Effects of stormwater ponds on microbial communities and subsequent ecosystem functioning in downstream receiving waters

Final Report

Prepared for Florida Department of Environmental Protection

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Executive Summary

The urbanization of Florida has contributed to ongoing water quality issues throughout the state. Increased impervious surfaces associated with development, coupled with anthropogenic nutrient inputs prevalent in urban areas, alters the hydrology and nutrient export from terrestrial landscapes into aquatic ecosystems. Stormwater wet ponds (SWPs) are a common management practice that are designed, constructed, and maintained to reduce the hydrologic impacts of urbanization while also protecting/improving downstream water quality by removing pollutants prior to water being discharged downstream. Despite the expectation for SWPs to protect natural aquatic ecosystems downstream, there has been relatively little research performed on biogeochemical processes within SWPs and even less focus on how SWPs affect downstream ecosystems. In this report, we describe the initiation of a research project designed to better understand the biogeochemical functioning of SWPs and a nearby natural stream, and to establish how SWP discharges into a natural aquatic ecosystem may alter biogeochemical and microbial dynamics. We report initial results from the first three months of the ongoing study.

Beginning in March 2022, we established seven sampling sites in the Braden River watershed in Bradenton, FL. Of the seven sites, three are ponds and four are streams. The four stream sites move from an undeveloped headwater region through a master-planned community with a larger number of SWPs, including the three in our study. At each of these sites, we deployed sensors to allow us to estimate gross primary production (GPP), ecosystem respiration (ER), and net ecosystem production (NEP) at the ecosystem scale. These metrics combined are often referred to as ecosystem metabolism and are fundamental biogeochemical components of aquatic ecosystems that drive nutrient cycling and food web dynamics. We found that ponds had higher GPP than streams during the dry season, whereas ER was comparable across ecosystems. Ponds were more likely to have positive NEP, which suggests that more carbon is being produced in the ecosystem than is being consumed.

In addition to metabolism, we conducted a one-day intensive field sampling campaign that involved sampling all seven sites for a range of biogeochemical parameters. We found variable patterns in water chemistry across all sites but dissolved organic matter dynamics further supported the metabolic results. Overall, it appears that ponds are more reliant on internally produced carbon than streams, but as streams flow downstream they may become more biogeochemically similar to ponds. Ongoing work to better understand the interactions between streams and SWPs is needed, particularly during the rainy season when SWPs and streams are more likely to be hydrologically connected. Understanding how SWP discharges may influence the microbial communities and the biogeochemical functions they perform will allow for a better idea of how effective these ubiquitous ecosystems are at protecting natural water bodies downstream.

Background and Justification

The river continuum concept (RCC) is a long-standing conceptual paradigm in stream ecology. The general tenets of the RCC are that as streams increase in size (as they flow downstream), there is a fundamental shift in the organic matter and energy sources driving the ecosystem (Vannote et al. 1980). This shift in organic matter and energy sources alters fundamental ecosystem processes like stream metabolism (McTammany et al. 2003). These shifts in organic matter and energy dynamics can in turn alter the macroinvertebrate community and shift the broader foodweb (Rosi-Marshall and Wallace 2002). These patterns predicted by the RCC, however, are based on forested watersheds in mountainous landscapes (Vannote et al. 1980). How these patterns play out in human-modified watersheds is less clear.

Although less clear, human modifications of the landscape have been shown to affect the RCC. For example, the Serial Discontinuity Concept postulates that the vast majority of lotic ecosystems are affected by hydrologic alterations driven by dam management, fundamentally re-setting the RCC below a dam (Ward and Stanford 1983). More recently, an improved understand of organic matter dynamics has shown that urbanization alters the quantity and quality of organic matter along the urban watershed continuum (Kaushal and Belt 2012, Hosen et al. 2014). Despite this improved understanding, this previous work has occurred primarily in temperate climates with fundamentally different stormwater management approaches. Only recently have we begun to investigate the potential role of stormwater ponds as biogeochemical regulators in urban watersheds (Gold et al. 2017a, Blaszczak et al. 2018, Hohman et al. 2021, Goeckner et al. 2022).

Stormwater wet ponds (SWPs; *note: we will use SWPs and ponds interchangeably; 'pond' refers to SWPs throughout unless otherwise noted*) are ubiquitous components of residential developments, particularly in Florida. In fact, there are more than 76,000 urban SWPs throughout Florida, covering more than 1% of the entire state's surface area (Sinclair et al. 2020). The expanding population of Florida, >90% of which currently resides in urban areas, will necessitate the expansion of urban areas (Carr and Zwick 2016) and associated stormwater infrastructure. Despite the variety of stormwater management options that exist, SWPs will inevitably remain an important tool for managing stormwater runoff from residential landscapes in Florida for the foreseeable future.

Typically, SWPs are built to protect natural downstream water bodies from flooding and water quality impairments. These human-made ecosystems are often expected to remove 80% or more of the incoming pollutant load from urban landscapes. However, empirical research has shown that SWPs in Florida typically remove far less than 80% of nitrogen (N) inputs, rarely even reaching 50% N removal (Harper and Baker 2007). Ongoing research investigating N cycling in SWPs shows that SWPs can exhibit net N_2-fixing activity rather than denitrification, indicating that SWPs can be N sources rather than sinks (Gold et al. 2017, Hohman et al. 2021, Goeckner et al. *in prep*). Despite this potentially large divergence between N removal expectations (i.e., 80% N removal) and reality (i.e., SWPs acting as a N source), there is little understanding of the mechanistic processes that drive the ultimate fate and transport of N within these ubiquitous aquatic ecosystems (Gold et al. 2019).

