THE INFLUENCE OF MACROPHYTES ON BENTHIC NUTRIENT DYNAMICS IN AN URBAN WETLAND

A THESIS SUBMITTED TO THE GRADUATE SCHOOL
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE
MASTER OF SCIENCE

BY
AARON MATTHEW MARTI

ADVISORS: MELODY J. BERNOT, Ph.D. & ALLISON R. ROBER, Ph.D.

BALL STATE UNIVERSITY
MUNCIE, INDIANA
JULY 2015
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BALL STATE UNIVERSITY
MUNCIE, INDIANA
JULY 2015
The influence of macrophytes on benthic nutrient dynamics in an urban wetland
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ABSTRACT

THESIS: The influence of macrophytes on benthic nutrient dynamics in an urban wetland

STUDENT: Aaron Matthew Marti

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Wetlands in urban-dominated watersheds can serve an important role in nutrient cycling, including removal of excess nutrients associated with anthropogenic inputs. These ecosystem processes can be mediated by macrophyte-microbial interactions. I measured benthic microbial nitrogen (N) and phosphorus (P) cycling, as well as benthic physicochemical characteristics associated with wetland macrophytes (individual species and functional groups) and unvegetated sediments (control) in a central Indiana urban wetland. Benthic physicochemical characteristics were measured in situ. Benthic microbial activity (as nutrient uptake and respiration) was measured in vitro. The benthic material of deep-rooted macrophyte species had higher organic matter content than the benthic material associated with shallow-rooted macrophyte species. Across macrophytes, benthic microbial activity was positively correlated with organic matter content. Benthic nutrient uptake rates were also correlated with benthic respiration rates. These data provide insight into the influence of macrophyte diversity on benthic microbial nutrient cycling which may be important in wetland restoration and nutrient mitigation in urban landscapes.
ACKNOWLEDGEMENTS

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Introduction

Freshwater wetlands support numerous ecosystem services which are valued from intrinsic (Nassauer 2004), ecological, cultural (Mitsch and Gosselink 2007), and economic (Costanza et al. 1997) standpoints. For example, wetlands can enhance biodiversity (Zedler and Kercher 2005) by providing temporary and permanent habitats for species of commercial, recreational and ecological importance (Mitsch and Gosselink 2007). Of the > 200 species endangered in the US, over half depend on wetlands for survival (Mitsch and Gosselink 2007). Wetlands can also provide hydrologic stability through water storage and groundwater discharge (Whigam and Jordan 2003; Mitsch and Gosselink 2007; Acreman and Holden 2013). Additionally, wetlands serve as “hotspots” of biogeochemical activity (McClain et al. 2003) with exchange of water, solutes, solids and gases with the atmosphere, groundwater, and surrounding ecosystem. On average, wetlands are more biogeochemically active than their respective uplands (Marton et al. 2015). Phosphorus sorption and burial can be facilitated through accumulation and stabilization of wetland sediments and organic matter (Cheesman et al. 2010; but see Aldous et al. 2007). Further, organic matter is a key resource supporting nitrogen cycling and removal of excess nitrogen via denitrification (Morrissey et al. 2013). Thus, wetlands play a key role in mediating watershed nutrient export (Whigam and Jordan 2003).

Although destruction and removal of wetlands on the landscape results in a direct loss of these ecosystem services, indirect anthropogenic disturbances to existing freshwater wetlands may also alter wetland structure and function, particularly in urban watersheds (Kaushal et al. 2014). For example, because of altered hydrology in urban ecosystems (Schramm et al. 2009), wetlands are less effective in nitrogen retention and can also serve as a phosphorus source (Harrison et al. 2014) rather than mitigating nutrient pollution. Thus, urban wetlands are more
susceptible to eutrophication and related adverse effects, particularly if receiving wastewater effluent (Anza et al. 2014). In addition to altered hydrology, urban wetlands are influenced by a myriad of anthropogenic contaminants (Wilcox 1986; Gaultier et al. 2009). In urban wetlands of Australia, agrochemicals and trace metals were present in ~60% of water and sediment samples collected across 24 sites (Allinson et al. 2015). Urban wetland sediments may also have high concentrations of polycyclic aromatic hydrocarbons (Kimbrough and Dickhut 2006) and trace organic compounds (Matamoros and Bayona 2008). Despite these known indirect disturbances to urban wetlands, assessments of the influence of these disturbances on wetland structure and function are limited.

