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The Unexpected Habitat in Sewer Pipes for Propagation of Microbial Communities and Their Imprint on Urban Waters

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Abstract

Modern urban sewer pipe infrastructure is a unique niche where microbes can thrive. *Arcobacter*, *Acinetobacter*, *Aeromonas*, and *Trichococcus* are among the organisms that dominate the microbial community of sewage influent, but are not major members of human fecal microbiome, drinking water, or groundwater. Pipe resident communities in untreated sewage are distinct from sewer biofilm communities. Because of their high biomass, these organisms likely have a role in biotransformation of waste during conveyance and could represent an important inoculum for treatment plants. Studies demonstrate stormwater systems act as direct conduits for sewage to surface waters, releasing organisms propagated in sewer pipes. Frequent occurrence of these pipe residents, in particular *Arcobacter*, demonstrates the extent that urban infrastructure impacts rivers, lakes, and urban coasts worldwide.

Graphical abstract

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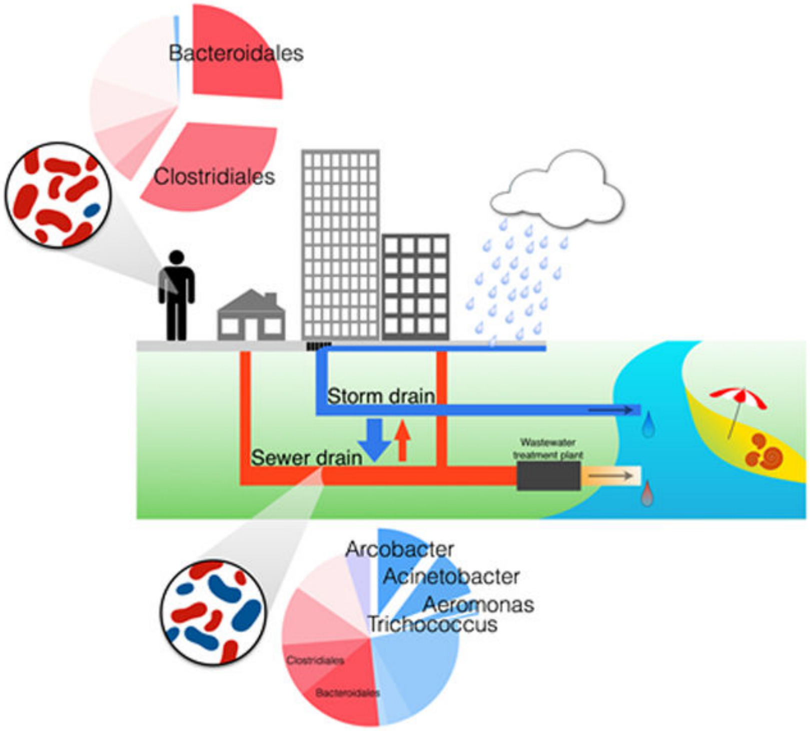

Introduction

Sewer infrastructure is a unique manmade environment that is a relatively new habitat for microbes in comparison to soil or oceans that have been around for millions of years. Only recently have the indigenous communities within these systems been defined [1-4]. Sewer pipe networks have been recognized as propagating rather than only conveying microorganisms [4,5]. Human fecal-associated bacteria account for only 15–20% of the sewage influent community which reflects an integrated profile of the human population microbiome within a city [6]. In contrast, approximately 80% of the total community appears to be residents within the sewer pipe and is dominated by a few genera that include *Arcobacter*, *Acinetobacter*, *Aeromonas*, and *Trichococcus* [4,5]. The sheer size of cities, and the thousands of kilometers of pipes within urban areas, suggest that the organisms that proliferate within pipes can have a large influence on the overall microbiome of the urban environment. This review will examine the environmental conditions and selective pressures within sewer pipes and the bacterial community composition. This review will also highlight some of the dominant organisms that thrive within sewer conveyance systems. The extensive release of pipe-derived organisms to the environment is illustrated by their imprint on receiving waters and urban coastal systems worldwide.

The environment within pipes, organic matter transformation, and selective pressures

The sewer conveyance system is an aqueous, dark, high nutrient environment with fluctuating oxygen conditions. Gravity sewers can be aerobic, but become anaerobic or microaerophilic depending on flow, turbulence, and organic matter [7,8]. Because these systems are generally buried two meters below the surface, temperatures are less variable then the daily fluctuating air temperatures. Temperature is a major factor that can select for organisms with different growth optimums. Colder temperatures also reduce microbial activity and favor aerobic conditions [7].

