

Microlayer enrichment in natural treatment systems: linking the surface microlayer to urban water quality

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> Natural treatment systems (NTS), such as constructed wetlands and stormwater ponds, are multibenefit, multidisciplinary approaches to sustaining water resources and reducing contaminant loading to urban streams. Surficial thin films (called surface microlayers) are not well characterized in NTS, but may have important implications for ecosystems, public health, and pollutant fate and transport. We present results from a case study evaluating microlayer contaminant partitioning across 4 NTS in Melbourne, Australia. To our knowledge, this study provides the first direct evidence for microlayer formation and contaminant enrichment (total petroleum hydrocarbons and trihalomethanes) in NTS. Contaminated microlayers were detected in the three most stable NTS, with stability defined relative to wind speed. Fluorescent-dissolved organic matter profiles differed between microlaver and subsurface water in these systems, suggesting that fluorescence-based techniques are useful for microlayer detection. Although individual fluorophores were not consistently associated with specific contaminants, fluorescence ratios were useful for identifying likely contaminant source waters, including road-runoff and irrigation water from nearby green spaces. We evaluate our case study in light of what is known about surface microlayers in analogous systems (e.g., oceans, estuaries, and lakes), in order to identify existing research gaps and future opportunities. © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

The surface microlayer is a naturally occurring surface film of water $1-1000 \ \mu m$ thick (average:

60 μ m^{1,2}). Microlayers are found in marine environments, estuaries, rivers, and lakes.^{2,3} They are unique ecosystems with complex physicochemical structure and distinct microbial, invertebrate, and larval vertebrate communities.^{3,4} Microlayers in urban-impacted water bodies often contain elevated concentrations of pollutants, including carcinogens and mutagens such as polycyclic aromatic hydrocarbons (PAH).^{2,5} Given that 10-fold contaminant enrichment in microlayer water is common (with > 1000-fold enrichment reported),^{2,3} microlayer dynamics may exert significant control on contaminant fate and transport in aquatic systems.

To date, the majority of surface microlayer research has focused on brackish or marine systems

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that are natural (as opposed to engineered).^{2,3} Freshwater microlayers are less well understood, and remain largely unevaluated in engineered systems such as constructed wetlands and stormwater ponds.^{2,3,6} Although engineered, these systems are referred to as natural treatment systems (NTS) because they treat urban runoff using natural, lowenergy, ecological, and physicochemical processes. They are intended to reduce water volume and pollutant loading to urban streams and, in some cases, increase urban water security by reclaiming freshwater for later reuse.^{7,8} Both constructed wetlands and stormwater ponds have freshwater ponding zones (although the latter may be transient), and thus have the potential for surface microlayer formation. Given that microlayer dynamics may exert significant control over contaminant fate and transport, and that pollutant removal is a primary goal of many NTS, there is a clear need to evaluate microlayer formation and contaminant enrichment in these systems. In this study, we review the state of the science concerning surface microlayers in natural aquatic systems, focusing on microlayer composition, formation/ stability, contaminant-enrichment, and ecological significance. We then present results from a case study in Melbourne, Australia, that expands the scope of microlayer research to include engineered systems for urban stormwater treatment.

THE SURFACE MICROLAYER IN MARINE AND FRESHWATER ANALOGUES

Composition, Formation, and Stability

The surface microlayer is a hydrated gelatinous film that is composed primarily of carbohydrates [including transparent ectopolymer particles (TEP)⁹], as well as proteins and lipids (Figure 1(a)).^{3,10} Lipids may be involved in the early stages of microlayer formation, providing nucleation sites for carbohydrate attachment.³ Hydrophobic proteins and dissolved amino acids either intermix with these lipids, or are displaced below them, depending on the magnitude of shear forces acting on microlayer water.^{3,11} TEP particles, rich in carbon and nutrients, are also important structural components of the microlayer. These particles tend to be 'sticky' because they are enriched in surface active compounds like sulfate half-ester groups that form metal ion bridges and hydrogen bonds.¹⁰ TEP stickiness increases the viscosity of microlayer water, promoting stability,¹⁰ and facilitates microorganism enrichment through providing favorable attachment sites, nutrients, and protection from photoinhibition.⁴

