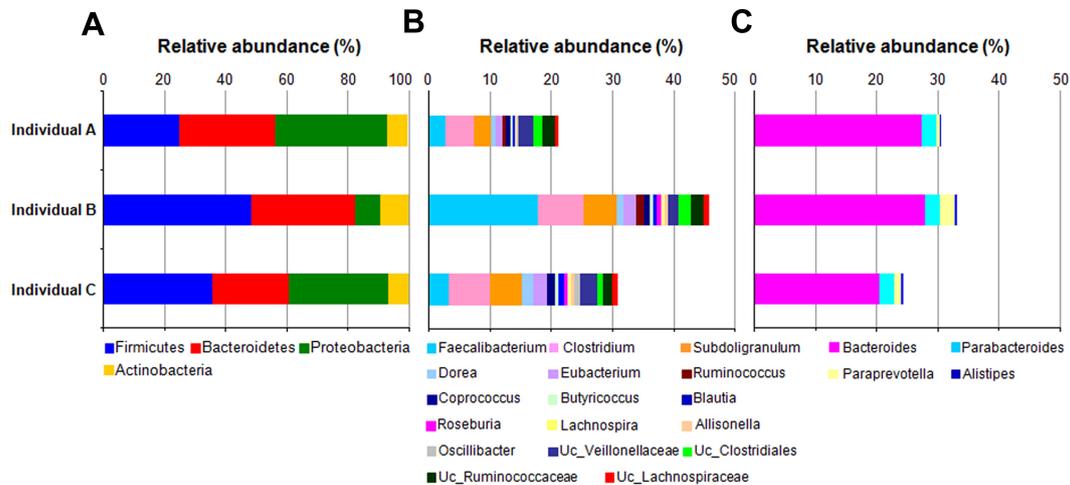
FIG 2 Comparison of fecal microbiotas from the three individuals studied in this investigation. Proportions of phyla (A), proportions of genera within *Firmicutes* (B), and proportions of genera within *Bacteroidetes* (C). Uc_Veillonellaceae, uncultured Veillonellaceae.

The impacts of oil and the oil-dispersant mixture on cultured fecal microbiota were also evaluated from altered phylotypes in pyrosequencing analysis. The relative abundances of phyla after exposure to crude oil alone and to the oil-dispersant mixture showed that exposure to the oil-dispersant mixture reduced the diversity of the intestinal microbiota, with increasing concentrations of the oil-dispersant mixture in cultures from all treated individual fecal samples (Fig. 3). Although the phylum *Bacteroidetes* from individuals B and C decreased with increasing oil concentration in cultures treated with crude oil and dispersed oil, the proportions were lower in the samples treated with oil alone than those in the samples treated with dispersed oil, and the proportions were not consistent with the concentration of crude oil (Fig. 3B and C). However, there were distinct changes in the phyla *Bacteroidetes* and *Proteobacteria* from all individuals after exposure to dispersed oil (Fig. 3A2, B2, and C2). The *Bacteroidetes* decreased significantly, from 0.5% of the oil-dispersant mixture concentration (from a mean value of 40.4% abundance in the control samples to 0.2% in the cultures treated with 2% oil dispersant), while the *Proteobacteria* increased (28% in the control samples to 72.8% in the cultures treated with 2% oil dispersant). Also, *Actinobacteria* from individual B showed a highly significant increase with an increased proportion of dispersed oil. The microbial compositions of control samples were different from each other, but the impact of dispersed oil was similar for all individuals.

The changes in genera caused by oil and dispersed oil were compared using heatmap analysis (Fig. 4). Most genera within *Bacteroidetes* decreased with increasing concentrations of oil and oil-dispersant mixture, while some genera within *Firmicutes* responded differently to increasing oil and oil-dispersant concentrations. Although *Proteobacteria* commonly increased in abundance with increasing concentrations of oil alone and oil-dispersant mixture, *Escherichia* in the cultures from individual A that were treated with oil alone did not increase. The trends of these changes were similar in oil- and dispersed-oil-treated cultures, but the influence of dispersed oil was greater than that of the oil-alone treatment. *Bacteroides* and *Escherichia* were commonly affected in all individuals, and the impacts on them due to dis-

persed oil were greater than those due to oil alone. *Faecalibacterium* spp. from individual B decreased more in cultures treated with dispersed oil than in cultures treated with oil alone.

