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eDNA-based bioassessment of coastal sediments impacted by an oil spill

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ABSTRACT

Oil spills offshore can cause long-term ecological effects on coastal marine ecosystems. Despite their important ecological roles in the cycling of energy and nutrients in food webs, effects on bacteria, protists or arthropods are often neglected. Environmental DNA (eDNA) metabarcoding was applied to characterize changes in the structure of micro- and macro-biota communities of surface sediments over a 7-year period since the occurrence of *Hebei Spirit* oil *spill* on December 7, 2007. Alterations in diversities and structures of micro- and macro-biota were observed in the contaminated area where concentrations of polycyclic aromatic hydrocarbons were greater. Successions of bacterial, protists and metazoan communities revealed long-term ecological effects of residual oil. Residual oil dominated the largest cluster of the community-environment association network. Presence of bacterial families (*Aerococcaceae* and *Carnobacteriaceae*) and the protozoan family (*Platyophryidae*) might have conferred sensitivity of communities to oil pollution. Hydrocarbon-degrading bacterial families (*Anaerolinaceae*, *Desulfobacteraceae*, *Helicobacteraceae* and *Piscirickettsiaceae*) and algal family (*Araphid pennate*) were resistant to adverse effects of spilt oil. The protistan family (*Subulatomonas*) and arthropod families (*Folsomia*, *Sarcophagidae Opomyzoidea,* and *Anomura*) appeared to be positively associated with residual oil pollution. eDNA metabarcoding can provide a powerful tool for assessing effects of anthropogenic pollution, such as oil spills on sediment communities and its long-term trends in coastal marine environments.

1. Introduction

Offshore spills of petroleum hydrocarbons, in the form of crude oil, alter structures of coastal ecosystems and thereby cause acute and chronic damages to their functions and services (Peterson et al., 2003; Mendelssohn et al., 2012). Due to its persistence, most of the oil remains in sandy soil of contaminated shorelines, where sedimentary refuges inhibit degradation and sequester persistently toxic oil along the gravel shore (Peterson et al., 2003). Spilled oils can cause various adverse effects on organisms, such as genotoxicity, reproductive toxicity, immunotoxicity, and modulation of endocrine function (Jeong et al., 2015; Ji et al., 2011; Hong et al., 2012, 2014; Barron, 2012; Paul et al., 2013). Spilled oil is often subjected to various physicochemical, biological weathering and degradation processes, and depending on the level of weathering and degradation, compositions of crude oil compounds and its toxicity change with time (Jeong et al., 2015). Effects of more persistent constituents of oil residues can cause long-term population-level effects (Peterson et al., 2003). However, historical assessments of oil spills were limited to short-term monitoring and use of acute toxicity testing of laboratory-tolerant taxa.

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Coastal ecosystems can be disturbed by spilt oils due to effects on the bottom of the food chain as well as bioaccumulation, transferring effects of hydrocarbons to higher-trophic-level organisms that can be more visible to and valued by humans (Peterson et al., 2003; Mendelssohn et al., 2012; Silliman et al., 2012). Most current practices for assessment of effects of oil spills focus on monitoring ecological responses of specific communities and/or populations, including microorganisms (King et al., 2015), terrestrial arthropods (Pennings et al., 2014), phytoplankton (Il Lee et al., 2009), zooplankton (McCall and Pennings, 2012), benthic fauna (Seo et al., 2011) and fishes (Jung et al., 2012). Due to the low efficiency of isolation-based morphology approaches, environmental surveys of effects of oil spills on communities of meiofauna cannot consider sufficient numbers of taxa to provide a comprehensive overview. Indirect effects of trophic interactions and interaction cascades can be as significant as direct trophic interactions in structuring ecosystems (Peterson et al., 2003). Despite their key positions in food webs relatively less is known about effects of oil on and recoveries of protists and arthropods (Atlas, 1981). Protists are ubiquitous, unicellular eukaryotes that are essential components of food webs. Protists graze on bacteria and link primary producers and detritivores with higher trophic levels (Clarholm, 1985; Bonkowski and Brandt, 2002; Rocke et al., 2015). Their greater rates of metabolism facilitate fluxes of carbon and energy through ecosystems (Bitencourt et al., 2014). Arthropods can also affect primary production and cycling of nutrients, thus providing valuable linkages and inflows of nutrients that connect disparate parts of ecosystems (Pennings et al., 2014). However, there are literally thousands, if not millions of taxa associated with microbiota, many of which are difficult to identify, classify and enumerate by use of traditional methods of taxonomy.

