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

3 **Authors:** Nicholas A Weller¹; Daniel L. Childers^{1,4}; Laura Turnbull²; Robert F. Upham³

4 ¹School of Sustainability, Arizona State University, Tempe, AZ, 85287

5 ²Department of Geography, University of Durham, UK

6 ³Water Services Department, City of Phoenix, Phoenix AZ

7 ⁴Corresponding Author

8 **ABSTRACT**

9 Urbanized areas increasingly rely on constructed treatment wetlands (CTW) for cost
10 effective and environmentally-based wastewater treatment. Constructed treatment wetlands are
11 particularly attractive treatment options in arid urban environments where water reuse is
12 important for dealing with scarce water resources. Emergent macrophytes play an important role
13 in nutrient removal, particularly nitrogen (N) removal, in CTW. However, the role of plant
14 community composition in nutrient removal is less clear. Numerous studies have shown that
15 macrophyte species differentially affect N uptake processes (e.g.: direct plant uptake, coupled
16 nitrification-denitrification, soil accretion). However, many of these studies have been based on
17 small-scale experiments and have been carried out in mesic environments, which means that
18 their findings are difficult to extrapolate to aridland CTW systems. Our study sought to examine
19 the relationships among emergent macrophyte productivity, plant community composition, and
20 N uptake [by both the plants and the entire ecosystem] at a 42 ha CTW in arid Phoenix, Arizona,
21 USA. We quantified above- and belowground biomass bimonthly and foliar N content annually
22 for four species groups (*Typha latifolia* + *T. domingensis*., *Schoenoplectus californicus* + *S.*
23 *tabernaemontani*, *Schoenoplectus acutus*, and *Schoenoplectus americanus*) from July 2011 to
24 September 2013. We quantified dissolved inorganic N fluxes into and out of the system and
25 compared plant N removal to total system fluxes. Additionally, we estimated monotypic N
26 content for each to compare the system's current community composition and plant N removal to
27 hypothetical scenarios in which the system was dominated by only one species.

28 Peak aboveground biomass ranged from 1586 ± 179 (SE) to 2666 ± 164 (SE) gdw m⁻² of
29 which *Typha spp.* accounted for an increasing portion (>66%). We observed widespread
30 ‘thatching’—the toppling of large stands of macrophytes—that was likely related to a decline in
31 peak biomass from July 2011 to July 2012. The foliar N content was similar among the species
32 groups and N content for all species combined, at peak biomass, was 31 ± 8 N g m⁻². This
33 measured foliar N content was higher than our estimates of the foliar N content in hypothetical
34 monotypic stands, suggesting that the system’s actual community composition performed better,
35 in terms of direct plant N uptake, than if the system had been planted with only one species
36 group. Overall, direct plant N uptake accounted for 7% of inorganic N inputs and 19% of whole-
37 system inorganic N removal. Our findings suggest that managers and designers should consider
38 diverse plant communities rather than monotypic stands when designing, constructing, and
39 managing CWT wetland systems. Future research is needed to elucidate those management
40 strategies that might best promote or preserve diverse plant communities in these systems in a
41 cost effective manner.

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

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INTRODUCTION

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

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

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

84 remove N from domestic wastewater because most surface waters in Arizona are N-limited
85 (Grimm & Fisher, 1986). Because N is the primary concern at our Arizona study site, we
86 focused this research on N removal and processing in this study.

87 Many CTW utilize emergent macrophyte vegetation to enhance nutrient removal or to
88 provide other desirable services. Emergent macrophytes contribute to N removal through direct
89 N assimilation into plant tissues, contributions to soil organic matter pools that fuel microbial N
90 processing, and the regulation of other soil conditions critical to coupled nitrification-
91 denitrification—such as oxygen availability (Faulwetter et al., 2009; Gebremariam & Beutel,
92 2008; Ingersoll and Baker, 1997; Reddy & Graetz, 1988). Thus, CTWs without emergent
93 macrophytes are often less effective at N removal than those with emergent macrophytes
94 (Brisson & Chazarenc, 2009). Plant culms within the water column also serve to reduce water
95 velocities, increasing the opportunity time for N-processing and surface sedimentation (Brix,
96 1997). Thus, the use of emergent macrophytes in CTWs to provide beneficial services is
97 becoming increasingly important to water resource managers (Thullen et al., 2005).

