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Changes in bacterial and eukaryotic communities during sewage decomposition in Mississippi river water

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ABSTRACT

Microbial decay processes are one of the mechanisms whereby sewage contamination is reduced in the environment. This decomposition process involves a highly complex array of bacterial and eukaryotic communities from both sewage and ambient waters. However, relatively little is known about how these communities change due to mixing and subsequent decomposition of the sewage contaminant. We investigated decay of sewage in upper Mississippi River using Illumina sequencing of 16S and 18S rRNA gene hypervariable regions and qPCR for human-associated and general fecal Bacteroidales indicators. Mixtures of primary treated sewage and river water were placed in dialysis bags and incubated *in situ* under ambient conditions for seven days. We assessed changes in microbial community composition under two treatments in a replicated factorial design: sunlight exposure versus shaded and presence versus absence of native river microbiota. Initial diversity was higher in sewage compared to river water for 16S sequences, but the reverse was observed for 18S sequences. Both treatments significantly shifted community composition for eukaryotes and bacteria ($P < 0.05$). Data indicated that the presence of native river microbiota, rather than exposure to sunlight, accounted for the majority of variation between treatments for both 16S ($R = 0.50$; $P > 0.001$) and 18S ($R = 0.91$; $P = 0.001$) communities. A comparison of 16S sequence data and fecal indicator qPCR measurements indicated that the latter was a good predictor of overall bacterial community change over time (rho: 0.804–0.814, $P = 0.001$). These findings suggest that biotic interactions, such as predation by bacterivorous protozoa, can be critical factors in the decomposition of sewage in freshwater habitats and support the use of Bacteroidales genetic markers as indicators of fecal pollution.

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1. Introduction

The advent of high-throughput DNA sequencing technologies makes it feasible to characterize the composition of microbial communities of both fecal pollution sources and indigenous aquatic communities. High-throughput sequencing data is available for human sewage (McLellan et al., 2013; Shanks et al., 2013), fecal microbiota from a variety of human, agricultural, and wildlife animal species (Unno et al., 2012, 2010) as well as various natural environments such as marine and freshwater systems (Humbert et al., 2009; Staley et al., 2013), groundwater (Lin et al., 2012) and intertidal/marine sediments (Lemke et al., 2009; Wang et al., 2012). Due to large differences between fecal-derived and indigenous aquatic microbial population structures, it may be possible to discriminate between these different populations when mixed in an environmental system (Cao et al., 2013; Unno et al., 2012, 2010). This strategy has been employed to characterize human fecal pollution in Lake Michigan during wet and dry weather events (Newton et al., 2013), to identify riverine intrusion to underground aquifers (Lin et al., 2012), to characterize wastewater impacted riparian buffer zones (Ducey et al., 2013), and to quantify sources of contamination by estimating the proportion of an invading community in a set of indoor environments (neonatal intensive care units, offices and molecular biology laboratories) (Knights et al., 2011).

To date, most of the information available about sewage decomposition in natural environments is based on the decay of specific fecal indicators such as *Escherichia coli*, enterococci, and host-associated Bacteroidales, a common choice for fecal source identification applications due to high concentrations in mammalian feces and evidence of coevolution with animal hosts (Harwood et al., 2014). These studies suggest that the decay of sewage in ambient waters is influenced by environmental factors such as the water type (marine or freshwater) (Green et al., 2011; Korajkic et al., 2013) and temperature (Okabe and Shimazu, 2007). Exposure to ambient sunlight has yielded somewhat conflicting results and there is a lack of concurrence on whether it has an impact on decay (Bae and Wuertz, 2009; Green et al., 2011; Korajkic et al., 2013, 2014). The effect of predation, competition and viral lysis is often overlooked, although recent studies suggest that these biotic interactions are important factors in decay (Dick et al., 2010; Korajkic et al., 2013, 2014; Wanjugi and Harwood, 2013, 2014). Furthermore, the extent of influence of any environmental factor can vary from one indicator to another and it remains unclear which member(s) of the aquatic microbial community play the most important role in sewage decomposition.

To address this research gap, we conducted an *in situ* experiment in the upper Mississippi River to characterize temporal changes in microbial communities associated with the decomposition of primary treated sewage over seven-days. We characterized bacteria and microbial eukaryotes using high-throughput DNA sequencing of partial 16S and 18S rRNA genes at depths of millions of sequences per sample using the Illumina HiSeq platform. These data combined with real-time quantitative PCR (qPCR) measurements of two Bacteroidales fecal indicators allowed us to characterize changes in microbial communities over time, evaluate the influence of solar radiation and biotic factors on decomposition, and examine the utility of high-throughput DNA sequencing for predicting levels of sewage pollution in a riverine environment.

2. Materials and methods

2.1. Field experiment and treatments

Mixtures of primary treated sewage and river water (final volume 200 mL) were placed in dialysis bags at a ratio of 1:1 and incubated *in situ* over a seven day period in the Upper Mississippi River, as previously described (Korajkic et al., 2014). A 1:1 ratio was selected to allow monitoring of decomposition for less abundant community members. Experimental treatments were designed to isolate the effects of ambient sunlight exposure (~3.08 kW h m^{-2} day^{-1} from http://eosweb.larc.nasa.gov) and indigenous riverine microbiota. Briefly, treatments included: A) exposure to both sunlight and river microbiota, B) exposure to sunlight while biotic interactions were reduced (river water filter-sterilized through 0.45 μm, 0.22 μm pore size nitrocellulose filters and a positively charged NanoCeram cartridge filter), C) exposure to river microbiota and reduced sunlight (shading), and D) reduced biotic interactions and shading. Sunlight exposed treatments were ensured by incubating dialysis bags approximately 1–2 cm below the surface of the water, while shaded treatments were incubated under a black tarp covering. The background changes (control samples: river only) in bacterial and eukaryotic communities were captured by incubating river water only under sunlight and shaded conditions. In addition, the primary treated sewage used to seed treatment samples was also characterized (control: sewage only). Triplicate dialysis bags were harvested per treatment at the beginning of experiment (T_{0h}), after 72 h (T_{72h}), and approximately every other day (T_{120h} and T_{168h}) for seven days. The potential blockage of the sunlight by the dialysis bag material was evaluated and found to be minimal (i.e. <10%) (Korajkic et al., 2014).

2.2. Sample processing

Fifty milliliters from each dialysis bag was filtered through a polycarbonate (0.40 μm pore size, 47 mm diameter) and nitrocellulose (0.45 μm pore size, 47 mm diameter) membrane filters for sequencing and qPCR analyses (Korajkic et al., 2014), respectively. Samples were stored at −80 °C until further processing (<6 months). Nucleic acids were extracted from each filter type using PowerSoil® DNA Isolation Kit (MoBio laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions except for the following: 1) additional 10 min incubation of bead beating tube containing filter and C1 reagent at 65 °C followed by: 2) utilization of FastPrep® homogenizer (MP Biomedicals, Santa Ana, CA) for 1 min at 60 ms^{-1} instead of vortexing.

2.3. 16S and 18S rRNA sample preparation, barcoding and Illumina sequencing

Briefly, samples were prepared for sequencing according to the earth microbiome project (EMP) standard protocols