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Structural and functional measures of marine microbial communities: An experiment to assess implications for oil spill management

Liz Morris[a,*], Allyson O'Brien[b], Siria H.A. Natera[c], Adrian Lutz[c], Ute Roessner[c], Sara M. Long[a]

[a] Centre for Aquatic Pollution Identification and Management (CAPIM), School of Biosciences, University of Melbourne, Parkville 3010, Victoria, Australia
[b] School of Biosciences, University of Melbourne, Parkville 3010, Victoria, Australia
[c] Metabolomics Australia, School of Biosciences, University of Melbourne, Parkville 3010, Victoria, Australia

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ABSTRACT

Microbial communities are ecologically important in aquatic environments and impacts on microbes have the potential to affect a number of functional processes. We have amended seawater with a crude oil and assessed changes in species composition as well as a measure of functional diversity (the ability of the community to utilise different carbon sources) and the community level metabolic signature. We found that there was a degree of functional redundancy in the community we tested. Oiled assemblages became less diverse and more dominated by specialist hydrocarbon degraders, carbon source utilisation increased initially but there was no change in metabolic signature in this small scale laboratory experiment. This study supports the decision framework around management of oil spills. This package of methods has the potential to be used in the testing and selection of new dispersants for use in oil spill response.

1. Introduction

Bioremediation of oil spills in the marine environment is a contentious issue (Kleindienst et al., 2015; Rahsepar et al., 2016) that can exert a huge financial burden on the remediators and be the source of ongoing conflict between all stakeholders (Walker et al., 2014). The choice of action resulting from a Spill Impact Mitigation Analysis (SIMA) usually includes complex deliberations around trade-offs between practical considerations (sea state, time since spill, location of spill etc) and impacts on different environmental compartments (surface water, water column, benthos, shoreline) (Baker, 1995; O'Brien et al., 2017). Incident response managers must weigh up the potential benefits of allowing the oil to degrade naturally (where natural attenuation is facilitated by the resident microbial assemblage) - or remediation actions such as applying chemical dispersants or oil-degrading microbial taxa (bioaugmentation).

A particular challenge to this type of decision making in environmental management is moving from an understanding of how a pollutant changes the species composition of a community (structure) to understanding how (or if) it changes the function of a community. As with other types of communities, we know that a disturbance can impact or change the composition of a microbial assemblage (Azarbad et al., 2015; Gutierrez et al., 2013; Mlejnkova and Sovova, 2010) and this type of structural change often leads us to infer that the functioning of the assemblage will also change. However, ecological assemblages can incorporate a degree of functional redundancy whereby even if the community composition changes following a disturbance, ecosystem process rates may not be altered; that is, they perform like the original community (Allison and Martiny, 2008; Rosenfeld, 2002; Walker, 1992). Alternatively, the loss of species may be associated with a loss of function (Fonseca and Ganade, 2001) and understanding the links between community structure and function is fundamental to environmental decision making (Hooper et al., 2005).

Microbial communities are ecologically important in aquatic environments; they form the base of food webs, play a major role in nutrient cycling, organic matter transformations and pollution degradation. Impacts on the microbial community composition therefore have the potential to affect a number of functional processes that microbes are involved in. Advances in technology have greatly increased our ability to gain insights into structure and function of microbial communities through the use of 'omics' approaches, which allow us to combine molecular and chemical measures (Vanwonterghem et al., 2014). Studies utilising genomics and transcriptomics for example have greatly increased our understanding of how changes in species composition have the potential to impact basic functions (Franzosa et al., 2015). Other measures, such as a direct measure of functional diversity (e.g. the ability of a community to utilise different carbon sources) and community metabolomics (community level metabolic signature) can

* Corresponding author.
E-mail address: e.morris@unimelb.edu.au (L. Morris).

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provide actual measures of community function following a disturbance.

Increasing our understanding of how oil spills and remediation strategies impact on microbial structure and function might feed directly into the decision making process around oil spill management and, in particular, decisions around remediation options. It is important to consider not only how oil pollution and remediation actions will affect the ability of the microbial community to degrade the oil, but also how it might impact on other significant ecological processes (Head et al., 2006). Numerous studies have documented the successional progression of oil degraders – where the community becomes sequentially dominated by specialist taxa able to degrade different components of oil, resulting in an overall reduction in diversity and an increase in species dominance (reviewed in McGenity et al., 2012). This reduction in diversity might, as stated above, result in altered community functions or, no net-loss of function due to functional redundancy. An experimental study by Delgado-Baquerizo et al. (2016) found that a reduction in microbial diversity affected general metabolic activity as well as the functional ability of the microbial community to degrade pollutants. Other studies investigating the impact of crude oils on the metabolic function of individual organisms have found that the amino acid and carbohydrate metabolic pathways were affected by exposure to a crude oil in tissues of salmon smolts and a polychaete worm (Fernández-Varela et al., 2015; Lin et al., 2009).

In this study we amended seawater with a crude oil to test the predictions that the microbial community will become less diverse and more dominated by oil degrading specialists, leading to a reduction in functional diversity (carbon source utilisation) and a change in metabolic signature indicating changes in microbial community function. There are no other studies that we are aware of, that combine these three measures at the scale of a whole community to assess the impacts of a crude oil on hydrocarbon degradation and other basic functions of a microbial community. We were particularly interested in how this combination of structural and functional measures could inform oil spill responders and its potential to contribute to the testing and selection of dispersants for use in spill response.

