TITLE: Aridland constructed treatment wetlands I: Macrophyte productivity, community composition, and nitrogen uptake.

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ABSTRACT

Urbanized areas increasingly rely on constructed treatment wetlands (CTW) for cost effective and environmentally-based wastewater treatment. Constructed treatment wetlands are particularly attractive treatment options in arid urban environments where water reuse is important for dealing with scarce water resources. Emergent macrophytes play an important role in nutrient removal, particularly nitrogen (N) removal, in CTW. However, the role of plant community composition in nutrient removal is less clear. Numerous studies have shown that macrophyte species differentially affect N uptake processes (e.g.: direct plant uptake, coupled nitrification-denitrification, soil accretion). However, many of these studies have been based on small-scale experiments and have been carried out in mesic environments, which means that their findings are difficult to extrapolate to aridland CTW systems. Our study sought to examine the relationships among emergent macrophyte productivity, plant community composition, and N uptake [by both the plants and the entire ecosystem] at a 42 ha CTW in arid Phoenix, Arizona, USA. We quantified above- and belowground biomass bimonthly and foliar N content annually for four species groups (Typha latifolia + T. domingensis, Schoenoplectus californicus + S. tabernaemontani, Schoenoplectus acutus, and Schoenoplectus americanus) from July 2011 to September 2013. We quantified dissolved inorganic N fluxes into and out of the system and compared plant N removal to total system fluxes. Additionally, we estimated monotypic N content for each to compare the system’s current community composition and plant N removal to hypothetical scenarios in which the system was dominated by only one species.
Peak aboveground biomass ranged from 1586±179 (SE) to 2666±164 (SE) gdw m⁻² of which *Typha spp.* accounted for an increasing portion (>66%). We observed widespread ‘thatching’—the toppling of large stands of macrophytes—that was likely related to a decline in peak biomass from July 2011 to July 2012. The foliar N content was similar among the species groups and N content for all species combined, at peak biomass, was 31±8 N g m⁻². This measured foliar N content was higher than our estimates of the foliar N content in hypothetical monotypic stands, suggesting that the system’s actual community composition performed better, in terms of direct plant N uptake, than if the system had been planted with only one species group. Overall, direct plant N uptake accounted for 7% of inorganic N inputs and 19% of whole-system inorganic N removal. Our findings suggest that managers and designers should consider diverse plant communities rather than monotypic stands when designing, constructing, and managing CWT wetland systems. Future research is needed to elucidate those management strategies that might best promote or preserve diverse plant communities in these systems in a cost effective manner.

**Keywords:** Constructed treatment wetlands, macrophyte productivity, arid, nitrogen, macrophyte community composition.
INTRODUCTION

Over the last 150 years, an ever-growing portion of the world’s population lives in cities. To deal with the wastes and problems associated with dense human populations, city managers, engineers, and policy-makers have crafted “sanitary cities” predicated on the separation of human populations from potential health hazards such as human waste (per Melosi, 2000). These highly engineered urban systems are energetically and monetarily expensive to build, maintain, and expand. Environmental and fiscal concerns place additional pressure on cities and municipalities to adopt alternative, cost effective, and environmentally-based approaches to managing problems associated with urban systems, such as water scarcity in arid climates.

Constructed treatment wetlands (CTW) are effective options for the treatment of domestic wastewater (Kadlec and Wallace, 2009). Constructed treatment wetlands are wetland ecosystems created to remove various forms of pollution and excess nutrients from influent waters (Fonder and Headley, 2013). There are a wide variety of CTW designs that encompass various hydrological and ecological configurations and that are designed for a range of applications (wastewater treatment, stormwater treatment, etc.), although the majority can be categorized as surface-flow treatment wetlands (Fonder and Headley, 2013). Surface-flow CTW are usually dominated by rooted macrophytes (i.e., not floating macrophytes) and characterized by horizontal water flow. Surface-flow CTW provide effective removal of pollutants and nutrients with low management, maintenance, and operating costs due their relatively simple design (Fonder and Headley, 2013; Kadlec and Wallace, 2009).

While CTW can be utilized to remove a variety of pollutants from wastewater, the macronutrients nitrogen (N) and phosphorus (P) are of particular interest due to concerns with eutrophication in recipient waters. In the United States, local, state, and federal laws often impose limits on N and P discharge into surface waters, mandating tertiary treatment (i.e., the removal of N and P from treated wastewater) in many places. Numerous biological and physical processes (e.g., nitrification-denitrification, soil accretion, assimilation into plant or microbial biomass) make CTW particularly suited for removing N and P from wastewater. Significant academic research in mesic systems and application of this knowledge has proven CTW to be reliable and cost effective for N and P removal (Huang et al., 2000; Kadlec & Knight, 2008). In Arizona, more than 40 CTW have been constructed over the last 20 years, mostly to treat and
remove N from domestic wastewater because most surface waters in Arizona are N-limited (Grimm & Fisher, 1986). Because N is the primary concern at our Arizona study site, we focused this research on N removal and processing in this study.