Environmental DNA (eDNA) metabarcoding provides more powerful tools for monitoring biodiversity in ecosystems (Gibson et al., 2014). eDNA obtained from environmental samples encompasses identification and enumeration of individual taxa that allows fine-scale analyses of ecosystems (Thomsen and Willerslev, 2015), which could allow a more comprehensive characterization of status and trends of communities and during longer-term weathering and recovery of oil spilled in coastal marine ecosystems. Here, we present results of a study that employed semi-quantitative eDNA metabarcoding to assess patterns of successions of bacterial, protistan, and benthic invertebrate communities in intertidal sediments of areas of the Taean coast (South Korea), that were contaminated by the *Hebei Spirit* oil spill (HSOS). The HSOS, which occurred on December 7, 2007, about 10 km off the coast, was the worst oil spill in Korean history. Approximately 10,900 tons of crude oil spread out along a wide stretch of the west coast of Korea. Within one day of the spill, approximately 70 km of the Taean shoreline was visibly contaminated with oil (Hong et al., 2014).

It was hypothesized that the *in situ* micro- and macro-biota could be altered by exposure to crude oil and changes of *in situ* communities (assemblages) could be related to the magnitude of oil pollution. To assess relationships between communities and oil pollution, relative abundances, Shannon diversity, and structure of sedimentary bacterial, protistan, and metazoan communities and abundant assemblages were compared to environmental variables. Diversities and structures of both micro- and macro-biotas were characterized by eDNA metabarcoding (Graphical abstract). This study was focused on sediments from inner parts of semi-closed small bays in the Taean area, where, due to the lack of flushing, relatively great concentrations of crude oil-derived PAHs are still found (Hong et al., 2014).

2. Materials and methods

2.1. Sampling sites and sample collection

A total of 26 surface sediments from heavily contaminated areas (Sinduri dune, Sinduri mudflat, and Sogeunri mudflat) along the Taean coast (Graphical abstract) were used to address long-term community changes over a 7-year period: Specifically, within about one month (December 2007 and January 2008), about one year (June and October 2008), about two years (June 2009), about three years (December 2010), about four years (September 2011 and January 2012), about six years (September 2013) and about seven years (October 2014) after the HSOS (Supplementary Table S1), following previously described sampling procedure (Hong et al., 2012). Four reference sediments were collected from the outer regions of the Taean coast (Naeri) in September 2011. Prior to sampling, residual oil in sediments was visually confirmed at all of the sites. All sediments collected in clean plastic bags were immediately transferred to the laboratory on ice, and then vacuum freeze-dried and stored at −20 °C until analysis.

2.2. Chemical analysis

Total organic carbon (TOC) content of sediments was analyzed using an Elementar Vario Microcube (Hanau, Germany) after removing inorganic carbon by 1M HCl. Concentrations of parent- and alkyl-PAHs were identified and quantified following a previously described method (Hong et al., 2012, 2015a, 2015b). A total of 45 parent- and alkyl-PAHs were measured and summarized in the Supplementary Table S1. To reduce the number of chemical variables, alkyl-PAHs (sum of C1-, C2-, C3- and C4-naphthalen, C1-, C2- and C3-fluorenes, C1-, C2-, C3- and C4-phenanthrenes (Phe), C1-, C2- and C3-dibenzothiophene (Dbthio), C1-, C2- and C3-chrysene (Chr)), parent-PAHs (sum of naphthalene, acenaphthylene, fluorene, Phe, Dbthio, fluoranthene, benz[*a*]anthracene, Chr, benzo[*b*]fluoranthene, benzo[k]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[ah]anthracene and benzo[ghi]perylene) and total concentration of PAHs (ΣPAHs; sum of alkyl- and parent-PAHs) were used in further analyses. Magnitudes of residual oil weathering in sediments were estimated with alkyl-PAHs double ratios (Hong et al., 2012, 2015a, 2015b; Douglas et al., 1996; Yim et al., 2011). To determine effects of residual oil to communities in sediments, contaminated sediments were grouped according to the magnitude of residual oil pollution; specifically, “greater pollution” group (ΣPAHs $\geq$ 5000 ng/g dry mass (dm)), “lesser pollution” group (ΣPAHs $<$ 5000 and $\geq$ 50 ng/g, dm). Concentrations of ΣPAHs in sediments from the reference site (Naeri) were less than 50 ng/g, dm.

2.3. eDNA extraction, PCR amplification and next-generation sequencing of eDNA

eDNA was extracted from a 0.25 g aliquot of homogenized sediment with the MoBio Power Soil DNA Kit (MoBio Laboratories Inc., CA, USA). Bacterial 16s rRNA genes (V3 fragment), protistan 18s rRNA genes (V9 fragment) and metazoan mitochondria Cytochrome Oxidase subunit I (COI) genes were amplified, purified and sequenced as described previously (Xie et al., 2017; Yang et al., 2017). Ribosomal products were sequenced using an ION proton sequencer (Life Technologies, CA, USA), and the COI products were analyzed with an Ion Torrent PGM sequencer (Life Technologies, CA, USA) according to the manufacturer's instructions.