Sanitary sewer pipes contain both biofilm and sediment [9] with distinct microbial assemblages in each compartment involved in the metabolism of organic compounds [2,8]. Organic matter is deposited into sediments during transport where it can be transformed into more readily degradable products and released back into sewage as it moves down the system [8,10]. The biotransformation of nutrients and other contaminants involves complex bacterial processes in both biofilm and sediments, which are shaped by dynamic environmental conditions [11,12]. Heterotrophic bacterial activity determines dissolved oxygen levels [7] and facilitates anaerobic conditions, which favor methanogenesis and sulfate reduction, two undesirable processes leading to methane production and sewer corrosion. In a pilot scale system, Shi et al. [13] demonstrated that fermentative bacteria were major members in the top layer sediments, while methanogens and sulfate reducing bacteria were more prominent in deeper layers. In this modelled system, abundant bacterial members linked to carbon transformation included *Trichococcus*, *Cloacibacterium*, *Aeromonas*, *Paludibacter*, *Arcobacter*, and *Moraxellaceae* (presumably *Acinetobacter*) [13]. Bacteria that can thrive in a broad range of conditions may have an advantage in an environment with changing oxygen availability and metabolism byproducts from other community members. By understanding the complex, interrelated conversions that take place in conveyance pipes, systems could be designed to favor beneficial microbes and limit undesirable reactions [10,13].

The ability to effectively compete with the large densities of viable bacteria, 10–100 times higher than surface fresh or saltwater [14], may also determine what organisms are dominant in the sewage. In addition, there is a nearly constant flow in sewer systems, and bacteria that are efficient at filling open niches may dominate. These organisms have been termed “microbial weeds” because of their rapid growth, their tolerance to diverse stresses, and inhibitory effect on other bacteria, *e.g.* production of surfactants or antimicrobial toxins, acidification of the environment, etc. [15].

Microbial communities in sewer systems can also be shaped by selective pressures including antimicrobial agents, disinfectants and heavy metals that create harsh conditions favoring well-adapted or specialized organisms [16,17]. Antibiotics directly favor the accumulation of antibiotic resistance genes, however, co-selection exerted by heavy metals or nutrients might also facilitate the acquisition and maintenance of antibiotic resistance genes in bacterial populations (see [18] and reference therein). The high biomass of organisms may be an important reservoir for resistance genes, making sewers a hot spot for antibiotic resistance accumulation and spread [19]. A large range of antibiotic gene resistance traits have been associated with *Acinetobacter* [16,20] and *Arcobacter* [20,21]. In one study, a metagenomic assembly of *A. cryaerophilus* from sewage recovered 25 categories of antibiotic resistance genes [21]. *Arcobacter* has also been suggested as a keystone species in shuttling antibiotic resistance genes among a broad group of phylogenetically distant organisms [16]. Metagenomics studies of untreated sewage detected 381 different bacterial resistance genes in China [22], and 303 different resistance subtypes in Singapore [23], which highlights the complex reservoirs of resistance genes within these systems.

Community structure in sewer pipe conveyance systems

The microbial community in wastewater treatment plant influent contains a unique assemblage of bacteria that are distinct from the human fecal microbiome (Figure 1), treated potable water [24], or urban groundwater [25] that may infiltrate the system. As sewage is transported from upstream portions of the conveyance system to the treatment plant, the non-fecal component increases and is dominated by *Arcobacter*, *Acinetobacter* and *Aeromonas* [5]. In addition, some facultative anaerobes associated with the human microbiome such as *Lactoccocus*, *Enterococcus*, or *Enterobacteriaceae* (the family that holds *Klebsiella*, *Enterobacter*, and *Escherichia coli*) have been observed 10–100 time more abundant in untreated sewage relative to human gut-microbiota [26]. The enrichment in sewage of these taxa, commonly associated with fecal pollution, suggests either these fecal-originated strains can thrive and grow in the pipe environment, or there are non-fecal, free living strains that colonized sewer pipes. The bacterial community in both biofilm and sediment share members but with distinct abundance patterns compared to wastewater [19,27]. The sewage influent community profiles suggest resident bacteria with the sediment can be mobilized, whereas the biofilm may be harder to detach. Many of the community members recovered from sewage appear in activated sludge [28], where they may be inoculum for wastewater treatment plants [29].