Wetland structure and function is closely connected to macrophyte community composition which is often used as an indicator of ecosystem health for regulatory (Lichvar et al. 2014) and management (Craft et al. 2007) decisions. Macrophyte community composition may change in response to natural (Brinson 1993; Zweig and Kitchens 2008) and anthropogenic disturbance (Johnson and Rejmankova 2005; Craft et al. 2007). Although macrophyte communities may respond to these disturbances, macrophytes can also influence their environment. For example, some macrophytes can transport oxygen to rhizospheres below the benthic-water interface, which may influence the oxidative state of benthic nitrogen and provide a direct benthic-atmospheric pathway of gas exchange (Dacey 1981; Reddy et al. 1989). Macrophytes also can provide labile exudates (Mann and Wetzel 1996) and long-term autochthonous carbon or nutrient subsidies to the benthic-water interface (Wetzel and Sondergaard 1998). These direct and indirect benthic-macrophyte interactions suggest that changes in macrophyte community composition may result in alterations to benthic
physicochemical conditions and ultimately microbial processes such as nutrient uptake and respiration.

In general, the influence of individual macrophyte species and macrophyte community composition on wetland microbial processes is poorly understood (Gutknecht et al. 2006). However, macrophyte community composition may not only drive benthic physicochemical properties in wetlands, but may also influence benthic microbial activity (Waldrop et al. 2012). In both Alabama lotic wetlands and Great Lakes coastal wetlands, macrophytes can directly influence microbially-mediated carbon dynamics (Mann and Wetzel 1996; Rothman and Bouchard 2007; Stanley and Ward 2010) and bacterial production (Stanley et al. 2003). While the effects of wetland macrophytes on benthic microbial dynamics have been sparsely documented in relatively pristine wetlands (e.g., Mann and Wetzel 1996; Stanley et al. 2003; Stanley and Ward 2010), no known studies have directly quantified how macrophytes or macrophyte communities may directly affect microbially-mediated benthic nutrient dynamics in urban wetlands.

To evaluate the influence of macrophytes on microbial nutrient dynamics in an urban wetland, I measured in situ benthic physicochemical conditions. Additionally, I quantified in vitro benthic microbial activity (nutrient uptake and respiration) associated with wetland macrophytes. I tested two hypotheses: 1) Benthic physicochemical conditions and microbial activity vary among macrophyte species and between macrophyte functional groups; and, 2) Patterns in benthic microbial activity are driven by resource availability (i.e., benthic physicochemical conditions such as nutrient concentrations or organic matter).
Methods

Site description

I examined benthic physicochemical variables, nutrient uptake dynamics and respiration of benthic microbial communities associated with different macrophytes and between macrophyte functional groups within the ~ 16,000 m² open water and littoral area of the Nina Mason-Pulliam Ecolab wetland located at Marian University, Marion County, Indianapolis, Indiana, USA (Fig. 1). The wetland was constructed in 1912 as part of a restoration project by Jens Jensen, a renowned landscape architect and restorationist of the early 20th century (Grese 1992). With the exception of a former land bridge that was dredged at an unknown time, the wetland received no known maintenance for over 90 years (Benson 2004). In the early 2000s, invasive woody plants including honeysuckle (*Lonicera* spp.) and common buckthorn (*Rhamnus cathartica*) were removed from the understory of upland areas surrounding the wetland and native seed was spread in the riparian zone, including *Carex* spp., *Scirpus* spp., and *Aster* spp. (Benson 2004).

Despite the accessibility of this urban wetland, there are no known publications to date which adequately describe the natural history, wetland bathymetry or surface watershed characteristics of the site. The surface watershed of the wetland is roughly 2.83 km², with 98% used for commercial or residential purposes, and over 35% of the area estimated as impervious surfaces (Purdue University 2013). The wetland is a permanently flooded, flow-through wetland with a water control structure on the eastern edge, constituting the only known surface export as very poorly drained soils allow only minimal groundwater export. Soils of the wetland are primarily comprised of the Sloan series (fine loamy, mixed, superactive, mesic, Fluvaquentic
Endoaquolls; Soil Survey Staff 2014). This series consists of very deep, very poorly drained soils which formed on low slopes (0–2%) in the loamy alluvium of floodplains. During this study, yellow-pond lily (*Nuphar advena*) covered approximately 80% the water surface area in May and June 2014. The remainder of the littoral zone was dominated by other macrophytes such as sedges (e.g. *Carex spp.*), bulrush (*Scirpus spp.*), broad-leaved cattail (*Typha latifolia*), and needle spikerush (*Eleocharis acicularis*). Minor inclusions of invasive and exotic species including narrow-leaved cattail (*Typha angustifolia*) and moneywort (*Lysimachia nummularia*) were also present.

**Sampling Methods**

Based on field observations, I assessed benthic conditions of six dominant macrophyte species (Table 1) and unvegetated (control) sediments in May and June 2014. I classified macrophytes into two basic functional groups broadly defined as interstitial species and matrix species (*sensu* Boutin and Keddy 1993; Table 1). Interstitial species consisted of shallow-rooted (< 0.2 m) macrophytes with clumped growth forms and small (fine roots and rhizomes) below-ground structures typical of shallow littoral areas (*L. nummularia, C. comosa and E. acicularis; sensu* Boutin and Keddy 1993). In contrast, matrix species were generally clonally-spreading with deep rooted (> 0.3 m), large (fleshy corms and adventitious roots) below ground structures which allow them to occupy deep-water (> 0.3 m) habitats (*N. advena, T. angustifolia and T. latifolia; sensu* Boutin and Keddy 1993).