Beyond internal dynamics of SWPs themselves, there is even less known about the actual downstream impacts of SWPs. Despite ancillary benefits from SWP aesthetics (e.g., enhanced property values; Monaghan et al. 2016), the primary purpose of SWPs and other stormwater control measures is to protect natural water bodies downstream. However, this may not always occur. For example, construction of stormwater ponds in a developed neighborhood in North Carolina has been shown to actually increase algal biomass and nitrate (NO_x) concentrations in

streams within the neighborhood (Gold et al. 2017a), further supporting the potential role of SWPs as sources of N. Despite this potential, though, there are no studies (to our knowledge) that have investigated biogeochemical impacts of SWPs on downstream ecosystems beyond a snapshot view of nutrient availability.

For this project, we built upon previous and on-going research focused on nutrient removal (Hohman et al. 2021, Goeckner et al. *in prep*), carbon sequestration (Goeckner et al. 2022), and algal community dynamics (Goeckner et al. *on going*) in SWPs. This project represents the initial stage of an ongoing research project that will expand on the results reported here. The objective of the ongoing research is to quantify the downstream effects of SWPs on various biogeochemical functions within natural water bodies and how these interactions between SWPs and the river differ seasonally and following storm events. This specific project and report focus on setting up the research project, initial sensor deployments, and the initial dry season field-intensive sampling campaign.

Methods

Study sites

To characterize physicochemical properties and ecosystem functions of ponds and streams as well as the influence pond discharge may have on streams, we identified four stream/river sites along the Braden River (Bradenton, Florida) and three adjacent SWPs (Figure 1). The headwaters of the Braden River begin in a relatively undeveloped region of the watershed. The river then flows west directly through a large master planned residential community, Lakewood Ranch, that contains hundreds of SWPs. Stream sites include a headwater site in the relatively undeveloped region (HW) and three urbanized sites downstream (U1, U2, U3). The HW site has substantial aquatic vegetation whereas U1 has a predominantly sandy
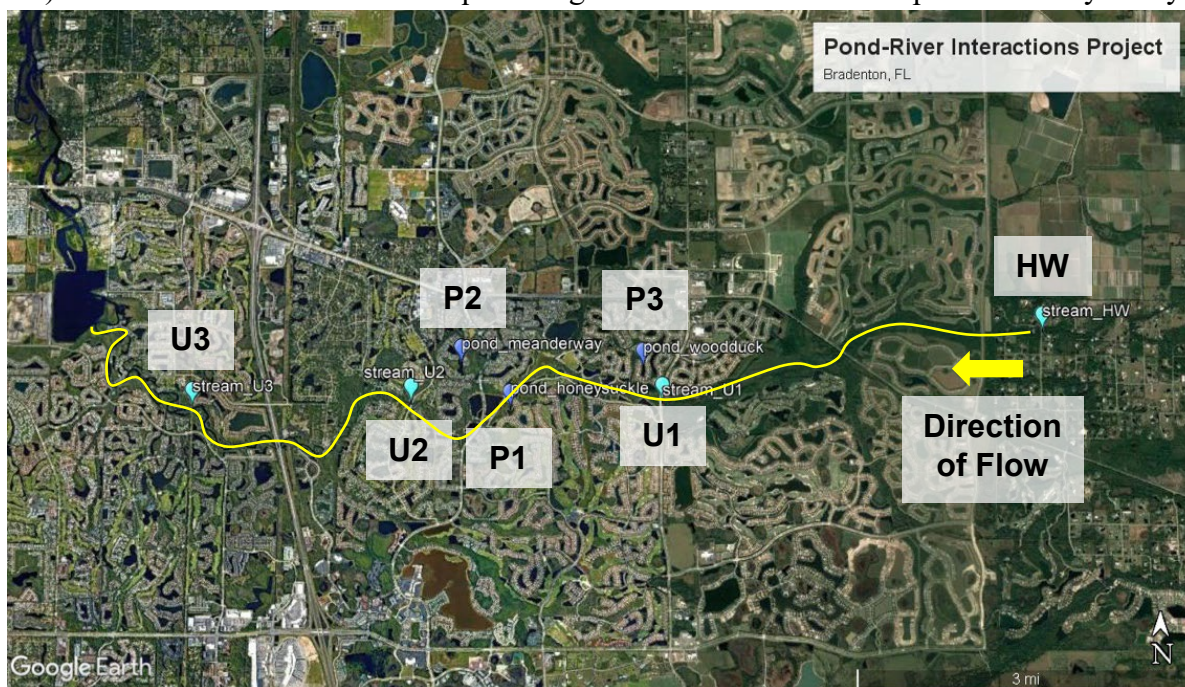

Figure 1. Location of pond (P1-P3) and stream/river (HW, U1-U3) sampling locations on or adjacent to the Braden River in Bradenton, FL. Pond sites are denoted by purple pins and stream/river sites are denoted by light blue pins. Labels were added to improve visualization. We added an approximation of the location of the Braden River (shown in yellow) with a yellow arrow denoting the direction of flow.

bottom and U2 and U3 have a more organic-rich muck bottom. Ponds adjacent to the river were considered if they contained no aeration system and three were randomly selected for this study (Honeysuckle Pond (P1), Meandering Way Pond (P2), and Wood Duck Pond (P3)). Morphological characteristics of all sites can be found in Table 1. We assume that as the stream sites move further downstream, they are increasingly under the influence of pond discharge during times of hydrologic connectivity (wet season or after rain events). Bradenton, FL receives an average of 56 in. of rainfall a year with the wet season defined as June to December and dry season from December to March (DelCharco and Lewelling 1997).

Table 1. Site coordinates and morphological characteristics for ponds and stream sites included in this study.

Site	Site ID	Coordinates (lat., long.)	Water Column Depth (m)*	Pond Area (m^2)	Pond Perimeter (m)	Stream Width (m)
Honeysuckle Pond	P1	27.416616, -82.421254	6.5	2,855	233	-
Meandering Way Pond	P2	27.423709, -82.429013	3.6	13,900	646	-
Wood Duck Pond	P3	27.421553, -82.402147	5.0	7,075	390	-
Braden River-headwaters	HW	27.427200, -82.343475	0.4	-	-	2.1
Braden River-urban 1	U1	27.418164, -82.399474	0.3	-	-	4.1
Braden River-urban 2	U2	27.418010, -82.435860	0.9	-	-	11
Braden River-urban 3	U3	27.417409, -82.468086	1.6	-	-	26

*Note: Water column depths are depths at the sensor location, and are not necessarily mean or max depth but are generally representative of each ecosystem.