Notable traits of dominant sewer pipe resident organisms

A study involving 71 U.S. cities sampled three times across a year [6] highlighted the consistency of the bacterial communities in sewers, which are dominated by a few members. Across 213 samples, *Arcobacter* averaged 9.2%, and *Acinetobacter* 8.1% of the community. *Aeromonas* was the third most abundant taxa comprising 1.8% of the community. *Trichococcus* represented on average 0.05% of the total community, but was found to comprise up to 1% for some cities. In this study, few spatio-temporal differences in the relative abundance of the most dominant genera were seen except for *Trichococcus* that appears more abundant in colder climates and in cooler months (Figure 2). This result is consistent with the optimal low-temperature growth reported for this genus [30,31]. Worldwide, these four genera have been reported to be prevalent in sewage [1,3,19,20,32] suggesting that urban sewer infrastructure provides a consistent environment in which they can thrive despite different physicochemical environments of specific systems or climates.

In sewer pipes, *Arcobacter* have been recovered from both sediment and biofilm communities [2,19], but comparatively, are major members in wastewater influent, suggesting *Arcobacter* are primarily planktonic. The water-sediment interface, so-called near-bed solids, may be a potential reservoir for members of this genus [33], but to date this has not been investigated. *Arcobacter* may be particularly suited to tolerate conditions near sediments. *Arcobacter* can use nitrate as a terminal electron acceptor [34,35]. They have been observed to compete efficiently with other sulfur-oxidizing bacteria by being able to tolerate high concentrations of hydrogen sulfide, and to grow in low or the absence of oxygen [35,36].

Arcobacter belong to the family *Arcobacteraceae* within the newly proposed phylum Epsilonbacteraeota [37] (formerly *Campylobacteraceae* within the Epsilon subdivision of the Proteobacteria). This genus holds about thirty species, among which, *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, are considered as potential human pathogens (see review [38] and references therein). Genome sequencing of *A. butzleri* demonstrated a large part of the genome is devoted to genes involved in growth and survival under diverse conditions [39]. Although *Arcobacter* are ubiquitous motile bacteria in terrestrial and aquatic habitats [38], they are not dominant members in the bacterial community in these environments. In contrast, in untreated sewage, *Arcobacter* are found with a density estimated around one million of cells per milliliter of wastewater [40]. The predominant species isolated from sewage include *A. butzleri and A. cryaerophilus* following by *A. thereius*, *A. defluvii*, *A. skirrowii*, *A. ellisii*, *A cloacae* and *A. nitrofigilis* [40,41]. In contrast, using 16S rDNA gene amplicon sequencing, the *Arcobacter* assemblage were found to be primarily assigned to *A. cryaerophilus*, while *A. butzleri* was only the eleventh most abundant member after *A. suis*; *A. cloacae/A. defluvii* ranked twenty-third [42]. Genome sequencing of 52 strains of *A. cryaerophilus* revealed four genomvars clusters, with human isolates only found in cluster 1 [43]. *A. butzleri* has been reported to be more easily cultured that *A. cryaerophilus* from the environment [44], and both have been found to be viable after wastewater treatment [40,45]. Temperature has been observed to shape *Arcobacter* densities and assemblages with warm and cold ecotypes observed across multiple cities [42,46]. Warm water seems to favor *A. butzleri* survival in comparison to *A. cryaerophilus* [47].

Similar to *Arcobacter*, *Acinetobacter* are motile and appear to be primarily planktonic as they are in higher proportions in influent samples relative to sediment or biofilm community members. *Acinetobacter*, within the family *Moraxellaceae*, holds about fifty species that are widely distributed in the environment from soil and water to humans [48], likely because of its ability to use a wide range of carbon sources. Sewage isolates were found to be more limited in the range of compounds they can utilize as sole carbon sources compared to soil isolates, suggesting they have been selected for with the sewer environment [49]. *Acinetobacter* is denoted as a “bacterial weed” and has developed strategies to inhibit other species though creation of an acid environment and production of biosurfactants [15]. Strong seasonal shifts in the relative abundance and density of *Acinetobacter* ecotypes have been observed across a 4-year survey of U.S. sewage [5]. Full length cloned 16S rRNA gene sequences suggested that changes were due to reoccurring cycles of dominant ecotypes within the genus rather than seasonal replacement by species [5].