98 The effect of macrophyte community composition on nutrient removal, however, is less well
99 understood. The interaction of community composition and nutrient removal is obscured by
100 confounding factors (e.g., climate, wastewater type and quality, wetland design) and is generally
101 only examined by comparing the performance of two species to each other (Brisson & Chazarenc,
102 2009). Nonetheless, there is reason to believe that community composition does influence CTW
103 performance (Brisson & Chazarenc, 2009; Miller & Fujii, 2010). Different species of emergent
104 macrophytes have varying nutrient uptake efficiencies and growth rates, suggesting differential
105 effects on their uptake of N. Different growth rates and physical characteristics influence the
106 quantity, quality, and timing of organic matter contributions to the soils, affecting denitrification
107 and other microbial processes (Bachand & Horne, 1999; Bastviken et al., 2007; Gebremariam &
108 Beutel, 2008; Hume et al., 2002). Wetland plants drive variable rates of oxygen diffusion and
109 active oxygen transport to soils, and thus have different influences on soil characteristics critical
110 to coupled nitrification-denitrification (Gebremariam & Beutel, 2008; Reddy & Graetz, 1988;
111 Tanner, 1996). Understanding the role community composition plays in CTW performance will
112 aid in improving CTW designs and management strategies while providing insight into the cost
113 effectiveness of planting and maintaining diverse macrophyte communities in CTWs.

114 While the interactions among specific wetland plant species, water, and soils have been
115 studied at the microcosm scale, fewer studies have examined them at the whole-system scale in
116 fully operational CTWs, and those studies that have been carried out at the whole-system scale
117 have been carried out in mesic climates where the water budget of the CTW is vastly different to
118 that of CTWs in arid climates where evapotranspiration rates are high (Dune et al., 2013;
119 Hernandez and Mitsch, 2007; Kadlec, 2006). The complex soil-water-plant interactions that take
120 place at the whole-system scale in arid climates may influence the relationship between
121 macrophyte community composition and nutrient removal in subtle ways that are not detectable
122 in more reductionist studies or in mesic climates. There are several mechanisms by which a hot,
123 arid climate may affect wetland function: 1) During hot summer months, extreme temperatures
124 may potentially inhibit plant or microbial activity; 2) conversely, warm winters may promote
125 plant growth and microbial activity; 3), different macrophyte species may be affected by a hot,
126 arid climate in different ways; 4) high temperatures may increase decomposition rates of
127 senesced plant material, potentially reducing the accumulation of nutrients in dead plant material
128 (Thullen et al., 2008), and; 5) high temperatures and low vapor pressure deficits may increase
129 evaporation and transpiration, with concomitant impacts on wetland hydrology (Ong et al., 1995;
130 Sanchez et al., this issue). Studying the dynamics and function of CTWs in arid environments
131 will thus build valuable knowledge for improving arid CTW management and design.

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

137 For this paper, our goal was to quantify the interaction between macrophyte community
138 composition and N dynamics at the 42 ha Tres Rios CTW in Phoenix, Arizona, USA. We sought
139 to quantify: 1) aboveground plant biomass, productivity, and community composition; 2)
140 assimilation of N into plant tissues, and; 3) N flux into and out of the wetland. Using
141 aboveground biomass, plant productivity, and plant N assimilation data, we developed estimates
142 of monotypic peak biomass N assimilation for each macrophyte for species group present (i.e.,
143 the mass of N assimilated by plants if the system was planted with or managed to maintain only

144 that species group). We compared plant N uptake from our direct observations and these
145 hypothetical monotypic estimates to total N flux into and out of the system to better understand
146 the role direct plant uptake plays in system N storage and removal. Our overall objective was to
147 better inform design and management decisions regarding the benefits and costs of planting and
148 maintaining diverse macrophyte communities in CTWs.

149 METHODS

150 2.1 Study site

151 We conducted this study at the Tres Rios CTW, located several kilometers west of downtown
152 Phoenix, Arizona, USA. Tres Rios receives partially-tertiary-treated wastewater from the City of
153 Phoenix's 91st Avenue Wastewater Treatment Plant, the largest in the Phoenix Metropolitan
154 Area. Construction of the system was completed in 2010. The CTW is comprised of 5 cells: 2
155 non-vegetated basins (Figure 1a) and 3 vegetated surface-flow wetlands. The non-vegetated
156 basins served to store and regulate water flow into vegetated cells. This study was carried out in
157 vegetated Flow Cell 1 (Figure 1b) as it has been in operation the longest (planted in late 2009
158 and receiving water since 2010). This vegetated flow cell contains 21 ha of open water and 21 ha
159 of vegetated marsh; the vegetated areas run along the margins of the system and extend 50-60 m
160 into the cell. Depth within vegetated marsh and open waters areas is approximately 25 cm and
161 1.5 m, respectively. The vegetation was relatively unmanaged through the duration of our study.
162 Seven native emergent macrophytes were originally planted in the system (exact proportions
163 unknown): *Schoenoplectus acutus*, *Schoenoplectus americanus*, *Schoenoplectus californicus*,
164 *Schoenoplectus maritimus*, *Schoenoplectus tabernaemontani*, *Typha domingensis*, and *Typha*
165 *latifolia*.

