EFFECT OF THREE EMERGENT MACROPHYTE SPECIES ON NUTRIENT RETENTION IN AQUATIC ENVIRONMENTS UNDER EXCESS NUTRIENT LOADING

Myrto Nikolakopoulou,^{,†,‡} Alba Argerich,[§] Susana Bernal,^{||} Esperança Gacia,^{||} Miquel Ribot,^{||}
Eugènia Martí,^{||} Albert Sorolla,[‡] and Francesc Sabater^{‡,‡}*

[†]Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals (BEECA), Universitat de
Barcelona, Av. Diagonal 643, 08028, Barcelona, Spain, [‡]Naturalea, Carrer de la Terra Alta 51,
08211, Castellar del Vallès, Spain, [§]School of Natural Resources, 303K Anheuser-Busch Natural
Resources Building, University of Missouri, Columbia, MO 65211, USA, ^{||}Integrative Freshwater
Ecology Group, Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Carrer Accés a la Cala St.
Francesc 14, 17300, Blanes, Spain, [‡]CREAF, Campus de Bellaterra Edifici C,
08193, Cerdanyola del Vallès, Spain

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19 **Abstract**

20 Emergent macrophyte species selection is critical for the effectiveness of nature-based engineered
21 solutions aiming to address excess nutrient concentrations in freshwater ecosystems. Yet, the
22 mechanisms with which macrophytes enhance nutrient retention need to be further understood.
23 Here, we compared nutrient retention among 12 artificial flumes fed with effluent from a
24 wastewater treatment plant and subjected to four treatments: absence of macrophytes (Control),
25 and presence of three different macrophyte species (*Iris pseudacorus* L., *Phragmites australis* L.,
26 and *Schoenoplectus lacustris* L.). We estimated net and gross nutrient uptake based on longitudinal
27 profiles of ambient concentrations and on pulse injections of ammonium (NH_4^+) and soluble
28 reactive phosphorus (SRP). Further, we investigated the influence of subsurface hydrological
29 retention, attributed to the architectural differences in the roots of these macrophytes, on nutrient
30 retention. Results showed a species-specific effect of macrophytes on nutrient retention and
31 confirmed root-associated subsurface hydrological retention as a driving factor. *Schoenoplectus*
32 showed both high net and gross NH_4^+ uptake, therefore being the most effective species to address
33 N loading, compared to *Iris* and *Phragmites*. This work contributes to improve our mechanistic
34 understanding of the role of emergent macrophytes on nutrient retention in aquatic environments.

35 **1. Introduction**

36 Urbanization and agriculture are responsible for elevated nutrient concentrations in aquatic
37 ecosystems.^{1, 2} Effluents from wastewater treatment plants, as well as agricultural and stormwater
38 runoff, deteriorate the ecological status of the receiving water by degrading water quality. Nature-
39 based engineered practices to address problems associated with elevated nutrient concentrations,

largely represented by constructed wetlands and stormwater biofiltration systems, use emergent macrophytes in the context of phytoremediation, as an alternative approach to conventional water treatment technologies (e.g. air stripping towers, break-point chlorination method).^{3, 4}

Emergent macrophytes contribute to nutrient removal by assimilating nutrients into plant tissues, and by facilitating microbially-mediated nutrient removal.^{5, 6} In particular, rooted macrophytes create ideal environmental conditions in the rhizosphere for microbial assimilation and coupled nitrification-denitrification. First, because the root surface serves as microbial habitat; and second, because root-tips release oxygen and labile carbon into the interstitial water.^{6, 7} Macrophyte species selection can be critical for the successful implementation of constructed wetlands and biofiltration systems aiming nutrient mitigation.^{8, 9} A good understanding of how plant morphological traits influence nutrient retention from freshwater systems would lead to optimum water treatment results by considering appropriate species selection. Yet, this type of investigation is usually overlooked, and most studies only report the influence of vegetation presence on nutrient retention, or in the case of comparative studies, the difference in retention effectiveness among species.¹⁰⁻¹² However, some studies have confirmed the hypotheses that increased root surface area and root oxygen release can enhance microbial activity, identifying these hypotheses as mechanisms that ultimately promote nutrient retention. Such hypothesis-driven studies have confirmed the influence of different plant morphological traits on nutrient retention, such as the number of adventitious roots, root surface area, fibrous type root architecture, total plant biomass, above ground biomass, root biomass, maximum root length, and total root length.^{9, 13-17} Still, even after identification of plant traits that correlate with nutrient retention, generalization of the results to guide species selection for nature-based water treatment technologies is still constrained by methodological limitations (i.e. poor replication and high variability of experimental conditions).¹¹