***Arcobacter* as a tracer of urban sewer impacts on receiving water.**

Urban sewer infrastructure is designed to carry harmful waste to the treatment plant. Over time, these systems deteriorate causing sewage to be released directly into the environment and groundwater, or indirectly through stormwater systems that act as a conduit to surface waters (Box 1). In addition, each year, millions of liters of untreated sewage are released during combined sewer overflows. Sewage contamination is evident after rainfall in the absence of known sewage overflows [50], and microbial communities from pipes can serve as an early warning that pipe are failing [2].

The two most dominant organisms in untreated sewage, *i.e. Arcobacter* and *Acinetobacter*, are prevalent in lakes, rivers, and coastal waters in or near urban areas. Several studies reported the presence of *Arcobacter* spp. in environmental waters was associated with high levels of fecal pollution [46]. In coastal waters where dilution reduces signals, *Arcobacter* was a sensitive measure of microbial footprint of the urban area [42,51]. *Arcobacter* may be the more specific indicator of sewage contamination given that *Acinetobacter* has also been observed to be prevalent in stormwater when there was no other evidence of sewage [52]. Worldwide, *Arcobacter* has been found in contaminated urban waters [53-57].

Conclusions

Modern sewer infrastructure has created a new habitat for microbial communities to develop and reach high biomass at a city-wide scale. The genomic potential and ecology of the dominant organisms within these communities may provide clues to the nutrient conversions and antibiotic resistance reservoirs within pipes, as well as illustrate more basic themes of fitness strategies in complex and highly competitive environments. These communities are not contained to the pipes of a city, and the impact due to the release of sewer-pipe organisms has not yet been explored. Further, these organisms are potentially ideal tracers of urban sewage contamination that release a plethora of chemicals, nutrients, and harmful microorganisms carried by sewage. Without question, increasing urbanization and hydrological pressures on urban infrastructure from climate change will make this a more relevant issue in the future.

Sewage exfiltration from pipes had previously been considered contained to surrounding soils and relatively harmless [58]. However, studies demonstrate sewage can be mobilized during rain events and reach groundwater and stormwater systems [59-62]. Sewage is released through joints or deteriorating pipes and exfiltration can be as high as 10% of dry weather flows [58,61,63]. If deteriorating sewage pipes are above groundwater elevations, the positive pressure in the pipe causes sewage to be released [64,65] (See Box 1 Figure). Researchers have developed a probability based model to predict exfiltration that relies on pipe attributes and groundwater elevation without prior knowledge of the locations of defects [61]. In other studies, Ly et al. [63] modelled local-scale migration of sewage leakage from a sewage pipe to nearby stormwater drains. Given the thousands of miles in cities though the U.S., modeling is useful to identify area for more labor intensive testing with closed circuit televising, dye flooding, or by molecular methods [59,60].

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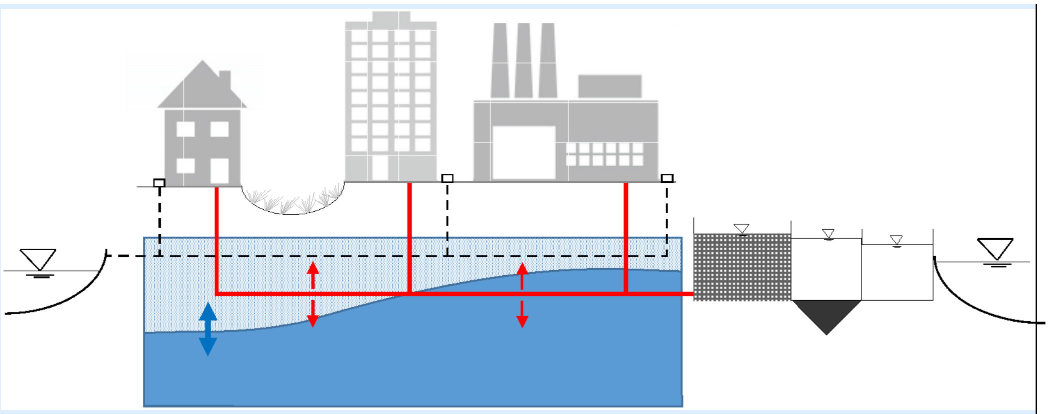
Highlights

