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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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ABSTRACT

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 covariation 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 90th 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

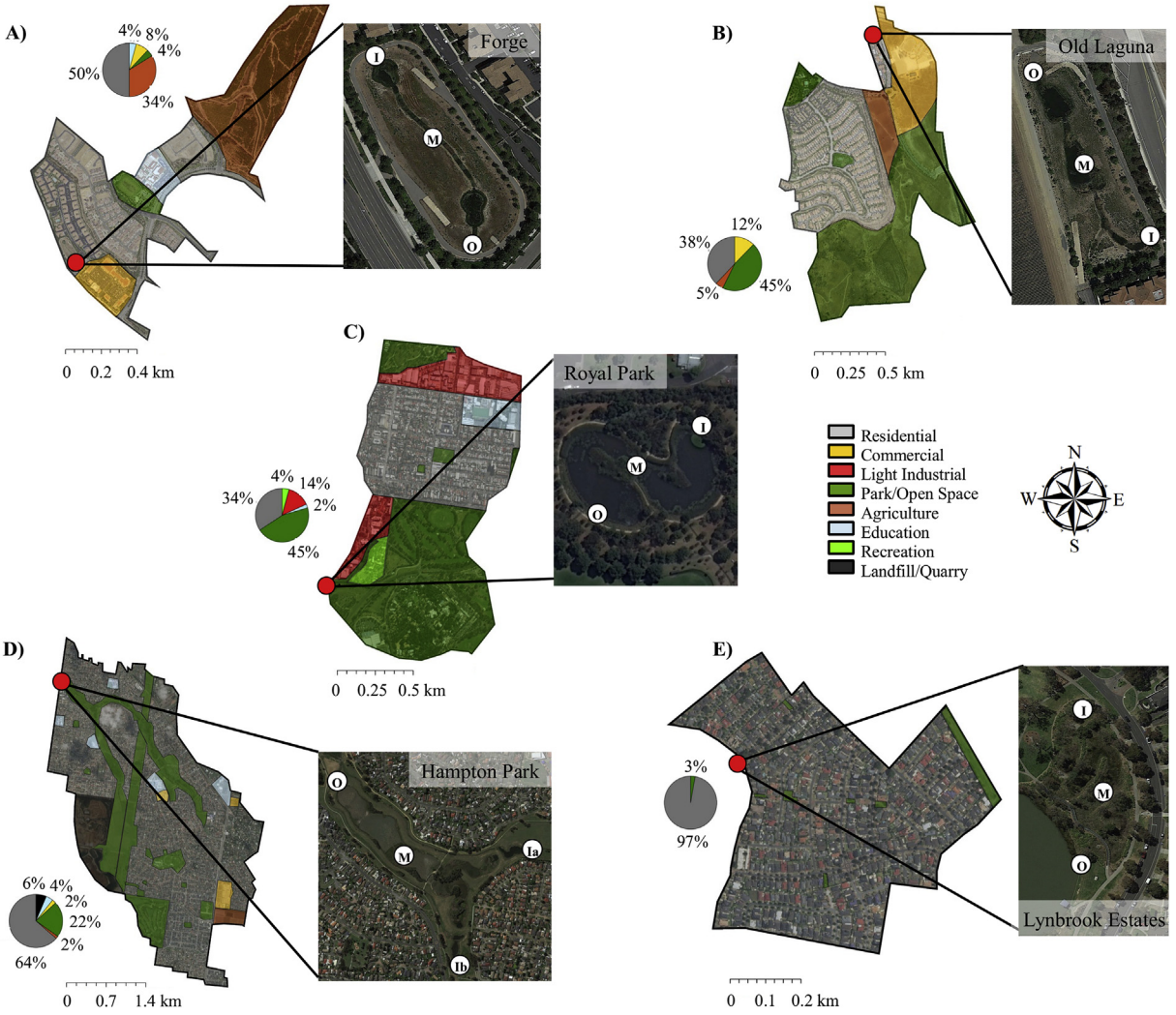

Fig. 1. Maps of catchment land-use are shown alongside wetland photographs for each wetland sampled during this study (A) Forge, (B) Old Laguna, (C) Royal Park, (D) Hampton Park, and (E) Lynbrook Estates. Wetland sampling locations are denoted on each wetland photograph (I = inlet, M = middle station, and O = outlet). Land-use characteristics are in color: grey (residential), yellow (commercial), red (light industrial), burnt orange (agricultural), dark green (parks and open space), light blue (educational), light green (recreational), and black (landfill/quarry). Pie charts detail the land-use breakdown of each catchment. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

wetland.

Royal Park wetland is managed by Melbourne Water. It came online in 2006, has a sigmoid footprint with a single inlet and outlet (0.8 ha), and a CR of 0.43% (Fig. 1C) (Pfleiderer, 2009). It is part of a stormwater treatment train whereby urban runoff is (1) diverted from the Royal Park drain by a high flow diversion weir, (2) passed through a silt trap and trash rack before entering the wetland, (3) drained to a secondary storage pond (capacity of 12000 m^3) through a submerged orifice outlet, and (4) UV treated and stored for later use as irrigation water (Pfleiderer, 2009). The wetland has no internal extended detention (all excess drains to the storage pond), and was designed to have a hydraulic residence time of at least 72-hrs, 90% of the time (MW, 2017).

Like Royal Park, Hampton Park is managed by Melbourne Water. It came online in 2000, has a footprint of 5.7 ha, and a CR of 0.42% (Fletcher et al., 2004). It is the only wetland that is Y-shaped, with two major inlets forming the arms of the Y (Fig. 1D). The wetland has extended detention (volume of 35300 m^3), and is drained by a perforated riser outlet and secondary spillway (Fletcher et al., 2004). As with Royal Park, Hampton Park was designed to have a hydraulic residence time of at least 72-hrs, 90% of the time (MW, 2017, Fletcher et al., 2004). Hampton Park was drained, dredged, and re-vegetated to improve hydrologic performance in 2014; draining and dredging operations were completed prior to our sampling efforts in July whereas re-vegetation work continued until November (see Fig. S1).

Lynbrook Estates, the oldest constructed wetland sampled during this study (online in 1999), is part of a water sensitive urban design demonstration project (the first neighborhood-scale project of its kind). The wetland is part of a stormwater treatment train whereby runoff from residential roads and rooftops is (1) pre-filtered and conveyed to the wetland by a network of bio-infiltration swales, (2) discharged to an ornamental lake via an overflow riser outlet, and (3) infiltrated to irrigate native trees or discharged to a regional floodway (Lloyd et al., 2002). The wetland has a sigmoid footprint (0.6 ha) and consists of a winding series of pools separated by riprap, with a single inlet and outlet, and a CR of 1.88% (Lloyd et al., 2002) (Fig. 1E).

2.2. Sample collection

2.2.1. Surface water samples

Surface water samples were collected in 2014 during dry-weather conditions (i.e., no precipitation during sampling and minimal (0.15 mm) to no precipitation 24-hrs prior to sampling; Table S2). Forge and Old Laguna were sampled in Boreal summer whereas Royal Park, Hampton Park and Lynbrook Estates were sampled in Austral winter. This means that sampling season was convolved with geographic location during this study. Additional samples were collected at Forge in winter 2015, before, during, and following a medium-sized rain event (6.6 mm of precipitation during and 13.7 mm over the preceding 72-hrs; Table S2). Water samples were collected at the inlet, middle, and outlet of each wetland (Fig. 1) and analyzed for: chlorophyll *a* (Chl, a proxy for phytoplankton), phaeophytin *a* (Phae, an indicator of detritus), total and volatile suspended solids (TSS and VSS, the total and organic fraction of suspended particles, respectively), the FIB *Escherichia coli* and *Enterococcus* (EC, ENT), the human-specific fecal marker *Bacteroides* HF183 (HF183), human pathogens (adenovirus, *Cryptosporidium parvum*), and microbial community composition (16S rRNA gene). Dissolved organic matter composition was also evaluated, but only at wetland inlets and outlets. All parameters were measured during dry-weather (2014), whereas only microbial parameters were measured during rain events (2015). Water samples were stored in coolers on ice and transported to the lab within 8 h for analysis (sample collection and processing details are in Fig. S2 and section 2.3).

2.2.2. Sensor measurements and climatic variables

Handheld dissolved oxygen meters (YSI ProODO, US; Thermofisher Orion Star, AU) were used to measure water temperature and dissolved oxygen at all sites in 2014 (average taken over ten measurements collected every 30 s). Cumulative precipitation during, 24-hrs, and 72-hrs prior to sampling was estimated from the following rain gages: (1) Melbourne Bureau of Meteorology (BoM) 86039 located 3 km from Royal Park; (2) BoM 86299, 86375, 86224, and 86210 located near equidistant (7–10 km) from Lynbrook Estates and Hampton Park; and (3) Orange County Public Works (OCPW) 61 located 4.1 and 9.2 km from Forge and Old Laguna, respectively. Site-specific daily maximum solar radiation (300–1100 nm wavelengths) was also estimated for each wetland using the simple model of the atmospheric radiative transfer of sunshine (SMARTS) (Gueymard, 2005) (Table S2).

