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The roles of biological interactions and pollutant contamination in shaping microbial benthic community structure

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HIGHLIGHTS

- Pollutants can severely impact structure and function of microbial communities.
- Biological interactions play a major role in structuring benthic food webs.
- We studied the impact of meiofauna on bacteria facing contamination by a mixture of 3 PAHs.
- Top down control by meiofauna was more effective than PAH in shaping bacterial diversity.
- The structural role of meiofauna disappeared when nutrients were added to enhance PAH biodegradation.

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ABSTRACT

Biological interactions between metazoans and the microbial community play a major role in structuring food webs in aquatic sediments. Pollutants can also strongly affect the structure of meiofauna and microbial communities. This study aims investigating, in a non-contaminated sediment, the impact of meiofauna on bacteria facing contamination by a mixture of three PAHs (fluoranthene, phenanthrene and pyrene). Sediment microcosms were incubated in the presence or absence of meiofauna during 30 days. Bioremediation treatments, nutrient amendment and addition of a hydrocarbon-degrading bacterium, were also tested to enhance PAH biodegradation. Results clearly show the important role of meiofauna as structuring factor for bacterial communities with significant changes observed in the molecular fingerprints. However, these structural changes were not concomitant with changes in biomass or function. PAH contamination had a severe impact on total meiofaunal abundance with a strong decrease of nematodes and the complete disappearance of polychaetes and copepods. In contrast, correspondence analysis, based on T-RFLP fingerprints, showed that contamination by PAH resulted in small shifts in microbial composition, with or without meiofauna, suggesting a relative tolerance of bacteria to the PAH cocktail. The PAH bioremediation treatments were highly efficient with more than 95% biodegradation. No significant difference was observed in presence or absence of meiofauna. Nutrient addition strongly enhanced bacterial and meiofaunal abundances as compared to control and contaminated microcosms, as well as inducing important changes in the bacterial community structure. Nutrients thus were the main structural factor in shaping bacterial community composition, while the role of meiofauna was less evident.

1. Introduction

The structure and species composition of benthic communities are controlled primarily by environmental and physical–chemical factors such as sediment particle size, solar radiation, organic matter content, temperature and salinity all of which shape the physical conditions in the sediment (First and Hollibaugh, 2010). In addition to these abiotic factors, contaminants can also impact the benthic community structure. Pollutants released to the environment, can settle and accumulate in bottom sediments of rivers, estuaries, lagoons, lakes and oceans. Thus, many coastal ecosystems are contaminated with high concentrations of anthropogenic pollutants such as organochlorines, heavy metals and polycyclic

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aromatic hydrocarbon (PAHs) compounds (Zaghden et al., 2005; Hack et al., 2007). PAHs introduced into the marine environment tightly adhere to sediments due to their low water solubility and hydrophobic nature. Consequently, very high concentrations of PAHs have been recorded in coastal sediments near urban and industrial environments. As many Mediterranean coastal lagoons, the Bizerte lagoon (Tunisia) is a polluted ecosystem subject to both urbanization and industrialization (cement works, metallurgical industry, boatyard, tire production factories), as well as naval and commercial shipping harbors. Runoff and discharges of urban and industrial wastes lead to significant pollution of sediments by PAHs, with concentration up to 876 $\mu g\ g^{-1}$ dry weight sediment (Ben Said et al., 2008). Many PAHs are recalcitrant compounds with toxic properties (Samanta et al., 2002). Numerous studies indicate that one-, two- and three-ring compounds are acutely toxic, while higher molecular weights are considered to be genotoxic (Cheung and Kinkle, 2001). These toxicity characteristics underline the importance of investigating the effects of PAHs on benthic sediment organisms. In this context, meiofauna community structure has been recognized as a sensitive bioindicator of pollution in benthic environments (Guo et al., 2001; Mahmoudi et al., 2007). Meiofauna are benthic metazoans of small size (all metazoans passing through a sieve of 1mm and retained on sieve of 40 μm), often associated to sediment particles (e.g. Kennedy and Jacoby, 1997). Meiofauna facilitate mineralization of organic matter, enhance nutrient regeneration and serve as food for a variety of higher trophic levels (Coull, 1999). Their small size, their high density and short generation times in addition to an easy laboratory maintenance encourage their use in biomonitoring experimental studies (Mahmoudi et al., 2007; Beyrem et al., 2010). Field and laboratory studies have documented that benthic meiofauna are sensitive to a wide range of organic toxic compounds such as hydrocarbons (Mahmoudi et al., 2005; Lindgren et al., 2012), lubricants (Beyrem et al., 2010), and diesel (Petersen et al., 2009).