2. Methods

2.1. Experimental set-up

Water accommodated fraction (WAF) of a Kuwait light crude oil was prepared using standard methods (Singer et al., 2000). Briefly, 200 mL of oil was added to 1800 mL of 0.45 μm filtered seawater in a 2 L shott bottle wrapped in aluminium foil (to keep the solution in the dark) and stirred using an electromagnetic stirrer for 23 h. Mixing energy was determined as the maximum stirrer rate that did not begin to form a vortex or visible droplet formation (approx. 200 rpm). After 23 h the mixture was allowed to settle for an hour and the WAF was then siphoned from under the oil layer to provide the WAF stock solution. Concentrations of TPH C10-C36 and BTEX C6-C9 were measured in the stock solution of WAF so that a nominal concentration of the oil treatment test solutions could be calculated.

Seawater was collected from a boat ramp adjacent to the Western Treatment Plant, Port Phillip Bay, Melbourne, Australia, and approximately 2 km from the nearest discharge point. Sea water was collected in two, 20 L plastic drums. Test solutions comprised of controls (seawater passed through a 100 μm mesh and with additional 0.45 μm filtered seawater) or oil treatments (seawater passed through a 100 μm mesh and with additional stock solution of WAF). There was a 1 in 5 dilution for the additions of WAF (oil treatments) or 0.45 μm filtered seawater (control treatments) in all cases. 30 mL of Class A recycled water (sourced from the WWTP and treated to meet high exposure usage standards) was added to each test bottle to ensure that there was an abundant microbial community present prior to incubation and to ensure test bottles did not become nutrient limited. Test solutions were then incubated in the dark for up to 3 or 5 days at 25 °C in 1 L amber glass bottles topped with aluminium foil prior to closing with lid. There were five replicates of each treatment and sampling time combination so a total of 40 bottles with each replicate comprising of 2 one litre bottles. Each complete replicate comprised 1 L from each of the seawater collecting drums to ensure there was no systematic bias in the microbial communities assigned to each treatment that might relate to collecting drum. Subsamples of water were randomly assigned to each measure i.e. chemical analysis, structural diversity, functional diversity and metabolic signature.

2.2. Sample processing

Test solutions were sampled at 3 and 5 days. For each replicate, test solutions were shaken well prior to siphoning off water for hydrocarbon analysis. Hydrocarbon sample bottles were stored on ice prior to analysis by ALS Environmental (http://www.alsglobal.com) for TPH C10-C36 and BTEX compounds C6-C9. A further 500 mL was filtered through a 45 μm nitrocellulose filter using a vacuum pump for each of the following analyses: diversity profiling, functional diversity and metabolomics. Filter papers for these analyses were stored at −20 °C (diversity profiling), −80 °C (metabolomics) or processed immediately (functional diversity).

2.3. Diversity profiling

DNA extraction was undertaken using the MoBio PowerWater DNA Isolation Kit following the kit protocol with an alternative lysis method. The samples were placed in a water bath at 65 °C for 10 min followed by 2 min of vortexing then a further 10 min of the water bath and another 1 min of vortexing. DNA samples were sent to the Australian Genome Research Facility (AGRF) Ltd. (www.agrf.org.au/services) for diversity profiling (16 s rDNA profiling) who amplified target amplicons 341F 806R and used a MiSeq platform utilising Illumina's Paired End Chemistry. Read lengths were 300bpPE and paired-ends reads were assembled by aligning the forward and reverse reads using PEAR1 (version 0.9.5). Primers were trimmed using Seqtk (version 1.0). Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8)4 USEARCH2,3 (version 8.0.1623) and UPARSE software. Using USEARCH tools sequences were quality filtered, full length duplicate sequences were removed and sorted by abundance. Singletons or unique reads in the data set were discarded. Sequences were clustered followed by chimera filtered using “rdp_gold” database as reference. To obtain number of reads in each Operational Taxonomic Unit (OTU), reads were mapped back to OTUs with a minimum identity of 97%. Using Qiime taxonomy was assigned using Greengenes database5 (Version 13_8, Aug 2013).

2.4. Functional diversity

Functional diversity was estimated using carbon source utilisation on Biolog™ Eco plates. Each plate contained 31 carbon sources and a blank well (water) with three subsamples of each carbon source or blank provided on each 96 well plate. A tetrazolium dye is included within each well which turns purple as the carbon source is utilised and the microbial community is respiring. Microbes were extracted from the filter papers by vigorous hand shaking with small glass balls in 100 mL of 10% sterile seawater. 150 μL of extract was added to each well and plates were incubated in the dark at 25 °C. Absorbance was measured in each well using a microplate reader (Thermo Scientific Multiscan Ex) at an absorbance of 595 nm at 24 h intervals for 10 days. The initial day zero readings were subtracted from each day's plate readings prior to averaging the three subsamples of each carbon source in each plate. The average colour development for the blank wells was also deducted from the average colour development for each carbon source and where this returned negative values these were coded as zeroes for further