2.3. Analytical methods

2.3.1. TSS, VSS, Chlorophyll a, and phaeophytin a

1.5-L water samples were collected in amber bottles at the inlet, middle, and outlet of each wetland. Upon arrival at the lab, each sample was subdivided (half for TSS/VSS and half for Chl/Phae). TSS analysis was performed in triplicate using pre-combusted glass fiber filters (ProWeigh, Charleston, SC) following APHA SM 2540D (APHA, 1998). After TSS analysis, all filters were combusted at 550 °C for 30 min and re-weighed to quantify VSS following APHA SM 2540E (APHA, 1998). Chl and Phae were analyzed in triplicate following APHA SM 10200H, omitting the sample grinding step (APHA, 1998). Spectrophotometric quantification was performed using a Thermo Scientific™ Genesys 20 spectrophotometer (Waltham, MA, USA).

2.3.2. Dissolved organic matter (DOM)

40-ml water samples were collected at wetland inlets and outlets using sterile borosilicate glass vials with silicone septa. All samples were wrapped in tinfoil to prevent photoreaction and filtered through 0.2 μm polyvinylidene fluoride syringe filters (Millipore, Billerica, MA) into sterile, foil wrapped vials. Samples were stored refrigerated (US sites) or shipped on dry ice and then refrigerated (AU sites) prior to analysis for DOM using excitation-emission fluorescence spectrometry (EEMs).

EEMs were evaluated using a FluorMax-4 spectrometer (Horiba Jobin Yvon, Inc.) with varied excitation and emission wavelengths (Ex: 250–550 nm, 5 nm interval; Em: 250–600 nm, 2 nm interval). Corrected EEMs were generated from raw scans following Rippy et al. (2016) (details in SI Methods) and used to estimate the following DOM components: (1) A-peak: UV humic-like, (2) C-peak: visible humic-like, (3) M-peak: marine humic-like (also observed in freshwater), (4) B-peak: tyrosine-like, and (5) T-peak: tryptophan-like (Coble et al., 2014). Several fluorescent indices were also calculated as per Coble et al. (2014): (1) the freshness index (BIX), which indicates if DOM is recently created or degraded, (2) the fluorescence index (FI), which indicates if DOM precursor material is of microbial or terrestrial (higher plant) origins, (3) two versions of the humification index (HIX_{SYN} and HIX_{EM}), which indicate the degree of DOM humification and recalcitrance, and (4) the T:C peak ratio, a proxy for sewage and algal DOM. See Fig. S3 (inspired by Coble et al., 2014) for a graphical illustration of these metrics.

2.3.3. Fecal indicator bacteria (FIB)

Water samples for FIB analysis were collected at each wetland (inlet, middle, and outlet) in 1.5-L sterile bags (Whirl-Pak, Fort Atkinson, WI). EC and ENT were enumerated using Colilert and Enterolert, respectively, implemented in a 97-well quantitray format (IDEXX, Westbrook, ME) following manufacturer's recommendations.

2.3.4. Pathogens, human fecal markers, and microbial community analysis

20-L water samples were collected at each wetland (inlet, middle, and outlet) using a battery powered, peristaltic pump and filtered on-site with a NanoCeram® cartridge filter (Argonide, Sanford, FL). The cartridge filter was transferred into a sterile bag containing 70 mL of elution buffer (Ikner et al., 2011) and refrigerated at 4 °C overnight. The following morning, elution buffer was collected, adjusted to pH 7.2, and concentrated by polyethylene glycol (PEG) precipitation as in Sánchez et al. (2012). Final PEG concentrates (2–3 mL/sample) were used for microbial community analysis and pathogen/human fecal marker analysis.

Microbial community structure was investigated using Tag-encoded 454 FLX-amplicon pyrosequencing. PEG concentrate (500 μL/sample) was shipped on dry ice to RTL Genomics (Lubbock, TX), where amplicon libraries were prepared and sequenced. Microbial diversity was evaluated with the 939f-1492r bacterial 16S rRNA gene assay, which corresponds to variable regions 6–9 of the 16S rRNA gene (Coats et al., 2014). Sequence data were analyzed using QIIME VERSION 1.9.0 (Kuczynski et al., 2012). Sequences that were fewer than 200 base pairs in length, contained ambiguous characters, had quality scores <25, or any mismatches to primer sequences were excluded from analysis. USEARCH (Edgar, 2013) was employed for chimeric sequence detection and operational taxonomic unit (OTU) selection at 97% sequence similarity. Taxonomic assignments were conducted using the 2013 Greengenes database (DeSantis et al., 2006).

Concentrations of HF183, *C. parvum*, and human adenovirus were analyzed using QX100 ddPCR (Bio-Rad, Hercules, CA). DNA

extraction was performed on 200 μL of PEG concentrate per sample using the PowerSoil® DNA Isolation Kit (Carlsbad, CA). ddPCR assays were performed as in Cao et al. (2015) using the primers and probes summarized in Table S3 (SI Methods).

2.4. Data analysis and statistical methods

2.4.1. DOM patterns

Principal component analysis (PCA) was used to identify dominant patterns in DOM composition across wetlands. Fluorescent components and indices from section 2.3.2 were used as inputs for this analysis. A resampling-based stopping rule detailed in Rippy et al. (2017) was used to identify dominant principal component (PC) modes that explained significantly more variance in DOM composition than expected by chance ($p < 0.05$ level). 95% confidence bounds about these modes and their corresponding scores were determined using non-parametric bootstrap analysis (Babamoradi et al., 2013).

2.4.2. Microbial patterns

Microbial community composition was compared within and across wetlands using principal coordinate analysis (PCoA) performed on a matrix of weighted UniFrac distances (Paily and Shankar, 2016). Samples collected during dry-weather conditions were analyzed separately and in combination with storm samples to determine if storm conditions introduced changes in microbial community structure not apparent during dry weather. Indirect bootstrapped correlation analysis of PCoA values vs microbial OTU scores was used to estimate the contribution of each OTU to PCoA axes (Paily and Shankar, 2016).

2.4.3. Relationships among weather, chemical, microbial, and engineering design variables

Bootstrapped Pearson's correlation analysis and agglomerative hierarchical clustering analysis were used to evaluate relationships among weather, chemical, microbial, and engineering design variables across wetlands, during dry-weather conditions. All measured variables, the first two DOM PC modes from *2.4.1*, and the first two microbial PCoA values from *2.4.2* were included in this analysis. Variables spanning three orders of magnitude or more were log-transformed to improve normality prior to analysis. Bootstrapped correlations were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (Benjamini and Hochberg, 1995). Hierarchical agglomerative clustering analysis was performed on all variables using R software (package Pvclust, Suzuki and Shimodaira, 2006). Clusters were determined using the between-group average method, where the proximity between clusters equals the arithmetic mean of the proximities between all constituents of each cluster. Multiscale bootstrap resampling was used to estimate the support for each cluster. The broadest possible clusters at a 90% or 95% confidence level were retained as coherent variable units.

2.4.4. Average wetland states and within-wetland treatment trajectories

PCA was performed on all variables included in the hierarchical clustering analysis (1) averaged across each wetland and (2) with wetland-specific means removed, isolating within-wetland variability. Dominant PC modes were evaluated relative to coherent variable units (section 2.4.3) to determine if clusters of weather, chemical, microbiological, or engineering design variables differentiate average wetland states or within-wetland treatment trajectories (e.g., patterns of pollutant removal from inlet to outlet within a wetland). This approach was employed in lieu of traditional methods for evaluating treatment performance because it integrates across multiple indicators of wetland function and facilitates inclusion of nontraditional information streams like microbial community composition. Furthermore, given that this study prioritized sampling breadth over depth, we believe that mass or load reduction estimates for individual wetlands would be misleading.

3. Results and discussion

3.1. Weather and chemistry

Only one wetland (Forge, winter 2015) received > 1 mm of precipitation during or 24-hrs prior to sampling (Table S2). Precipitation was more variable 72-hrs prior to sampling (0–13.7 mm), occurring at all sites sampled in winter, but not summer (Table S2). Wetland catchment ratios ranged from 0.4 to 1.9%, and were similar at US and AU sites (Table S2). Only one wetland (Lynbrook Estates) had a catchment ratio > 1% (a minimum of 1–3% is recommended (Carleton et al., 2001)), suggesting that most wetlands were undersized.

Maximum solar radiation, surface water temperature, and dissolved oxygen were approximately two-fold higher at sites sampled in Boreal summer than in Austral winter (Tables S2 and S4). Average concentrations of Chl also differed at sites sampled in summer versus winter (311.6 μg/L in summer; 20.2 μg/L in winter; Table S4), and were positively correlated with maximum solar radiation, dissolved oxygen, and surface water temperature (Fig. 2), consistent with the expectation of enhanced primary production in the summer.