A variety of processes contribute to surface microlayer formation in aquatic systems, including scavenging by bubbles, atmospheric deposition, molecular or turbulent diffusion, buoyant particle accumulation, photodegradation and transformation, secretion and biodegradation by microlayer fauna, and migration of motile organisms into or out of the microlayer, amongst others (Figure 1(b)).^{3,12} Bubble scavenging is thought to be one of the most important mechanisms controlling microlayer formation in marine systems.^{3,10} Bubbles are formed at depth (up to several meters deep) by wind and breaking waves. Subsequently, surface active compounds (dissolved material and small particles) adsorb to bubble walls and rise toward the air-water interface, where they are released into microlayer water (or the atmosphere) upon bubble bursting.^{3,4,10}

Although frequently disrupted by wind, waves, rainfall, and other environmental perturbations, surface microlayers are remarkably persistent. Indeed, stirred tank experiments suggest that microlayers can reform in less than a minute.³ Following reformation, however, their composition may differ: enzyme activity and surfactant concentrations may be lower, and bacterial communities may change.³ In some cases, wind driven mixing may facilitate desorption of particle-associated compounds (such as PAH), which may not be reincorporated into the microlayer when winds cease.⁵ However, stable surface microlayers have been observed at a variety of wind speeds in marine systems (up to 36 km/h in some instances).³ It has also been suggested that winds may cease to effect microlayer processes such as air-water gas exchange in small lakes/ponds at speeds <13.3 km/h.¹³

Contaminant Enrichment

Although contaminant concentrations in the surface microlayer vary due to high frequency fluctuations in physical and biogeochemical processes at the airwater interface, the microlayer is frequently enriched in petroleum hydrocarbons (TPH), PAH, heavy metals, pthalates, pesticides, polychlorinated biphenols, and sewage markers.^{2,5,13-16} For instance, fecal sterol enrichment factors (EFs), where EF > 1 indicates elevated concentrations in microlayer versus subsurface water, have been reported in coastal microlayers impacted by stormwater and/or outfall plumes (EF: 0.82-40).¹⁶

Volatility is expected to affect microlayer enrichment of hydrocarbons, with more volatile compounds like BTEX (benzene, toluene, ethyl benzene,



FIGURE 1 (a) A schematic representation of surface microlayer structure inspired by figure 1(b) in Ref 9, showing intermixed organics (carbohydrates, proteins, and lipids), soil particles, nutrients, metals, and biotic communities (bacteria, viruses, phytoplankton, and protozoa/ zooplankton). Although depicted as particles (red dots), the authors emphasize that microlayer-associated nutrients and metals can be particulate, colloidal, dissolved, or some combination of the three. Note that microlayer constituents are not drawn to scale and are not proportional to one another. Although the microlayer shown here is approximately $400-1000 \mu m$ thick, microlayers can have a variety of thicknesses typically defined by the sampling method used ($\leq 6 \mu m$ thick: polytetrafluoroethylene filter method to $\geq 1000 \mu m$ thick: original 1974 floating tray method). (b) The processes involved in microlayer formation: (1) bubble scavenging; (2) atmospheric deposition; (3) diffusion; (4) buoyant transport by TEP; (5) photodegradation [illustrated as solar-induced splitting (dashed red line) and separation (opposing red arrows) of two protein subunits], (6) biodegredation and exudate release by phytoplankton or grazer feeding, (7) transport of motile organisms (and fecal pellets) into and out of the microlayer, and (8) other external biotic/abiotic processes such as foraging activity by birds and other wildlife, rainfall, and winds, which can effect microlayer composition and thickness.

and xylenes) partitioning out of the microlayer and into the air phase. While this loss may deplete the microlayer relative to subsurface water, enrichment can still occur if subsurface-microlayer partitioning is more favorable than volatilization. Indeed weak microlayer enrichment has been reported for benzene (EF: 1.1) and ethyl benzene (EF: 1.7) in drinking water reservoirs in China.¹⁷ Interestingly, this same study reported strong enrichment of chloroform, a volatile trihalomethane (THM), in microlayer water (EF: 13.4).¹⁷ Heavy metals (Cu, Pb, Zn, and Hg) and pesticides can also be enriched in the microlayer. In marine systems, EFs ranging from <1 to 3215 have been reported for metals.² Enrichment has also been reported for pyrethroid insecticides in agricultural ponds (EF: 15-150).¹⁸