I measured benthic physicochemical variables at five sites within the observed distribution of each macrophyte species in the wetland [\(n = 35; (6 \text{ macrophyte species + control}) \times 5 \text{ replicate sites}\)]. In general, water depth where samples and measurements were taken
was shallow (0.05 – 0.3 m). All measurements and samples described hereafter were taken immediately adjacent to an individual stem or a cluster of stems within a site. Dissolved measurements and water samples were taken directly above (< 1 cm) the sediment-water surface as a proxy for benthic conditions. Benthic material was sampled directly below the location where water samples and measurements were taken.

I measured pH using an Extech Instruments pH300 pH meter, as well as dissolved oxygen (DO; mg L$^{-1}$) and temperature (°C) using an Oakton DO 6 Acorn Series Dissolved Oxygen Meter. Filtered (Whatman GF/F; 0.7 μm pore size) and unfiltered water samples were also collected at each site into acid-washed containers. Samples were placed on ice (< 10 h) and transported back to the laboratory where samples were frozen at -20 °C until subsequent analyses. Filtered samples were analyzed for nitrate and phosphate using ion chromatography (Dionex ICS-3000, detection limit = 0.001 mg L$^{-1}$; APHA 1995) and ammonium using the colorimetric phenol-hypochlorite technique (detection limit = 0.01 μg L$^{-1}$; APHA 1995; Aminot et. al 1997).

Benthic samples (~ 150 cm$^3$ from upper 5 cm of profile) were collected at each site in acid-washed 200 ml HDPE specimen jars and transported on ice back to the laboratory (< 10 h) where they were refrigerated (4 °C) until subsequent processing. Within 24 h, samples were sieved through a #10 (2 mm) standard mesh sieve to remove coarse debris (Megonigal and Schlesinger 1997) and homogenized. Benthic material was then dried (65 °C; > 72 h) and ground using a mortar and pestle. A subsample was combusted (550 °C; 3 h) to quantify percent benthic organic matter (BOM %) similar to standard soil analysis methods (i.e., Nelson and Sommers 1982).
Microbial assays

Microbial nutrient uptake assays were performed in vitro within 48 h of benthic material and water collection during May 2014 sampling (Kemp and Dodds 2002). For the assay, ~10 cm$^3$ of sieved, homogenized benthic material and 30 mL of unfiltered water from the site were added to a pre-weighed, acid-washed 50 ml polypropylene conical tube. Tubes were capped, vortexed, incubated in the dark at room temperature (27 °C) for 3 d, and subsequently centrifuged for 10 min to settle any remaining suspended material. Supernatant was extracted, filtered (as above) and frozen at -20 °C until analyzed for dissolved nutrients (as described above). Tubes with the remaining benthic material were dried (65 °C; 3 d) and weighed for determination of uptake rates per mass. Uptake rates were calculated as changes in concentrations of nitrate, ammonium and phosphate in the supernatant over time per mass according to the following equation (Bunch and Bernot 2012; Elias and Bernot 2014):

$$\text{Nutrient uptake rate} = \frac{(C_f - C_i) \cdot V}{T \cdot gdm}$$

where $C_f$ = final concentration of the assay supernatant (mg L$^{-1}$), $C_i$ = initial concentration of the filtered water sample (mg L$^{-1}$), $V$ = volume (L) of water used for assay, $T$ = time (d), and gdm = g dry mass of benthic material (g). Net assimilation of nutrients was indicated by positive uptake rates, whereas negative uptake rates indicated net mineralization of nutrients (Elias and Bernot 2014).

Microbial respiration assays were performed in vitro in conjunction with nutrient uptake assays using the dehydrogenase activity assay (Bunch and Bernot 2011). Briefly, this rapid enzymatic assay measures microbial respiration as the reduction of tetrazolium salts (electron
acceptors) which form a deep red colored formazan that can be measured using spectrophotometry (Bunch and Bernot 2011). Benthic microbial respiration was expressed as $\mu$g $O_2$ gdm$^{-1}$ d$^{-1}$.

Statistical analysis

Data for physicochemical variables, nutrient uptake rates, and respiration rates were log-transformed to meet assumptions of normality, if necessary, and analyzed using one-way analysis of variance (ANOVA) tests with Tukey’s HSD post-hoc pairwise comparisons of means to determine differences in benthic physicochemical conditions and benthic microbial function among macrophytes. To determine differences in environmental conditions and benthic microbial activity between macrophyte functional groups, I performed Welch’s t-tests between interstitial and matrix macrophyte species on these same variables. To explore environmental drivers of benthic microbial processes, I performed regressions of physicochemical (independent) variables and nutrient uptake and respiration rates (dependent). Regression models were developed using SigmaPlot v. 12.3 Dynamic Fit Wizard. To assess potential coupling of microbial processes, I performed Pearson correlations between nutrient uptake and respiration rates. All analyses and graphing were performed using SigmaPlot/SigmaStat v. 12.3.

