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# Shifts in dissolved organic matter and microbial community composition are associated with enhanced removal of fecal pollutants in urban stormwater wetlands

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A B S T R A C T

Constructed stormwater wetlands provide a host of ecosystem services, including potentially pathogen removal. We present results from a multi-wetland study that integrates across weather, chemical, microbiological and engineering design variables in order to identify patterns of microbial contaminant removal from inlet to outlet within wetlands and key drivers of those patterns. One or more microbial contaminants were detected at the inlet of each stormwater wetland (*Escherichia coli* and *Enterococcus* > *Bacteroides* HF183 > adenovirus). *Bacteroides* HF183 and adenovirus concentrations declined from inlet to outlet at all wetlands. However, co-removal of pathogens and fecal indicator bacteria only occurred at wetlands where microbial assemblages at the inlet (dominated by Proteobacteria and Bacteriodetes) were largely displaced by indigenous autotrophic microbial communities at the outlet (dominated by Cyanobacteria). Microbial community transitions (characterized using pyrosequencing) were well approximated by a combination of two rapid indicators: (1) fluorescent dissolved organic matter, and (2) chlorophyll *a* or phaeophytin *a* fluorescence. Within-wetland treatment of fecal markers and indicators was not strongly correlated with the catchment-to-wetland area ratio, but was diminished in older wetlands, which may point to a need for more frequent maintenance.



## 1. Introduction

Surface flow constructed wetlands have been an accepted method of water pollution control since the 1950's (Vymazal, 2011). They gained widespread popularity in North America between the 1970's and 1990's for tertiary treatment of municipal wastewater, and more recently (early 2000's and on) for treating urban storm and dry-weather runoff (Vymazal, 2011; Adyel et al., 2017). Constructed wetlands confer hydrologic (e.g., peak flow reduction) as well as water quality benefits (e.g., removal of suspended solids, microbial pathogens, nutrients, and heavy metals) (Carleton et al., 2001; Karim et al., 2004; Vymazal, 2011). They also perform notable ancillary ecosystem services (e.g., related to aesthetics, recreation, habitat provisioning, biodiversity, and public health (Hsu et al., 2017)) as well as disservices (e.g., greenhouse gas emission, among others (Mehring et al., 2017)).

Compared to wastewater wetlands, relatively little is known about the water quality performance and ecosystem services provided by stormwater wetlands. This is particularly true for public health services and the fate and transport of microbial

*Abbreviations:* A-peak, UV humic-like fluorescence; BIX, freshness index; B-peak, tyrosine-like fluorescence; Chl, chlorophyll *a*; C-peak, visible humic-like fluorescence; CR, catchment ratio; DOM, dissolved organic matter; EC, *Escherichia coli*; ENT, *Enterococcus*; FI, fluorescence index; FIB, fecal indicator bacteria; $HIX_{EM}$ and $HIX_{SYN}$, humification indices; HF183, human-specific fecal marker *Bacteroides* HF183; M-peak, marine-type DOM fluorescence; OTU, operational taxonomic unit; PCA, principal component analysis; PCoA, principal coordinate analysis; Phae, phaeophytin *a*; T-peak, tryptophan-like fluorescence; TSS, total suspended solids; VSS, volatile suspended solids.

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contaminants (Jiang et al., 2015; Hsu et al., 2017). Indeed, despite widespread acknowledgment that fecal indicator bacteria (FIB) and human pathogens behave differently in surface waters (reflecting regrowth of FIB, inputs from non-human sources, and differential fate and transport behavior (Savichtcheva and Okabe, 2006)), FIB remain the primary indicator of microbial water quality. FIB removal has been reported to be more variable in stormwater wetlands than wastewater wetlands (Hsu et al., 2017), ranging between −20% (i.e., increasing from inlet to outlet) to 96% for the most frequently reported indicator, *Escherichia coli* (EC) (Hsu et al., 2017; Hathaway et al., 2009). This variability is a challenge from a stormwater management perspective, and it remains unclear the extent to which it reflects true variability in underlying health risk.

Contaminants more closely related to public health (namely pathogens, human-specific fecal markers like *Bacteroides* HF183, and antimicrobial resistance genes) are less frequently evaluated in stormwater wetlands than FIB, with recent work by Hsu et al. (2017) being a notable exception. Hsu et al. (2017) found that shiga toxin-producing EC, *Arcobacter*, *Bacteroides* HF183 and tetracycline and sulfonamide resistance genes were all prevalent in a stormwater wetland in Ohio (present in 16.9–98.3% of samples). Furthermore, minimal attenuation was observed from inlet to outlet, suggesting that some stormwater wetlands confer little treatment of human pathogens and fecal markers. Given these results, there is an urgent need for additional information on fecal marker and FIB removal in stormwater wetlands, and the chemical, climatic, microbiological, and engineering design characteristics that underlie treatment variability.