- *Arcobacter*, *Acinetobacter*, and *Aeromonas* dominate communities in sewage.
- Pipe organisms in sewage influent have abundance patterns distinct from biofilm.
- The high stress environment selects for organisms with unique fitness strategies.
- Sewer pipe organisms carry a wide range of antibiotic resistance genes.
- Urban sewer pipes leak and during rain, sewage is mobilized to stormwater systems.
- *Arcobacter* is an indicator of urban impact and readily detected in waters worldwide.

Box 1

Sewage exfiltration from urban infrastructure



Box 1 Figure.

Illustration of the dynamic between urban sanitary sewers and stormwater systems with changing groundwater levels.

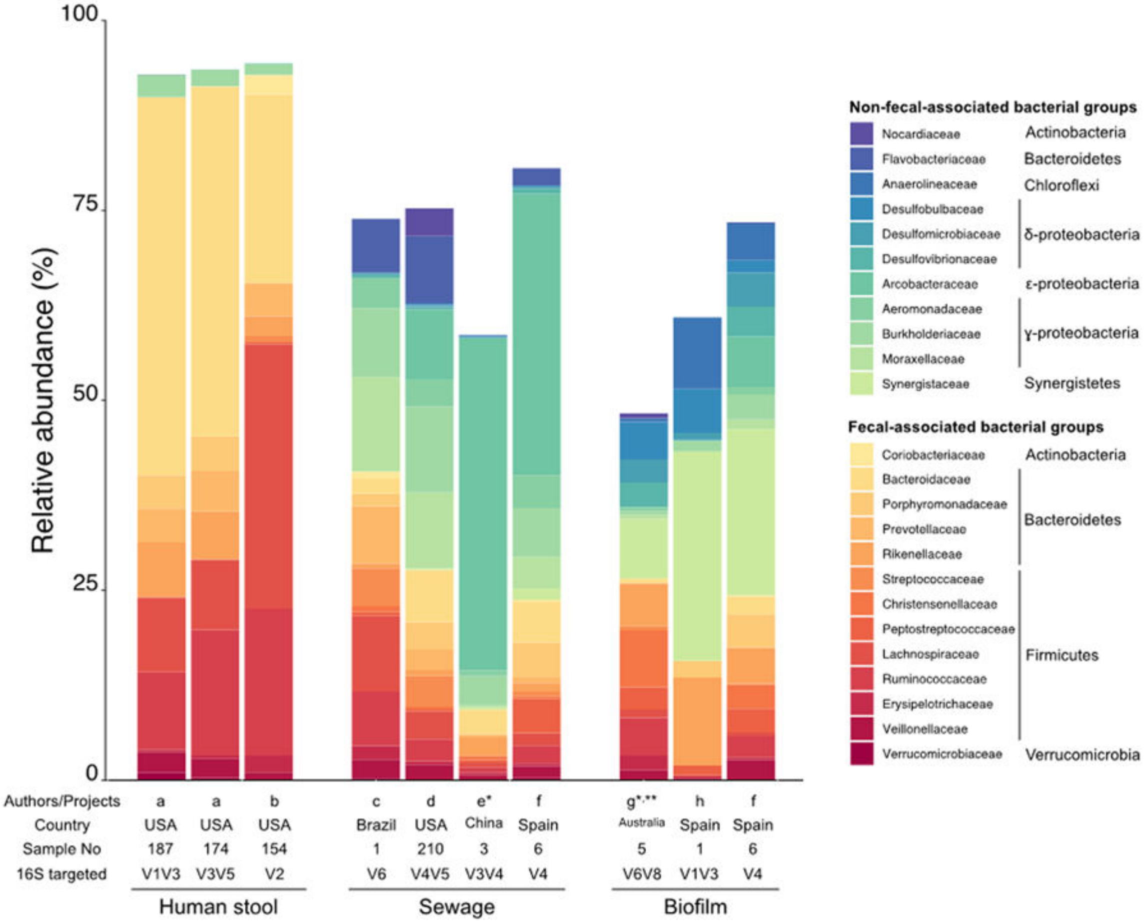


Figure 1.