TSS and VSS concentrations were lowest at Hampton Park and highest at Royal Park (Table S4). TSS and VSS were significantly positively correlated with each other ($r = 0.8$) and Chl ($r = 0.51–0.63$), but not with other variables exhibiting strong seasonality (for instance, solar radiation, $r = 0.04–0.2$; Fig. 2). This suggests that TSS and VSS patterns across wetlands were not strictly seasonal. However, the positive relationship between solids and Chl suggests that algal biomass contributes to the suspended solids pool.

3.2. DOM composition

Across all wetlands, the primary fluorescent DOM components were (in order of decreasing intensity): A-peak > C and M peaks > T-peak > B-peak (see Fig. S3 for typical peak intensities). The prevalence of peaks A and C was unsurprising given their near ubiquity in freshwater systems, often associated with humified, terrestrial DOM (Coble et al., 2014). Indeed, average DOM composition across wetlands was primarily terrestrial, humified and old, as indicated by the low average FI index (1.4; common for plant litter or soil-derived DOM), high humic index (0.94; indicative of recalcitrant, humified DOM), and moderate-low BIX index and T:C ratio (0.52 and 0.36, respectively; indicative of old, allochthonous DOM) (Coble et al., 2014; Hansen et al., 2016) (Table S4; details in SI Methods).

Although wetland DOM (on average) was terrestrially-derived, humified, and old, its composition varied both within and between wetlands. This variability was captured by two PCs (DOM PC1: 63% variance explained and DOM PC2: 27% variance explained), both significant at a $p < 0.05$ level (resampling-based stopping rule; Fig. S4). Both PCs are shown as a biplot (Fig. 3). Biplot vectors (thick black lines) indicate the relative contribution of

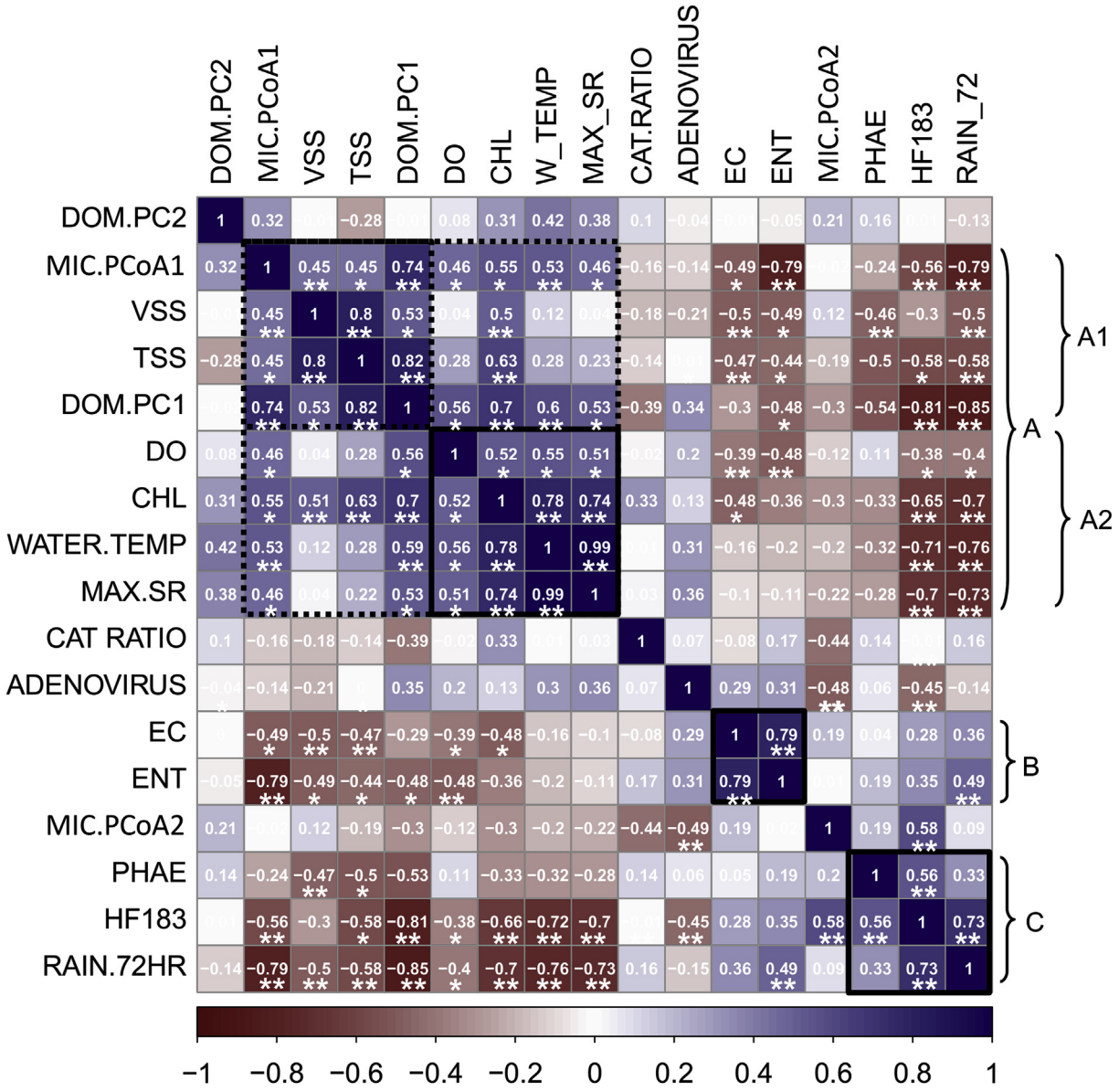

Fig. 2. Bootstrapped Pearson's correlation coefficients for all combinations of measured variables (positive = blue, negative = red). Correlations that are significant at a $p < 0.05$ or $p < 0.1$ level following correction for multiple comparisons are marked with ** or *, respectively. Groups of variables that cluster significantly according to hierarchical agglomerative clustering analysis are grouped using solid ($p < 0.05$) or dashed ($p < 0.1$) black lines, and labeled as groups A1 through C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

individual fluorescent components or indices to each DOM PC: vectors angled along the x-axis (y-axis) contribute primarily to DOM PC1 (DOM PC2). The location of individual wetland samples in different biplot quadrants indicates which fluorescent components or indices (and therefore DOM PCs) best characterize the sample.

DOM PC1 was defined by fluorescent components and indices, with FI, BIX, the T:C ratio, and B-peak fluorescence constituting significant positive loadings and HIX_{EM}, HIX_{SYN}, A, C, and M-peak fluorescence constituting significant negative loadings (Fig. 3). This implies that wetland sites with negative DOM PC1 scores had more allochthonous, older, and humified DOM whereas wetland sites with positive DOM PC1 scores had mixed allochthonous and autochthonous (e.g., algal and microbial) DOM that was fresher and more biodegradable. The significant positive correlation between DOM PC1 and Chl ($r = 0.7$, $p < 0.05$; Fig. 2) was consistent with this interpretation. DOM PC2 was defined by fluorescent components only, with high (low) intensity fluorescence constituting significant positive (negative) loadings (Fig. 3). This suggests that wetland sites with positive DOM PC2 scores had more fluorescent DOM than sites with negative scores. DOM PC2 was not significantly correlated with any other measured variables (Fig. 2).

Taken together, these PC modes defined four quadrants in PC space with distinct DOM characteristics that typify specific wetlands and/or wetland sites. For instance, all sites at Hampton Park clustered tightly in the −/− PC quadrant, suggesting little DOM variability inlet to outlet, and that available DOM was terrestrial, old, and humified (cyan symbols, Fig. 3). This is typical of winter DOM patterns in wetlands (Singh et al., 2014). In contrast, Forge lay in the +/+ PC quadrant, suggesting that DOM was abundant, fresh, biodegradable, and to some extent algal or microbial in origin,