Biological interactions between metazoans and the microbial community play a major role in structuring food webs in aquatic sediments (Montagna et al., 1995; Nascimento et al., 2012). Meiofaunal groups such as nematodes can influence directly (by predation) or indirectly the activity and/or species composition of microbial communities (De Mesel et al., 2004; Moens et al., 2005). Generally, mineralization of organic matter is enhanced and bacterial production is stimulated by the presence of meiofauna (Rysgaard et al., 2000). In deep-sea sediments, a significant positive correlation between bacterial biomass and meiofauna biomass was observed along a gradient of organic matter (Raghukumar et al., 2001), suggesting a tight coupling between both compartments. Recently, using a microcosm approach, Naslund et al. (2010) have observed that meiofauna can be an important regulating factor for the microbial mineralization of organic contaminants such as PAHs. Bioturbating meiofauna exert a top-down impact on bacterial community structure by changing the physicochemical conditions of sediment thus enhancing nutrient and carbon supplies (De Mesel et al., 2004; Cuny et al., 2007). Nevertheless, meiofauna and bacteria can also be in competition for food sources depending on the carbon supply conditions (Danovaro et al., 1999). Therefore, meiofauna has both positive and negative impacts on bacteria, which complicates the interactions between these two essential compartments of the benthic food web.

Microorganisms play an important role in the marine food chain as degraders of organic matter, and producers and recyclers of inorganic nutrients (Lindgren et al., 2012). Thus, bacterial degradation represents a potential route for the ultimate elimination of PAHs from sediments under both aerobic and anaerobic conditions (Yuan et al., 2000). Since natural degradation of PAHs is a slow process, bioremediation can represent a safer and more efficient approach to restore polluted sites as compared to conventional chemical and physical methods (Alexander, 1999). In order to enhance the biodegradation efficiency, two main remedial strategies, namely biostimulation and bioaugmentation, are used in bioremediation treatments (Goni-Urriza et al., 2013). Biostimulation, by adding nutrients and/or a terminal electron acceptor, enhances the low activity of indigenous microbial populations. Bioaugmentation involves the addition of microbial strains (indigenous or exogenous), which have the ability to degrade the target toxic molecules (Goni-Urriza et al., 2013). Bioremediation of PAHs in benthic sediments shows contrasted results, with an efficient biodegradation, but also sometimes inefficient strategies due to competition for nutrients (Yu et al., 2005; Louati et al., 2012). This competition for nutrients within the microbial community is increased when microorganisms also have to compete with organisms from higher trophic levels such as meiofauna for nutrients and organic matter.

The aim of the present study was to investigate, in a non-contaminated sediment (Bizerte lagoon), the impact of meiofauna on microorganisms facing contamination from a mixture of three PAHs (fluoranthene, phenanthrene and pyrene) as a function of the bioremediation treatment used for PAH biodegradation. For that purpose, sediment microcosms were settled in the presence or absence of meiofauna. Bioremediation schemes included biostimulation and the combination of biostimulation with bioaugmentation by the inoculation of a marine PAH degrading bacterium, *Bacillus megaterium*, previously isolated from Bizerte lagoon contaminated sediment (Ben Said et al., 2010).

2. Materials and methods

2.1. Collecting site

Natural sediment including the meiobenthos was collected from Echaraà in Bizerte lagoon (Tunisia) during April 2010. More details on the sampling site can be found in Louati et al. (2012). On the sampling day, the water salinity was 35 PSU, the dissolved oxygen concentration was 6.7 $mg\ l^{-1}$ and the water pH was 8.1. The sediments had a median particle diameter of 43 μm, organic carbon content of 0.79% and were largely composed of fine sand (70% < 63 μm). Sediment total nitrogen content was 1.03%.

Handcores of 10 cm^2 were used to a depth of 15 cm to transfer sediment into a bucket. On return to the laboratory, sediments were homogenized by gentle hand stirring with a large spatula before they were used for toxicant sediment spiking or microcosms filling. No detectable contamination (all concentrations < detection limit) of the sediment was observed for the three studied PAHs (fluoranthene, phenanthrene and pyrene). Buckets and spatula were all acid rinsed before use. Three sediment replicates were sampled to measure the *in situ* abundance of meiofauna and bacteria, determine the bacterial community structure, and for TOC and PAH analysis.

2.2. PAH contamination of sediments

Sediment used for the contamination experiments was frozen (−80 °C) and thawed three times to defaunate it following the recommendations of Gyedu-Ababio and Baird (2006). It was then wet sieved to remove the larger particles (>63 μm). Fluoranthene, pyrene and phenanthrene were selected as PAH congeners as all three are found in relatively high concentration in contaminated marine sediments as well as for their apparent toxicity to sediment-dwelling invertebrates. Moreover, these PAHs are present in high concentrations in some areas of the Bizerte lagoon (Ben Said et al., 2010). Stock solutions of high purity (Sigma–Aldrich Chemical) fluoranthene (Fl), pyrene (Pyr) and phenanthrene (Phe) were