Aquatic Ecosystems and Stormwater Treatment

Surface microlayers perform important ecological and biogeochemical roles in aquatic ecosystems,

including providing habitat for neustonic (surface associated) larval invertebrates and fish,^{2,5} and regulating air-water gas exchange.³ In some cases, contaminant enrichment in the microlayer may impair ecosystem health. For instance, many TPH, PAH, THM, heavy metals, and pyrethroid insecticides are toxic to phytoplankton, invertebrates, and birds. PAH concentrations exceeding 1 µg/L impair DNA synthesis in the common phytoplankter Prochlorococcus,¹⁹ whilst pesticides such as atrazine and carbaryl can alter sex ratios and swimming behavior in zooplankton, impacting reproduction and predator encounter rates.²⁰ Furthermore, many early life history stages of aquatic fauna are more sensitive to toxicants than adult organisms, and have a pelagic phase in and around microlayer water.^{2,20} Mortality, reduced growth or hatch rates, and developmental abnormalities have all been reported in larvae and fish eggs exposed to microlayer contamination.²

The microlayer can also impact air-water gas exchange. Soluble surfactants (e.g., detergents, personal care products, and ingredients in petroleum products or pesticides)²¹ can increase microlayer viscoelasticity, suppressing air-water gas exchange.³ Synthetic surfactants such as oleyl alcohol have been shown to reduce gas transfer velocities up to 55% in marine systems.³ While contaminant-induced suppression of air-water gas exchange may have implications for biogeochemical cycling in aquatic ecosystems, the nature and magnitude of these effects remains unknown.

Microlayer contaminant enrichment has the potential to improve stormwater treatment in aquatic systems as well as impact biogeochemistry or ecosystem function. For instance, UV radiation is higher in the microlayer than subsurface water, promoting photodegradation and photoinactivation. This may be particularly important for bacterial and viral pathogens, many of which are UV-sensitive. Indeed, the microlayer is reported to contain more damaged (or low activity) microorganisms than subsurface waters.⁴ Biodegradation of contaminants can also be elevated in the microlayer, which can improve water quality.²² It should be noted, however, that some degradation products are more toxic than parent compounds and that UV rays can induce phototoxicity of some contaminants, including PAH.^{2,23}

MICROLAYERS IN STORMWATER NTS: A CASE STUDY

It is currently unclear how the above-stated results translate to NTS. Indeed, to our knowledge, the case

study presented here is the first to evaluate surface microlayer contaminant enrichment in NTS. Specifically, we addresses three questions: (1) Are microlayers detectable in NTS? (2) Are microlayers enriched in contaminants relative to subsurface water? and (3) Can excitation-emission (EEM) fluorescence spectroscopy be used either for rapid detection of microlayers or identification of likely pollutant sources? Question 3 is prompted by the reported utility of fluorescent-dissolved organic matter (FDOM), and FDOM ratios, for tracking anthropogenic contaminants such as sewage in urban rivers.²⁴ Indeed, many substances (sewage, recycled water, PAH, and select aromatic pesticides) exhibit distinct FDOM signatures that are readily identified using EEMs, suggesting fluorescent methods may for prove useful detecting microlayer contamination.²⁴⁻³¹ The contaminants evaluated in this case study include hydrocarbons (TPH and PAH) and disinfection byproducts (THM). These were chosen because they span a range of volatilities (Table 1), and are likely to be present in stormwater runoff.

We emphasize that this study is not intended to be a comprehensive evaluation of NTS microlayers, but rather a first look at what may be an important (and hitherto unevaluated) control on contaminant fate and transport in these systems.

Methods

Site Description

Four freshwater NTS were sampled during the winter storm season (August, 2013), in the Melbourne metropolitan area (Victoria, Australia). Three types of systems were evaluated: two multipart treatment trains (Banyan Reserve; BAN, Monash Ornamental Pond; OP), a retention basin (Monash North Pond; NP), and a treatment wetland (Huntingdale Rd. Wetland; HRW; Figure 2(a)).