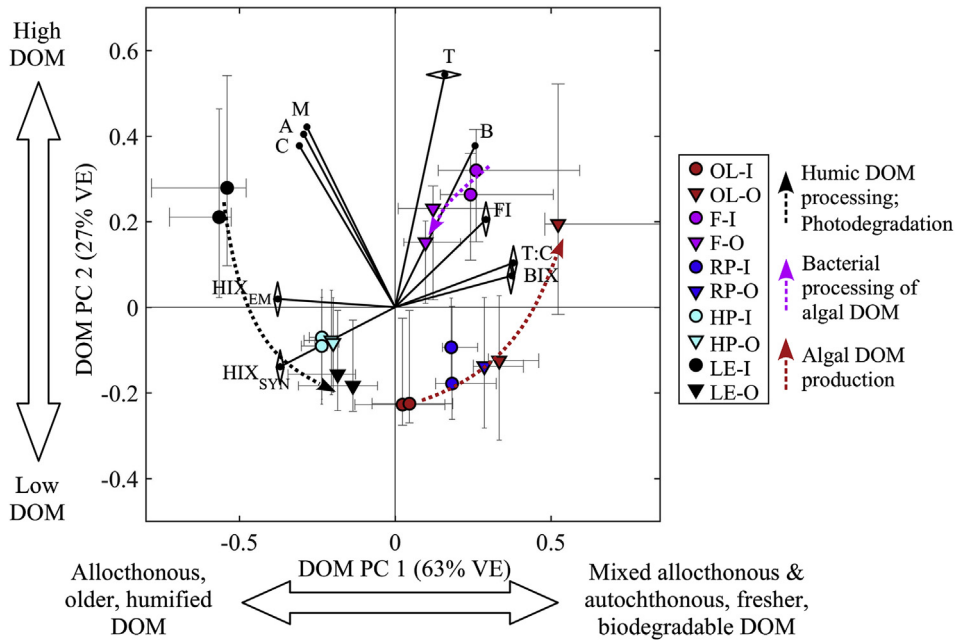

Fig. 3. Biplot of fluorescent dissolved organic matter (DOM) components and indices in principal component (PC) space. The dominant mode (DOM PC1) is on the *x*-axis and the secondary mode (DOM PC2) is on the *y*-axis. Loading vectors for different fluorescent components and indices are shown in black, with vector significance ($p < 0.05$ level) shown using vector markers (dot: significant in both PC modes, vertical diamond: significant in DOM PC1 only, horizontal diamond: significant in DOM PC2 only). Scores for individual wetland samples are shown using colored symbols (red = Old Laguna, pink = Forge, blue = Royal Park, cyan = Hampton Park, and black = Lynbrook Estates). Within-wetland trajectories of DOM transformation are shown using arrows of corresponding color. Circles denote samples collected at inlets, and triangles at outlets. Grey error bars indicate 95% bootstrapped confidence bounds about sample scores. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

typical of summer wetland DOM (pink symbols, Fig. 3) (Singh et al., 2014). Interestingly, some sites (namely Lynbrook Estates and Old Laguna) spanned multiple quadrants, with inlet and outlet samples exhibiting different DOM signatures. These quadrant-quadrant transitions may point to changes in abiotic or biotic generation/processing of DOM within wetlands. For instance, inlet waters at Lynbrook Estates had more abundant, humic DOM than outlet waters. This likely reflects low inputs of terrestrial humics at the outlet (located along the shoreline of a sparsely vegetated lake) as well as increased importance of coupled photodegradation and bacterial processing of humic DOM in open waters, the primary sink for humics in most aquatic systems (black arrow, Fig. 3) (Coble et al., 2014).

DOM composition at Old Laguna also differed from inlet to outlet, but along a separate trajectory, with inlet sites having less abundant DOM of intermediate age and biodegradability, and outlet sites having higher DOM abundance, particularly fresh, algal/microbial DOM (red arrow, Fig. 3). This increase in algal DOM was consistent with the elevated Chl concentrations measured at Old Laguna's outlet (Table S4). Royal Park fell along the same trajectory as Old Laguna (blue symbols, Fig. 3), but the increase in autochthonous production at the outlet was minor, perhaps a consequence of reduced winter productivity. Forge exhibited a slight counter trajectory to Old Laguna and Royal Park in which algal/microbial DOM was abundant at the inlet and declined somewhat towards the outlet (pink arrow, Fig. 3). This signature was consistent with microbial processing of algal DOM, which is often more bioavailable than humic DOM, and therefore preferentially degraded (Coble et al., 2014).

3.3. FIB, human-specific fecal markers, and pathogens

3.3.1. Dry-weather conditions (2014)

EC and ENT concentrations were significantly positively correlated across all wetlands ($r = 0.79$; Cluster B, Fig. 2). Dry-weather patterns are shown in Fig. 4; symbol size indicates the total concentration of each microorganism, and symbol location indicates which sites contained a higher fraction of this total (inlet: lower-left corner, middle: lower-right corner, or outlet: upper corner). On average, FIB concentrations were lowest at Royal Park (~110 MPN-per-100 ml EC and ENT) and highest at Hampton Park for EC (1091 MPN-per-100 ml) or Hampton Park, Forge, and Old Laguna for ENT (>2500 MPN-per-100 ml; Fig. 4, Table S4). Two opposing within-wetland patterns were observed: (1) FIB concentrations were highest at the outlet (Hampton Park; EC, ENT) or (2) FIB concentrations were highest at inlet or middle sites (Forge, Old Laguna, and Royal Park (EC, ENT); Lynbrook Estates (EC only); Fig. 4).

Although constructed wetlands are rarely intended for recreational use, their discharge can impact downstream recreational waters. All wetlands where FIB concentrations declined from inlet to outlet exceeded AU ANZACC (2003) and/or US EPA (2012) single sample recreational standards at the inlet, and fell below those standards at the outlet (AU: EC > 260 MPN-per-100 ml; US: EC (ENT) > 235 (70) MPN-per-100 ml) (Table S4). In contrast, wetlands with comparable or higher FIB concentrations near the outlet relative to the inlet (Hampton Park: EC, ENT; Lynbrook Estates: ENT) exceeded recreational water quality standards throughout. Pathogens and human-specific fecal markers were also more prevalent at these wetlands, with Hampton Park being the only system where adenovirus and HF183 were co-detected (Fig. 4). In all wetlands, adenovirus and HF183 were more prevalent at inlet than outlet sites (Fig. 4), suggesting that wetlands can perform a public health service (i.e., pathogen removal) even if FIB concentrations remain high. This discrepancy between FIB and other health risk indicators is often reported in the literature, and can reflect environmental regrowth or non-human sources of FIB like animal feces (Savichtcheva and Okabe, 2006). Given the abundance

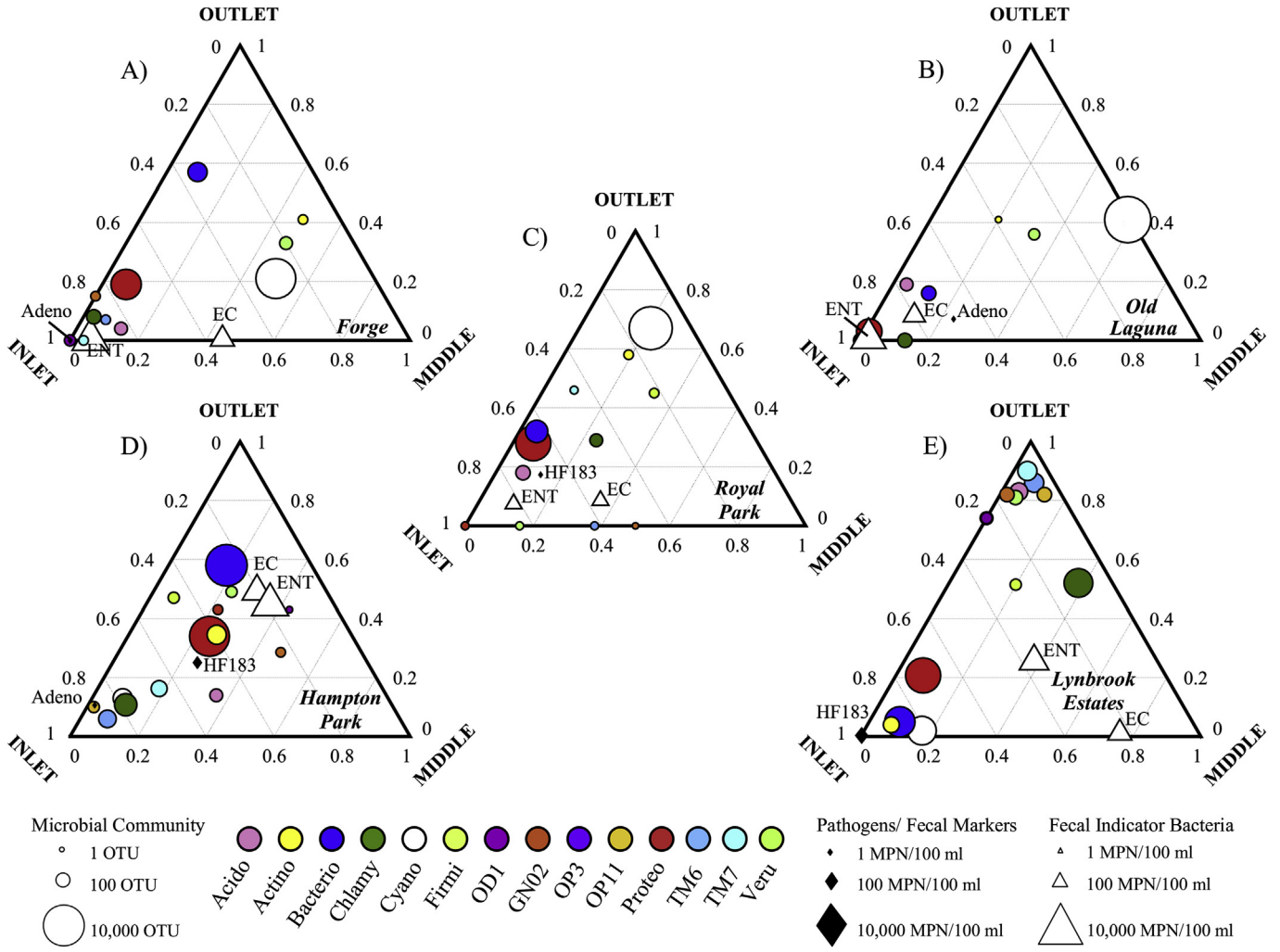

Fig. 4. Ternary diagrams depicting the relative abundance of microbial community constituents (phylum level OTU; colored circles), fecal indicator bacteria (white triangles), and pathogens and human-specific fecal markers (black diamonds) during dry-weather conditions at (A) Forge, (B) Old Laguna, (C) Royal Park, (D) Hampton Park, and (E) Lynbrook Estates. Symbol size denotes total within-wetland abundance. The following abbreviations are used in the figure: Acido (Acidobacteria), Actino (Actinobacteria), Adeno (adenovirus), Bacterio (Bacteriodetes), Chlamy (Chlamydiae), Crypto (*Cryptosporidum parvum*), Cyano (Cyanobacteria), Firmi (Firmicutes), Proteo (Proteobacteria), and Veru (Verrucomicrobia).

of waterfowl and gulls at Hampton Park and Lynbrook Estates, an avian FIB source was probable.