Many CTW utilize emergent macrophyte vegetation to enhance nutrient removal or to provide other desirable services. Emergent macrophytes contribute to N removal through direct N assimilation into plant tissues, contributions to soil organic matter pools that fuel microbial N processing, and the regulation of other soil conditions critical to coupled nitrification-denitrification—such as oxygen availability (Faulwetter et al., 2009; Gebremarium & Beutel, 2008; Ingersoll and Baker, 1997; Reddy & Graetz, 1988). Thus, CTWs without emergent macrophytes are often less effective at N removal than those with emergent macrophytes (Brisson & Chazarenc, 2009). Plant culms within the water column also serve to reduce water velocities, increasing the opportunity time for N-processing and surface sedimentation (Brix, 1997). Thus, the use of emergent macrophytes in CTWs to provide beneficial services is becoming increasingly important to water resource managers (Thullen et al., 2005).

The effect of macrophyte community composition on nutrient removal, however, is less well understood. The interaction of community composition and nutrient removal is obscured by confounding factors (e.g., climate, wastewater type and quality, wetland design) and is generally only examined by comparing the performance of two species to each other (Brisson & Chazarenc, 2009). Nonetheless, there is reason to believe that community composition does influence CTW performance (Brisson & Chazarenc, 2009; Miller & Fujii, 2010). Different species of emergent macrophytes have varying nutrient uptake efficiencies and growth rates, suggesting differential effects on their uptake of N. Different growth rates and physical characteristics influence the quantity, quality, and timing of organic matter contributions to the soils, affecting denitrification and other microbial processes (Bachand & Horne, 1999; Bastviken et al., 2007; Gebremarium & Beutel, 2008; Hume et al., 2002). Wetland plants drive variable rates of oxygen diffusion and active oxygen transport to soils, and thus have different influences on soil characteristics critical to coupled nitrification-denitrification (Gebremarium & Beutel, 2008; Reddy & Graetz, 1988; Tanner, 1996). Understanding the role community composition plays in CTW performance will aid in improving CTW designs and management strategies while providing insight into the cost effectiveness of planting and maintaining diverse macrophyte communities in CTWs.
While the interactions among specific wetland plant species, water, and soils have been studied at the microcosm scale, fewer studies have examined them at the whole-system scale in fully operational CTWs, and those studies that have been carried out at the whole-system scale have been carried out in mesic climates where the water budget of the CTW is vastly different to that of CTWs in arid climates where evapotranspiration rates are high (Dune et al., 2013; Hernandez and Mitsch, 2007; Kadlec, 2006). The complex soil-water-plant interactions that take place at the whole-system scale in arid climates may influence the relationship between macrophyte community composition and nutrient removal in subtle ways that are not detectable in more reductionist studies or in mesic climates. There are several mechanisms by which a hot, arid climate may affect wetland function: 1) During hot summer months, extreme temperatures may potentially inhibit plant or microbial activity; 2) conversely, warm winters may promote plant growth and microbial activity; 3), different macrophyte species may be affected by a hot, arid climate in different ways; 4) high temperatures may increase decomposition rates of senesced plant material, potentially reducing the accumulation of nutrients in dead plant material (Thullen et al., 2008), and; 5) high temperatures and low vapor pressure deficits may increase evaporation and transpiration, with concomitant impacts on wetland hydrology (Ong et al., 1995; Sanchez et al., this issue). Studying the dynamics and function of CTWs in arid environments will thus build valuable knowledge for improving arid CTW management and design.

Our research used a whole-system approach to study an operational CTW where vegetation has been relatively unmanaged since planting, lending insights into how changes in plant community composition may have impacted whole-system N uptake. Studying the impact of community composition at this holistic scale provides a valuable context for extrapolating small-scale experimental findings to ecosystem-level management practices.

For this paper, our goal was to quantify the interaction between macrophyte community composition and N dynamics at the 42 ha Tres Rios CTW in Phoenix, Arizona, USA. We sought to quantify: 1) aboveground plant biomass, productivity, and community composition; 2) assimilation of N into plant tissues, and; 3) N flux into and out of the wetland. Using aboveground biomass, plant productivity, and plant N assimilation data, we developed estimates of monotypic peak biomass N assimilation for each macrophyte for species group present (i.e., the mass of N assimilated by plants if the system was planted with or managed to maintain only
that species group). We compared plant N uptake from our direct observations and these hypothetical monotypic estimates to total N flux into and out of the system to better understand the role direct plant uptake plays in system N storage and removal. Our overall objective was to better inform design and management decisions regarding the benefits and costs of planting and maintaining diverse macrophyte communities in CTWs.

METHODS

2.1 Study site

We conducted this study at the Tres Rios CTW, located several kilometers west of downtown Phoenix, Arizona, USA. Tres Rios receives partially-tertiary-treated wastewater from the City of Phoenix’s 91st Avenue Wastewater Treatment Plant, the largest in the Phoenix Metropolitan Area. Construction of the system was completed in 2010. The CTW is comprised of 5 cells: 2 non-vegetated basins (Figure 1a) and 3 vegetated surface-flow wetlands. The non-vegetated basins served to store and regulate water flow into vegetated cells. This study was carried out in vegetated Flow Cell 1 (Figure 1b) as it has been in operation the longest (planted in late 2009 and receiving water since 2010). This vegetated flow cell contains 21 ha of open water and 21 ha of vegetated marsh; the vegetated areas run along the margins of the system and extend 50-60 m into the cell. Depth within vegetated marsh and open waters areas is approximately 25 cm and 1.5 m, respectively. The vegetation was relatively unmanaged through the duration of our study. Seven native emergent macrophytes were originally planted in the system (exact proportions unknown): Schoenoplectus acutus, Schoenoplectus americanus, Schoenoplectus californicus, Schoenoplectus maritimus, Schoenoplectus tabernaemontani, Typha domingensis, and Typha latifolia.