The BAN treatment train consists of a sedimentation basin, a wetland, and a stormwater pond, in series.³⁶ BAN captures and treats runoff from a primarily residential catchment (catchment area ~ 2.4 km²).^{37,38} OP, also part of a stormwater treatmenttrain, is a clay-lined basin that lies at the terminus of a sedimentation basin-biofilter series on the Monash, Clayton Campus. It receives runoff from a multistory parking lot (catchment area: 4500 m²) and irrigates/receives runoff from a sports oval.^{39,40} OP's sister pond, NP, is a stand-alone stormwater retention basin that drains the northern half of the Clayton campus, including several sports fields, buildings,

	Analytes Detected	$\log K_{\rm ow}^{32}$	${H_{ m pc}}^{ m 33}$ (atm m ³ /mol)	Detect Lim. (µg/L)	BAN-M (µg/L)	OP-M (µg/L)	NP-M (µg/L)	HRW-M (µg/L)
TPH	C6-C9 (BTEX)	2.1-3.2 ³²	$2.7 \times 10^{-3} - 1.0 \times 10^{-2}$	123	a	—		_
	C10-C14	6.3-8.0 ³²	1.1–21	164	—	492		_
	C15–C28	7.3–13.0 ³⁴	3.8-1700	410	—	17,220	410	_
	C29-C36	n/a	2500-6600	410	—	1230	_	—
PAH	Total PAH	3.2-7.4	$1.7 \times 10^{-9} - 1.2 \times 10^{-2}$	4.1	—	—	_	—
THM	Chloroform	2.0 ³⁵	$2.1 \times 10^{-3} - 7.0 \times 10^{-3}$	4.1	—	—	4.1	7.8
	Total THM	2.0-2.4 ³⁵	$2.6 \times 10^{-4} - 7.0 \times 10^{-3}$	4.1	—	—	4.1	7.8

TABLE 1 | Micropollutant Concentrations Detected in the Surface Microlayer

 $Log K_{ow}$, octonal water partition coefficient (higher = more hydrophobic); H_{pc} , Henry's volatility constant (lower = more volatile); n/a, reliable data are not available. ^a Micropollutant concentrations < methodological detection limits (this was true for all bulk water samples, results not shown).



FIGURE 2 | (a) Study area map; Melbourne, Australia. Red dots mark NTS sampling sites (BAN: Banyan Creek, OP: Ornamental Pond, NP: North Pond, and HRW: Huntingdale Road Wetland). White dots mark weather stations where rainfall and wind speed data were collected for BAN (ERD and FAWS) or HRW, NP, and OP (MA). (b) Cumulative rainfall (mm) at each NTS site for the period during sampling (red), 12 h prior to sampling (black), or 24 h prior to sampling (white). Note that rainfall is higher at OP and NP than BAN and HRW. (c) Average wind speed (km/h) at each LID site. Color coding is the same as in panel (b).

and roads. The final sampling site, HRW, is a retrofitted sedimentation basin within Scotchmans creek (catchment area ~ 8.1 km^2).^{41,42} The wetland

receives runoff from major roads and freeways and is located in a high-density residential area downstream of a park. 41,42

Precipitation and Wind Speed

Cumulative precipitation and average wind speeds were measured across the study area, both during sampling, and in the periods 12 and 24 h antecedent. Rainfall and wind speed data were compiled from three gages; the Bureau of Meteorology Moorabbin Airport gage (MA; station 86077), the Melbourne Water Eel Race Drain gage (ERD; station 228371A), and the Bureau of Meteorology Frankston AWS gage (FAWS; station 86371; Figure 2(a)). For BAN, rainfall data are from ERD (~5 km from the sampling site), while wind speed data are from FAWS (~8 km away). All data for OP, NP, and HRW are from MA (located < 9.7 km away).

Field Sampling

Paired surface microlayer and bulk water samples were collected at all sites on August 7, 2013 (BAN, HRW) or August 8, 2013 (OP, NP). All samples were collected in <2 m water depth. Microlayer samples were always collected prior to bulk water samples to minimize microlayer disturbance.

Samples of surface microlayer water were collected using the glass plate method,⁴³ which samples a microlayer thickness of $50-60 \ \mu m$.^{1,44} In total, approximately 600 mL of microlayer was collected from each site; 10 mL was reserved for EEM fluorescence spectroscopy, and the remainder was analyzed for pollutants. Subsurface (bulk) water samples were collected in sterile amber bottles, uncapped at depth, and recapped prior to extraction, to avoid contamination of bulk water with the microlayer. As with microlayer samples, 10 mL of bulk water was reserved for fluorescence spectroscopy, and the remainder was analyzed for pollutants.