Sensor deployments

At each of the pond and stream sites, we deployed sensors on 17 March 2022 to collect up to 5-min. interval data used for estimating ecosystem metabolism. These sensor stations are expected to remain deployed until at least March 2023. Stream and pond ecosystem metabolism are estimated differently, reflected by their unique sensor set ups. At stream sites, we deployed a single dissolved oxygen (DO) and temperature logger (HOBO-Onset, Bourne, MA) protected by PVC housing and anchored to a cinderblock on the stream bed. At each pond, we deployed a thermistor chain near the center of the pond, which is a chain that spans the length of the water column anchored to the sediment and hoisted up by a surface buoy. A DO and temperature logger is situated underneath the buoy and vertically-aligned temperature loggers are held at various increments along the chain to observe water column thermal profiles. In addition to the thermistor chains, a HOBO Micro Station Data Logger was installed on the banks of each pond equipped with HOBO Smart Sensors for barometric pressure, temperature, relative humidity, wind speed, and wind direction. Data is downloaded from sensors approximately monthly.

Field sampling campaign

We conducted a field sampling campaign for all sites on 21 April 2022. Using two teams of personnel, ponds and streams were sampled concurrently between the hours of 9am and 4pm. During sampling, water physicochemical parameters (temperature, specific conductivity, pH, and DO) were measured using a YSI ProDSS. At each site, we collected samples for chlorophyll-a

(chl-a), bacterial community composition, dissolved nutrient ions (NO_x, NH_4^+, PO_4^{3-}), dissolved organic matter (DOM) quality, dissolved organic carbon (DOC), total dissolved nitrogen (TN), dissolved N_2 gas, water samples for net N_2 rate incubations, and greenhouse gases (GHG; CO_2, CH_4, & N_2O). Water samples for nutrient ions, DOM, DOC, and TN were filtered through a 0.22µm polyethylene sulfonate (PES) filter in the field. Nutrient, DOC, and TN samples were frozen at -20°C and DOM samples were refrigerated until analysis. Dissolved N_2 gas samples were preserved immediately with 7M $ZnCl_2$ to halt biological activity and stored refrigerated. GHG samples were stored at room temperature. Chl-a samples were filtered through a 0.45µm GF/F filter and bacterial samples through 0.22µm Sterivex filters within 10 hours and frozen at -20°C until further processing. Net N_2 rate incubation water was stored on ice until incubations were performed (on the same day of sampling). Chl-a, bacterial community, and dissolved gas sample data are currently awaiting further analysis and these data are not present in this report.

Water chemistry analyses

Nutrient ion samples were thawed to room temperature and analyzed with a Seal AQ400 discrete nutrient analyzer (Southampton, United Kingdom) colorimetrically for NH_4^+ using the phenol-hypochlorite method (Solorzano 1969), for $NO_2^- + NO_3^-$ (hereafter referred to as NO_x) using the cadmium reduction method (APHA 2005), and for PO_4^{3-} using the ascorbic acid method (Murphy and Riley 1962). DOC & TN samples were analyzed with a Shimadzu TOC-L total organic carbon analyzer coupled with a TNM-L total nitrogen unit (Kyoto, Japan). DOC was analyzed as non-purgeable organic carbon and samples were acidified with 2% HCl to purge carbonates. Detection limits were 0.006 mg/L for NO_x^--N, NH_4^+-N, and PO_4^{3-}-P, 4 ug/L DOC and 5 ug/L TN. DOM samples were brought to room temperature and analyzed on a HORIBA Aqualog spectrofluorometer (within 2 weeks of collection). Using generated excitation-emission matrices and absorbance data, we calculated two indices representative of autochthonous and recently produced biological material (BIX; Huguet et al. 2009), and the degree of humic, complex, and aromatic compounds (HIX; Zsolnay et al. 1999, Huguet et al. 2009).

N_2 rate incubations

We estimated water column net N_2 production/consumption rates using dark and light-exposed glass vial incubations to evaluate denitrification (positive net N_2 rate) and N fixation (negative net N_2 rate). Three replicate groups of vials were used for each site and each group consisted of a time-zero vial, a light incubation vial, and a dark incubation vial (9 vials per site). Using a 50mL syringe with attached tygon tubing, we filled 12mL exetainer vials with site water from the bottom-up and overfilled the vials 3x, leaving a positive meniscus (to reduce atmospheric contamination). Time-zero vials were preserved with 7M $ZnCl_2$ to halt biological activity. Light and dark incubations were held at room temperature in a water bath. Light vials were exposed to a grow-lamp while dark-vials were wrapped in foil. Incubations lasted approximately 5 hours after which vials were removed from their bath, de-foiled (if necessary), and preserved with $ZnCl_2$ as described above. Samples were refrigerated until analyzed for dissolved N_2 and argon (Ar) gas on a membrane inlet mass spectrometer (MIMS; Kana et al. 1994)). We use Ar as a biologically-inert gas to estimate gas solubility and to calculate N_2 concentrations from the MIMS. The net N_2 rate was calculated for light and dark conditions as the difference between final and initial N_2 concentrations divided by the incubation time.

Metabolism

We used DO and temperature data collected at the four stream/river sites to estimate daily stream metabolism. In addition to DO and temperature data, we estimated photosynthetically active radiation using the "calc_light" function in the *streamMetabolizer* package in R (Appling et al. 2017), which calculates photosynthetically active radiation based on site coordinates. We also accessed Braden River discharge data collected at a USGS hydrological monitoring gage (gage #02300033) using the "readNWISdata" function in the *dataRetrieval* package in R (De Cicco et al. 2022). We used discharge data and the "calc_depth" function within *streamMetabolizer* to estimate mean stream depth of the Braden River at our U2 site, which is co-located with the USGS gage. The "calc_depth" function estimates mean stream depth based on established relationships between discharge and depth (Leopold and Maddock 1953, Raymond et al. 2012). We then estimated depth at the remaining three stream sites using U2 estimates scaled by relative depths we measured during our sensor maintenance visits.