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

171 INSERT FIGURE 1 HERE

172 2.2 Experimental design

173 We used a point intercept transect method to monitor aboveground biomass and
 174 community composition (similar to Doren et al., 1997 and Childers et al., 2003). We established
 175 10 transects (50-60 m long) perpendicular to the shoreline in the vegetated marsh (Figure 1b),
 176 with the objective of spatially representing the various vegetated units and capturing the open
 177 water-to-shoreline and inflow-outflow gradients of the whole system. Every two months, from
 178 July 2011 through September 2013, we quantified live biomass in five 0.25 m² quadrats located
 179 randomly along each transect. We measured every plant culm in these quadrats for key
 180 morphometric characteristics and converted these measurements to dry weight for each plant
 181 using phenometric biomass models. The morphometric characteristics measured and
 182 phenometric models are described in section 2.3. Plant weights were summed for each quadrat,
 183 yielding 50 independent bimonthly estimates of aboveground biomass for all species present
 184 across the system. The following equation was used to scale quadrat biomass estimates to the
 185 entire system:

$$AG_{system} = 4 * A * \overline{AG}_{quadrat} \quad 186 \quad \text{Equation 1}$$

187 where AG_{system} is the total above ground biomass across the entire system, A is the total area of
 188 the system (m²), and $\overline{AG}_{quadrat}$ is the average of all 50 aboveground biomass estimates in
 189 g(0.25m²)⁻¹. The constant 4 scales the quadrats (0.25 m²) to 1 m².

190 2.3 Non-destructive phenometric models

191 Aboveground biomass was quantified bimonthly using a non-destructive technique based
 192 on multiple regression phenometric models that related measurements of plant structure (e.g.,
 193 leaf height, culm diameter at base) to dry weight biomass (e.g., Daoust & Childers, 1998;
 194 Gouraud et al., 2008; Miller & Fujii, 2010). In July 2011 and September 2011, approximately 30
 195 individuals representing various sizes and growth stages (e.g., flowering, seeding) of the seven
 196 macrophyte species present at the study site were harvested and measured for all physical
 197 characteristics likely to contribute to the phenometric models: culm diameter at base (CDB), leaf
 198 length, stem height, and seed dimensions. The individual plants were then dried at 50° C to
 199 constant weight. We used standard least squares stepwise regression models (JMP[®], Version 8.

200 SAS Institute Inc., Cary, NC, 1989-2007) to determine which characteristics were significant
 201 predictors of dry weight biomass for each species. Stepwise models were run until only
 202 characteristics that were significant at an alpha level of 0.01 remained. These characteristics
 203 were then used as input data to the appropriate regression equations to estimate bimonthly
 204 aboveground biomass. We used a single phenometric model for *Typha latifolia* and *T.*
 205 *domingensis* (hereby referred to as *Typha*. spp) because model parameters for these species were
 206 not different from each other. *Schoenoplectus acutus*, *S. tabernaemontani* (hereby referred to as
 207 *Schoenoplectus* spp.), and *S. californicus* individuals without seeds were similarly grouped.
 208 *Typha* spp., *Schoenoplectus* spp., and *S. californicus* individuals with seeds heads produced a
 209 different phenometric model from those without seed heads. We generated separate models for *S.*
 210 *americanus* and *S. maritimus* to represent their unique morphology.

211 2.4 Belowground biomass

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

$$\frac{BG}{AG}(AG_{system}) = BG_{system} \quad 223 \quad \text{Equation 2}$$

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

226 2.5 Plant tissue nutrients

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

233 2.6 Plant productivity and N uptake

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

$$N_{content} = PAG_{spp} \times N_{spp} \quad 237 \quad \text{Equation 3}$$

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

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

$$N_{mono} = PAG_{mono} \times N_{spp} \quad 246 \quad \text{Equation 4}$$

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

249 We utilized the Maximum Biomass method to calculate NAPP, where NAPP is equal to
 250 the difference between minimum and maximum biomass (per Morris and Haskin, 1990), and
 251 thus assumed biomass turnover and herbivory were negligible. Because the Maximum Biomass
 252 method has been shown to underestimate net primary productivity of wetlands plants (Cronk and