Results

Benthic physicochemical characteristics

Benthic physicochemical characteristics (BOM %, pH and DO) varied among vegetation types ($p < 0.001$; Fig. 2). Typha spp. benthic material had 2.5-5 times greater BOM % (mean = 14.8 to 17.9 %) compared to other macrophytes ($F_{6,62} = 45.51$, $p < 0.001$; Fig. 2A). Benthic pH (7.5 to 7.7) was similar among macrophyte species with the exception of E. acicularis (mean =
7.98), which had a more alkaline pH (\(F_{6,62} = 8.58, \ p < 0.001;\) Fig. 2B). *E. acicularis* benthic material also had the highest DO concentrations among macrophyte species (mean = 11.4 mg L\(^{-1}\); Fig. 2C), approximately 2 times greater than benthic material of other macrophytes and the unvegetated control (4.9 to 7.0 mg L\(^{-1}\); \(F_{6,62} = 7.50, \ p < 0.001;\) Fig. 2C). Benthic ammonium and phosphate concentrations did not differ among vegetation type (\(p > 0.05;\) Figs. 3A and 3B).

However, benthic nitrate concentrations did vary (\(F_{6,63} = 3.93, \ p = 0.002;\) Fig. 3C). *T. angustifolia* (mean = 2.6 mg NO\(_3^-\) L\(^{-1}\)) had benthic nitrate concentrations up to 7 times greater than *C. comosa* and *T. latifolia* (\(p < 0.01;\) Fig. 3C).

**Benthic microbial activity**

Among macrophyte species, benthic net NH\(_4^+\) uptake was greatest in *T. angustifolia* benthic material (0.26 µg NH\(_4^+\) gdm\(^{-1}\) d\(^{-1}\); Fig. 4A). *T. angustifolia* benthic net NH\(_4^+\) uptake was over eight times greater than *E. acicularis* (0.03 µg NH\(_4^+\) gdm\(^{-1}\) d\(^{-1}\); \(p = 0.001\)), which had the lowest uptake rates among all macrophyte species. Benthic net NH\(_4^+\) uptake of *E. acicularis* was also six times lower than *T. latifolia* (\(p < 0.01\)) or control sediments (\(p = 0.001;\) \(F_{6,27} = 7.29, \ p < 0.001;\) Fig. 4A). Similar patterns were observed for benthic net NO\(_3^-\) uptake (\(F_{6,27} = 12.68, \ p < 0.001;\) Fig. 4B). *T. angustifolia* had the highest benthic net NO\(_3^-\) uptake rate (18.07 µg NO\(_3^-\) gdm\(^{-1}\) d\(^{-1}\), which was double the rate of *T. latifolia* benthic material and control sediment (\(p < 0.01\)) and 4-7 times greater than benthic material of other macrophytes (\(p < 0.001;\) Fig. 4B).

Benthic net PO\(_4^{3-}\) uptake rates varied nearly an order of magnitude among macrophyte spp. (-0.09 to -0.77 µg PO\(_4^{3-}\) gdm\(^{-1}\) d\(^{-1}\)), but no differences were observed among macrophyte species (\(F_{6,27} = 0.92, \ p = 0.50;\) Fig. 4C). Finally, benthic microbial respiration varied almost an order of
magnitude among macrophyte species ($F_{6.27} = 4.27, p = 0.004$; Fig. 4D), ranging from 218 $\mu$g O₂ gdm⁻¹ d⁻¹ ($E. acicularis$) to 1555 $\mu$g O₂ gdm⁻¹ d⁻¹ ($T. latifolia; p = 0.02$).

**Differences between functional groups**

Benthic material of matrix species had over three times greater BOM % (mean = 12.3 %) than interstitial species (mean = 3.67 %; $df = 29, t = 6.38, p < 0.0001$; Fig. 5A). However, benthic DO concentrations of matrix species (mean = 5.91 mg L⁻¹) were 29 % lower than benthic DO concentrations of interstitial species (mean = 7.58 mg L⁻¹; $df = 44, t = 2.08, p < 0.05$; Fig. 5C). Matrix and interstitial species benthic pH did not differ (mean pH = 7.60 and 7.71, respectively; $df = 55, t = 1.94, p = 0.057$; Fig. 5B). Benthic nutrient concentrations (nitrate, ammonium and phosphate) also did not differ between interstitial and matrix species ($p > 0.05$).