The Tres Rios CTW is located in the arid and hot Sonoran Desert with monthly average temperatures ranging from 11.2° C in December to 33.5° C in July (National Oceanic and Atmospheric Administration, 2013). Annual precipitation averages 231 mm yr⁻¹ with most rainfall from December to March and from July to September. Precipitation amounts and timing vary widely from year to year (National Oceanic and Atmospheric Administration, 2013).
2.2 Experimental design

We used a point intercept transect method to monitor aboveground biomass and community composition (similar to Doren et al., 1997 and Childers et al., 2003). We established 10 transects (50-60 m long) perpendicular to the shoreline in the vegetated marsh (Figure 1b), with the objective of spatially representing the various vegetated units and capturing the open water-to-shoreline and inflow-outflow gradients of the whole system. Every two months, from July 2011 through September 2013, we quantified live biomass in five 0.25 m$^2$ quadrats located randomly along each transect. We measured every plant culm in these quadrats for key morphometric characteristics and converted these measurements to dry weight for each plant using phenometric biomass models. The morphometric characteristics measured and phenometric models are described in section 2.3. Plant weights were summed for each quadrat, yielding 50 independent bimonthly estimates of aboveground biomass for all species present across the system. The following equation was used to scale quadrant biomass estimates to the entire system:

\[ AG_{\text{system}} = 4 \times A \times \bar{AG}_{\text{quadrat}} \]  

where \(AG_{\text{system}}\) is the total above ground biomass across the entire system, \(A\) is the total area of the system (m$^2$), and \(\bar{AG}_{\text{quadrat}}\) is the average of all 50 aboveground biomass estimates in g(0.25m$^2$)$^{-1}$. The constant 4 scales the quadrats (0.25 m$^2$) to 1 m$^2$.

2.3 Non-destructive phenometric models

Aboveground biomass was quantified bimonthly using a non-destructive technique based on multiple regression phenometric models that related measurements of plant structure (e.g., leaf height, culm diameter at base) to dry weight biomass (e.g., Daoust & Childers, 1998; Gouraud et al., 2008; Miller & Fujii, 2010). In July 2011 and September 2011, approximately 30 individuals representing various sizes and growth stages (e.g., flowering, seeding) of the seven macrophyte species present at the study site were harvested and measured for all physical characteristics likely to contribute to the phenometric models: culm diameter at base (CDB), leaf length, stem height, and seed dimensions. The individual plants were then dried at 50° C to constant weight. We used standard least squares stepwise regression models (JMP®, Version 8.
SAS Institute Inc., Cary, NC, 1989-2007) to determine which characteristics were significant predictors of dry weight biomass for each species. Stepwise models were run until only characteristics that were significant at an alpha level of 0.01 remained. These characteristics were then used as input data to the appropriate regression equations to estimate bimonthly aboveground biomass. We used a single phenometric model for Typha latifolia and T. domingensis (hereby referred to as Typha. spp) because model parameters for these species were not different from each other. Schoenoplectus acutus, S. tabernaemontani (hereby referred to as Schoenoplectus spp.), and S. californicus individuals without seeds were similarly grouped. Typha spp., Schoenoplectus spp., and S. californicus individuals with seeds heads produced a different phenometric model from those without seed heads. We generated separate models for S. americanus and S. maritimus to represent their unique morphology.

2.4 Belowground biomass

We collected four root cores (14.5 cm in diameter and approximately 30 cm deep) for each species present in November 2011 and 2012. Belowground biomass was only sampled twice because we assumed annual estimates would reveal any change in long-term storage of N. Notably, belowground biomass is generally not the focus of plant management techniques used to improve N retention, such as biomass harvesting. Cores were rinsed, dead root material removed, and the live biomass was dried to a constant weight at 50° C and weighed. When we collected root cores, all corresponding aboveground biomass was also harvested, dried, and weighed. We used these data to generate aboveground:belowground biomass ratios, and used these ratios to estimate whole-system belowground biomass. One-way ANOVA tests were run (JMP Pro 8) to determine statistical differences in aboveground:belowground biomass ratios among species. System-wide belowground biomass for the entire system was calculated as:

$$\frac{BG}{AG}(AG_{system}) = BG_{system}$$  \hspace{1cm} Equation 2

Where $$\frac{BG}{AG}$$ was the belowground:aboveground biomass ratio and $$AG_{system}$$ was system-wide aboveground biomass for each species.

2.5 Plant tissue nutrients
To determine foliar N content, we collected live aboveground biomass samples from leaves and stems at various times during the year. Belowground samples were collected in November of 2011 and 2012. Samples were dried at 50° C, ground in a Wiley Mill, then ball-milled. Milled samples were analyzed for C and N content using a Perkin Elmer 2400 CHN Analyzer. One-way ANOVA tests were run (JMP Pro 8) to determine statistical differences among species.