Therefore, identification of the controlling factors and underlying mechanisms with which different macrophyte species contribute to nutrient retention requires further investigation. Currently, this lack of information limits the capacity to successfully restore impaired aquatic ecosystems.¹⁷

Moreover, macrophyte root architecture influences subsurface solute transport.¹⁸ Solute transport with increased hydrological retention (i.e. long water residence time and large regions of slower water velocity than the average channel, termed transient storage) provides sufficient contact time between bioreactive sediments and solutes for biogeochemical processing, critical for nutrient retention.^{19, 20} Therefore, one would expect that plant-induced changes in subsurface hydrology could influence nutrient retention. However, results about the influence of hydrology on nutrient retention are equivocal^{21, 22} most likely because the extent of transient storage is not always proportional to its metabolic activity.²³ Although difficult to quantify, the contribution of different transient storage regions to a system's metabolic activity can vary substantially.²³ This is a critical question for understanding the potential benefits of phytoremediation technologies, which have not been empirically investigated yet.

Here, we seek to examine whether subsurface hydrological retention attributed to the presence of different macrophyte species can cause ecologically relevant changes in nutrient retention, and to identify the most effective species to enhance nutrient retention. To do so, we used three macrophyte species with contrasting nutrient requirements²⁴ and root architecture-mediated influence on subsurface solute transport.¹⁸ We hypothesized that macrophyte roots would influence pore water chemistry by altering subsurface flow. We expected that nutrient uptake metrics, used as an index of nutrient retention, would be positively related with hydrological metrics describing hydrological retention. This work contributes to improve our mechanistic

understanding of the role of emergent macrophytes in nutrient loads mitigation in aquatic environments.

2. Methods

2.1 Experimental setting

The study was conducted at an outdoor research facility with 12 artificial flumes (length: 12 m, width: 0.6 m, depth: 0.4 m) containing a 25-cm-thick layer of granitic gravels and fed with treated municipal wastewater (<http://www.urbanriverlab.com>; Ribot et al.²⁵). The inlet and outlet of the flumes are 40-cm-long sediment free spaces to facilitate the water inflow and outflow (Figure 1). Inflow rates were adjusted at $5.02 \pm 0.01 \text{ L min}^{-1}$, resulting in a continuous subsurface flow through the gravel bed. To sample subsurface water along the flumes, we installed five PVC wells per flume (L: 50 cm, diameter: 2.5 cm) into the gravel at 1, 3, 5, 7, 9 m from the inlet. The wells were perforated over the last 15 cm from the well bottom.

Figure 1. a) Scheme of a vegetated flume at the Urban River Lab experimental platform. Flumes were fed with WWTP effluent at an inflow rate of 5 L min^{-1} , resulting in a continuous subsurface flow through the gravel bed. Five PVC wells were placed along each flume to collect subsurface water. b) Diagram summarizing the sampling strategy at each flume.

The experimental setting consisted of three flumes without macrophytes (Control), and flumes with emergent macrophytes (three flumes for each of the three studied species; that is *Iris pseudacorus* L. (common yellow flag, hereafter as *Iris*), *Phragmites australis* L. (common reed, hereafter as *Phragmites*), and *Schoenoplectus lacustris* L. (formerly known as *Scirpus lacustris* L., common club-rush, hereafter as *Schoenoplectus*)). The selected macrophyte species are autochthonous, perennial, with contrasting root system architecture and evapotranspiration capacity,¹⁸ and are commonly used in constructed wetlands and river restoration projects.³ *Iris* is

characterized by high % volume of rhizomes to volume of soil, *Phragmites* by low % volume of fine roots and rhizomes, and *Schoenoplectus* by high % volume of fine roots.¹⁸ The three replicates per each treatment were randomly distributed among the 12 available flumes on the facility to ensure random exposure to the environmental conditions. Saplings were planted in February 2015, at a density of 6.7 shoots m⁻². By the time of the study (July 30th – August 27th, 2015) the vegetation covered approximately 90% of the surface of each flume.

In a previous study we showed that these species differentially increased hydrological retention along the flumes (Table 1).¹⁸ Hydrological retention was described by hydrological transient storage (M_1/t_{peak}), which is the ratio between the mean water arrival time (M_1) and the time of the preferential flow path (t_{peak}), the maximum observed water residence time (RT_{max}), and the slope of the tail of the power-law residence time distribution which is described by the time-fractional exponent ($0 < \gamma < 1$). Values of γ close to 0 correspond to stronger power-law tails and are indicative of longer residence times. The hydrological metrics used derived from statistical moments and temporal metrics analyses on conservative tracer breakthrough curves²⁶.