3.3.2. Rain event sampling (Forge, 2015)

FIB concentrations during winter at Forge were low before the rain event, high during the rain event (35-fold higher on average), and returned to low levels three days after the rain event (Fig. 5). Rain elevated FIB concentrations throughout Forge (see increased size and central position of triangles in Fig. 5B), whereas FIB declined from inlet to outlet during non-storm conditions (Figs. 4A and 5A,C). This suggests that Forge acts as a FIB sink during dry weather conditions, but behaves like a pipe during wet weather (conveying FIB directly to the storm sewer system with minimal treatment).

Adenovirus and *C. parvum* concentrations were two or more orders of magnitude higher at Forge during winter 2015 (compare Figs. 4A and 5). *C. parvum* (only present during winter) was more prevalent at middle and outlet sites before, and inlet and outlet sites during, the rain event (>100 copies-per-100 ml; Fig. 5). Adenovirus, in contrast, was only detected at the inlet, and at lower concentrations than *C. parvum* (approximately 30 copies-per-100 ml; Fig. 5). Thus, different measures of fecal pollution (pathogens and FIB) had different patterns of occurrence in these wetlands: adenovirus concentrations declined from inlet to outlet, FIB concentrations declined pre- and post- (but not during) rain events, and *C. parvum* attenuation was never observed, perhaps reflecting the presence of within-wetland animal hosts and/or sediment reservoirs of this parasite (Harkinezhad et al., 2009; Lagkouvardos et al., 2014).

3.4. Microbial community composition

3.4.1. Dry-weather conditions (2014)

More than 90% of sequences in most surface water samples were assigned at the phylum level. The exception was inflow water at Old Laguna, where ~40% of sequences were unassigned (Table S5). Rarefaction curves plateaued at a sequencing depth of 700 per sample, demonstrating that the bacterial community was well captured at the sampling depth evaluated (Fig. S5).

Across all five wetlands the most abundant phyla were Cyanobacteria (primary producers), Proteobacteria (particularly classes γ and β), and Bacteroidetes, with Cyanobacteria being most prevalent

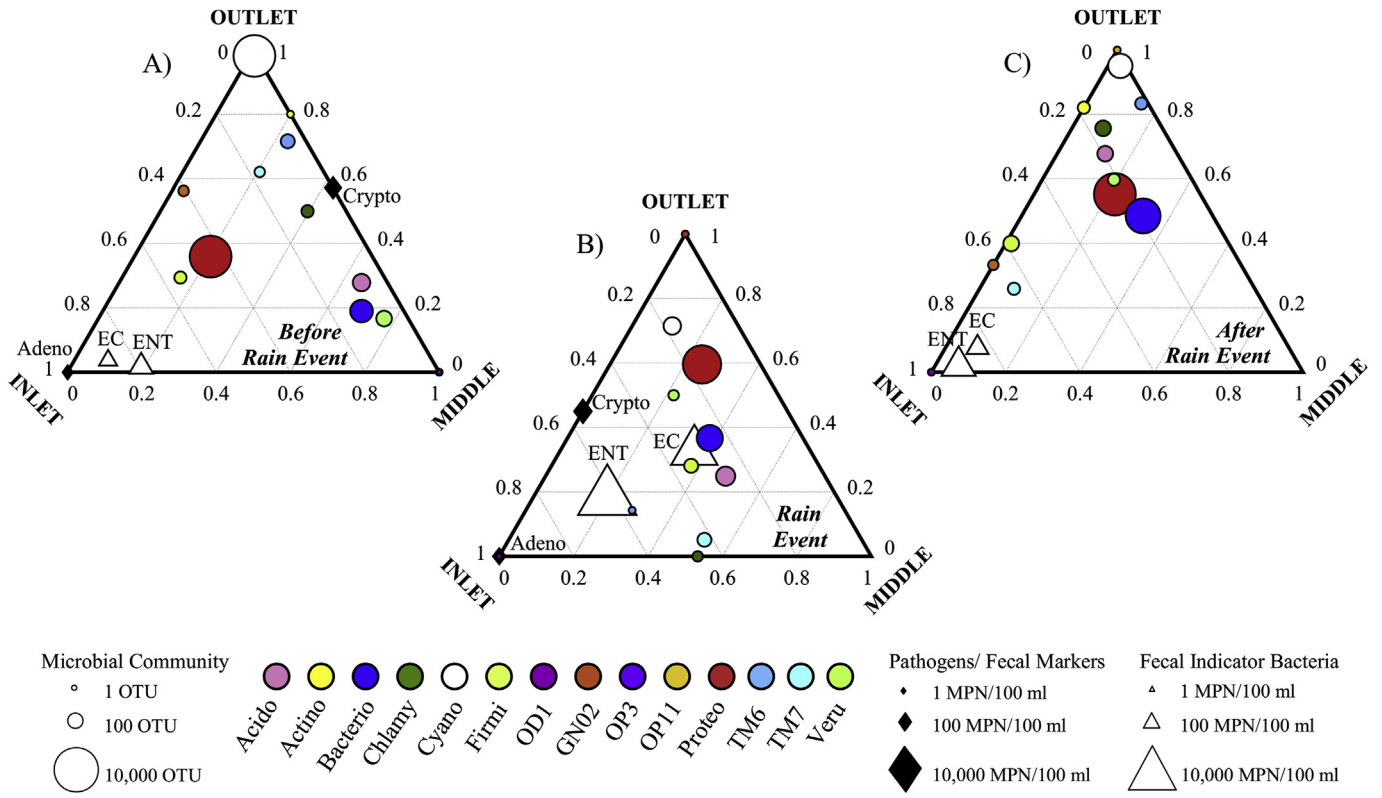

Fig. 5. Ternary diagrams depicting the relative abundance of microbial community constituents, pathogens, human-specific fecal markers, and fecal indicator bacteria at Forge in winter 2015 (A) before a rain event, (B) during a rain event, and (C) after a rain event. Axes and symbols are as described in Fig. 4. Pathogens and human fecal markers were not measured post-rain event and are thus not included in panel (C).

at Forge, Royal Park, and Old Laguna, and Bacteroidetes and Proteobacteria being most prevalent at Hampton Park and Lynbrook Estates, respectively (Fig. 4). The fourth most abundant phyla, Chlamydiae, was only common at Hampton Park and Lynbrook Estates (Fig. 4 D,E). The prevalence of these four phyla is consistent with reports from other constructed wetlands, with Proteobacteria and Cyanobacteria variously dominating surface water assemblages (Ibekwe et al., 2007; Bai et al., 2014), and Proteobacteria, Acidobacteria, Firmicutes, and Bacteroidetes all prevalent in rhizosphere and sediment communities (Sanchez, 2017). Chlamydiae are also frequently reported in freshwater systems, reflecting an abundance of environmental strains as well as human and animal (particularly avian and ruminant) pathogens (Lagkouvardos et al., 2014).

Ten additional “low-abundance” phyla were also detected in surface water samples during this study, including the aforementioned soil-associated bacterial groups (Firmicutes and Acidobacteria) as well as Verrucomicrobia, Actinobacteria, TM6, TM7, OP11, GN02, OP3, and OD1. The majority of these phyla were observed at Lynbrook Estates or Hampton Park, the two most microbially diverse wetlands (average Shannon diversity > 3.6; Fig. 4, Table S5).