Dominant bacterial families in fecal human microbiome, untreated sewage and sewer biofilm based on 16S rRNA gene amplicon data. Only the top 10 dominant families among at least one of the three environments are displayed. Fecal-derived taxa are represented using red/orange hue colors, while non-fecal-derived taxa are colored in blue/green hue. List of the publications: a [26], b [66], c [67], d [6], e [3], f [19], g [68], h [69]. *: data reprocessed from the SRA files using the 454 Mothur SOP and Silva132 database. **: sewer pilot.

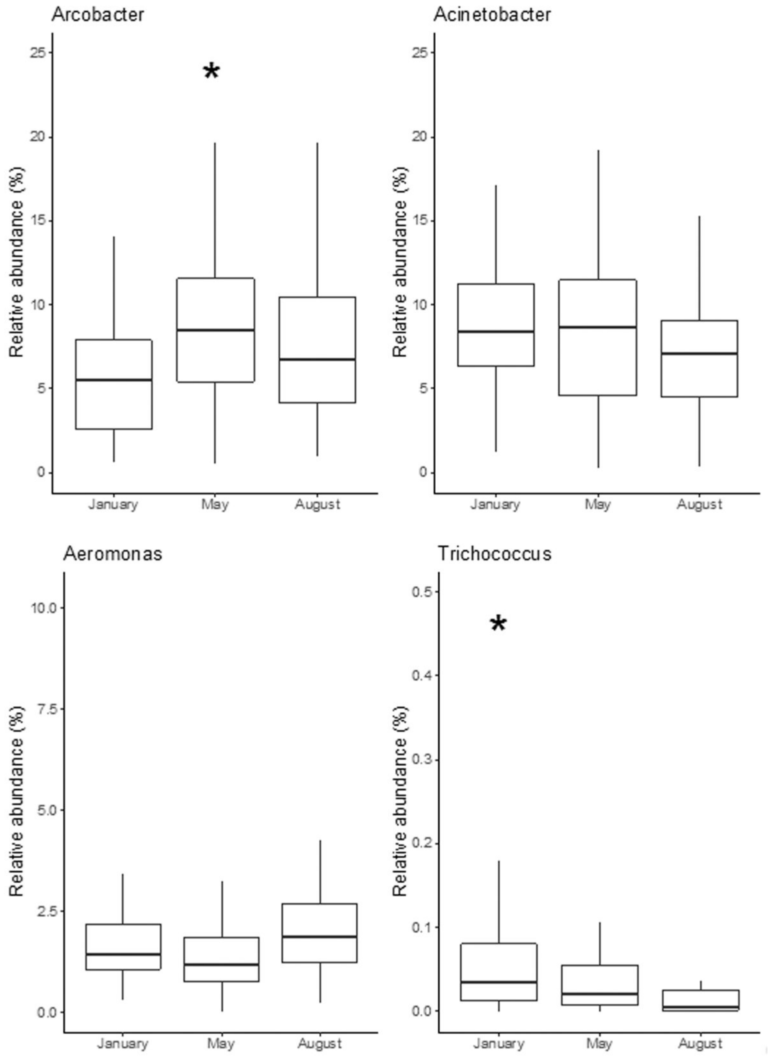


Figure 2.

Box plot depicting the relative abundance of the bacterial groups *Arcobacter*, *Acinetobacter*, *Aeromonas* and *Trichococcus* within sewage influent from 71 U.S. cities [6]. A total of 31 cold cities (blue dots, average air temperature over the campaigns ≤ 10°C), and 40 warm cities (red dots, average air temperature over the campaigns > 10°C) were sampled in January, May and August between 2012 and 2013. *Six outliers were removed from the plot for *Arcobacter* (88.9, 85.5, 84.4, 73.1, 58.9, and 44.6%) and one for *Trichococcus* (1.5%).

Figure 3.

Log2 fold change comparison between the relative abundance of bacterial families in untreated sewage and biofilm. Families more abundant in sewage are represented using green bars, while families more abundant in biofilm are in orange. Relative abundance of the families per compartment are listed on the left of the plot. The relative abundance per family was computed by averaging the abundances pondered with the number of sample from the publications described in the Figure 1. Relative abundance higher than 10% are in bold. Only the families with a relative abundance higher than 1% in at least one of the two compartments were considered.