Table 1. Subsurface solute transport parameters in flumes without macrophytes (Control) and with presence of *Iris*, *Phragmites*, and *Schoenoplectus*^a

	M_1/t_{peak}	RT_{max}	γ
	(unitless)	(h)	(unitless)
Control	1.42 ± 0.09	14.15 ± 2.58	0.78 ± 0.04
<i>Iris</i>	1.93 ± 0.19	25.25 ± 3.08	0.69 ± 0.05
<i>Phragmites</i>	2.42 ± 0.19	34.37 ± 2.58	0.70 ± 0.02

<i>Schoenoplectus</i>	3.66 ± 0.34	47.78 ± 6.6	0.59 ± 0.04
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^a Values are means $\pm$ standard error. Data reported in Nikolakopoulou et al.¹⁸ *Schoenoplectus* was formerly known as *Scirpus*.

2.2 Measurement of nutrient uptake

We estimated net uptake rate coefficient per unit of distance (k_{net} , m^{-1}) and gross uptake rate coefficient per unit of time (k_{gross} , min^{-1}) for ammonium (NH_4^+) and soluble reactive phosphorus (SRP), as indices of nutrient retention, based on the nutrient spiraling concept. Sampling for nutrient retention consisted in longitudinal samplings of subsurface water from the wells and the inlet and outlet (seven locations in total), followed by solute pulse injections. Given the low flowrates in the flumes in order to ensure subsurface flow, constant rate additions that would provide comparable k_{net} and k_{gross} estimates, were not feasible, as several hours would be needed for the systems to reach steady-state conditions. Nevertheless, the assessment of both k_{net} and k_{gross} , even in different units, allows a more complete understanding of the influence of each macrophyte species on N and P cycling.²⁷ Nutrient spiraling methods for the estimation of nutrient retention have been commonly used by stream ecologists. Estimation of k_{net} , based on the longitudinal trend of nutrient concentrations across several sampling points, provides more robust results than the mass-balance approach (i.e. Inflow – Outflow), which is commonly used for the evaluation of wastewater treatment performance systems.²⁷ Further, estimation of k_{gross} using nutrient injections considers both biogeochemical and transport conditions of a system, resulting to reliable results, since nutrient concentrations and solute transport are tightly coupled.²⁸ We performed longitudinal samplings and solute injections in sets of three flumes. Each set of flumes was sampled twice resulting in a total of 24 longitudinal samplings and 24 solute injections.

k_{net} was estimated from the longitudinal samplings. Water samples were collected using a 100 ml syringe with an attached silicone tube. k_{gross} was estimated using a pulse co-injection of 1 L solution containing 12 g of NH_4Cl , 0.875 g of $\text{NaH}_2\text{PO}_4\text{H}_2\text{O}$, and 80 g NaCl at the inlet of each flume. Temperature-corrected electrical conductivity (EC, $\mu\text{S}/\text{cm}$) was measured continuously at the outlet of each flume at 30 sec intervals. EC was considered a proxy for Cl^- concentration and the change in EC during time (breakthrough curve, or BTC) was used to characterize the transport of the conservative tracer. EC measurements started 30 minutes before the injection until EC returned to background conditions (approx. 7h after the pulse injection). Simultaneously, water samples were collected at the outlet of each flume to characterize the BTC of the reactive tracers.

To account for fluctuations in the solute concentrations of the inflow water, we measured EC at 30 s intervals and collected water samples hourly at the inlet of one flume. For all EC measurements we used portable 3310 Proline EC meters (WTW; Germany). Concentration of dissolved oxygen (DO) was monitored continuously at 10 min intervals using ProODO DO meters (YSI; USA) deployed at the outlet of each flume, and at the inlet of one flume. All water samples were immediately filtered through ashed Whatman GF/F glass-fiber filters (0.7 μm pore size) into 10 mL plastic tubes and stored frozen until nutrient analysis.

All water samples were analyzed for concentrations of elemental nitrogen as nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$) and as NH_4^+ , and of SRP using the cadmium reduction,²⁹ salicylate–nitroprusside,³⁰ and the acidic molybdate³¹ methods, respectively. Water samples from longitudinal profiles were analyzed with an automatic continuous flow analyzer (Futura AMS Alliance; Italy). Samples from solute pulse injections were analyzed using a Technicon autoanalyzer (Technicon; USA) for $\text{NO}_3^- + \text{NO}_2^-$ and a spectrophotometer (PharmaSpec UV-1700 Shimadzu; Japan) for NH_4^+ and SRP. Inflow water samples from longitudinal profiles were further analyzed for Total dissolved nitrogen

(TDN) on a Shimadzu (Tokyo, Japan) TOC-VCSH analyzer. Dissolved organic nitrogen (DON) was estimated by subtracting dissolved inorganic nitrogen (DIN) from TDN concentrations.