253 Fennessy, 2001), our estimates of plant N uptake are conservative. Total plant N uptake was
 254 calculated by multiplying average foliar N content by NAPP for each species present:

$$Plant\ Uptake = NAPP \times N_{spp} \quad 255 \quad Equation\ 5$$

256 2.7 Water quality and flow

257 Triplicate surface-water grab samples were collected bimonthly at the inflow and outflow
 258 of the system using acid-washed 1 L Nalgene bottles. Samples were chilled on ice until they
 259 were returned to the lab for processing and analysis. Unfiltered samples were centrifuged at
 260 8,000 RPM for 10 minutes and analyzed for inorganic N (nitrate, NO_3^- ; nitrite, NO_2^- , and;
 261 ammonium, NH_4^+) via flow injection analysis on a Lachat QC 8000 Quickchem Flow Injection
 262 Analyzer (method detection limits were $0.85\ \mu g\ NO_3/NO_2-N\ L^{-1}$ and $3.01\ \mu g\ NH_4-N\ L^{-1}$). We
 263 selected inorganic N to represent nitrogen dynamics within the system because these are the
 264 forms taken up by macrophytes. Concentration data were multiplied by total monthly water
 265 flows to determine inorganic N fluxes. This research was carried out in partnership with the City
 266 of Phoenix Water Department, who provided daily inflow and outflow data, measured by
 267 acoustic Doppler flow meters, from January 2012 – August 2013—with a few exceptions (e.g.,
 268 the flow data from March, April, and May of 2012 may not be reliable due to metering
 269 problems). Flow data were unavailable prior to January 2012. We calculated inorganic N
 270 uptake and processing by the system as the difference between the inorganic N in surface water
 271 influx and efflux plus estimates of atmospheric N deposition. We used a conservative estimate
 272 of $12\ kg\ N\ ha^{-1}\ yr^{-1}$ for dry + wet N deposition of $NH_4^+ + NO_3^-$ for the Phoenix Metro area
 273 (Lohse et al., 2008). The following equations were used in our N budget calculations:

$$N_{influx} = Influx + Deposition_{dry+wet} \quad 274 \quad Equation\ 6$$

$$N_{uptake} = N_{influx} - Efflux \quad 275 \quad Equation\ 7$$

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277 where *Influx* was the mass of inorganic N, as $NH_4^+ + NO_3^-$, entering the system via system
 278 water inputs, *Deposition_{dry+wet}* was dry and wet deposition from Lohse et al. (2008), and
 279 *Efflux* was the mass of inorganic N leaving the system via effluent waters.

280

RESULTS AND DISCUSSION

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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.

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3.2 Phenometric models

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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.

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INSERT TABLE 1 HERE

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3.3 Aboveground biomass and community composition

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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

308 and constructed treatment wetlands that ranged from 790-2200 gdw m⁻², (Kadlec & Knight,
309 2008; Miller & Fujii, 2010; Tanner, 2001; van der Valk & Davis, 1978).

310 INSERT FIGURE 2 HERE

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

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

335 *3.4 Belowground biomass*

336 Belowground biomass averaged 1056 ± 233 (SE) and 815 ± 163 (SE) gdw m^{-2} for all
 337 species in November of 2011 and 2012, respectively (Table 2). Our observations were
 338 considerably lower than those reported for *T. latifolia* (2900gdw m^{-2}) by Kadlec and Wallace
 339 (2009) but comparable to estimates for *S. tabernaemontani* from Tanner (1996; 2001). In mixed
 340 stands of *Typha* spp. and *S. acutus*, Miller & Fujii (2010) reported belowground biomass values
 341 between $1000\text{-}1800 \text{gdw m}^{-2}$, higher than our mixed-stand estimates (Table 2). Our estimates
 342 translated to a total system-wide belowground biomass of approximately 222 Mgdw in 2011 and
 343 171 Mgdw in 2012. Aboveground:belowground biomass ratios were not significantly different
 344 among species within each year; however, ratios in *Typha* spp. and *Schoenoplectus* spp. varied
 345 considerably from 2011 to 2012 (Table 2). *Typha* spp. accounted for the largest portion of
 346 belowground biomass.

347 INSERT TABLE 2 HERE

348 3.5 Macrophyte N content

349 Aboveground tissue N content averaged 1.64 ± 0.11 (SE) %N for all species, with no
 350 significant difference among the species groups (Table 3). Belowground tissue N content was
 351 also not significantly different among species and averaged slightly lower ($1.29 \pm 0.08\%$ SE).
 352 Our observations were consistent with those reported in the literature for *Typha* spp. (Pratt et al.,
 353 1980; Kadlec and Wallace, 2009) and *S. tabernaemontani* (Tanner, 2001).