2.6 Plant productivity and N uptake

We estimated the N content of each plant species by multiplying peak aboveground biomass \( (PAG_{spp}) \) by the corresponding aboveground foliar N content \( (N_{spp}) \) as a percentage of dry weight:

\[
N_{content} = PAG_{spp} \times N_{spp} \tag{3}
\]

Because plants of different species often occupied the same quadrats, we summed, rather than averaged, these species-specific \( N_{content} \) estimates to estimate whole-system N content.

Hypothetical estimates of monotypic N content were calculated by averaging all observations of aboveground biomass from quadrats in which only one species group was present for each species \( (PAG_{mono}) \) during the months just before, during, and just after peak biomass (May, July, and September) of 2011, 2012, and 2013. These averages were then multiplied by average N content \( (N_{spp}) \) for that species group to estimate total monotypic N.

Monotypic N calculations were summarized as:

\[
N_{mono} = PAG_{mono} \times N_{spp} \tag{4}
\]

Note that our species-specific, whole-system, and hypothetical \( N_{mono} \) estimates were based on peak aboveground biomass and not net aboveground primary production (NAPP).

We utilized the Maximum Biomass method to calculate NAPP, where NAPP is equal to the difference between minimum and maximum biomass (per Morris and Haskin, 1990), and thus assumed biomass turnover and herbivory were negligible. Because the Maximum Biomass method has been shown to underestimate net primary productivity of wetlands plants (Cronk and
Fennessy, 2001), our estimates of plant N uptake are conservative. Total plant N uptake was calculated by multiplying average foliar N content by NAPP for each species present:

\[
Plant \, Uptake = NAPP \times N_{spp} \quad \text{Equation 5}
\]

2.7 Water quality and flow

Triplicate surface-water grab samples were collected bimonthly at the inflow and outflow of the system using acid-washed 1 L Nalgene bottles. Samples were chilled on ice until they were returned to the lab for processing and analysis. Unfiltered samples were centrifuged at 8,000 RPM for 10 minutes and analyzed for inorganic N (nitrate, NO$_3^-$; nitrite, NO$_2^-$, and ammonium, NH$_4^+$) via flow injection analysis on a Lachat QC 8000 Quickchem Flow Injection Analyzer (method detection limits were 0.85 µg NO$_3^-/NO_2^-$ N L$^{-1}$ and 3.01 µg NH$_4^+$ N L$^{-1}$). We selected inorganic N to represent nitrogen dynamics within the system because these are the forms taken up by macrophytes. Concentration data were multiplied by total monthly water flows to determine inorganic N fluxes. This research was carried out in partnership with the City of Phoenix Water Department, who provided daily inflow and outflow data, measured by acoustic Doppler flow meters, from January 2012 – August 2013—with a few exceptions (e.g., the flow data from March, April, and May of 2012 may not be reliable due to metering problems). Flow data were unavailable prior to January 2012. We calculated inorganic N uptake and processing by the system as the difference between the inorganic N in surface water influx and efflux plus estimates of atmospheric N deposition. We used a conservative estimate of 12 kg N ha$^{-1}$ yr$^{-1}$ for dry + wet N deposition of NH$_4^+$ + NO$_3^-$ for the Phoenix Metro area (Lohse et al., 2008). The following equations were used in our N budget calculations:

\[
N_{influx} = Influx + Deposition_{dry+wet} \quad \text{Equation 6}
\]

\[
N_{uptake} = N_{influx} - Efflux \quad \text{Equation 7}
\]

where \textit{Influx} was the mass of inorganic N, as NH$_4^+$ + NO$_3^-$, entering the system via system water inputs, \textit{Deposition}_{dry+wet} was dry and wet deposition from Lohse et al. (2008), and \textit{Efflux} was the mass of inorganic N leaving the system via effluent waters.
RESULTS AND DISCUSSION

To examine the impact of community composition on N processing in an aridland CTW, we first generated phenometric models relating structural plant characteristics to dry weight. These models were used to estimate macrophyte biomass and productivity. We then compared macrophyte N content and water quality to approximate overall system performance (i.e., N concentration reductions). Finally, we examined macrophyte productivity, community composition, and macrophyte N content to estimate plant N uptake. These plant N uptake estimates were compared to estimates of whole-system N removal to understand the role played by plants in whole-system N dynamics.

3.2 Phenometric models

Our phenometric models predicted dry weight reasonably well: Adjusted R-squared values ranged from 0.73 – 0.90 across the species groups (Table 1). Stem volume of *Schoenoplectus* spp. and *S. californicus*, which was assumed to be conical, and stem height were significant parameters both with and without seeds, though parameter estimates differed between these species depending on whether or not seeds were present. Curiously, parameter estimates for leaf count and the length of the longest leaf were negative in *Typha* spp. without flowers. Culm diameter at base (CDB), stem height, and pistillate length and width were significant parameters for *Typha* spp. with flowers. Culm diameter at base and stem height were significant characteristics for *S. americanus. Schoenoplectus maritimus* dry weight was predicted by CDB, stem height, and seed head count.