The differences between the impacts of crude oil alone and the oil-dispersant mixture on fecal microbiota were clearly shown at the species level. The abundance of *Escherichia coli* from all individuals was maintained with an increasing proportion of crude oil, while the abundance increased significantly in cultures treated with the oil-dispersant mixture (Fig. 5). *Bacteroides uniformis* and uncultured *Faecalibacterium* EF402172 from all individuals had more significant effects when treated with dispersed oil than when treated with oil alone. The abundances of *Bifidobacterium adolescentis* and *Eubacterium biforme* from individual B were higher in cultures treated with dispersed oil cultures than in cultures treated with crude oil. Most species were more influenced by the oil-dispersant mixture than by the oil-alone treatment.

Impact of oil and oil-dispersant mixture on diversity of microbiota. The comparison of communities by principal coordinate analyses (PCoA analyses using UniFrac distance is shown in Fig. 6. The communities from all individuals were clearly influenced by the concentrations of the oil-dispersant mixture, while the communities were not influenced by the concentration of crude oil. The distribution of the bacterial community treated with oil alone does not relate to concentration. The bacterial communities in cultures with 0.5 and 2% of oil-dispersant mixtures formed a distinct group from the other concentrations of cultures treated with the oil-dispersant mixture. These results were consistent with DGGE results and phylum changes for each individual.

The community similarities between control and treated cultures clearly showed the significant impact of dispersed oil on the intestinal microbiota (Fig. 7). The similarities of cultures treated with dispersed oil were lower than those of cultures treated with crude oil alone. The Bray-Curtis similarities showed a significantly greater decrease than the Jaccard similarities. The community differences between samples were evaluated by Libshuff analysis, and the changes in the intestinal microbiota caused by exposure to crude oil and dispersed oil were significant ($P < 0.05$ in all samples). These analyses were conducted to evaluate the shift in the
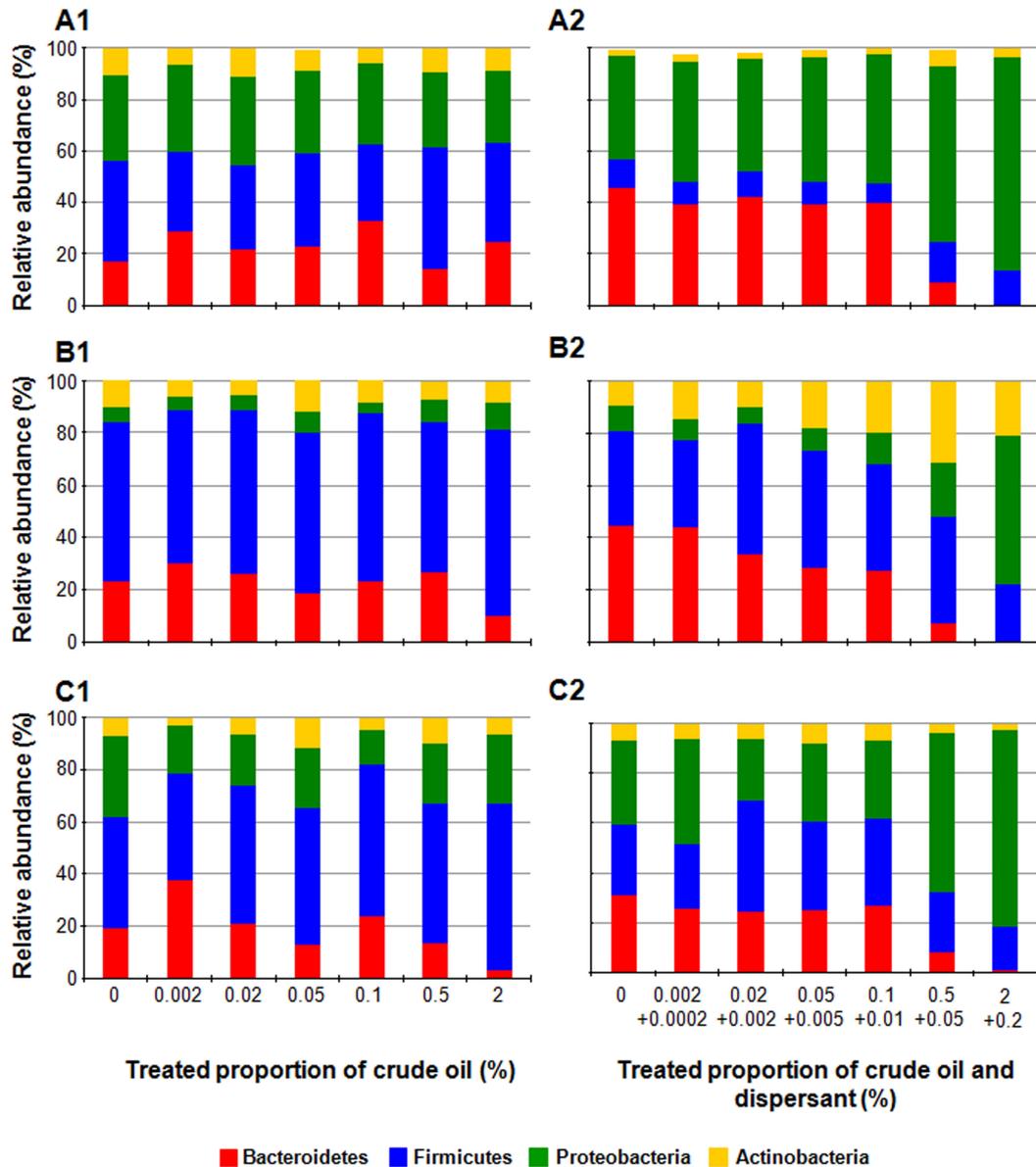
FIG 3 Changes of microbial composition after exposure of three human fecal microbiota cultures to crude oil (A1, B1, and C1) or to dispersed oil(A2, B2, and C2). Relative abundance was obtained from 16S rRNA pyrosequencing. The compositions of intestinal microbiota from individual A (A1 and A2), individual B (B1 and B2), and individual C (C1 and C2) were compared with different concentrations of crude oil, crude oil-dispersant mixtures, and control incubations.