Here we present results from a multi-wetland study across two countries (the US and Australia) that addresses the knowledge gap described above. Our study combines pathogen detection with structural analysis of the microbial community to provide a more complete picture of wetland microbiological state than is typically reported. Integrated wetland analysis is used to evaluate co-variation across multiple indicators of wetland performance (pathogen and phytoplankton abundance, suspended solids concentration, microbial community composition, and fluorescent indicators of dissolved organic matter presence and processing) as well as chemical, weather, and engineering design-related variables, in order to characterize the key public health services provided by stormwater wetlands and their drivers. The study focuses on dry-weather wetland performance, but also demonstrates the capacity of rain events to fundamentally alter a wetlands microbiological state, impacting community composition, organic matter processing, and treatment performance.

## 2. Methods

### 2.1. Site description

Five stormwater wetlands were sampled during this study, two in Orange County, California, USA (Forge and Old Laguna), and three in Melbourne, Victoria, AU (Royal Park, Hampton Park, and Lynbrook Estates). These wetlands were selected because they are typical stormwater wetlands in their respective areas and were built in response to similar water quality initiatives. The three AU wetlands are part of a large stormwater treatment system (407 wetlands total), intended to reduce nitrogen pollution to Port Phillip Bay (Carew et al., 2012), whereas Forge and Old Laguna are part of the first wave of stormwater treatment wetlands in Orange County, also intended to improve local water quality (IRWD, 2005). 47 wetlands are currently planned for Orange County and as of 2017, only 27 have been built. This makes it an opportune time to compare these constructed wetlands to others with similar design goals, and inform ongoing stormwater management efforts.

#### 2.1.1. Catchment characteristics and pollutants of concern

All wetlands sampled during this study drain urban catchments with 28–71% total imperviousness (Lynbrook Estates > Royal Park > Hampton Park > Forge > Old Laguna; Table S1) (SI Methods). Most catchments are primarily comprised of residential land (34–97%) and open space/parkland (3–45%; Fig. 1, Table S1). Three catchments have agricultural and commercial land-use (Forge: 34% agricultural, 8% commercial; Old Laguna: 5% agricultural, 12% commercial; and Hampton Park: ~2% each). One catchment (Royal Park) has light industrial land-use (14%).

In all catchments, runoff is expected to occur year-round, with stormwater runoff occurring primarily in winter and spring, and dry-weather runoff from over irrigation of residential landscape and car washing (IRWD, 2005) occurring when precipitation is low (Table S1). An additional source of dry-weather runoff (exfiltration of perched groundwater into the storm drain system) likely occurs at Forge and Old Laguna, where the groundwater table is shallow (IRWD, 2005). Dry-weather runoff can be a significant fraction of total runoff in Southern California, ranging from 45% in dry years to 25% in wet years (e.g., years in the 10th and 90$^{th}$ percentile of rainfall, respectively) (Stein and Ackerman, 2007). It also contributes to total pollutant loading, particularly during dry years, when up to 25% of coliform and 47% of heavy metal loads can be from dry-weather runoff (Stein and Ackerman, 2007). Dry-weather runoff is expected to constitute a smaller fraction of total runoff in Melbourne, which receives more continuous rainfall (Ambrose and Winfrey, 2015). Our study focuses primarily on dry-weather conditions (see section 2.2.1) to minimize this anticipated difference.

Despite the above noted differences in runoff and land-use, major pollutants of concern across all five catchments are similar, and include suspended solids, nitrogen, and phosphorus (Table S1). Pathogens are also a concern, particularly at Royal Park, where treated stormwater is used for irrigation (Pfleiderer, 2009). Other contaminants of concern include pesticides (organophosphate pesticides at Forge and Old Laguna (IRWD, 2005), and pyrethroid insecticides at Lynbrook Estates (Amis, 2016)), as well as heavy metals, particularly cadmium, copper, lead, and zinc. Metals are of particular concern at Old Laguna and Forge, which drain to downstream waterbodies that are under total maximum daily load restrictions for heavy metals (IRWD, 2005).

#### 2.1.2. Wetland design characteristics

Design and maintenance details for all wetlands are reported in Table S2. Forge and Old Laguna are managed by the Irvine Ranch Water District. Both wetlands are linear with a single major inlet and outlet, although Old Laguna has a nonoperational secondary inlet (Fig. 1A and B). Forge came online in June 2007, has a 1.2 ha footprint, and a catchment ratio (CR; (Wetland Area/Catchment Area) x 100) of 0.95%. Old Laguna is slightly older and smaller; it came online in February, 2006, has a 1.0 ha footprint, and a CR of 0.57% (IRWD, 2005). Both wetlands primarily treat dry-weather and small storm flows, and were designed to have a low-flow hydraulic residence time of 10 days (calculated assuming plug flow from inlet to outlet (IRWD, 2005)). They have extended detention capacity (controlled by perforated riser outlets) to detain first flush storm flows (Forge: 9967 $m^3$, Old Laguna: 13322 $m^3$) (IRWD, 2005). Extended detention hydraulic residence times are 36- and 48-hrs for Forge and Old Laguna, respectively (IRWD, 2005). Large storm flows in excess of the first flush are routed through major storm drain channels by manhole diversion weirs at the inlet of each