PERIODICALLY SPILLED-OIL INPUT AS A TRIGGER TO STIMULATE THE DEVELOPMENT OF HYDROCARBON-DEGRADING CONSORTIA IN BEACH ECOSYSTEM

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The marine coastal environment is highly susceptible to oil spills due to the expansion of offshore oil and gas exploration and transportation. Previous studies demonstrated that biodegradation is mainly constrained by the complex interactions between microbial diversity and the local environmental complexity, typically as the bio-availability of the residue oil and geochemical properties such as temperature, nutrients and the presence of oxygen. Therefore, understanding the interactions of microbial species and physiochemical properties is a prerequisite to make an effective remediation strategy in respect to the spilled oil. In this study, time-series samples were taken from a gravel beach to ascertain whether a periodic oil input induced by tidal action at the early stage of an oil spill can be a trigger to stimulate the development of hydrocarbon-degrading bacteria under natural *in situ* attenuation.

The originally spilled oil is heavy oil with an API Gravity of 16^{o}, although light oil was added to increase its mobility. Therefore, a whole oil gas chromatogram, on the one hand, displays the full series of normal alkanes ranging from *n*-C_7 to *n*-C_{35} and, on the other hand, is still characterized by significant unresolved complex mixtures (UCM), which is a typical indicator of heavy oil. However, the first sample (D0) collected from the gravel beach 34 days after the spill accident shows the typical characteristics of biodegraded oil, with the almost total removal of *n*-alkanes, enrichment in branched and cyclic alkanes, and high UCM. Thereafter, although the gravel beach is periodically affected by tidal action with fresh inputs of spilled-oil, the residue oils, depending on the time sequence, exhibit a full depletion of *n*-alkanes, a decreasing trend of isoprenoids, and no discernible changes on saturated biomarker distributions (*e.g.*, steranes, hopanes). This suggests that the oil patch from the beach has undergone a variety of natural weathering processes, including emulsification, dispersion, dissolution and biodegradation while floating on the sea surface, leaving behind the viscous residues like the tar balls and other recalcitrant compounds. Overall, the time sequence of the sample series identified by days can be roughly equivalent to the biodegradation indices PM=3-5 in terms of petroleum geochemistry.

High-throughput sequencing shows that the microbial community in beach sediments is characterized by enrichment of well-known hydrocarbon-degrading bacteria including *Alcanivorax*, *Dietzia*, and *Marinobacter*. Accompanying the periodic floating-oil input, successions of the microbial community and

corresponding fluctuations of functional genes (*alk*B and RDH) are clearly indicated in a time sequence, which keep pace with the ongoing biodegradation of the spilled oil components (Fig.1). The microbial succession that accompanies tidal action could benefit, on the one hand, from the enhanced exchange of oxygen and nutrients, and on the other, from regular inputs of the floating oil, which can be a trigger to stimulate an *in-situ* "seed bank" of hydrocarbon-degrading bacteria. Therefore, it leads to the continued blooming of hydrocarbon-degrading consortia in beach ecosystem. The results provide new insights on our understanding of the beach microbial community structure and function in response to oil spills.

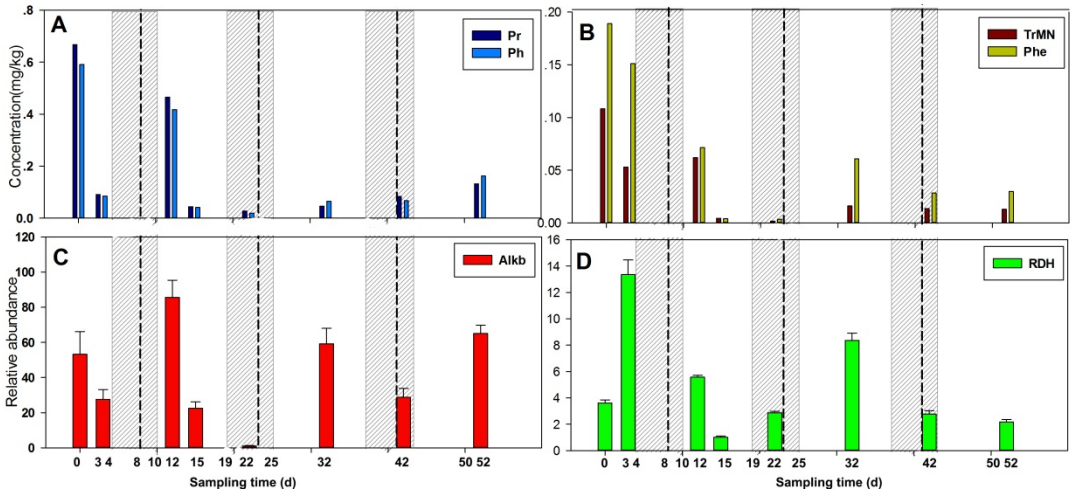

Figure 1. *Time-series shifts of selected geochemical and biological parameters during the sampling campaign. A = concentrations of Pristane and Phytane; B = concentrations of trimethylnaphthalene and Phenanthrene; C = relative abundance of alkane 1-monooxygenasegene (alkB) gene; D = relative abundance of Ring-hydroxylating dioxygenase (RDH) gene. The dashed lines represent the maximum tidal action at every tide period and shadow area for the time duration of every tide period.*