microbial community caused by treatment with oil and dispersed oil.

Growth of total bacteria in samples treated with oil alone, dispersant alone, or oil-dispersant mixture. The effects of crude oil, dispersant, and mixtures of crude oil and dispersant on the abundance of total bacteria from three fecal samples compared to control culture incubations (samples not treated) were determined by quantitative PCR (qPCR) (see Fig. S1 in the supplemental material). The 16S rRNA genes of cultured bacteria were increased in controls (274 times higher than the value at 0 h) and 0.05% dispersant (183 times higher than the value at 0 h) over the 18-h incubation time (Fig. S1A). However, the 16S rRNA genes of dispersed oil and oil alone were substantially decreased after 18 h on 0.5% dispersed oil alone (155 times lower than that of the

controls), 0.5% dispersed oil plus 0.05% oil-dispersant mixture (579 times lower than that of the controls at 18 h), 0.2% dispersant alone (129 times lower than that of the controls at 18 h), and 2% dispersed oil plus 0.2% oil-dispersant mixture (844 times lower than that of the controls at 18 h), while bacterial growth on 2% dispersed oil was inhibited as the number of 16S rRNA gene copies was approximately 437 times lower than that of the controls. The abundance of 16S rRNA genes increased over the incubation time in the control (176 times higher than the value at 0 h), 0.05% dispersant (239 times), 0.5% dispersed oil plus 0.05% oil-dispersant mixture (376 times), 0.5% oil alone (242 times), and 0.2% dispersant alone (640 times) (Fig. S1B). However, the total 16S rRNA genes were substantially decreased about 15-fold after 18 h of treatment with 2% dispersed oil plus 0.2% oil-dispersant
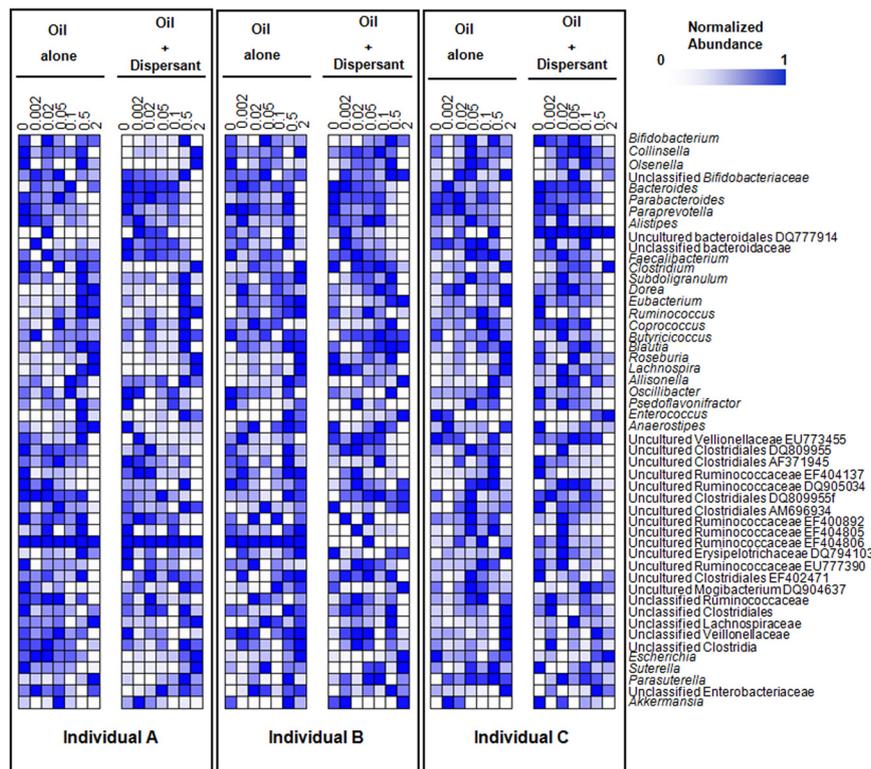
FIG 4 Changes in the genera detected by pyrosequencing of three human fecal microbiota cultures treated with crude oil or dispersed oil and control cultures incubated without treatment. The numbers above the columns indicate the concentrations of crude oil and crude oil-dispersant mixture. The color panels show the relative abundance of each genus with raw-normalized sorting.