3.3 Aboveground biomass and community composition

Average aboveground peak total biomass declined steadily from 2666±164 (SE) gdw m$^{-2}$ in July 2011 to 2123±182 (SE) gdw m$^{-2}$ in July 2012 to 1586±179 (SE) gdw m$^{-2}$ in July 2013 (Figure 2). System-wide, these estimates equated to 561 Mg dw, 447 Mg dw, and 334 Mg dw of peak aboveground biomass, respectively, for the 21 ha marsh. Minimum aboveground biomass was 265±50 (SE) gdw m$^{-2}$ and 136±31 (SE) gdw m$^{-2}$ in March of 2012 and 2013, respectively. Our maximum aboveground biomass values were comparable to observations from other natural...
and constructed treatment wetlands that ranged from 790-2200 gdw m\(^2\), (Kadlec & Knight, 2008; Miller & Fujii, 2010; Tanner, 2001; van der Valk & Davis, 1978).

‘Thatching,’ which is the phenomenon where large stands of macrophytes topple over, was likely responsible for part of the decline in biomass from 2011 to 2013. Large areas of wetland were covered with a deep (>0.5 m) mat of senesced vegetation after the first thatching event between July and September 2011. This thick mat of wrack likely inhibited the growth of new shoots. Managing aboveground biomass through harvesting near peak summer biomass could potentially prevent thatching and thus promote biomass growth in the following year. This would also allow managers to remove the maximum amount of N bound in aboveground plant tissues, where it could be composted and used in various urban applications. However, the process of aboveground biomass removal could result in disturbance to soil microbial communities, adversely affecting microbial N processing. In arid climates that have warm autumn temperatures, harvesting at peak biomass might also encourage the growth of a second crop in fall. Thatching also occurred in late Summer 2012 and 2013, although to a lesser extent because of the lower peak aboveground biomass in these two years.

*Typha* spp. accounted for 70.5% of peak biomass across July 2011, 2012, and 2013 (Figure 2). The thatching phenomenon mentioned above generally took place in areas were *Typha* spp. was dominant, and this resulted in the sharp decline of *Typha* spp. aboveground biomass between July and September, particularly in 2011. Aboveground biomass of *Schoenoplectus* spp. was approximately one third that of *Typha* spp. at peak biomass while aboveground biomass of *S. americanus* and *S. californicus* was orders of magnitude lower than *Typha* spp. *Schoenoplectus maritimus* was not observed after July 2011, likely because it was outcompeted by other species. We thus excluded *S. maritimus* from most analyses. *Typha* spp. steadily dominated total biomass during our sampling (Figure 2), suggesting an ongoing community compositional shift to what may ultimately be a *Typha* spp. dominated system. We discuss the ramifications of this shift for plant N uptake in Section 3.6.

### 3.4 Belowground biomass
Belowground biomass averaged 1056±233 (SE) and 815±163 (SE) g dw m\(^{-2}\) for all species in November of 2011 and 2012, respectively (Table 2). Our observations were considerably lower than those reported for *T. latifolia* (2900 g dw m\(^{-2}\)) by Kadlec and Wallace (2009) but comparable to estimates for *S. tabernaemontani* from Tanner (1996; 2001). In mixed stands of *Typha* spp. and *S. acutus*, Miller & Fujii (2010) reported belowground biomass values between 1000-1800 g dw m\(^{-2}\), higher than our mixed-stand estimates (Table 2). Our estimates translated to a total system-wide belowground biomass of approximately 222 Mg dw in 2011 and 171 Mg dw in 2012. Aboveground:belowground biomass ratios were not significantly different among species within each year; however, ratios in *Typha* spp. and *Schoenoplectus* spp. varied considerably from 2011 to 2012 (Table 2). *Typha* spp. accounted for the largest portion of belowground biomass.

### 3.5 Macrophyte N content

Aboveground tissue N content averaged 1.64±0.11 (SE) %N for all species, with no significant difference among the species groups (Table 3). Belowground tissue N content was also not significantly different among species and averaged slightly lower (1.29±0.08% SE). Our observations were consistent with those reported in the literature for *Typha* spp. (Pratt et al., 1980; Kadlec and Wallace, 2009) and *S. tabernaemontani* (Tanner, 2001).

Nitrogen content per unit area of wetland at peak biomass provided further insights into the role of each species in whole-system N uptake. *Typha* spp. had the highest N content (21.5±4.1 (SE) N g m\(^{-2}\)), accounting for more than two-thirds of the whole-system plant N (Table 3). The N content of *Schoenoplectus* spp., *S. californicus*, and *S. americanus* was 5.3±2.6 (SE) g m\(^{-1}\), 0.7±0.5 (SE) g m\(^{-1}\), and 2.9±2.1 (SE) g m\(^{-2}\), respectively. The average system-wide N content (i.e., the average sum of all species’ N contents) at peak biomass was 30.5±8.3 (SE) N g m\(^{-2}\) from 2011-2013. This level of plant N was higher than the N content of 24.3 g m\(^{-2}\) reported by Kadlec & Knight (2008) for a mesic, *Typha* spp. wetland receiving water of similar quality to Tres Rios, but it was lower than the N content of approximately 40 g m\(^{-2}\) reported by Tanner (1996) from a mesic mesocosm experiment using *S. tabernaemontani* and influent water with substantially higher N concentrations (TN ~100 mg L\(^{-1}\)) than at Tres Rios. The wide variation of
macrophyte N content reported for various species by Kadlec and Wallace (2009), Tanner (1996), and our study could be due to differences in wastewater type, climate, and research approach.