Benthic material of matrix species had higher rates of microbial activity, including net NH₄⁺ uptake ($df = 15, t = 3.34, p < 0.01$), net NO₃⁻ uptake ($df = 14, t = 3.69, p < 0.01$) and respiration ($df = 13, t = 2.80, p < 0.05$) as compared to interstitial species (Figs. 6A, 6B, 6D). However, no differences in benthic net PO₄³⁻ uptake were observed between functional groups ($df = 23, t = 0.51, p = 0.61$; Fig. 6C). Net NH₄⁺ uptake (0.16 $\mu$g NH₄⁺ gdm⁻¹ d⁻¹; Fig. 6A) and net NO₃⁻ uptake (10.38 $\mu$g NO₃⁻ gdm⁻¹ d⁻¹; Fig. 6B) in matrix species benthic material were over three-fold greater than interstitial species (0.05 $\mu$g NH₄⁺ gdm⁻¹ d⁻¹ and 2.92 $\mu$g NO₃⁻ gdm⁻¹ d⁻¹, respectively). Benthic respiration rates of matrix species (998.33 $\mu$g O₂ gdm⁻¹ d⁻¹) were also over three times greater than those of interstitial species (300.87 $\mu$g O₂ gdm⁻¹ d⁻¹; Fig. 6D).
**Physicochemical drivers and coupling of benthic microbial processes**

Among all physicochemical variables measured across vegetation types, BOM % explained the most variation in benthic nutrient uptake rates ($r^2 = 0.43$ to $0.67$, $p < 0.001$) and respiration rates ($r^2 = 0.45$, $p < 0.001$; Fig. 7). Benthic net nutrient uptake and respiration rates were positively correlated with BOM % except net $\text{PO}_4^{3-}$ uptake, which was negatively correlated with BOM % indicating a net release or mineralization of benthic $\text{PO}_4^{3-}$ (Fig. 7). Benthic net $\text{NO}_3^-$ and $\text{NH}_4^+$ uptake rates were positively correlated ($r = 0.72$ and 0.78, respectively) with benthic respiration rates across macrophyte species ($p < 0.001$; Figs. 8C and 8B). In contrast, benthic net $\text{PO}_4^{3-}$ uptake rates were negatively correlated with benthic respiration rates across all macrophyte species ($r = 0.82$, $p < 0.001$; Fig. 8A).

**Discussion**

*Benthic physicochemical conditions*

Consistent with hypotheses, benthic physicochemical conditions differed among macrophyte species with BOM % having the most pronounced differences. The range of BOM % observed (~3 - 20 %) was comparable to sediment organic matter content observed in Alabama lotic wetlands (2 - 38 %; Mann and Wetzel 2000) and wetlands of central and northern Indiana (~4 - 15 %; Marton et al. 2013), but greater than Lake Huron lacustrine marshes (~2-8 %; Angeloni et al. 2006) and 2-4 times lower than Indiana Dunes wetlands (51-87 %; Geddes et al. 2014). Similar to this study, organic matter content associated with *Typha* spp. benthic material has been shown to differ relative to native macrophyte communities, ranging from 5 - 6 % greater content in marshes of Lake Huron (Angeloni et al. 2006) and 10 - 35 % greater content in Indiana Dunes wetlands (Geddes et al. 2014). Once established, *Typha* spp. contribute a
refractory litter layer of senesced biomass to underlying benthic material (Vaccaro et al. 2009), which may contribute a long-term source of autochthonous material to wetland sediments (Rothman and Bouchard 2007).

Benthic dissolved oxygen concentrations also differed among vegetation types. In contrast to organic matter, dissolved oxygen differences were most pronounced with the presence of *E. acicularis*. *E. acicularis* can oxidize sediments and increase sediment redox potential through radial oxygen loss (Freeman and Urban 2012), which may explain why *in situ* benthic DO concentrations associated with *E. acicularis* were elevated compared to other macrophyte species. Despite having the highest benthic DO concentrations, mean benthic nitrogen uptake was lowest in *E. acicularis* benthic material (Fig. 4); this may suggest that high DO concentrations limit uptake of nutrients by benthic microbial communities in favor of more energetically favorable respiratory pathways (i.e., aerobic respiration; D’Angelo and Reddy 1999). However, no significant correlations between DO concentrations and microbial activity rates were observed across macrophyte species (data not shown), and rates of benthic respiration were lowest in *E. acicularis* benthic material (Fig. 4).

In general, benthic nutrient concentrations observed in this study are comparable to concentrations observed in other wetlands across Indiana (Craft et al. 2007). *T. angustifolia* had elevated benthic NO$_3^-$ concentrations as compared to the benthic-water interface of other macrophyte species, which is also consistent with previous studies of freshwater wetlands in Indiana (Geddes et al. 2014). However, I found no effect of macrophyte species or functional groups on benthic NH$_4^+$ and PO$_4^{3-}$ concentrations, which is contradictory to previous results in Indiana wetlands (Geddes et al. 2014) and Lake Huron lacustrine marshes (Angeloni et al. 2006).
No other known studies describe wetland benthic nutrient concentrations in relation to specific macrophyte species or functional groups.