mixture and about 13-fold in the culture treated with 2% oil alone compared to the copy numbers of control cultures after 18 h. On the other hand, no significant impacts of dispersant, dispersed oil, and oil alone on the abundance were observed (Fig. S1C). Although the influence of treatments varied between individuals, the abundance of bacterial 16S rRNA genes within each individual showed that the growth of bacteria was influenced more by the high concentration of oil and dispersed oil than by the dispersant alone or by the lower concentration of oil and dispersed oil. However, differential responses of impacted bacterial populations to dispersed oil and oil alone among the three individual fecal samples, compared to control *in vitro* incubations, may result from the physicochemical properties and microbiological compositions of the feces, which could affect bioavailability of the crude oil and dispersant mixtures.

DISCUSSION

The potential influences of Deepwater Horizon light Louisiana crude oil and oil dispersed by Corexit 9500 on human fecal microbiota were investigated in this study. We analyzed the shifts of bacterial communities and bacterial numbers from *in vitro* cultures of feces exposed to oil, dispersant, or dispersed oil using pyrosequencing and quantitative real-time PCR molecular approaches. Using the identical microbial communities in *in vitro* incubations of fecal suspensions treated with different concentrations of crude oil and dispersant provided us with comparative results for the concentrations tested for each individual.

Dispersed oil affected the intestinal microbiota more than ei-

ther oil or dispersant alone (Fig. 1 and 3). This may be due to the increased solubility of dispersed oil, which could provide more surface area of hydrophobic and toxic compounds for microbial contact than oil alone. Therefore, dispersed oil may be more bioavailable to the microbiota than oil alone. Previous studies reported that chemical dispersants may increase the concentration of PAHs in the water column (20, 21). The toxicity of dispersed oil showed that chemically dispersed oil increased the toxicity and concentrations of TPHs and PAHs in fish more than