Analytical Procedures

Concentrations of TPH, PAH, and THM (chloroform and total) were quantified in all water samples. All analyses were performed by the ALS Water Resources Group, VIC, Australia, using National Association of Testing Authorities, Australia (NATA) accredited in-house methods. To meet minimum volume requirements for analysis, samples were diluted to 2.2 L with DI water prior to processing. Volatile TPH (C₆-C₁₀) and THM concentrations were quantified using purge and trap GC-MS (VIC-CM047). Longer-chain TPH fractions (C₁₀-C₃₆) were quantified using GC-FID (VIC-CM039), and PAHs were quantified using standard GC-MS (VIC-CM043).

EEM spectra were generated for all samples, allowing comparison of fluorescence signatures between microlayer and bulk water. Samples were filtered through a 0.2- μ m syringe filter prior to analysis. Fluorescence was measured using a Cary Eclipse Fluorescence Spectrophotometer with varied excitation and emission wavelengths (Ex: 250–550 nm, 5 nm interval; Em: 250–600 nm, 2 nm interval). Corrected EEM spectra were generated from raw scans as follows: Raman scattering was removed by subtracting the EEM of a DI water blank, spectra were normalized to the Raman peak of the blank (20 a.u.—Ex: 350/Em: 396), Rayleigh scattering bands were excised and replaced with missing values, and both the region of no fluorescence (Em < Ex) and the high scattering region above the second Rayleigh band were set to 0.^{24,25,45,46}

Following correction, EEM spectra were analyzed using parallel factor (PARAFAC) analysis (DOMFluor 1.7).^{46,47} PARAFAC analysis is an analytical tool that decomposes datasets of 3D EEM spectra into individual fluorescent components. The proper number of PARAFAC components for our NTS data was determined using residual analysis, the core consistency diagnostic, and random initialization, as recommended in the literature.46,48,49 These components were subsequently used to calculate protein-like to fulvic-like FDOM ratios for each water sample. Ratios were compared to measured pollutant concentrations in microlayer or bulk water (to assess the utility of EEM spectroscopy for detecting contaminant enrichment), as well as literature values for various source waters (to assess its utility for identifying likely sources of NTS contamination).

Results and Discussion

Physical Environment: Precipitation and Wind Speed

Although no rainfall occurred during sampling, precipitation was measured 12 h prior to sampling (OP and NP) and 24 h prior to sampling (all sites) (Figure 2(b)). Overall, rainfall was lower at HRW and BAN than OP and NP, which were sampled following a small storm (<0.5 mm vs ~15 mm, respectively). As a result, OP and NP may have received more stormwater runoff than the other two sites during our sampling campaign.

Average wind speed across all sites was 13.9 km/h, below the range of speeds reported to impact microlayer persistence in the literature (15-36 km/h).³ OP winds were steady (during 12 and 24 h prior to sampling), with wind speeds clustering around the average (Figure 2(c)). During sampling, NP had lower than average winds (~8 km/h), and HRW had higher than average winds

(~21 km/h). Only BAN had wind speeds that were above average (>19 km/h) and within reported ranges for microlayer disturbance at two time periods, during and 24 h prior to sampling. Thus, based on wind information alone, microlayer stability may have increased as follows; BAN < HRW < OP < NP.

Pollutant Partitioning: Microlayer Versus Bulk Water

TPH, PAH, and THM, were not observed in bulk water samples from any NTS. TPH and chloroform were observed, however, in microlayer samples from OP, NP, and HRW (Table 1). Of the TPH measured, only high molecular weight compounds $(C_{10}-C_{36})$ were detected. These compounds are hydrophobic and exhibit low volatility, which may explain their enrichment in microlayer water. Chloroform enrichment is harder to explain from chemistry alone, although it has been observed in other systems (discussed above).¹⁷ Chloroform is less hydrophobic (and just as volatile) as several contaminants that were not detected in the microlayer (e.g., BTEX and PAH). Thus, chloroform enrichment in NTS may have other explanations, for instance, high chloroform concentrations in NTS inflow water.