354 Nitrogen content per unit area of wetland at peak biomass provided further insights into
 355 the role of each species in whole-system N uptake. *Typha* spp. had the highest N content
 356 (21.5 ± 4.1 (SE) N g m^{-2}), accounting for more than two-thirds of the whole-system plant N (Table
 357 3). The N content of *Schoenoplectus* spp., *S. californicus*, and *S. americanus* was 5.3 ± 2.6 (SE) g
 358 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
 359 content (i.e., the average sum of all species' N contents) at peak biomass was 30.5 ± 8.3 (SE) N g
 360 m^{-2} from 2011-2013. This level of plant N was higher than the N content of 24.3g m^{-2} reported
 361 by Kadlec & Knight (2008) for a mesic, *Typha* spp. wetland receiving water of similar quality to
 362 Tres Rios, but it was lower than the N content of approximately 40g m^{-2} reported by Tanner
 363 (1996) from a mesic mesocosm experiment using *S. tabernaemontani* and influent water with
 364 substantially higher N concentrations (TN $\sim 100 \text{mg L}^{-1}$) than at Tres Rios. The wide variation of

365 macrophyte N content reported for various species by Kadlec and Wallace (2009), Tanner (1996),
366 and our study could be due to differences in wastewater type, climate, and research approach.

367 INSERT TABLE 3 HERE

368 3.6 Hypothetical monotypic N content

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

386 Our data do not allow us to speculate on management strategies that would promote or
387 preserve more diverse macrophyte communities in established CTW systems. In existing
388 monotypic systems, the removal of vegetation and planting of other species could prove costly
389 and would likely require temporarily halting CTW operations. Maintaining existing diverse
390 plant communities in established systems may also prove difficult when one species begins to
391 dominate the system, as we observed at Tres Rios with *Typha* spp. In theory, different species
392 might respond differently to common management techniques, such as burning, harvesting, or

393 periodic drying of CTW marshes. However, little research exists on the use of these techniques
394 for managing community composition.

395 Planting diverse macrophyte communities in new CTW systems is likely much easier
396 than bringing diverse macrophyte communities into existing systems. In new CTW systems,
397 planting diverse communities would likely cost no more than planting monotypic marshes
398 because the costs of planting are more driven by plant density than by the species or number of
399 species planted (Environmental Protection Agency, 1999). Given this, we argue that planting
400 more than one species is a simple way to improve nutrient retention in new CTW systems.
401 System-specific variables, such as climate, wastewater type, and other services desired of CTW,
402 should be considered when determining what plant species should be used in new systems. The
403 planting design across a CTW might influence the ability of one species to take over a system
404 and should also be considered. At Tres Rios, macrophyte species were dispersed amongst each
405 other with the exception of *S. americanus*, which grew in dense monotypic stands. This highly
406 mixed arrangement may have contributed to the trend towards *Typha* spp. domination that we
407 observed because the *Typha* spp. plants grew taller than most other species and likely shaded out
408 individuals of other species. Dividing vegetated areas into species group zones could deter or at
409 least slow the encroachment of one species across the entire system. Another approach might be
410 single species management through harvesting or other means. However, the separation of
411 species or species groups in zones might negate the benefits of macrophyte diversity.

412 3.7 Water quality

413 Water quality measurements lend insight into whole-system N retention and are the basis
414 for our N budget calculations. We restricted our analysis to NO_3^- and NH_4^+ as these species of N
415 are most readily taken up by plants. Nitrite was a relatively small portion of inflow and outflow
416 N ($<0.30 \text{ mg L}^{-1}$), and is thus not presented. Inflow concentrations of NO_3^- ranged from 1.5 – 7.5
417 mg L^{-1} while outflow ranged from 1.2 – 6.8 mg L^{-1} (Figure 3). Ammonium concentrations
418 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).
419 Nitrate concentration was, on average, 15% lower (0.59 mg L^{-1}) in the outflow water compared
420 with the inflow while NH_4^+ concentrations were 51% lower (0.63 mg L^{-1}). With the exception of
421 January 2012 and March 2013, NO_3^- concentrations were consistently lower in the outflow;
422 NH_4^+ was always lower in the outflow. Because N flux accounts for both concentration and

423 water flow, it may be a better measure of system effectiveness. However, effluent concentrations
424 are important for estimating downstream environmental impact, and most CTW water quality
425 regulations and management are based on outflow water quality. Sanchez et al. (this issue)
426 provide further insight to N concentrations and flux in the Tres Rios CTW.