_Benthic microbial activity_

No other known studies in lentic and wetland ecosystems have used methods employed in this study to quantify nutrient uptake or reported results on a per mass basis using other methods. However, rates of benthic nutrient uptake observed in this study were partially consistent with previous studies of lotic ecosystems which used the same methods (Kemp and Dodds 2002; Elias and Bernot 2014). Elias and Bernot (2014) found similar rates of net NH$_4^+$ uptake (0.24-0.96 µg NH$_4^+$ gdm$^{-1}$ d$^{-1}$), but observed PO$_4^{3-}$ uptake (0.002-105 µg PO$_4^{3-}$ gdm$^{-1}$ d$^{-1}$) and mineralization of NO$_3^-$ (~38.8-35.8 µg NO$_3^-$ gdm$^{-1}$ d$^{-1}$). Rates of net NH$_4^+$ uptake associated with benthic organic material in a Kansas Prairie stream were orders of magnitude greater (4.0-4.24 mg NH$_4^+$ gdm$^{-1}$ d$^{-1}$) than net NH$_4^+$ uptake observed in this study (0.03-0.26 µg NH$_4^+$ gdm$^{-1}$ d$^{-1}$; Kemp and Dodds 2002). Rates of benthic NO$_3^-$ uptake in the Kansas stream were comparable (~28.4 µg NO$_3^-$ gdm$^{-1}$ d$^{-1}$) to uptake rates observed in this study (2.6-18.07 µg NO$_3^-$ gdm$^{-1}$ d$^{-1}$). Differences in nutrient uptake may reflect benthic microbial community nutrient preferences or response of microbial communities to inherent physicochemical differences between lentic and lotic/wetland ecosystems.

Benthic microbial activity was partially affected by resource availability (i.e., sediment physicochemical conditions) as hypothesized, though results varied depending on the microbial function measured. Macrophytes may drive microbial activity through several potential mechanisms including direct alteration in resource availability or facilitating shifts in the microbial community. *Typha* spp. benthic material in this study had the highest respiration and
nitrogen uptake rates which were correlated with benthic organic matter content. From a resource availability perspective, *Typha* spp. generally have more refractory leaf litter relative to other macrophytes (Vaccaro et al. 2009). However, *Typha* spp. benthic material can support up to 40% greater microbial biomass compared to other macrophytes (Rothman and Bouchard 2007). Microbial biomass is often measured by CO₂ production from microbial respiration (Rothman and Bouchard 2007). Like Rothman and Bouchard (2007), benthic microbial respiration associated with *Typha* spp. in this study was also elevated compared to microbial respiration within the benthic material of other macrophytes. Because *Typha* spp. produce more aboveground biomass per area, on average, compared to other common macrophytes, continual litter deposition to the benthic-water interface may form a large pool of refractory biomass with some small fractions of labile C (Vaccaro et al. 2009), ultimately supporting larger benthic microbial communities. Additionally, greater diversity of benthic microbial communities, specifically denitrifying bacteria, has been previously demonstrated with the presence of *Typha* spp. (Angeloni et al. 2006; Geddes et al. 2014). Microbial communities of *Typha* spp. benthic material may have a greater demand or affinity for nitrogen and higher respiration rates as compared to benthic material associated with other macrophytes due to greater benthic microbial diversity or larger benthic microbial populations, which may explain why the highest rates of respiration and nitrogen uptake in *Typha* spp. were observed in *Typha* spp. benthic material.

In contrast to benthic respiration and nitrogen uptake, no differences in net PO₄³⁻ uptake were measured among macrophyte species. Across macrophyte species, there was a net gain in dissolved PO₄³⁻ indicating net mineralization or release of PO₄³⁻ from the benthic material. Consistent with this study, sediment organic matter was negatively correlated with PO₄³⁻ sorption in created wetlands of Virginia (Bruland et al. 2009). In contrast, Grace et al. (2008) found that
additions of *Typha* spp. leaf litter to oxic, inundated *Typha* sediment cores resulted in no net mineralization of phosphorus; however, *Typha* sediment cores without *Typha* litter amendments incubated under the same conditions led to mineralization of phosphorus, suggesting that microbial phosphate uptake on *Typha* spp. litter may potentially mitigate any mineralization of sediment phosphorus. Benthic material used in this study was sieved to remove the coarse debris which likely removed leaf litter deposited by *Typha* spp. (Vaccaro et al. 2009), potentially leading to a release of PO$_4^{3-}$ similar to observed results in Grace et al. (2008). Alternatively, PO$_4^{3-}$ mineralization from the benthic material may be due to reduced redox potential facilitating geochemical release. Cation-bound and ligand-bound PO$_4^{3-}$ may be released under anaerobic conditions (Brady and Weil 2002; Mitsch and Gosselink 2007). Biotic release may occur when benthic microbes use oxidized cations bound to PO$_4^{3-}$ as terminal electron acceptors, or when biotic oxidation of benthic organic matter leads to a reduced redox potential (Reddy et al. 1999). In this study, *Typha* spp. had the highest benthic organic matter content and lowest benthic DO concentrations coupled with the highest rates of benthic respiration and PO$_4^{3-}$ mineralization; this suggests that benthic microbial respiration may have reduced the benthic redox state, ultimately facilitating PO$_4^{3-}$ release to the overlying water column.