mechanically dispersed oil, dispersant alone, water-soluble oil fractions, or seawater alone (22). In this study, significantly greater influences of dispersed oil on fecal bacteria than oil alone were shown at the genus and species levels (Fig. 4 and 5). Exposure to dispersed oil increased the abundance of *E. coli* in all three individuals, while the abundance of *B. uniformis* and uncultured *Faecalibacterium* were reduced. The increased abundance of *E. coli* could be an important health concern, because high densities of *E. coli* have been associated with increased susceptibility to *Salmonella enterica* infections (18). *B. uniformis*, a prominent species in the intestinal microbiota, is correlated with host urinary metabolites, such as citrate and taurine (10). The reduction of *Bacteroidetes* by dispersed oil in fecal microbiota could affect barrier disruption with potential susceptibility to protection against pathogens. It is difficult to know the effect of uncultured *Faecalibacterium* reduction in samples treated with dispersed oil, because of limited information on uncultured *Faecalibacterium*. The most abundant *Faecalibacterium* species in the human intestine is *Faecalibacterium prausnitzii*, which is related to anti-inflammatory factors and reduced in pa-
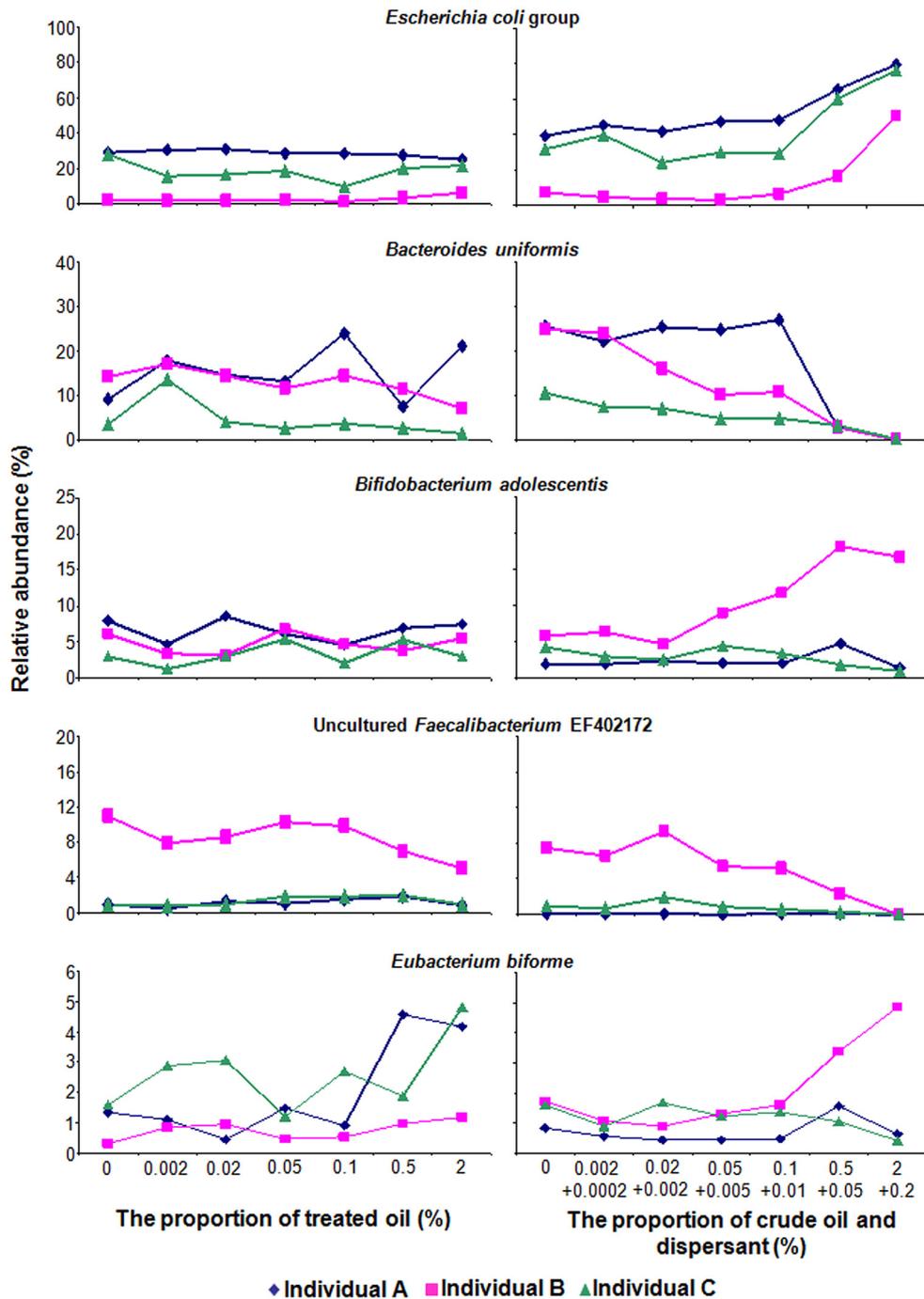
FIG 5 Changes in species abundance due to treatment with crude oil or dispersed oil and control incubations (no treatment) analyzed from pyrosequencing data.

tients with inflammatory bowel disease and Crohn's disease (23, 24). Most species were more influenced by the oil-dispersant mixture than by the oil-alone treatment. The changes of bacterial population could change nutrient compositions that could affect other biological groups in the gastrointestinal tract, as in previous reports of microeukaryote shifts in mangrove sediments (25). Although the concentration of oil-dispersant mixtures that significantly influenced microbiota was over the level of previously detected concentrations in seafood, the study provides a conservative estimate of impact on human intestinal microbiota with regard to potential food safety concerns (see Table S1 in the supplemental material).