427 INSERT FIGURE 3 HERE

428 3.8 Whole-System nitrogen flux

429 Water inflow data were unavailable prior to January 2012, so we calculated a system-
430 wide N budget from January 2012 – August 2013. From January 2012 to August 2013, the
431 system received a total of 147.4 ± 4.1 (SE) Mg $\text{NO}_3^- + \text{NH}_4^+$, which equated to a loading rate of
432 0.61 ± 0.02 (SE) $\text{g m}^{-2} \text{d}^{-1}$ or 6.1 ± 0.2 (SE) $\text{kg ha}^{-1} \text{d}^{-1}$ (Table 4). During this period, the system
433 removed 52.3 ± 4.2 (SE) Mg N (equivalent to 0.22 ± 0.02 (SE) $\text{gN m}^{-2} \text{d}^{-1}$), or 35% of the inorganic
434 N load. These results showed that the system was performing an important nutrient removal
435 service. Our N removal rates were comparable to NH_4^+ and NO_3^- removal rates by an arid CTW
436 in Southern California studied by Sartoris et al. (2000). We estimated that plant N uptake totaled
437 10.0 ± 0.8 Mg. This plant uptake equated to 7% of inorganic N loading and 19% of system-wide
438 inorganic N uptake. Notably, during the growing season (March – September), plants in the Tres
439 Rios CTW accounted for a relatively large portion of whole-system N removal (33-51%). Our
440 observations fell well within previously reported plant N uptake estimates of 2-18% of inflow N
441 for mesic CTW systems (Faulkner & Richardson, 1990; Kadlec & Knight, 2008; Meers et al.,
442 2008). *Typha* spp. accounted for 70% of all plant N uptake, *Schoenoplectus* spp. 18%, *S.*
443 *americanus* 10%, and *S. californicus* 2%. Our estimates did not account for the remineralization
444 of foliar N during the decomposition of senesced plant biomass. Decomposition rates for these
445 species may differ, which would also suggest that community composition is important for CTW
446 performance.

447 INSERT TABLE 4 HERE

448 The portion of whole-system N sequestration that we were able to account for by
449 quantifying direct plant uptake was smaller than we expected. As is typical in many CTW,
450 coupled nitrification-denitrification likely accounts for the majority of N processing and removal

451 at Tres Rios, as has been shown in similar aridland wetlands (Kadlec, 2008). Specific plant
452 species do have an impact on coupled nitrification-denitrification through the active transport of
453 oxygen to soils and through contributions to labile organic matter pools in soils. Hume et al.
454 (2002) found that *Typha* spp. litter contained less lignin and had lower C:N ratios than
455 *Schoenoplectus* spp., suggesting that organic matter contributed by *Typha* spp. may be more
456 available for denitrifying bacteria. Gebremariam & Beutel (2008) found that *Typha* spp. plants
457 transported less oxygen to soils than *Schoenoplectus* spp., leading to higher rates of
458 denitrification in *Typha* spp. plots (notably, nitrification was not examined and this is the process
459 that requires oxygen). As our research in the Tres Rios CTW continues we will expand our
460 whole-system N budget by including measurements of nitrification-denitrification and trace gas
461 fluxes, as well as experiments on the effects of various drying-wetting regimes on soil N
462 processing. Ultimately this information will help inform best management practices of this
463 important urban CTW.

464 CONCLUSIONS

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

475 Based on our findings, we argue that planting a diverse macrophyte community in new
476 CTW systems may lead to increased whole-system N uptake without significant costs
477 (Environmental Protection Agency, 1999). However, strategies to manage existing CTWs in
478 order to promote or preserve diverse macrophyte communities need to be better investigated. Our
479 study adds to a growing body of literature examining the role of community composition and
480 CTW performance but further study in full-scale CTW systems is clearly necessary to help

481 inform more effective management strategies. While our study examined only one pathway for
482 N processing in CTW marshes (direct plant uptake), further investigation of N processing at Tres
483 Rios is underway. This further investigation includes quantifying coupled nitrification-
484 denitrification, trace gas flux, and plant decomposition. These projects will shed further light on
485 the role of macrophyte community composition in CTW N processing.

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FIGURE LEGENDS

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

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

677 **Figure 3.** Nitrate and ammonium concentrations at system inflow and outflow.

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FIGURES

680 Figure 1a.



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691 Figure 1b.