TPHs were detected at sites that received >0.5 mm of rainfall prior to sampling (OP and NP) (Figure 2(b)). OP, which drains a parking lot, had measurable concentrations of three heavy hydrocarbon fractions typically found in diesel and engine oil: $C_{10}-C_{14}$, $C_{15}-C_{28}$, and $C_{29}-C_{36}$.^{50,51} NP also had detectable $C_{15}-C_{28}$ hydrocarbons, which may reflect runoff from nearby roads. The relative concentrations of heavy TPHs detected during this study ($C_{15}-C_{28} > C_{29}-C_{36} > C_{10}-C_{14}$) are consistent with the concentrations reported in Australian road runoff.⁵²

Chloroform, like TPH, was detected in the surface microlayer at two NTS sites (NP and HRW). Measured concentrations were consistent with those reported in stormwater across the United States $(0.2-7 \mu g/L)$,⁵³ but lower than published drinking water guidelines (AU NHMRC: 250 µg/L, US EPA: 80 µg/L).^{54,55} This suggests that THM enrichment in NTS is unlikely to pose a health risk to humans. Notably, the chloroform concentrations measured at HRW were higher than NP, despite limited rainfall (Figure 2 (b)). This suggests that chloroform sources (unlike TPH sources) may be rainfall independent. Because chloroform is a common byproduct of chlorine disinfection,⁵⁶ these rainfall-independent sources may be linked to the use of recycled wastewater for irrigation.⁵³ Interestingly, brominated THMs, which can be more common in recycled water than chloroform,⁵⁶ were not detected in any NTS. This may reflect the

elevated chloroform to brominated THM ratios reported in Australian recycled water,⁵⁶ and/or the higher volatility of brominated THMs, which may limit their environmental persistence (Table 1).³³

EEM Fluorescence: PARAFAC Components

PARAFAC modeling of EEM spectra identified five primary FDOM components, explaining 99.02% of dataset variability (Figure 3). The five components correspond well to fulvic-, humic-, and protein-like components in other systems.^{24,25,57-60} Following the nomenclature of Ref 57, the observed peaks are defined as follows: a tyrosine-like (B) peak (Ex: 270, Em: 292), a tryptophan-like (T) peak (Ex: 290, Em: 346), and a UV humic-like (A) peak (Ex: 250, Em: 452). The other two peaks, grouped together by Coble as visible humic-like (C), were defined as fulvic-like (C1; Ex: 340, Em: 412), and humic-like (C2; Ex: 365, Em: 484), as described in Ref 20. Different fluorophore patterns were observed in microlayer and bulk waters at OP, NP, and HRW (Figure 4). This is consistent with our pollutant analyses, which revealed chemically distinct surface microlayers in all systems except BAN (Table 1).

Protein-Like Fluorescence and Anthropogenic Inputs

Protein-like fluorescence (B or T peak) was only detected in NTS exhibiting TPH or THM contamination (OP, NP, and HRW) (Table 1 and Figure 4). This is in accord with other studies, where proteinlike fluorescence has been linked to anthropogenic pollutants.^{26,27} However, no consistent relationships between TPH or chloroform and specific fluorophores were observed within the microlayer itself. This suggests that EEMs are better suited for detecting the presence of microlayers than the partitioning of specific contaminants. However, EEM analysis can, in some cases, point to pollutant sources that may be impacting receiving waters.²⁴ We apply this approach to our NTS sites by comparing fluorescence ratios (protein-like T or B peak to fulvic-like C1 peak) to those reported for different source waters sampled around the world (Figure 5).

Our cleanest site, BAN, had T:C1 and B:C1 ratios similar to clean rural river water with limited agricultural inputs.^{25,61-63} At all other sites microlayer or bulk waters clustered with specific pollutant sources. At NP, T:C1 ratios were consistent with rural rivers and manure, while B:C1 ratios clustered with urban rivers (bulk water) and manure or landfill leachate (surface microlayer).^{24,25,28,31,63,64} Notably, both ratios point to a manure source at NP. Given the chloroform detected at this site, the source may



FIGURE 3 Best-fit PARAFAC model with five components. (a–e) Excitation–emission spectra of model components; *x*-axis is excitation (nm) and *y*-axis is emission (nm). The spectra in panel (a) is consistent with UV humic-like fluorescence (e.g., A peak), panel (b) is consistent with visible fulvic-like fluorescence (e.g., C1 peak), panel (c) is consistent with visible humic-like fluorescence (e.g., C2 peak), and panels (d) and (e) are consistent with tyrosine and tryptophan-like protein fluorescence (e.g., B and T peaks, respectfully). (f–j) Excitation (solid line) and emission (dashed line) loadings of the components shown in panels (a–e).

be fertilizer that was mobilized by irrigation of nearby green-spaces with chlorinated wastewater.