*Application and management implications*

These data provide insight into the influence of wetland macrophytes on benthic dynamics and microbial processes, which may be important to future management of urban wetlands. In particular, these data demonstrate that macrophytes may influence physicochemical conditions (i.e., benthic organic matter) and ultimately benthic microbial processes such as nutrient uptake and respiration, which were correlated with BOM %. Although anaerobic conditions commonly observed in wetlands lead to faster accretion of organic matter as
compared to upland environments (Mitsch and Gosselink 2007), accretion of organic matter in wetlands is still a relatively slow process (100s of years; Ballantine and Schneider 2009; Hossler and Bouchard 2010). Macrophyte species such as Typha may contribute large amounts of refractory organic matter to the benthic-water interface through biomass turnover, providing a potential long-term carbon source for microbial communities (Vaccaro et al. 2009); comparatively, wetlands containing other macrophytes (i.e., native species or communities) may accrete organic matter slower due to lower biomass turnover and more labile litter (e.g., Rothmann and Bouchard 2007) — a potentially shorter-term carbon subsidy for benthic microbial communities. Thus, artificial additions of organic matter to these ecosystems may enhance biogeochemical functioning, particularly in restored wetlands (Bruland et al. 2009; Ballantine et al. 2015). However, organic matter additions may result in tradeoffs in ecosystem services. For example, increased organic matter may increase benthic microbial nitrogen cycling, but may also lead to higher emissions of greenhouse gases (Ballantine et al. 2015) or reduced capacity of wetland benthic material to retain phosphorus (Bruland et al. 2009). These tradeoffs, as well as potential implications of invasive macrophytes (e.g., Typha spp.) on ecosystem structure (i.e., benthic physicochemical alterations) and function (i.e., microbial cycling) as observed in this study, should be taken into account when evaluating the management objectives and desired functional values for a particular wetland.
References


### Table 1

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Class</th>
<th>Type</th>
<th>Height</th>
<th>Water depth</th>
<th>Rhizomes (structure, depth)</th>
<th>Wetland Status</th>
<th>Ecological Status</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carex comosa</td>
<td>Longhair sedge</td>
<td>Monocot</td>
<td>Emergent</td>
<td>0.5 - 1.5</td>
<td>&lt; 0.1</td>
<td>Fine, many Shallow</td>
<td>OBL</td>
<td>Native</td>
<td>Interstitial, Tussock</td>
</tr>
<tr>
<td>(Boott)</td>
<td>Bristly sedge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eleocharis acicularis</td>
<td>Needle spikerush</td>
<td>Monocot</td>
<td>Emergent</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>Fine, many Shallow</td>
<td>OBL</td>
<td>Native</td>
<td>Interstitial, Reed</td>
</tr>
<tr>
<td>(L.) Roem. &amp; Schult.</td>
<td>Needle spike-rush</td>
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<td></td>
<td></td>
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<tr>
<td>Lysimachia nummularia</td>
<td>Creeping jenny Moneywort</td>
<td>Dicot</td>
<td>Creeping, Variable</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>Fine, few Shallow</td>
<td>FACW</td>
<td>Introduced Exotic, Invasive</td>
<td>Interstitial, Reed</td>
</tr>
<tr>
<td>L.</td>
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<td></td>
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</tr>
<tr>
<td>Nuphar advena</td>
<td>Yellow pond-lily</td>
<td>Dicot</td>
<td>Floating-Leaved</td>
<td>0 - 0.3&quot;</td>
<td>0 - 2.0</td>
<td>Stout, Deep</td>
<td>OBL</td>
<td>Native</td>
<td>Matrix, Clonal Dominant</td>
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<tr>
<td>(Aiton) W.T. Aiton</td>
<td>Yellow water-lily</td>
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<tr>
<td>Typha angustifolia</td>
<td>Narrowleaf cattail</td>
<td>Monocot</td>
<td>Emergent</td>
<td>1.0 - 3.0</td>
<td>0 - 1.0</td>
<td>Stout, Deep</td>
<td>OBL</td>
<td>Invasive</td>
<td>Matrix, Clonal Dominant</td>
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<tr>
<td>L.</td>
<td>Narrow-leaf cattail</td>
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<tr>
<td>Typha latifolia</td>
<td>Common cattail</td>
<td>Monocot</td>
<td>Emergent</td>
<td>1.0 - 3.0</td>
<td>0 - 0.5</td>
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<td>OBL</td>
<td>Native</td>
<td>Matrix, Clonal Dominant</td>
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<tr>
<td>L.</td>
<td>Broad-leaved cat-tail</td>
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<td></td>
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</tr>
</tbody>
</table>

**a** Interpreted from USDA-NRCS PLANTS database, November 2014 (http://plants.usda.gov)

**b** Interpreted from Robert W. Freckmann Herbarium database, University of Wisconsin- Stevens Point, November 2014 (http://wisplants.uwsp.edu/index.html)