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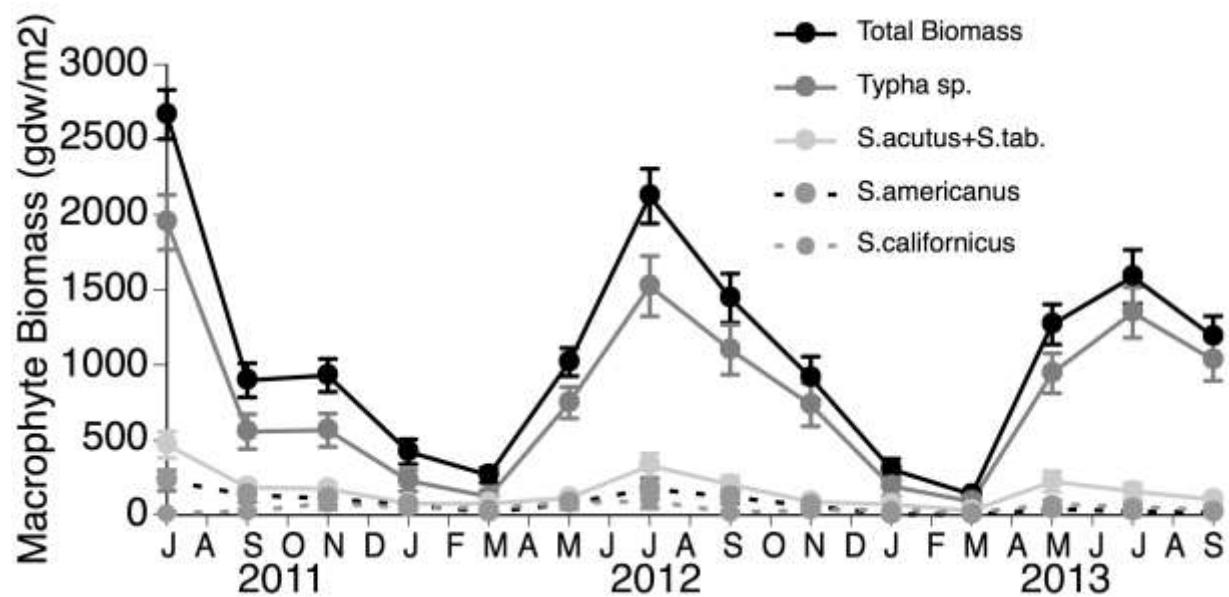
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704 Figure 2.



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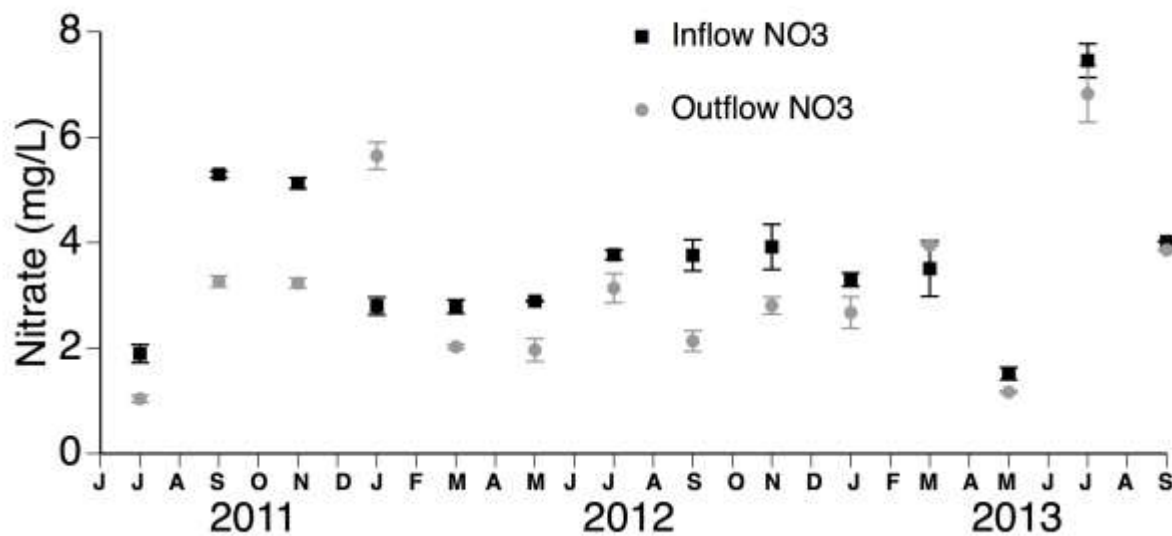
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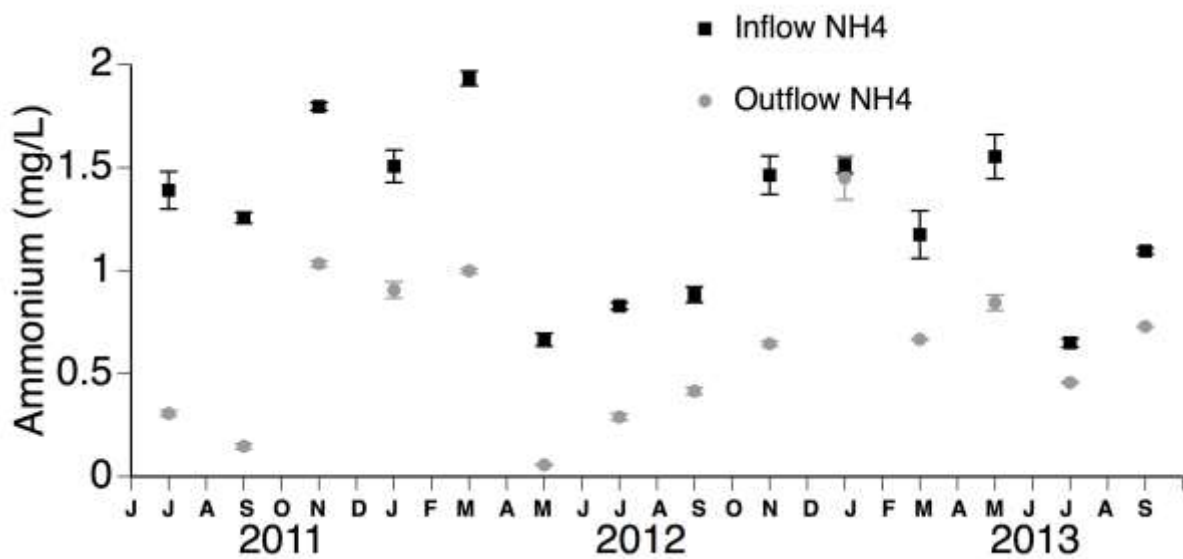
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716 Figure 3.