Microbial variability across all five wetlands was captured by two principal coordinates (MIC PCoA1: 50% variance explained and MIC PCoA2: 15% variance explained; Fig. 6). Indirect correlation analysis between MIC PCoA 1 values and microbial OTU scores suggests that Cyanobacteria contributed most strongly to MIC PCoA1 (significant positive correlation of 0.83). Most other OTUs contributed more weakly, and negatively (Fig. 6). This implies that wetland sites with negative MIC PCoA1 scores had predominantly heterotrophic bacterial communities in surface water, whereas sites with positive MIC PCoA1 scores had autotrophic bacterial communities (especially towards the outlet). This interpretation is supported by the positive correlation between MIC PCoA1, Chl, and DOM PC1 (positive equals fresh, algal DOM) and the negative correlation between MIC PCoA1, fecal heterotrophs (EC, ENT, and HF183), and antecedent rainfall (Fig. 2). MIC PCoA2, in contrast, was associated with poor water quality. Bacteroidetes, Proteobacteria, and Actinobacteria contributed positively to MIC PCoA2, whereas Chlamydiae, TM6 and OP11 contributed negatively (Fig. 6). Three of these groups include known human pathogens. MIC PCoA2 was also significantly correlated with HF183 and adenovirus (positively and negatively, respectively; Fig. 2).

Taken together, these principal coordinates reveal how microbial communities change from inlet to outlet across all wetlands evaluated here. Most inlet stations were negative for MIC PCoA1 (colored circles in Fig. 6 biplot) reflecting the dominance of heterotrophic (perhaps stormwater-associated) microbial assemblages in water flowing into wetlands. In contrast, outlet and middle stations were either (1) positive for MIC PCoA1, indicating an increase in autotrophic Cyanobacteria from inlet to outlet (Forge, Old Laguna, and Royal Park; Fig. 4A–C and pink, red, and blue arrows, Fig. 6) or (2) negative for MIC PCoA1 (Hampton Park and Lynbrook Estates; Fig. 6). Hampton Park and Lynbrook Estates were distinguished by opposing trajectories in MIC PCoA2 space, with increasing concentrations of Chlamydiae and TM6 (and decreasing Bacteriodetes) observed from inlet to outlet at Lynbrook (black arrow, Fig. 6), and the inverse observed at Hampton Park (cyan arrow, Fig. 6).

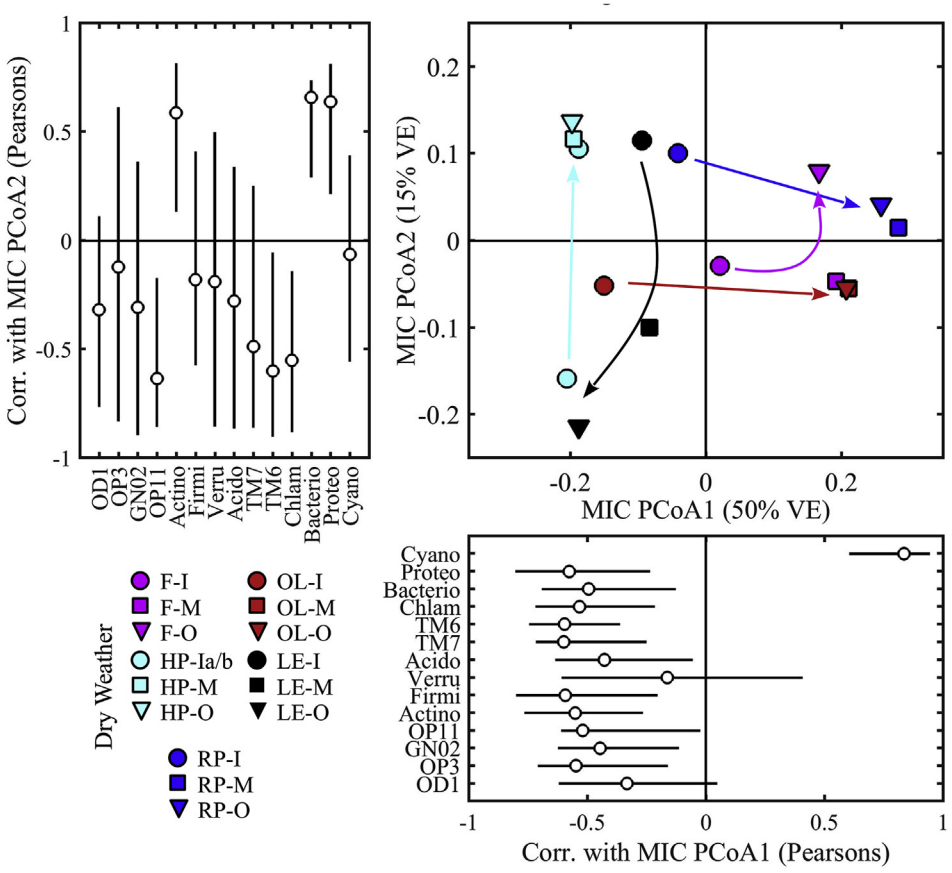

Fig. 6. Principal coordinate analysis of microbial community composition during dry-weather conditions performed using the weighted UniFrac distance method (main plot). The dominant coordinate (MIC PCoA1) is on the *x*-axis and the subordinate coordinate (MIC PCoA2) is on the *y*-axis. Arrows delineate microbial community transitions inlet to outlet. Symbol colors and shapes are the same as in Fig. 3. Side plots depict the relative contribution (Pearson's correlation coefficient) of each microbial OTU from Figs. 4 and 5 to MIC PCoA1 (bottom plot) and MIC PCoA2 (upper left plot). Error bars are 95% bootstrapped confidence bounds. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4.2. Rain event sampling (2015)

The microbial community at Forge was more diverse during rain event sampling than summer dry-weather conditions (average Shannon's diversity of 3.4 before rain, 4.7 during rain, 4.0 after rain, and 2.6 during dry-weather conditions; Table S5). Microbial communities at Forge during summer, and before winter rains, were dominated by the same phyla (Cyanobacteria, Proteobacteria, and to a lesser extent Bacteroidetes), whereas microbial communities during and after rain events had fewer Cyanobacteria (Fig. 5).

Within-wetland microbial patterns before the rain event were similar to those observed at Old Laguna, Royal Park and Forge during dry-weather conditions; namely, surface water microbial communities transitioned from net heterotrophic at the inlet to autotrophic at the outlet (Fig. 5A and pink, blue and red arrows in Fig. 7). During the rain event, however, the microbial community at Forge became more evenly mixed inlet to outlet (see central clustering of circles in Fig. 5B), and stayed heterotrophic throughout the wetland (i.e., in negative PCoA1 space; bicolor pink-and-red symbols in Fig. 7). After the rain event, microbial communities also clustered in negative PCoA1 space (bicolor pink-and-black symbols, Fig. 7), with most phyla being more prevalent at the outlet, indicative of a perturbation that was passing through (Fig. 5C). Taken together these findings suggest that (1) rain-induced perturbations to the microbial community are rapid onset, making entire wetlands appear like inlets, and (2) that recovery of the microbial community takes time (in some cases more than what is required for individual indicators like FIB, which did recover post-rain event; Fig. 5). This slow recovery likely reflects the complexity of the microbial community and the wetland functions they provide.

3.5. Average wetland states and within-wetland treatment trajectories

Across all wetlands, hierarchical clustering analysis revealed four groups of interrelated variables that delineated average wetland states and/or within-wetland treatment trajectories. Group A clustered with 90% confidence and includes two subgroups (1) MIC PCoA1, VSS, TSS, and DOM PC1 (Group A1), and (2) the season-associated variables from section 3.1 (solar radiation, water temperature, Chl, and dissolved oxygen; Group A2) (Fig. 2). The next two groups were the FIB (Group B, 95% confidence) and HF183, antecedent precipitation, and Phae (Group C, 95% confidence). Group C may indicate stormwater runoff, as it links precipitation to the delivery of human fecal markers (HF183) and detritus (Phae) to wetland surface waters. The exclusion of FIB from group C suggests that FIB have additional (non stormwater) sources, perhaps dry-weather runoff or animal feces.