3.6 Hypothetical monotypic N content

Based on our hypothetical estimates, the mixed stands of vegetation at our study site performed better than scenarios in which the site was planted with only one species, or was dominated by only one species. The observed system-wide N content (30.5±8.3 (SE) g m⁻²) outperformed hypothetical monotypic stands of *S. americanus* (17.9±3.3 (SE) g m⁻²), *Schoenoplectus* spp. (11.0±2.0 (SE) g m⁻²), and *Typha* spp. (22.2±2.3 (SE) g m⁻²; Table 3). We had only one sampling quadrat where the only plant species was *S. californicus*, and thus excluded it from our hypothetical monotypic N estimates. Our peak monotypic biomass N values are lower than those reported for single-species stands in other studies (Hunt et al., 2002; Kadlec & Knight, 2008; Tanner, 1996; Tanner, 2001) but many of these studies—with the exception of Kadlec & Knight (2008)—received considerably more N loading than our system. Surprisingly, our estimates of monotypic N content and actual observed N content for *Typha* spp. are very similar, which implied that *Typha* spp. performed as well in the current six-species community as it would if the entire wetland was *Typha* spp. While *Typha* spp. accounted for 70.5% of peak biomass, the remaining biomass of the other species appeared to considerably increase system-wide macrophyte N content. Based on our estimates, we argue that CTWs should be designed and managed for several emergent macrophyte species rather than for a single species, even if that single species is *Typha* spp.

Our data do not allow us to speculate on management strategies that would promote or preserve more diverse macrophyte communities in established CTW systems. In existing monotypic systems, the removal of vegetation and planting of other species could prove costly and would likely require temporarily halting CTW operations. Maintaining existing diverse plant communities in established systems may also prove difficult when one species begins to dominate the system, as we observed at Tres Rios with *Typha* spp. In theory, different species might respond differently to common management techniques, such as burning, harvesting, or...
periodic drying of CTW marshes. However, little research exists on the use of these techniques for managing community composition.

Planting diverse macrophyte communities in new CTW systems is likely much easier than bringing diverse macrophyte communities into existing systems. In new CTW systems, planting diverse communities would likely cost no more than planting monotypic marshes because the costs of planting are more driven by plant density than by the species or number of species planted (Environmental Protection Agency, 1999). Given this, we argue that planting more than one species is a simple way to improve nutrient retention in new CTW systems.

System-specific variables, such as climate, wastewater type, and other services desired of CTW, should be considered when determining what plant species should be used in new systems. The planting design across a CTW might influence the ability of one species to take over a system and should also be considered. At Tres Rios, macrophyte species were dispersed amongst each other with the exception of S. americanus, which grew in dense monotypic stands. This highly mixed arrangement may have contributed to the trend towards Typha spp. domination that we observed because the Typha spp. plants grew taller than most other species and likely shaded out individuals of other species. Dividing vegetated areas into species group zones could deter or at least slow the encroachment of one species across the entire system. Another approach might be single species management through harvesting or other means. However, the separation of species or species groups in zones might negate the benefits of macrophyte diversity.

3.7 Water quality

Water quality measurements lend insight into whole-system N retention and are the basis for our N budget calculations. We restricted our analysis to NO$_3^-$ and NH$_4^+$ as these species of N are most readily taken up by plants. Nitrite was a relatively small portion of inflow and outflow N (<0.30 mgL$^{-1}$), and is thus not presented. Inflow concentrations of NO$_3^-$ ranged from 1.5 – 7.5 mg L$^{-1}$ while outflow ranged from 1.2 – 6.8 mg L$^{-1}$ (Figure 3). Ammonium concentrations ranged from 0.65 – 1.9 mg L$^{-1}$ at the inflow and 0.56 – 1.5 mg L$^{-1}$ at the outflow (Figure 3). Nitrate concentration was, on average, 15% lower (0.59 mg L$^{-1}$) in the outflow water compared with the inflow while NH$_4^+$ concentrations were 51% lower (0.63 mg L$^{-1}$). With the exception of January 2012 and March 2013, NO$_3^-$ concentrations were consistently lower in the outflow; NH$_4^+$ was always lower in the outflow. Because N flux accounts for both concentration and
water flow, it may be a better measure of system effectiveness. However, effluent concentrations are important for estimating downstream environmental impact, and most CTW water quality regulations and management are based on outflow water quality. Sanchez et al. (this issue) provide further insight to N concentrations and flux in the Tres Rios CTW.