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TABLE LEGENDS

- 721 **Table 1.** Stepwise multiple regression phenometric models developed for each species and
722 subcategory. Estimates for each significant parameter are given in parenthesis. CDB is
723 culm diameter measured at the soil surface. Volume was calculated from CDB and stem
724 height, assuming that culms were conical. All length, width, and diameter parameters
725 were measured in centimeters.
- 726 **Table 2.** Above-to-belowground biomass ratios and estimates of belowground biomass.
- 727 **Table 3.** Nitrogen content in plant tissues and estimates of monotypic (i.e., a system planted with
728 only one species) nitrogen content for the 4 species groups.
- 729 **Table 4.** Whole-system surface water N budget for January 2012 – August 2013 and for the
730 2012 and 2013 growing seasons. The difference between inflow and retained $\text{NO}_3^- +$
731 NH_4^+ is outflow $\text{NO}_3^- + \text{NH}_4^+$.
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TABLES

734 Table 1.

Species	n	Cate -gory	Significant parameters & regression coefficients				Adjusted R- squared and p-value
<i>Typha</i> spp.	49	w/ flower	CDB (7.976)	Stem height (0.2021)	Pistillate length (0.0804)	Pistillate diameter (6.651)	0.75, p<0.0001
		w/o flower	Sum of all leaf lengths (0.0667)	Leaf count (-2.059)	Longest leaf (-0.1640)		0.88, p<0.0001
<i>S. americanus</i>	60	all	CDB (1.5821)	Stem height (0.0106)			0.76, p<0.0001
<i>S. acutus</i> , <i>S.</i> <i>californicus</i> , <i>S.</i> <i>tabernaemontani</i>	31	w/o seeds	Stem height (0.0369)	Volume (0.0226)			0.73, p<0.0001
<i>S. acutus</i> <i>S. tabernaemontani</i>	35	w/ seeds	Stem height (0.0264)	Volume (0.036)			0.90, p<0.0001
<i>S. californicus</i>	22	w/ seeds	Stem height (0.0237)	Volume (0.0576)			0.84, p<0.0001
<i>S. maritimus</i>	33	all	CDB (1.001)	Stem height (0.0162)	Seed head count (0.107)		0.80, p<0.0001

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745 **Table 2.**

		<i>Schoenoplectus</i> spp.	<i>S. americanus</i>	<i>S. californicus</i>	<i>Typha</i> spp.	Total
2011	Above:below ratio	0.85±0.13	0.59±0.09	0.80±0.06	1.00±0.31	
	(n)	(4)	(3)	(2)	(4)	
	Belowground biomass (gdw m⁻²)	207±54	186±80	99±50	564±206	1056±233
2012	Above:below ratio	0.36±0.02	0.62±0.08	0.80±0.20	1.71±0.42	
	(n)	(3)	(3)	(3)	(6)	
	Belowground biomass (gdw m⁻²)	248±79	99±42	40±23	428±134	814±163

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761 **Table 3.**

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

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778 **Table 4.**

	January 2012 - August 2013²	2012 growing season (Mar-July)	2013 growing season (Mar-July)
NO₃⁻ + NH₄⁺ Inflow + Deposition (Mg)	147.4±4.1	33.0±0.3	44.1±1.0
NO₃⁻ + NH₄⁺ retained¹ (Mg)	52.3±4.2 (35%)	17.9±0.5 (54%)	8.4±1.3 (19%)
Net Aboveground Primary Productivity (Mg)	-	395	305
Total N retained by plant growth (Mg)	10.0±0.8 (7%)	5.8±0.6 (9%)	4.2±0.5 (5%)
Plant uptake as a percentage of whole- system N Uptake	19%	33%	51%

¹Percentage represents proportion of inflow N retained

²Excludes December 2012 as inflow data was not available

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