Seventy-five percent of the variability in average wetland state was captured by two PC modes, well defined by Groups A1, A2, B, and C (Fig. 8A). PC1 explained 49% of the variance ($p < 0.05$;

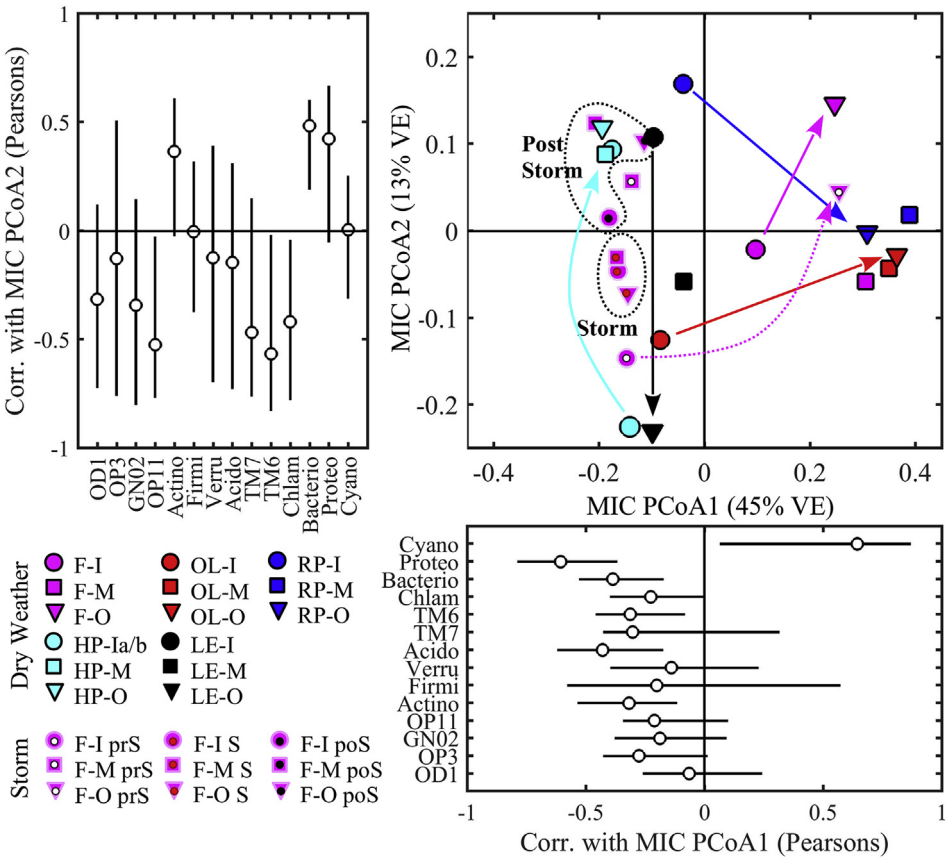

Fig. 7. Principal coordinate analysis of microbial community composition performed across all surface water samples (dry-weather and rain event). Figure layout, axes, and dry weather symbols are identical to Fig. 6. Additional symbols are included to indicate samples collected at Forge in winter 2015, before a rain event (bicolor pink-and-white), during a rain event (bicolor pink-and-red), and after a rain event (bicolor pink-and-black). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. S6A). It distinguished older (14–15 yr, B and C group) wetlands with high concentrations of microbial contaminants (Lynbrook Estates and Hampton Park) from younger (7–8 yr, A1 and A2 group) wetlands that were less contaminated and supported autotrophic microbial communities (Royal Park, Forge, and Old Laguna) (Fig. 8A, Table S2). PC 2 primarily differentiated among group A wetlands, with Royal Park (A1 group) separating out from Old Laguna (A2 group), and Forge located at an intermediate position between the two (Fig. 8A). This separation primarily reflects characteristics of Old Laguna and Royal Park (the presence of adenovirus and elevated suspended solids concentrations, respectively) that were not shared with one another or with Forge.

The same variable clusters (Groups A-C) that differentiated average wetland states also defined within-wetland treatment trajectories in PC space (73% variance explained by 2 PC modes; significant at a $p < 0.05$ level; Fig. 8B, Fig. S6B). However, MIC PCoA1, which was originally clustered with group A1, became aligned with group A2, reflecting differences in the underlying processes captured by these variables that only manifested within specific wetlands (Fig. 8B). For instance, Lynbrook Estates transitioned from a stormwater runoff contaminated inlet (Group C) to an outlet containing Chlamydiae, ENT, and signatures of humic DOM processing, suggesting the establishment of a unique, heterotrophic outlet community (and perhaps additional within-wetland pollution sources) (Fig. 8B and Fig. 9). Although this trajectory was well described by an increased loading on DOM PC1 because fresh, microbial DOM can be heterotrophic as well as autotrophic in origin (+/− quadrant, Fig. 8B), it did not align with MIC PCoA1, which was closely tied to the autotrophic community (+/+ quadrant, Fig. 8B). Forge, Old Laguna and Royal Park, however, did have outlets that loaded on MIC PCoA1 (as well as Chl, and dissolved oxygen; Fig. 8B), pointing to a true heterotrophic to autotrophic transition. This transition was weakest at Royal Park, likely reflecting reduced autotrophic productivity during Austral winter. Hampton Park was unique in that no treatment trajectory was observed for this site (see tight cluster of points at the origin of Fig. 8B). This is consistent with the microbial contamination detected throughout this wetland (Fig. 3D), as well as Melbourne Water's decision to retrofit Hampton Park in 2014 to improve hydrologic performance.

All equations necessary to calculate within wetland PC modes are provided in Table S6 in order to allow other wetlands to be evaluated relative to the treatment trajectories presented here.

3.6. Implications for monitoring and management

This study demonstrates that, although wetlands provide varying degrees of public health services and disservices, the signature of high performance systems (e.g. Forge, Old Laguna, and Royal Park) transcends both seasons and continents, being well described by metrics related to productivity, microbial community composition, and DOM processing (red to green axis in Fig. 9). When constructed wetlands function properly, microbial

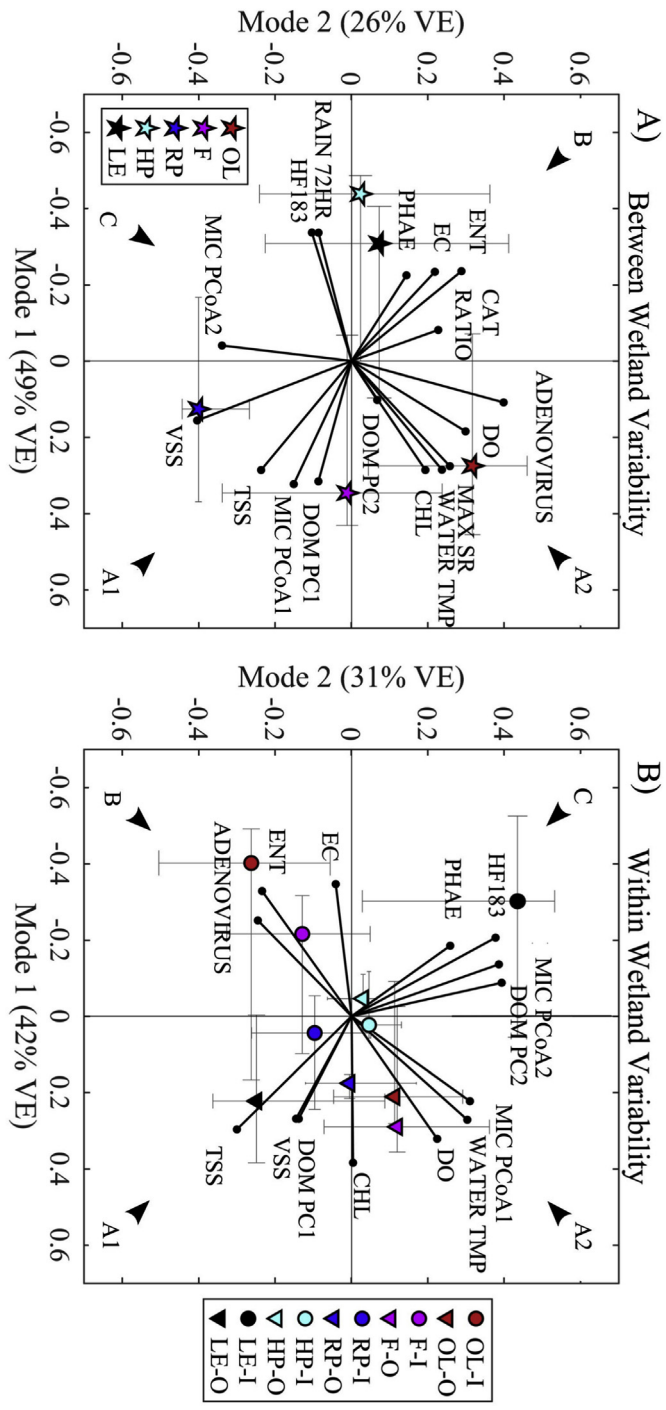

Fig. 8. Biplots of (A) average wetland state and (B) within wetland variability evaluated across sixteen variables pertaining to chemistry, climate, engineering design and the microbial community. (A, B) Loading vectors for each variable are in black, and coherent variable clusters defining specific PC quadrants (groups A1 through C from the hierarchical correlation analysis in Fig. 2) are labeled. Sample scores are color coded as in Fig. 3, with stars used to indicate average wetland states (A), and circles and triangles marking inlets and outlets, respectively (B). Grey error bars show 95% bootstrapped confidence bounds about sample scores. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

community structure changes as water circulates through, reflecting replacement of microorganisms from runoff with indigenous wetland microbes (for instance the replacement of pathogens, FIB, and fecal markers by Cyanobacteria at Forge, Royal Park and Old Laguna; Fig. 4A–C). However, microbial transitions are not always positive (e.g., Chlamydiae increased at Lynbrook Estates' outlet, perhaps from additional pollution sources such as waterfowl feces; Harkinezhad et al., 2009) (Fig. 4E). Even the above-noted shift from net heterotrophy to autotrophy can have costs, as some Cyanobacteria are toxic, and algal biomass can clog outlets, alter wetland hydrology, and (when degraded) cause odor. Proper management and maintenance are necessary to keep these (and other microbial-associated disservices) at a minimum.