In conclusion, our molecular, bioinformatic, and statistical results indicated that when comparing the toxicity of Deepwater Horizon crude oil alone, Corexit 9500 alone, and dispersant-oil mixtures, the human fecal microbiota was impacted more by dispersed oil than by either oil or dispersant alone, which were found not to be highly toxic. Although the results have the typical limi-
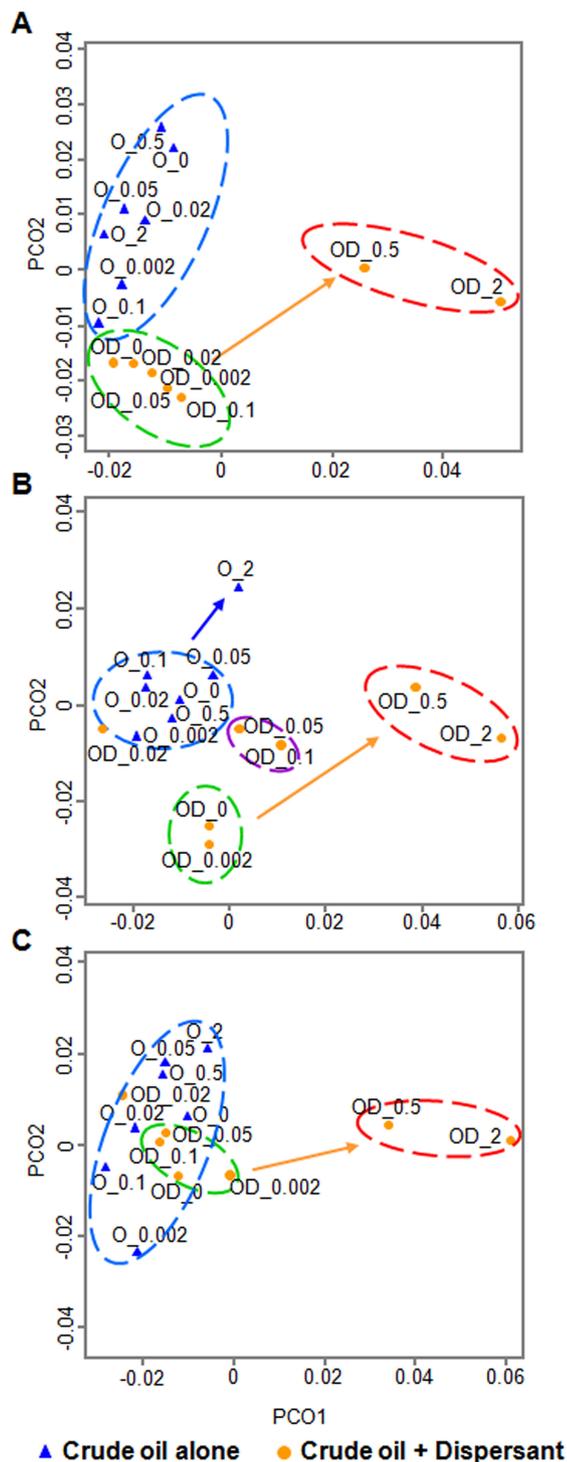
FIG 6 Principal coordinate analyses (PCoA) of microbial community structures, obtained using a Fast UniFrac distance matrix. (A to C) Distributions of microbial community structures in fecal microbiota cultures treated with crude oil alone) and oil-dispersant mixture originating from individual A (A), individual B (B), and individual C (C). The concentrations of crude oil (O) and the oil/dispersant ratios of the oil-dispersant mixture (OD) are shown for the points. The communities treated with crude oil were distributed in the blue circle. The green circle included samples treated with dispersed oil (D), which included bacterial communities that were similar to those in the control (not treated with dispersed oil). The red circle indicates the most diverse community group when comparing the control samples to cultures treated with dispersed oil.

tations of *in vitro* studies extrapolated to *in vivo* exposure and we used fecal samples representative of the lower gastrointestinal microbiota that show individual variation in microbial composition, this pilot study provides new information on the potential influences of oil and dispersed oil on the human intestinal microbiota. This topic should be further explored when assessing the potential risk of exposure to petrochemical contaminated food products.

MATERIALS AND METHODS

Cultural conditions for fecal suspensions treated with crude oil, dispersant, and dispersed oil. Fecal samples were obtained from six healthy males, 50 to 60 years old, and used immediately after donation. On the basis of a previous study (19), fecal suspensions (3% [wt/vol]) were inoculated into 10 ml of low-concentration carbohydrate medium with 1% fecal supernatant and incubated under anaerobic conditions at 37°C for 18 h.

Deepwater Horizon Source light Louisiana crude oil and the dispersant Corexit 9500 were obtained from the Gulf Coast Seafood Laboratory, Center for Food Safety and Applied Nutrition, FDA. Concentrated stock solutions (10×) of crude oil, Corexit 9500 dispersant, and an oil-dispersant mixture were prepared with anaerobic dilution buffer in an oxygen-free anaerobic mixture of gas (85% N_2, 10% H_2, and 5% CO_2 gas mixture; Nexair, Memphis, TN) and used for dosing the fecal microbiota cultures. The effects of crude oil, dispersant, and oil-dispersant mixture on the human fecal microbiota were investigated with triplicate samples of controls (not treated) and 6 different concentrations (0.002, 0.02, 0.05, 0.1, 0.5, and 2%) of oil (vol/vol) added to the low-concentration carbohydrate medium. Dispersant was added to the medium in a 1:10 ratio to oil for oil-dispersant mixtures as well as at the manufacturer's oil/dispersant ratios recommended for environmental treatment (0.0002, 0.002, 0.005, 0.01, 0.05, and 0.2% [vol/vol] of dispersant). The concentrations of crude oil used were based on previous data for PAH levels detected as residues in seafood (see Table S1 in the supplemental material) (8, 26, 27). Dispersant and dispersed oil were soluble in anaerobic buffer, whereas the crude oil was insoluble in buffer. Solutions were mixed by vortexing before each addition to the growth culture medium. The cultures were incubated with shaking to mix well for 18 h.