We used these DO, temperature, light, and depth data to estimate daily stream metabolism from the four stream/river sites using the *streamMetabolizer* model framework (Appling et al. 2018a). A detailed description of the model framework is available in Appling et al. (2018a, b). Briefly, *streamMetabolizer* uses an inverse modeling approach with a Bayesian Markov chain Monte Carlo fitting procedure to estimate DO concentrations and oxygen change over time at each time point of data collection throughout the day. Concentrations and rates of DO change are estimated by iteratively testing the best combination of estimates of daily gross primary production (GPP; g O_2 m^{-2} d^{-1}), ecosystem respiration (ER; g O_2 m^{-2} d^{-1}), and K600$_d$, which is the daily average value of the standardized gas exchange rate coefficient (d^{-1}) scaled to a Schmidt number of 600, to best fit DO observed over the course of the day. We note that ER is conventionally reported as a negative number as it represents oxygen consumption within the ecosystem. For this initial analysis of stream metabolism, we used the default constraints and prior estimates for each factor in the model.

We used DO and temperature data, gas transfer velocity (air-water physical gas exchange), surface water mixing layer depth, and atmospheric pressure, temperature and windspeed collected at the three pond sites to estimate daily pond metabolism. Surface layer mixing depth was estimated with the "ts.thermo.depth" function of the RLakeAnalyzer package in R (Winslow et al. 2019) at every time interval using density gradients calculated from the temperature recorded at various depths of the water column. The gas exchange coefficient was estimated using the "k.cole.base" function of the *LakeMetabolizer* package, which uses wind speed scaled to 10 meters above the water body surface ("wind.scale.base" function) (Cole and Caraco 1998, Winslow et al. 2016). Photosynthetically active radiation was used to designate time points as night or day. With the aforementioned variables, pond metabolism was modeled using the "metab.mle" function of the *LakeMetabolizer* package (Winslow et al. 2016). This function uses maximum likelihood estimates of linear regression model parameters and calculates the mean GPP and ER per day using DO concentrations and supporting parameters. For both stream and pond metabolism, we also calculate NEP as the sum of GPP and ER (which is traditionally reported as a negative value). A positive NEP value signifies autotrophic ecosystem metabolism (GPP>ER), and a negative value indicates heterotrophic ecosystem metabolism (GPP<ER). We also present the $\log_{10}$(GPP/ER) as an alternative way to conceptualize metabolic trophic state, with positive values of $\log_{10}$(GPP/ER) signifying autotrophic conditions and negative values of $\log_{10}$(GPP/ER) signifying heterotrophic conditions.

Statistical analyses

Results are interpreted both qualitatively (e.g., identification of visual trends) and quantitatively (i.e., statistical analyses). To assess differences among sites, we use analysis of variance (ANOVA) with site as the factor and our various response variables as the response. We set $\alpha=0.05$. Therefore, any statistical analysis with $p<0.05$ is interpreted as a significant difference. For any statistically significant ANOVA, we performed a post-hoc Tukey's HSD test to identify which individual sites differed. We analyzed pond and stream metabolism both separately (e.g., ponds only and streams only) and together. For each analysis, we report our F-statistic (along with the numerator and denominator degrees of freedom provided as subscripts for the F-statistic) and the p-value.

Results

Field sampling campaign

Nutrient concentrations varied among sites and analytes. Nitrate (NO_x^--N) was below detection (detection limit = 0.006 mg N L^{-1}), ammonium (NH_4^+-N) was generally higher in ponds than streams, whereas phosphate (PO_4^{3-}-P) was higher in streams than ponds, which were consistently below detection (DL = 0.006 mg P L^{-1}; Table 2, Figure 2). Dissolved organic carbon (DOC) and total nitrogen (TN) were more variable in ponds than streams. Within stream sites, PO_4^{3-}-P generally decreased with urbanization while NH_4^+-N showed a consistent decreasing pattern. DOC and TN were more variable among streams, but the headwater site (HW) generally had higher nutrient concentrations than any of the urban sites (Figure 2).

Table 2. Chemical parameters measured from the seven sampling locations during the April field sampling campaign.

Site	Site ID	pH	Conductivity (uS/cm)	DOC (mg/L)	TN (mg/L)	NO_x^- (mg/L)	NH_4^+ (mg/L)	PO_4^{3-} (mg/L)
Honeysuckle Pond	P1	7.69	1153	9.64	0.59	BD	0.013	BD
Meandering Way Pond	P2	7.54	542	15.2	0.83	BD	0.015	BD
Wood Duck Pond	P3	8.02	825	8.67	0.56	BD	0.071	BD
Braden River-headwaters	HW	6.87	453	11.3	0.68	BD	0.018	0.62
Braden River-urban 1	U1	6.89	1154	10.1	0.57	BD	0.015	0.03
Braden River-urban 2	U2	7.24	937	10.2	0.60	BD	0.013	0.20
Braden River-urban 3	U3	7.56	817	12.2	0.63	BD	0.003	0.05

Dissolved organic matter composition clearly differed between sites (Figure 3). Both the Biological Index of Recent Origin (BIX; ANOVA; $F_{6,33}=249$, $p<0.001$) and the Humification Index (HIX; ANOVA; $F_{6,33}=433$, $p<0.001$) differed among sites. When comparing across streams and ponds, ponds had higher BIX and lower HIX values than streams, indicating that DOM in ponds was composed of newer, more autochthonously produced organic matter than in streams. When comparing just among stream sites, DOM became increasingly autochthonous and less humic moving from headwaters to the most downstream site (Figure 3).