HRW, unlike NP, had no manure signature, and its FDOM ratios were generally consistent with urban rivers and municipal landfill leachate.^{24,25,31,63,64} The exception was HRW bulk water, which had a B:C1 ratio similar to some green algae.⁶⁵ Benthic biofilms of green filamentous microalgae have been reported in the HRW watershed, ⁶⁷ suggesting that the observed fluorescence could have been caused by algal DOM. Algal exudates may also have caused the elevated T:C1 ratio in microlayer waters at OP. A dense algal bloom was observed in the microlayer at this site, and the fluorophores detected were consistent with cyanobacterial DOM.⁶⁶ Other possible sources for the elevated T: C1 florescence detected in the OP microlayer include sewage and the low molecular weight (LMW) PAH fraction in diesel.^{24,25,27,29} A diesel source is consistent with the drainage characteristics of OP (a parking lot), and the detection of TPH in the microlayer. The B:C1 ratio in OP bulk water also points to a diesel source, as BTEX compounds and some LMW PAHs produce intense B peak fluorescence.²⁹ However, pollutant analyses revealed no PAH or BTEX contamination at OP (Table 1). This apparent contradiction may stem from high detection limits (>4.1 µg/L: PAH and >123 µg/L BTEX). In short, the fluorescence spectra may have revealed contaminants at OP that the pollutant analyses were not sensitive enough to detect.

CONCLUSIONS AND FUTURE DIRECTIONS

Harkening back to the three questions that motivated our case study, we provide the following answers: (1) Surface microlayers are detected in NTS, but not ubiquitously; (2) These microlayers can be enriched in contaminants (including volatiles, like chloroform); and (3) EEM fluorescence spectroscopy shows promise, both for signaling the presence of microlayers and for identifying likely sources of contamination. This last point is important because it suggests that fluorescent EEMs could be used as a first-cut source tracking method to identify and mitigate contaminant inputs to NTS, reducing pollutant loading.

Intriguingly, BAN, the only site where a microlayer was not detected (based on fluorescent EEMs, and TPH or THM concentrations) was also the only site where average wind speeds were consistently above the range known to disrupt microlayer formation (both during and 24 h prior to sampling, Figure 2(c)). This may be a case where studies of microlayer stability in natural analogues (oceans, estuaries, and lakes) inform microlayer stability in NTS. However, factors other than wind could have been responsible for the lack of a microlayer at BAN,



FIGURE 4 Percent fluorescence at each LID site for the five fluorophores identified by the best-fit PARAFAC model (see Figure 3). Protein-like fluorophores (tyrosine-like B peak and tryptophan-like T peak) are shown in hot tones (reds). Humic-like fluorophores (UV humic-like A peak and visible humic-like C2 peak) and fulvic-like fluorophores (C1 peak) are shown in cool tones (blue and white). Microlayer samples are at the top of the figure and bulk water samples are at the bottom. The acronyms above each pie diagram correspond to the stations in Figure 2 (BAN: Banyan Creek, OP: Ornamental Pond, NP: North Pond, and HRW: Huntingdale Road Wetland). Note that BAN is the only site with identical fluorescent signatures in microlayer and bulk waters.



FIGURE 5 Cross plots of fulvic-like (C1 peak) fluorescence intensity to (a) tryptophan-like (T-peak) or (b) tyrosine-like (B peak) fluorescence intensity (arbitrary fluorescence units; a.f.u.). A black line shows the 1:1 fluorescence ratio in (a) and (b). Water samples from different NTS sites (this study) are marked by black circles and labeled with site names. Labels with -M and -B indicate samples from microlayer and bulk waters, respectively. Colored symbols show fluorescence ratios from samples (other studies) of various pollutants or water types; dark blue (clean rural river water), cyan (urban river water), light green (algae), gray (diesel fuel), purple (landfill leachate), brown (manure), yellow (recycled water), orange (secondary treated waste water effluent), pink (combined sewer overflow water), red (raw sewage). Where provided, error bars show the standard deviation in fluorescence intensity. Symbol shape relates specific fluorescence ratios to their respective studies. These are defined in the legend using the first letter of the first authors last name, and the study year; B-05,²⁵ B-08,⁶¹ N-01,⁶² BS-04,⁶³ B-03,²⁴ N-07,⁶⁴, N-05,⁶⁵, Q-12,⁶⁶ A-08,²⁹ Y-12,³¹ B-02,²⁸ H-10,³⁰ H-07,²⁶ and H-09²⁷. Most samples (this study and others) were filtered or centrifuged to remove particulates prior to analysis (exceptions: N-01, B-02, H-10, A-08, and Y-12). Most samples (this study and others) were normalized to the Raman Intensity of a DI water blank (Intensity: 19 \pm 1 a.f.u.). Exceptions include A-08 (hexane blank required due to diesel hydrophobicity) and N-05 (Super-Q water + KCI blank).