The study was conducted with the approval of the FDA Research Involving Human Subjects Committee (approval number 09-033T).

Nucleic acid extraction and DGGE analysis. Genomic DNA was extracted from 1 ml of each sample using a DNA elution accessory kit with a total RNA extraction kit (MoBio Laboratories, Carlsbad, CA). The V3 region of the 16S rRNA genes was amplified using a Mastercycler gradient instrument (Eppendorf, Hauppauge, NY) in a final volume of 50 μl (19, 28). The PCR product was confirmed by using 2% agarose gel electrophoresis and a Gel Doc system (Bio-Rad, Hercules, CA). The amplified products were purified with the QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA), and the concentration was determined on a NanoDrop 1000 spectrophotometer (Nanodrop Tech, Wilmington, DE). The extraction and amplification of genomic DNA from triplicate culture samples were conducted independently. Equal amounts of purified amplicons were loaded on the DGGE gels. The DGGE analysis was conducted using the Dcode system (Bio-Rad) (19), and the profiles of DGGE gels were photographed using the Gel Doc system (Bio-Rad). Normalization and analysis of gel profiles were conducted using the BioNumerics program, version 6.0 (Applied Maths, St.-Martens-Latem, Belgium). The DGGE profiles of triplicate samples were compared to each other to evaluate the reproducibility of experiments.

Quantitative real-time PCR. The numbers of total bacteria were compared between treatments to evaluate the effects of treatments with oil and dispersant solutions on fecal sample incubations. Real-time PCR was performed in a final 12-μl volume containing 2× SYBR Green PCR master mix (Applied Biosystems), 1 μl of template DNA (10-fold dilution series of standard and sample DNA) or sterilized water (negative control) with 10 μM of each primer (MWG-Biotech). Bact349F (5′-AGG CAG CAG
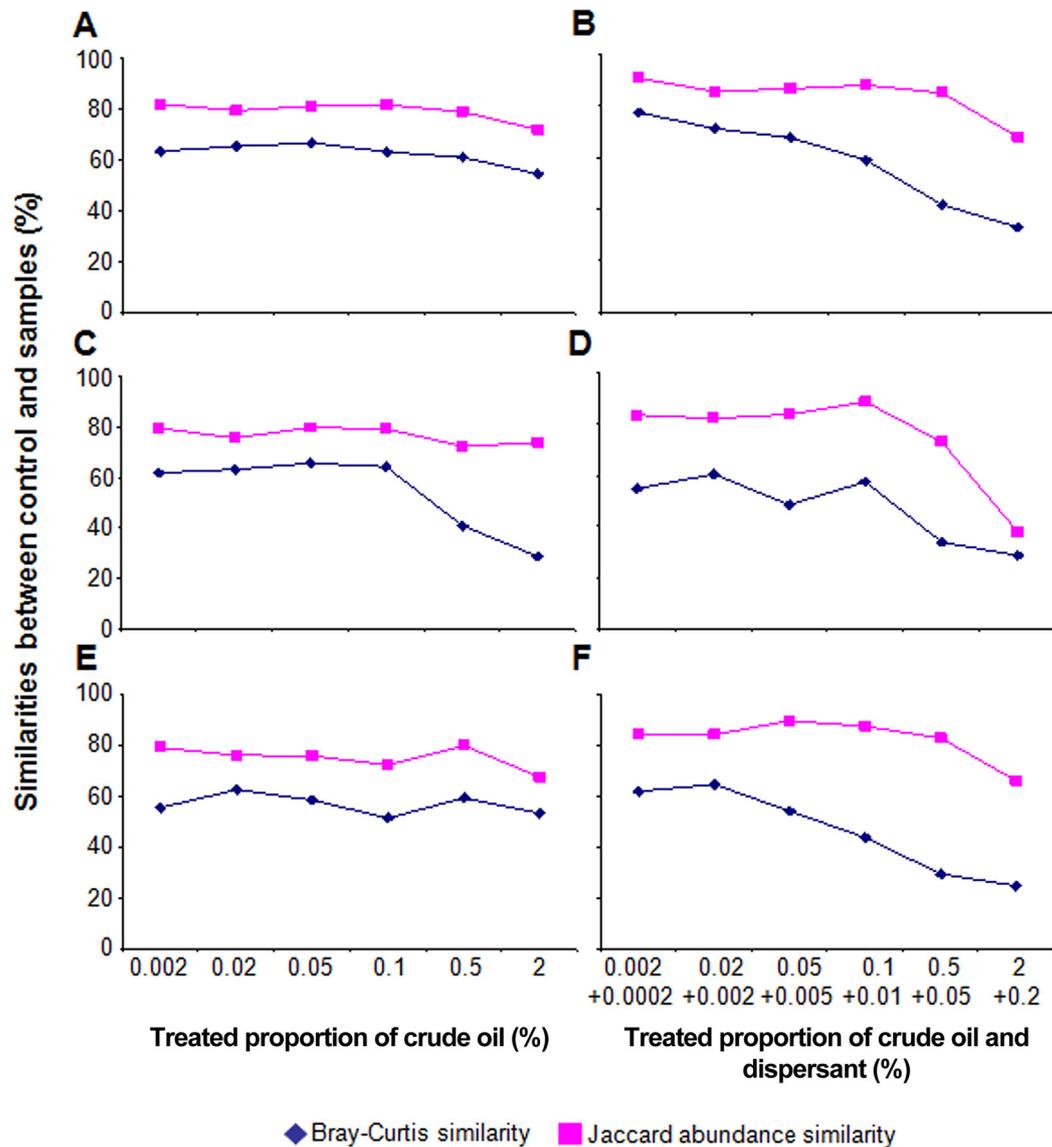
FIG 7 Comparison of the similarities between microbial communities from cultures treated with different concentrations of crude oil alone and with dispersed oil. (A to F) Similarities obtained from fecal samples from individual A (A and B), individual B (C and D), and individual C (E and F). The similarities of each pair of concentration samples were calculated by comparison with control samples from the same individual.