There were no obvious patterns in water column N_2 production rates across all sites, and no obvious differences between light and dark incubations ($p>0.05$ for both; Figure 4). Under

light conditions, only one pond showed evidence of net denitrification (positive net N_2 rate with error bars not overlapping zero) and only one stream site (the most urban) showed evidence of net N-fixation (negative net N_2 rate with error bars not overlapping zero). Under dark conditions, one stream was net denitrifying whereas one pond and one stream were net N-fixing. The remaining sites did not show clear denitrification or N-fixation (Figure 4).

Continuous ecosystem metabolism

Stream metabolism was estimated from 19 March through 13 June 2022. At the first urban site (U1), the sensor was buried by sand at the end of April (despite multiple counter-burial deployment strategies).

Figure 2. Total nitrogen (TN), ammonium (NH_4^+-N), and dissolved organic carbon (DOC) concentrations were more variable in ponds (P1-P3) than streams (HW-U3) while phosphate (PO_4^{3-}-P) concentrations were more variable in streams. Increasing or decreasing trends along the urbanization/stream size gradient (shown by the arrows below the x-axis) are evident for each analyte. Site abbreviations are as in Table 1. Cool colors denote ponds, warm colors denote streams.

Therefore, we have metabolism data from 19 March through 30 April for U1 whereas the other three sites have data for the entire time period. Across all stream sites and sampling dates, GPP averaged 1.71 (min=0, max=8.85) g O_2 m^{-2} d^{-1} (Figure 5), ER averaged -6.33 (0, -23.6) g O_2 m^{-2} d^{-1} (Figure 5), and NEP averaged -4.64 (-16.32, 0.34) g O_2 m^{-2} d^{-1}. When comparing just among stream sites, there were significant differences among sites for GPP (ANOVA; $F_{3,282}$=162.7; p<0.001; Figure 5), ER (ANOVA; $F_{3,281}$=90.0; p<0.001; Figure 5), and NEP (ANOVA; $F_{3,278}$=37.1; p<0.001). Across the four stream sites, the most downstream site (U3) was the most metabolically active, showing the highest rates of GPP and ER (most negative) and was the most heterotrophic (most negative NEP) of the four stream sites. The first urban site (U1) was the least metabolically active site, exhibiting the lowest GPP (although GPP at U1 did not significantly differ from U2) and GPP. The non-urbanized headwater site (HW) exhibited similar metabolic activity as the middle urban site (U2) (Figure 5).

Pond metabolism was estimated from 18 March to 20 July 2022. Across all pond sites and sampling dates, GPP averaged 7.27 (min=0.74, max=22.8) g O_2 m^{-2} d^{-1} (Figure 6), ER averaged -7.08 (-0.04, -26.2) g O_2 m^{-2} d^{-1} (Figure 6), and NEP averaged 0.19 (-12.0, 7.9) g O_2 m^{-2} d^{-1}. There were significant differences in GPP (ANOVA; $F_{2,255}$=30.8; p<0.001) and ER (ANOVA; $F_{2,255}$=33.3; p<0.001) among ponds, with Honeysuckle (P1) and Meandering Way (P2) ponds exhibiting higher rates of GPP and ER than Wood Duck (P3) pond (Figure 6). NEP
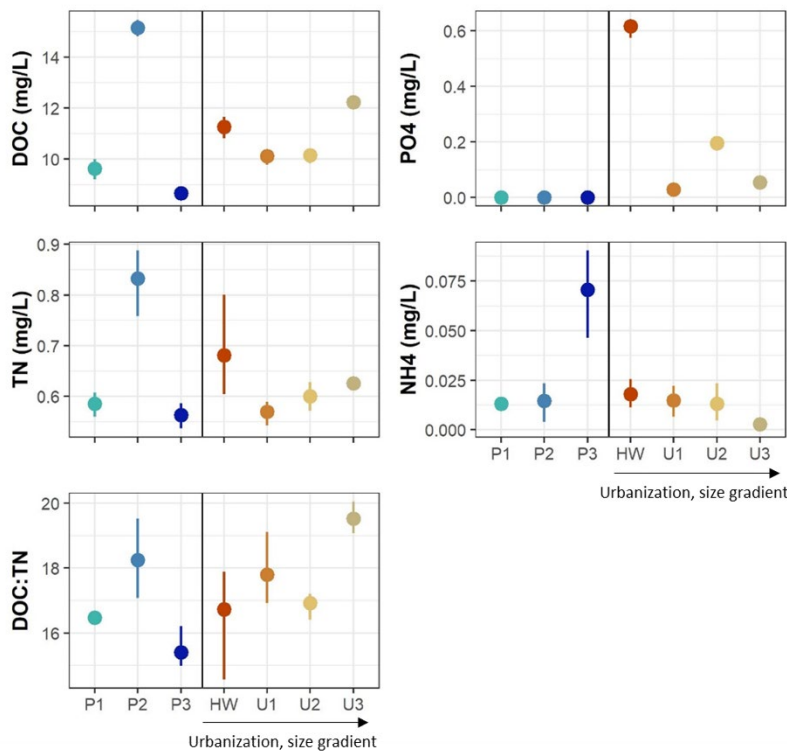
also differed among ponds (ANOVA; $F_{2,255}=7.35$; $p<0.001$) with Meandering Way (P2) pond (heterotrophic on average) having lower NEP than Honeysuckle (P1) or Wood Duck (P3) ponds (both autotrophic on average).

When comparing among all sites (ponds and streams), there were differences among sites for GPP (ANOVA; $F_{6,537}=103.6$, $p<0.001$; Figure 7), ER ($F_{6,536}=45.28$, $p<0.001$; Figure 7), and NEP ($F_{6,533}=89.8$, $p<0.001$; Figures 7, 8). Overall, ponds tended to have higher GPP throughout the study period (although the most downstream stream site was similar to the least productive pond; Figure 7) and were more likely to be autotrophic (Figure 8). Ecosystem respiration was generally more similar between ponds and streams, with Wood Duck Pond (P3) and the first urban stream (U1) showing similar rates of ER, ER from the headwater and U2 sites were similar, Honeysuckle (P1) and Meandering Way (P2) ponds did not differ, and U3 had the highest (most negative) rates of ER (Figure 7). Although ponds were generally more autotrophic than streams, certain periods (potentially associated with rain events) led to overlap in NEP between streams and ponds (e.g., late April and late May in Figure 8).