3.8 Whole-System nitrogen flux

Water inflow data were unavailable prior to January 2012, so we calculated a system-wide N budget from January 2012 – August 2013. From January 2012 to August 2013, the system received a total of 147.4±4.1 (SE) Mg NO$_3^-$+NH$_4^+$, which equated to a loading rate of 0.61±0.02 (SE) g m$^{-2}$ d$^{-1}$ or 6.1± 0.2 (SE) kg ha$^{-1}$ d$^{-1}$ (Table 4). During this period, the system removed 52.3±4.2 (SE) Mg N (equivalent to 0.22±0.02 (SE) gN m$^{-2}$ d$^{-1}$), or 35% of the inorganic N load. These results showed that the system was performing an important nutrient removal service. Our N removal rates were comparable to NH$_4^+$ and NO$_3^-$ removal rates by an arid CTW in Southern California studied by Sartoris et al. (2000). We estimated that plant N uptake totaled 10.0±0.8 Mg. This plant uptake equated to 7% of inorganic N loading and 19% of system-wide inorganic N uptake. Notably, during the growing season (March – September), plants in the Tres Rios CTW accounted for a relatively large portion of whole-system N removal (33-51%). Our observations fell well within previously reported plant N uptake estimates of 2-18% of inflow N for mesic CTW systems (Faulkner & Richardson, 1990; Kadlec & Knight, 2008; Meers et al., 2008). *Typha* spp. accounted for 70% of all plant N uptake, *Schoenoplectus* spp. 18%, *S. americanus* 10%, and *S. californicus* 2%. Our estimates did not account for the remineralization of foliar N during the decomposition of senesced plant biomass. Decomposition rates for these species may differ, which would also suggest that community composition is important for CTW performance.

The portion of whole-system N sequestration that we were able to account for by quantifying direct plant uptake was smaller than we expected. As is typical in many CTW, coupled nitrification-denitrification likely accounts for the majority of N processing and removal.
at Tres Rios, as has been shown in similar aridland wetlands (Kadlec, 2008). Specific plant species do have an impact on coupled nitrification-denitrification through the active transport of oxygen to soils and through contributions to labile organic matter pools in soils. Hume et al. (2002) found that *Typha* spp. litter contained less lignin and had lower C:N ratios than *Schoenoplectus* ssp., suggesting that organic matter contributed by *Typha* spp. may be more available for denitrifying bacteria. Gebremarium & Beutel (2008) found that *Typha* ssp. plants transported less oxygen to soils than *Schoenoplectus* ssp., leading to higher rates of denitrification in *Typha* spp. plots (notably, nitrification was not examined and this is the process that requires oxygen). As our research in the Tres Rios CTW continues we will expand our whole-system N budget by including measurements of nitrification-denitrification and trace gas fluxes, as well as experiments on the effects of various drying-wetting regimes on soil N processing. Ultimately this information will help inform best management practices of this important urban CTW.

**CONCLUSIONS**

Our objectives were to quantify macrophyte biomass and productivity, plant N content and uptake, and whole-system N removal in order to understand how community composition influenced N removal in an aridland CTW. Based on our findings, macrophyte community composition did affect N processing at Tres Rios. Our estimates of system-wide plant N content were substantially higher than our hypothetical monotypic estimates, demonstrating that the six-species community outperformed hypothetical monotypic systems. *Typha* spp. accounted for the largest portion of aboveground biomass and plant N uptake but the other species present did contribute to N uptake. Overall, direct plant N uptake accounted for a small but not inconsequential portion of system-wide N removal; this contribution was considerably higher during the summer growing season.

Based on our findings, we argue that planting a diverse macrophyte community in new CTW systems may lead to increased whole-system N uptake without significant costs (Environmental Protection Agency, 1999). However, strategies to manage existing CTWs in order to promote or preserve diverse macrophyte communities need to be better investigated. Our study adds to a growing body of literature examining the role of community composition and CTW performance but further study in full-scale CTW systems is clearly necessary to help
inform more effective management strategies. While our study examined only one pathway for N processing in CTW marshes (direct plant uptake), further investigation of N processing at Tres Rios is underway. This further investigation includes quantifying coupled nitrification-denitrification, trace gas flux, and plant decomposition. These projects will shed further light on the role of macrophyte community composition in CTW N processing.
ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1. Aerial images of the Tres Rios constructed treatment wetlands. (A) Red letters denote non-vegetated basins that distribute water to vegetated basins and red numbers denote vegetated free water surface cells. (B) Cell number 1 from (A) that was used in this study. Red lines denote the locations of the 10 wetland monitoring transects. Inflow and outflow are marked with blue arrows indicating the direction of flow.

Figure 2. Total aboveground biomass and aboveground biomass by species.

Figure 3. Nitrate and ammonium concentrations at system inflow and outflow.
Figure 1a.
Figure 1b.

Approx. 150 m
Figure 2.
Figure 3.
Table 1. Stepwise multiple regression phenometric models developed for each species and subcategory. Estimates for each significant parameter are given in parenthesis. CDB is culm diameter measured at the soil surface. Volume was calculated from CDB and stem height, assuming that culms were conical. All length, width, and diameter parameters were measured in centimeters.

Table 2. Above-to-belowground biomass ratios and estimates of belowground biomass.

Table 3. Nitrogen content in plant tissues and estimates of monotypic (i.e., a system planted with only one species) nitrogen content for the 4 species groups.