TDR GGA AT-3′) and Bact518R (5′ ATT ACC GCG GCT GCT GG 3′) were used as primers for bacterial quantification. Quantification was performed with three independent real-time PCR runs, using the CFX96 Real-Time PCR Detection system (Bio-Rad), associated with CFX manager interface software (version 1.0.1035.131; Bio-Rad). Amplification was conducted by the following steps: 2 min at 50°C, 10 min at 95°C, and 40 cycles, with 1 cycle consisting of 15 s at 95°C and 30 s at 60°C. Fluorescent signals were detected after the 60°C step of each cycle. Melting curve data were obtained from 60°C to 95°C, at a rate of 0.5°C s^{-1}, with continuous measurements of the SYBR Green I signal intensities. Melting point analysis allowed confirmation of the specificity of the amplification products. DNA extracted from cultures of *Escherichia coli* ATCC 25922 was used to construct standard curves for quantification by plotting the threshold cycle (C_T) values obtained from amplification of dilution series.

Pyrosequencing analysis of 16S rRNA genes. Changes in specific groups of the microbial community were determined using pyrosequencing. 16S rRNA gene fragments (from V1 to V3) were amplified from the genomic DNA from 42 samples (genomic DNA from a culture treated with crude oil alone and a culture treated with the oil-dispersant mixture for each individual), using bar-coded primers. The amplification, sequencing, and basic analysis of reads were conducted following previous descriptions (29) using a 454 GS Junior Sequencing system (Roche, Branford, CT). After excluding low-quality reads (average score of <25), short reads (<300 bp), ambiguous reads (Ns ≥2), and potentially chimeric sequences, the taxonomic classification of each sequence was assigned to the extended EzTaxon database (http://eztaxon-e.ezbiocloud.net/). The statistical analyses of phylotypes in each sample were performed using the Mothur program (30). The similarity of community structure (Bray-Curtis similarity and Jaccard similarity) between samples was used to evaluate community shift, and the significance of community differences was determined by Libshuff analysis. These analyses were conducted, with 3% difference of sequences, by the Mothur program. The sizes of different samples were normalized for comparison of samples with different read numbers. Principal coordinate analyses (PCoA), based on Fast UniFrac

matrices, were conducted using the CLcommunity software (Chunlab, Inc., Seoul, South Korea). The obtained pyrosequencing reads are available at the EMBL SRA database under the study accession number ERP001036 (http://www.ebi.ac.uk/ena/data/view/ERP001036).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org /lookup/suppl/doi:10.1128/mBio.00376-12/-/DCSupplemental.

Figure S1, DOCX file, 0.1 MB.
Table S1, DOCX file, 0 MB.

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