Samples in progress

We collected and filtered water to analyze the water column microbial communities across all seven sites during the April field sampling event. We will quantify algal biomass (as chlorophyll *a*) from these sites during summer 2022. Additionally, we will establish the microbial community composition across these sites using molecular sequencing approaches to identify the microbial and algal species present at each site. We will begin processing these microbial samples in July 2022. These microbial community data will be used to more firmly establish whether apparent similarities between more downstream urban streams and ponds are due to pond influences on the stream microbial communities or other environmental drivers.
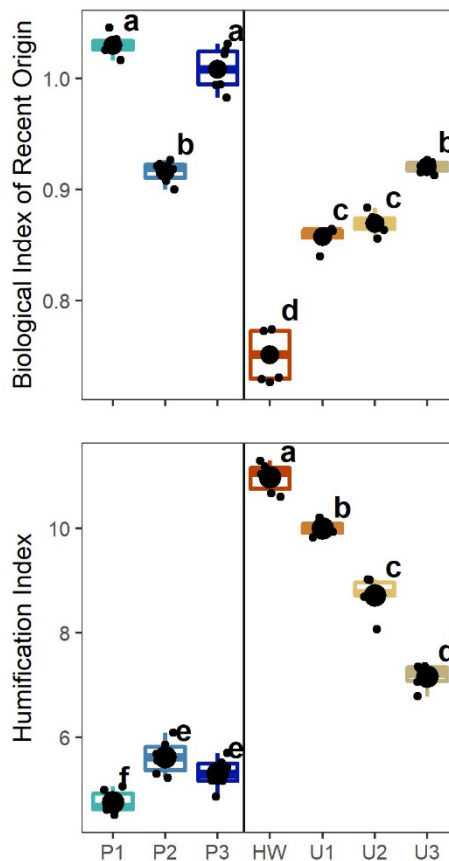

Figure 3. Dissolved organic matter composition revealed clear differences between ponds (P1-P3; cool colors) and streams (HW-U3; warm colors), with pond DOM being more autochthonous (higher BIX) and less humic (lower HIX). Stream DOM became increasingly autochthonous and less humic moving downstream. Site abbreviations are as in Table 1.
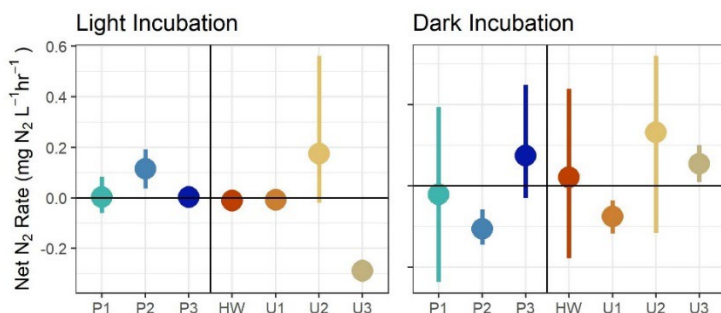
Discussion and Implications

Relatively little is known about the interactions between stormwater ponds (SWPs, also referred to as ponds herein) and natural streams and rivers in the same watershed. Our results show that similarities and differences between SWPs and streams differ between response metrics. Streams had more variability in phosphorus concentrations whereas ponds were more variable in their nitrogen and dissolved organic carbon concentrations. During the dry season, SWPs are more reliant on internally produced (autochthonous) carbon than streams, as evidenced by dissolved organic matter indices and ecosystem metabolism estimates. These differences

Figure 4. Net N_2 transformation rates from the water column of three pond sites (P1-P3) and four stream sites (HW-U3) did not exhibit any clear patterns under either light (left panel) or dark (right panel) conditions. Positive net N_2 rates are indicative of denitrification whereas negative net N_2 rates are indicative of N fixation. Site abbreviations are as in Table 1. Cool colors represent ponds, warm colors represent streams. The vertical line demarcates ponds from streams in each panel.

between SWPs and streams are in-line with general differences that we might expect between lentic (still water; ponds, lakes, wetlands) and lotic (flowing water; streams, rivers) ecosystems. However, various results suggest that the larger and more urbanized stream sites are more biogeochemically similar to ponds than smaller headwater streams. Understanding the drivers of these patterns can provide insight into how stormwater ponds are influencing downstream ecosystems and the consequences for natural ecological processes therein.

In general, ponds and lakes are expected to retain more P than N due to a combination of physical, chemical, and biological factors (Wu et al. 2022). For example, P adsorbs more strongly to suspended particles than N. When these particles reach lentic ecosystems (including SWPs), they settle out to the bottom, bringing the adsorbed P along with the settling sediment. These physicochemical conditions are why P is often assumed to be more limiting than N in lentic ecosystems, and are likely why P was below detection in SWPs within this study. In contrast to SWPs, we found that P in streams decreased along the urbanization gradient. This decrease may have been driven by the preponderance of SWPs in the watershed increasing with stream size, but it may also be driven by natural conditions. In contrast to P, N (as NH_4^+ and total N) was more variable in SWPs than streams, with NH_4^+ decreasing in the downstream direction despite an increase in TN (after moving into the urbanized portion of the watershed).
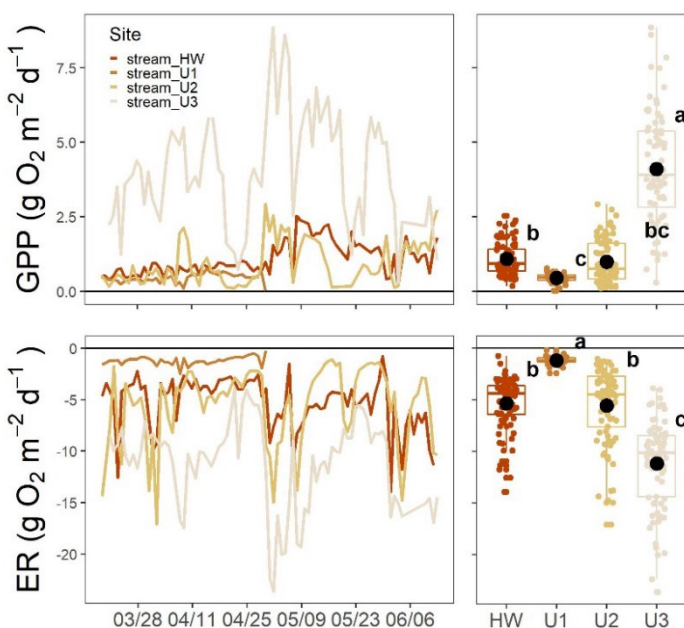