**c** Refers to “Wetland Indicator Status” categories used for wetland delineation in United States; “OBL” = Obligate Wetland Plant, “FACW” = Facultative Wetland Plant (Lichvar et al.2014)

**d** Functional groups as determined by Boutin and Keddy (1993); species not listed were placed into appropriate functional group as interpreted from group descriptions

**w** Indicates height above water surface
Fig. 1

Howland Ditch Watershed

Nina Mason Pulliam EcoLab
at Marian University

Delaware Creek-Crooked Creek Watershed

Upper EcoLab

Cold Springs School

EcoLab

Howland Ditch Watershed
Fig. 2

A. % Benthic organic matter

B. pH

C. Dissolved oxygen (mg/l)

* p < 0.001
Fig. 3

A. $P_{O_2}^2$ (mg l$^{-1}$)

B. $NH_4^+$ (µg l$^{-1}$)

C. $NO_3^-$ (mg l$^{-1}$)

$p > 0.05$

$p = 0.02$
Fig. 4

A

Net $NH_4^+$ uptake (µg g$^{-1}$ d$^{-1}$)

- Control
- Carex comosa
- Eleocharis acicularis
- Lysimachia nummularia
- Nuphar advena
- Typha angustifolia
- Typha latifolia

$p < 0.001$

B

Net $NO_3^-$ uptake (µg g$^{-1}$ d$^{-1}$)

- Control
- Carex comosa
- Eleocharis acicularis
- Lysimachia nummularia
- Nuphar advena
- Typha angustifolia
- Typha latifolia

$p < 0.001$

C

Net $PO_4^{3-}$ uptake (µg g$^{-1}$ d$^{-1}$)

- Control
- Carex comosa
- Eleocharis acicularis
- Lysimachia nummularia
- Nuphar advena
- Typha angustifolia
- Typha latifolia

$p > 0.05$

D

Respiration (µg O$_2$ g$^{-1}$ d$^{-1}$)

- Control
- Carex comosa
- Eleocharis acicularis
- Lysimachia nummularia
- Nuphar advena
- Typha angustifolia
- Typha latifolia

$p < 0.004$
Fig. 5

A

% Benthic organic matter

\[ p < 0.001 \]
\[ t = 6.38 \]
\[ df = 29 \]

B

pH

\[ p = 0.057 \]
\[ t = 1.94 \]
\[ df = 55 \]

C

Dissolved oxygen (mg l\(^{-1}\))

\[ p < 0.05 \]
\[ t = 2.08 \]
\[ df = 44 \]

Functional group

Interstitial

Matrix
Fig. 6

A

\[ \text{Net NH}_4^+ \text{ uptake (µg gdm}^{-1} \text{ d}^{-1}) \]

\[ p < 0.01 \\
\text{t} = 3.34 \\
\text{df} = 15 \]

B

\[ \text{Net NO}_3^- \text{ uptake (µg gdm}^{-1} \text{ d}^{-1}) \]

\[ p < 0.01 \\
\text{t} = 3.69 \\
\text{df} = 14 \]

C

\[ \text{Net PO}_4^{3-} \text{ uptake (µg gdm}^{-1} \text{ d}^{-1}) \]

\[ p = 0.61 \]

D

\[ \text{Respiration (µg O}_2 \text{ gdm}^{-1} \text{ d}^{-1}) \]

\[ p < 0.05 \\
\text{t} = 2.80 \\
\text{df} = 13 \]
Fig. 7

A. Net $\text{NH}_4^+$ uptake (µg gdm$^{-1}$ d$^{-1}$) vs. % Benthic organic matter

B. Net $\text{NO}_3^-$ uptake (µg gdm$^{-1}$ d$^{-1}$) vs. % Benthic organic matter

C. Net $\text{PO}_4^{2-}$ uptake (µg gdm$^{-1}$ d$^{-1}$) vs. % Benthic organic matter

D. Respiration (µg O$_2$ gdm$^{-1}$ d$^{-1}$) vs. % Benthic organic matter

$p < 0.001$

$r^2 = 0.44$

$r^2 = 0.67$

$r^2 = 0.43$

$r^2 = 0.45$
Fig. 8

A

Net $\text{PO}_4^{2-}$ uptake
($\mu$g gdm$^{-1}$ d$^{-1}$)

Net $\text{NH}_4^+$ uptake
($\mu$g gdm$^{-1}$ d$^{-1}$)

B

Net NO$_3^-$ uptake
($\mu$g gdm$^{-1}$ d$^{-1}$)

C

Respiration (\(\mu\text{g O}_2 \text{gdm}^{-1} \text{d}^{-1}\))

\(\rho < 0.001\)
\(r = 0.82\)

\(\rho < 0.001\)
\(r = 0.78\)

\(\rho < 0.001\)
\(r = 0.72\)