Only wetlands that developed autotrophic microbial communities performed both pathogen and FIB removal services (Fig. 9). The signature of this transition was more intense (and readily identifiable) in the summer, but remained detectable in winter. Its single best indicator was MIC PCoA1, a master variable during this study; no other variable was significantly correlated with more wetland variables (see Fig. 2). However, microbial community analysis based on next generation sequencing may be cost prohibitive for routine monitoring, making alternative indicators attractive. Fluorescent DOM indices (HIX, BIX, FI, and the T:C ratio) are likely candidates, as they are rapid and relatively inexpensive, and DOM PC 1 was more significantly and positively correlated with MIC PCoA1 than any other variable (Fig. 2). However, care must be taken when using fluorescent DOM as an indicator, as algal and heterotrophic bacterial DOM can look alike, confounding interpretation of DOM transitions from inlet to outlet (indeed, the T:C ratio is routinely used as a marker for microbial signatures in sewage (Coble et al., 2014)). One solution to this problem (drawn from the urban stream literature (Baker et al., 2003)), would be to couple fluorescent DOM measurements with another indicator of autotrophy. Indeed, in our study, when DOM PC1 was paired with Phae (as a ratio) or Chl (as a two-parameter multiple linear regression model), we improved our ability to predict MIC PCoA1 over fluorescent DOM alone (see reduced root mean squared error of approximation estimated using leave-one-out cross validation; Table S7 and SI Methods). This suggests that simple models combining DOM and autotrophic indices may prove useful for monitoring heterotrophic to autotrophic microbial community transitions in wetlands.

Most wetlands in the current study, autotrophic or not, performed a pathogen removal service (Fig. 9). This is consistent with the literature for wastewater wetlands (Karim et al., 2004), but is more promising than recent reports for stormwater wetlands (Hsu et al., 2017). Pathogen and human fecal marker concentrations declined to below 1 copy-per-100 ml at the outlet of all wetlands except: (1) Forge during winter for *C. parvum*, and (2) Hampton Park, where HF183 was present at inlet, middle, and outlet sites (50%, 25%, and 25%, respectively; Fig. 4D). The persistence of HF183 at Hampton Park may reflect its retrofit, which was only partly complete when the wetland was sampled (it had been drained and dredged, but not fully re-vegetated, and was likely not operating at peak performance). Indeed, Hampton Park's treatment trajectory (minimal services performed; Fig. 8B) was most similar to Forge's rain event trajectory, where the wetland conveyed microbial contaminants like a pipe (Fig. 5B). The latter may have had hydrologic underpinnings, as Forge was primarily designed to improve water quality during dry-weather and small rain events, not medium-large storms like the one that occurred in winter 2015, which overtopped the wetland's permanent ponds and filled its extended detention zone (Table S2). During larger rains, Forge's hydraulic residence time is expected to drop to 36-hrs (IRWD, 2005) (Table S2), well below the minimum residence time of 50-hrs recommended for effective treatment of fecal microbes like FIB (Struck et al., 2008).

Catchment ratio has also been reported to control pollutant removal efficacy in wetlands. Carleton et al. (2001) found that

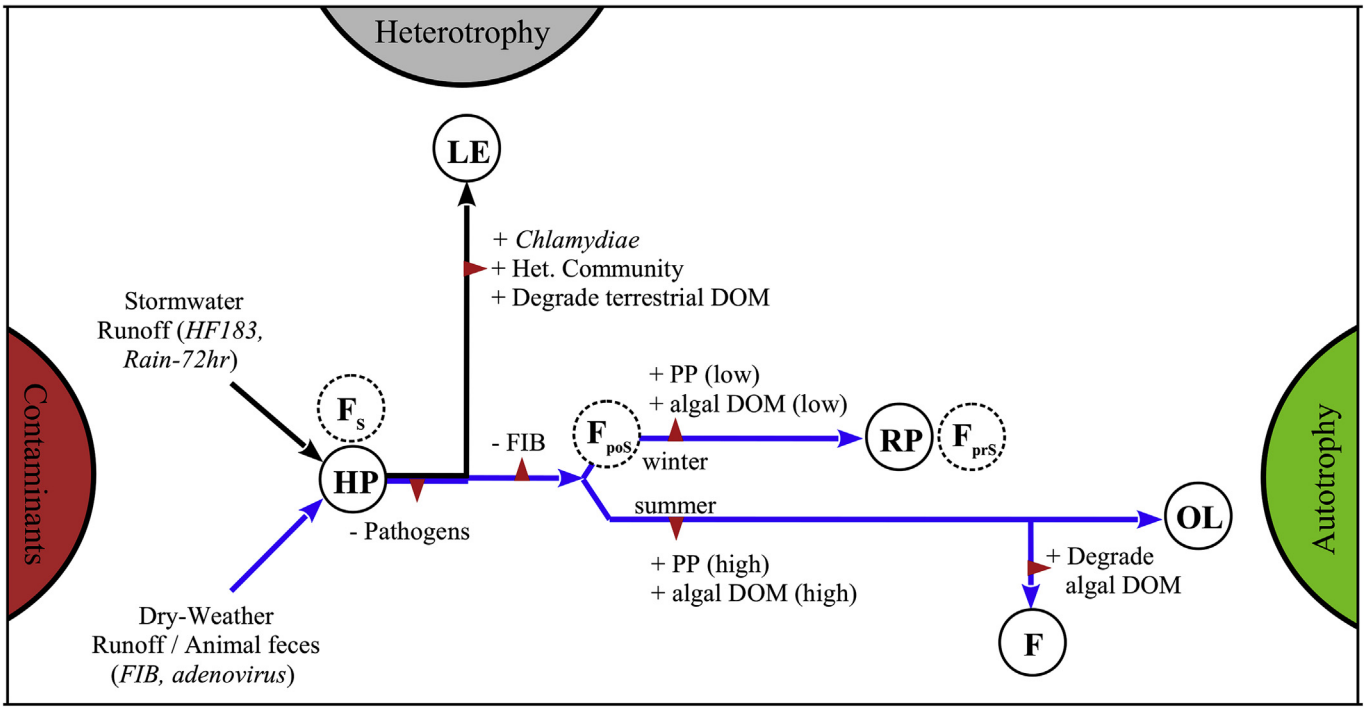

Fig. 9. A schematic of within-wetland treatment trajectories progressing from contaminated (red pole) to autotroph or heterotroph dominated (green or grey pole, respectively). Black arrows denote trajectories that begin with traces of stormwater runoff whereas blue arrows denote trajectories that begin with signatures of dry-weather runoff and/or animal feces. Additions or subtractions along each trajectory are marked with red arrows and individual wetland positions are denoted using circles (solid for dry weather conditions, and dashed for the winter 2015 rain event series (prS: pre-storm, S: storm, poS: post-storm), where position is inferred from microbiological variables alone). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

catchment ratio was significantly, positively correlated with percent removal of TSS, nutrients, and heavy metals across 49 stormwater wetlands. However, in our study, catchment ratio was not correlated with any microbial contaminant (or indeed any other measured variable; Fig. 2). This lack of correlation may reflect variable treatment performance at the smallest catchment ratios, as all of our wetlands were somewhat undersized (<2% of catchment area). It also suggests that other, perhaps volume based metrics like hydraulic residence time, may be more reliable indicators of treatment performance in small urban wetlands.

Wetland performance also declined with age, with older wetlands (Lynbrook Estates and Hampton Park) exhibiting elevated microbial contamination relative to younger wetlands (Royal Park, Forge and Old Laguna) (Fig. 8, Table S1). This points to a need for careful maintenance and restoration activities that rejuvenate aging wetlands. However, these activities can have hidden costs. For instance, Adyel et al. (2017) observed that sediment removal and channel redirection at a constructed wetland in Australia reduced nitrate attenuation because the removal of organic-rich sediments limited the carbon available to denitrifiers. Similarly, replanting efforts necessary to restore hydraulic residence time can reduce solar exposure, increasing microbial contaminant persistence and reducing treatment performance (Kadlec and Wallace, 2009). Management and maintenance efforts must proceed with these trade-offs in mind to achieve the best balance of desired ecosystem services across wetlands as they age.

4. Conclusions

- Under dry-weather conditions, wetland age and within-wetland microbial community transformations (from heterotrophic at the inlet to predominantly autotrophic at the outlet) are stronger indicators of microbial treatment performance than the catchment ratio, sampling season (summer vs winter) and wetland location (US vs AU).
- Wetland maintenance or restoration programs that rejuvenate aging wetlands and monitoring programs that track microbial community shifts (either directly or using proxies like fluorescent DOM and algal pigments) are important for extending the performance of wetlands.
- The performance of wetlands during wet weather can differ substantially from dry weather, where a moderate storm can transform the wetland from a pollutant sink to a transport conduit. This difference underscores the importance of utilizing caution when extrapolating the findings of dry-weather observations to wet-weather conditions.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.03.020.

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