Table 4. Whole-system surface water N budget for January 2012 – August 2013 and for the 2012 and 2013 growing seasons. The difference between inflow and retained $\text{NO}_3^- + \text{NH}_4^+$ is outflow $\text{NO}_3^- + \text{NH}_4^+$. 
### Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Category</th>
<th>Significant parameters &amp; regression coefficients</th>
<th>Adjusted R-squared and p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Typha spp.</em></td>
<td>49</td>
<td>w/ flower</td>
<td>CDB (7.976) Stem height (0.2021) Pistillate length (0.0804) Pistillate diameter (6.651)</td>
<td>0.75, p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>w/o flower</td>
<td>Sum of all leaf lengths (0.0667) Leaf count (-2.059) Longest leaf (-0.1640)</td>
<td>0.88, p&lt;0.0001</td>
</tr>
<tr>
<td><em>S. americanus</em></td>
<td>60</td>
<td>all</td>
<td>CDB (1.5821) Stem height (0.0106)</td>
<td>0.76, p&lt;0.0001</td>
</tr>
<tr>
<td><em>S. acutus, S. californicus, S. tabernaemontani</em></td>
<td>31</td>
<td>w/o seeds</td>
<td>Stem height (0.0369) Volume (0.0226)</td>
<td>0.73, p&lt;0.0001</td>
</tr>
<tr>
<td><em>S. acutus</em></td>
<td>35</td>
<td>w/ seeds</td>
<td>Stem height (0.0264) Volume (0.036)</td>
<td>0.90, p&lt;0.0001</td>
</tr>
<tr>
<td><em>S. californicus</em></td>
<td>22</td>
<td>w/ seeds</td>
<td>Stem height (0.0237) Volume (0.0576)</td>
<td>0.84, p&lt;0.0001</td>
</tr>
<tr>
<td><em>S. maritimus</em></td>
<td>33</td>
<td>all</td>
<td>CDB (1.001) Stem height (0.0162) Seed head count (0.107)</td>
<td>0.80, p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>2012</td>
<td></td>
<td></td>
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<tr>
<td>----------------</td>
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<tr>
<td></td>
<td>Above:below ratio</td>
<td>Above:below ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n)</td>
<td>(n)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.85±0.13</td>
<td>0.59±0.09</td>
<td>0.80±0.06</td>
<td>1.00±0.31</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(3)</td>
<td>(2)</td>
<td>(4)</td>
</tr>
<tr>
<td>Belowground biomass (g dw m$^{-2}$)</td>
<td>207±54</td>
<td>186±80</td>
<td>99±50</td>
<td>564±206</td>
</tr>
<tr>
<td></td>
<td>0.36±0.02</td>
<td>0.62±0.08</td>
<td>0.80±0.20</td>
<td>1.71±0.42</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(6)</td>
</tr>
<tr>
<td>Belowground biomass (g dw m$^{-2}$)</td>
<td>248±79</td>
<td>99±42</td>
<td>40±23</td>
<td>428±134</td>
</tr>
</tbody>
</table>
Table 3.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Belowground N content % (n)</th>
<th>Aboveground Foliar N content % (n)</th>
<th>Observed N content (g m⁻²)</th>
<th>Monotypic N content estimate (g m⁻²) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Schoenoplectus</em> spp.</td>
<td>1.316±0.145 (15)</td>
<td>1.653±0.141 (11)</td>
<td>5.3±2.6</td>
<td>11.0±2.0 (24)</td>
</tr>
<tr>
<td><em>S. americanus</em></td>
<td>1.230±0.095 (9)</td>
<td>2.014±0.302 (6)</td>
<td>2.9±2.1</td>
<td>17.9±3.3 (16)</td>
</tr>
<tr>
<td><em>S. californicus</em></td>
<td>0.974±0.036 (3)</td>
<td>1.352±0.230 (3)</td>
<td>0.7±0.5</td>
<td>-</td>
</tr>
<tr>
<td><em>Typha</em> spp.</td>
<td>1.402±0.152 (11)</td>
<td>1.341±0.185 (5)</td>
<td>21.5±4.1</td>
<td>22.2±2.3 (123)</td>
</tr>
<tr>
<td>All species</td>
<td>1.293±0.076 (38)</td>
<td>1.641±0.111 (25)</td>
<td>30.5±8.3</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 4.

<table>
<thead>
<tr>
<th></th>
<th>January 2012 - August 2013&lt;sup&gt;2&lt;/sup&gt;</th>
<th>2012 growing season (Mar-July)</th>
<th>2013 growing season (Mar-July)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NO\textsubscript{3}\textsuperscript{-} + NH\textsubscript{4}\textsuperscript{+} Inflow + Deposition (Mg)</strong></td>
<td>147.4±4.1</td>
<td>33.0±0.3</td>
<td>44.1±1.0</td>
</tr>
<tr>
<td><strong>NO\textsubscript{3}\textsuperscript{-} + NH\textsubscript{4}\textsuperscript{+} retained&lt;sup&gt;1&lt;/sup&gt; (Mg)</strong></td>
<td>52.3±4.2 (35%)</td>
<td>17.9±0.5 (54%)</td>
<td>8.4±1.3 (19%)</td>
</tr>
<tr>
<td><strong>Net Aboveground Primary Productivity (Mg)</strong></td>
<td>-</td>
<td>395</td>
<td>305</td>
</tr>
<tr>
<td><strong>Total N retained by plant growth (Mg)</strong></td>
<td>10.0±0.8 (7%)</td>
<td>5.8±0.6 (9%)</td>
<td>4.2±0.5 (5%)</td>
</tr>
<tr>
<td><strong>Plant uptake as a percentage of whole-system N Uptake</strong></td>
<td>19%</td>
<td>33%</td>
<td>51%</td>
</tr>
</tbody>
</table>

<sup>1</sup>Percentage represents proportion of inflow N retained

<sup>2</sup>Excludes December 2012 as inflow data was not available