including (but not limited to) contaminant removal by the upstream treatment train and low rainfall prior to sampling (Figure 2(b)). A mechanistic evaluation of microlayer formation and dissipation in NTS would help identify the specific conditions (or disturbance criteria) under which microlayers are likely to persist.

The public health implication of microlayer contaminant enrichment in NTS is an interesting topic for future research. While it is clear that microlayer contaminant concentrations can be below human health criteria (as was the case for chloroform), evaluation of additional contaminant suites is needed, including pesticides and emerging contaminants, such as personal care products and perfluorinated compounds.

The ecosystem effects of NTS microlayer contamination also require attention. Although contaminant enrichment may appear minor from a pollutant loading perspective (given the microlayer is <60 μ m thick on average), the absolute concentrations of toxicants may prove damaging to neustonic biota in NTS, as observed in marine systems.² This makes toxicity testing of NTS microlayer water a logical next step toward evaluating the ecological effects of microlayer contaminant enrichment. Special attention should also be paid to PAHs, as these compounds can be particularly toxic to aquatic life.

A final important unknown regarding NTS microlayers is their role in pollution treatment. Given that NTS tend to be shallow, connectivity between sediment-associated contaminant reservoirs and the microlayer may be high. This connectivity could enhance pollutant treatment by NTS if contaminants (shuttled upward by bubbles or downward in aggregates and fecal pellets) experience a broader range of degradation pathways (e.g., aerobic, anaerobic, and photo-induced). In light of the microlayer contaminant enrichment observed in our case study, NTS design modifications that target microlayer water at the inlet might be a useful means of improving treatment performance while protecting sensitive habitat. Absorbent booms (located at the surface of NTS) are one such technology, as they are low energy, inexpensive, and remove a variety of chemicals from solution including TPHs and PAHs.⁶⁸ Absorbents can also be combined with other technologies suitable for pond inlets, like oil-water separators. Such systems have been successfully deployed in constructed wetlands to treat oilfield effluent, reducing inflow concentrations of oil and grease by approximately 50%.⁶⁹ Many alternate configurations and design possibilities exist. Further research is called for to identify those designs that best suit the diverse pollutant fingerprints and NTS configurations present in different countries and climatic regimes.

In summary, although our current understanding of surface microlayers in NTS is nascent, much can be learned from prior studies in analogous natural systems. Key targets for future research include: (1) spatial and temporal variability of the microlayer, including expected disturbance frequency and changes in contaminant composition pre and post disturbance; (2) common drivers of disturbance, starting with winds and convective overturning, which are known to be important in oceans and lakes; (3) EFs across the full range of potential microincluding nutrients, bacteria/viruses, pollutants, PAHs, and contaminants of emerging concern; (4) microlayer toxicity, focusing on common neustonic NTS organisms and multiple life history stages; (5) contaminant reactivity in the microlayer, including photodegradation, phototoxicity, and volatilization; and (6) mechanisms controlling microlayer contaminant enrichment, emphasizing those that link different pollutant reservoirs within NTS. Insights from all of these research areas are necessary to identify the primary roles of microlayers in NTS: are they areas of enhanced pollutant treatment? areas of enhanced toxicity? or some combination of the two? Addressing these questions is likely to require expertise (and cutting edge technologies) from multiple disciplines, including surface layer physics, engineering, genomics, aquatic ecology, and chemistry. These questions may also point toward a brighter future for NTS, as answering them requires advancing our process-based understanding, a necessary first step toward the development of new NTS designs (informed by microlayer science) that better meet pollutant removal targets without becoming ecological traps.

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