Figure 5. Stream gross primary production (GPP) and ecosystem respiration (ER) differed spatially and temporally. There was a decrease in metabolic activity from the headwater site (HW; dark orange) to the most upstream urban site (U1; light orange). Both GPP and ER increased as the Braden River flowed through more urbanized locations at U2 (darker yellow) and U3 (lighter yellow). The line chart on the left represents all data collected thus far for GPP (positive) and ER (negative). Letters on the boxplots denote statistically significant differences based on ANOVA. Individual boxplots show all data points, the mean (solid dot), median (line), 25th and 75th percentiles (boxes) and the hinges extend to the highest and lowest values that are 1.5 times the inter-quartile range from the median.
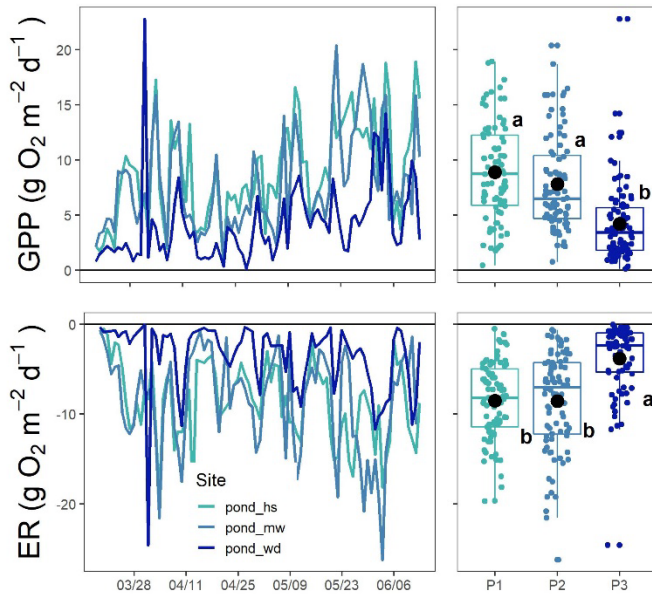
Figure 6. Pond gross primary production (GPP) and ecosystem respiration (ER) exhibited more temporal variability than variability among sites. The line chart on the left represents all data collected thus far for GPP (positive) and ER (negative). Both Honeysuckle Pond (P1; blue-green) and Meandering Way Pond (P2; light blue) exhibited higher rates of GPP and ER than Wood Duck Pond (P3; dark blue). Different letters on the boxplots denote statistically significant differences based on ANOVA. Individual boxplots show all data points, the mean (solid dot), median (line), 25th and 75th percentiles (boxes) and the hinges extend to the highest and lowest values that are 1.5 times the inter-quartile range from the median.

Ponds and lakes are more likely to be autotrophic than streams and rivers (Hoellein et al. 2013), which is consistent with both the DOM and ecosystem metabolism results shown here. The relative contribution of autochthonous and allochthonous organic matter has implications for greenhouse gas emissions, food web dynamics, and nutrient cycling. Nutrient cycling is tightly linked with metabolic processes. In particular, nitrogen removal in streams has been closely linked with GPP and ER (Hall and Tank 2003, Tank et al. 2018), although N and P assimilated by autotrophic or heterotrophic processes may only be temporarily removed from the ecosystem.

In addition to these broad linkages between energy and nutrient cycling in aquatic ecosystems, specific metabolic processes and drivers can further influence the impacts of ponds and streams on downstream nutrient export. For example, both water column and sedimentary processes contribute to nutrient cycling in large rivers (Reisinger et al. 2021). Although permanent N removal via denitrification can occur in the water column (Liu et al. 2013, Reisinger et al. 2016), N uptake in the water column is more likely to be driven by phytoplankton. This N assimilated by phytoplankton is likely exported downstream as organic N, simply shifting the form of N rather than the ultimate fate.
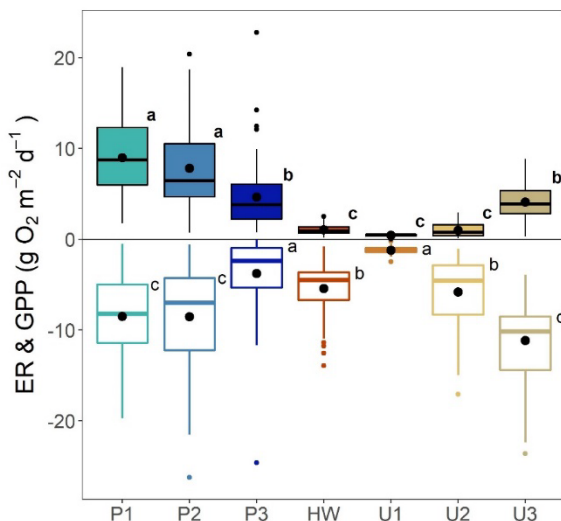

Figure 7. Gross primary production (GPP; positive values; solid boxes) and ecosystem respiration (ER; negative values; open boxes) differed between ponds (P1-P3; cool colors) and streams (HW-U3; warm colors) and within ponds and streams as well. Different letters on the boxplots denote statistically significant differences based on ANOVA. Individual boxplots the mean (solid dot), median (line), 25th and 75th percentiles (boxes) and the hinges extend to the highest and lowest values that are 1.5 times the inter-quartile range from the median.
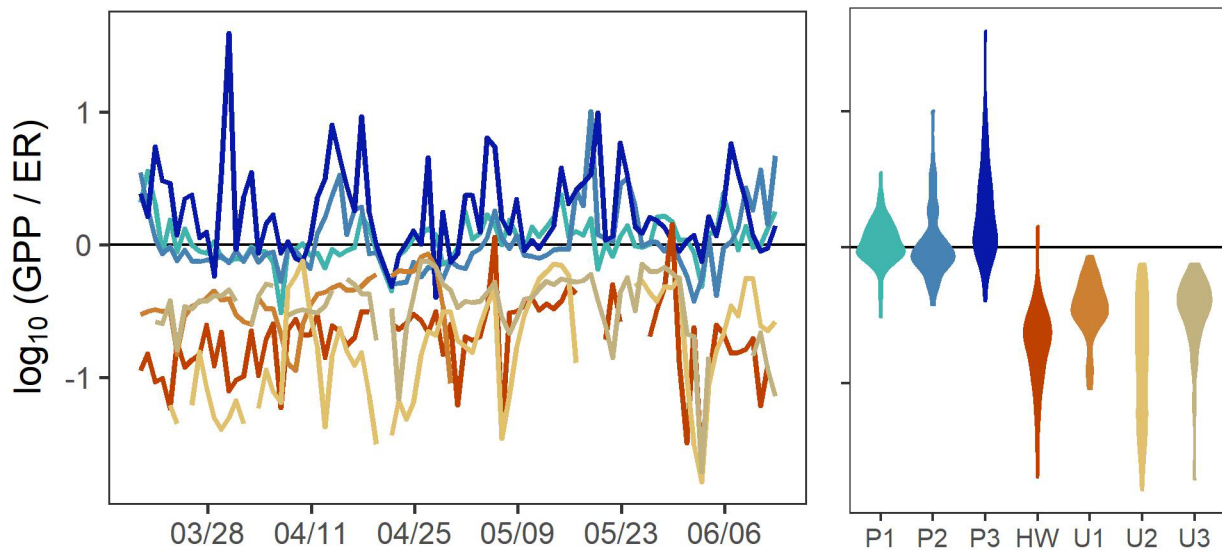
Figure 8. The logarithm (base-10) of the GPP/ER ratio improves our ability to visualize differences between ponds and streams. Any value of $\log_{10}(\text{GPP/ER}) > 0$ indicates an autotrophic ecosystem, whereas negative values indicate heterotrophic ecosystems. The line chart on the left shows all daily metabolism data collected to this point. The violin plot on the right shows the distribution of all metabolism data collected thus far, clearly showing that ponds (P1-P3; cool colors) are more autotrophic than stream sites (HW-U3; warmer colors).

The river continuum concept (RCC) predicts an increase in GPP and NEP with increasing stream size driven by hydrologic and geomorphic conditions of larger rivers (Vannote et al. 1980). These patterns have been shown to carry over to urban watersheds as well (Kaushal et al. 2014). The metabolic results we have found thus far generally support the patterns predicted by the RCC, with an increase in GPP from site U1 to U3. The somewhat unexpected result was the decrease in metabolic rates from the headwater (non-urbanized) to the first urbanized (U1) stream site. Both GPP and ER significantly decreased from HW to U1. The urbanized portion of the stream (U1 – U3) then followed the predictions of the RCC. These results suggest that urbanization may initially reset the metabolic signature of a stream, while the RCC patterns are enhanced moving downstream in the urbanized portion of the watershed.

The results presented here are exclusively from the spring of 2022 during the dry season. The dry season is when we would expect the least interaction between SWPs and streams because they are rarely hydrologically connected. As the rainy season progresses and SWPs fill up and start to discharge into the river, we expect the impact of SWPs on the Braden River to become more evident. Furthermore, the microbial community composition data that we have yet to analyze can provide insight into whether the Braden River becomes more similar to nearby SWPs than its own headwaters. Ongoing work for this project will continue to monitor ecosystem metabolism continuously. We will also perform additional field sampling campaigns under different hydrologic conditions (e.g., during the peak of the wet season, during the early dry season). These ongoing results will be coupled with additional research and extension/education projects we have planned focused on 1) the role of littoral plants for improving water quality; 2) the effect of homeowner perceptions on SWP biogeochemical functioning; 3) an experiment testing the effects of combining pond and stream microbial communities on nutrient and energy cycling; and 4) the role of stormwater ponds, canals, and other anthropogenic ecosystems for protecting coastal water quality throughout Florida.

Conclusions

Ultimately, SWPs are designed, constructed, and maintained to reduce flooding and protect downstream water quality. The high rates of primary production within SWPs shows that these SWPs are biogeochemically active and are at a minimum transforming nutrients before discharging them downstream. There appears to be an increase in the similarities between SWP and stream metabolism as the stream/river gets larger and is exposed to more SWPs. It is unclear if these similarities are driven by connections between SWPs and the river or if they are both responding to similar environmental drivers (e.g., increasing light and nutrient availability). Future work during the wet season along with an in-depth look at microbial communities at these sites will provide more details on these interactions.

A shift in biogeochemical and microbial dynamics along the river continuum of the Braden River will highlight the impacts of SWPs on natural downstream ecosystems. If SWPs are strongly influencing the river, these results would suggest that not only do SWPs have the potential to discharge nutrients (and other pollutants) to streams and rivers, but they are fundamentally altering the ecological functioning of these natural environments. Understanding these interactions between natural and anthropogenic ecosystems is essential to allow for improved management of both SWPs and urban streams to protect and improve sensitive coastal environments downstream.

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