2.3 Parameter Estimation

We estimated k_{net} from longitudinal profiles of nutrient concentrations at ambient levels; and as such it reflects the balance between uptake and release processes occurring along the flumes, which determines whether a system currently acts as a net sink or source of nutrients. k_{gross} was estimated from a mass balance approach to infer the capacity of the system to uptake the mass of added nutrients from the solute injections.²⁷ The method of solute injections allows measurement of nutrient uptake, while precluding the effect of remineralization on the results, and thus is considered appropriate for gross uptake characterization³²⁻³⁴.

2.3.1 Net uptake rate coefficient

k_{net} for NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$, and SRP was calculated based on changes in subsurface water nutrient concentrations along the flumes following Merseburger et al.:³⁵

$$C_x = C_{\text{in}} e^{k_{\text{net}} x} \quad (1)$$

where C_x and C_{in} are ambient concentrations of NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$, or SRP (mg N or P L^{-1}) at distance x (m) from the inlet and at the inlet, respectively. We calculated k_{net} as the statistically significant slope ($p < 0.05$) of the linear relationship between the natural logarithm of nutrient concentrations and distance along the flume. We considered flumes with $k_{\text{net}} > 0$ to act as net nutrient sinks (i.e., gross uptake $>$ release), whereas flumes with $k_{\text{net}} < 0$ were considered to act as net nutrient sources (i.e., gross uptake $<$ release). We assigned $k_{\text{net}} = 0$ to flumes with no significant linear regression, considering that uptake and release processes balanced each other.

2.3.2 Gross uptake rate coefficient

Three of the 24 nutrient BTCs were excluded from the analysis because of sample contamination resulting in a final dataset of 21 BTCs. We used the nutrient BTCs measured at the outlet and the temporal variation of nutrients at the inlet to compute k_{gross} for each nutrient injected (NH_4^+ , SRP) following the approach by Wilcock et al.³⁶ We used a mass-based approach to estimate k_{gross} as it is considered more accurate than approaches using only nutrient concentration.³⁷

For each nutrient, we compared the observed mass of injected nutrient retrieved at the outlet of the flume (M_{obs}), with the expected mass of injected nutrient at the outlet if nutrient transport along the flume behaved as a conservative tracer (M_{exp}). We calculated M_{obs} as:

$$M_{obs} = Q_{out} \int_0^7 C_{out} dt \quad (2)$$

where Q_{out} is discharge at the outflow calculated from the background-corrected BTCs of EC using the dilution gauging approach.³⁸ C_{out} is the nutrient concentration measured at the outlet over the 7-hour course of the BTC.³⁶ M_{exp} was calculated as the sum of the mass of nutrient entering through the inflow (M_{in}) and the mass of nutrient injected (M_{carboy}):

$$M_{exp} = M_{in} + M_{carboy} \quad (3)$$

M_{in} is the sum of the nutrient mass of the inflow water over the 7 hours of the solute injection:

$$M_{in} = Q_{in} \int_0^7 C_{in} dt \quad (4)$$

where Q_{in} is the discharge of the inflow water and C_{in} is the nutrient concentration at the inlet. We calculated k_{gross} by linearizing the following first-order equation:

$$M_{exp} = M_{obs} e^{k_{gross} t_{peak}} \quad (5)$$

where t_{peak} is the time from the start of the injection until the arrival of the peak of the conservative tracer at the outlet of the flume. Additionally, the relative k_{gross} of N to P was calculated as the ratio between $k_{gross-NH_4^+}$ and $k_{gross-SRP}$.

2.4 Statistical analysis

Differences in inflow water across treatments were tested using analysis of variance (ANOVA) and the post-hoc Tukey's test, or non-parametric Kruskal–Wallis test and post-hoc Dunn's test, when assumptions for normality and homoscedasticity were not met. The same non-parametric method was used to detect differences in k_{net} among treatments. We compared k_{gross} , $k_{\text{gross}} - \text{NH}_4^+ / k_{\text{gross}} - \text{SRP}$ ratio, and outflow DO concentration across treatments using the log likelihood ratio test in a linear mixed model. Treatment was considered a fixed factor, and flume a random effect. Post-hoc Tukey's test was used for pairwise comparisons. To explore how root-induced changes in subsurface hydrology influenced nutrient uptake rate coefficients (k_{net} and k_{gross}) we used Spearman correlations. Finally, we used Spearman correlations to examine if variation in background nutrient concentrations of the inflow water influenced nutrient uptake in the flumes. All statistical analyses were carried out using R, version 3.3.1³⁹ setting $\alpha = 0.05$.

3. Results

3.1 Physicochemical characterization of inflow water in the flumes

All flumes received inflow water with high EC, ranging from 2161 to 2416 $\mu\text{S}/\text{cm}$, and high temperature, ranging between 27.2 and 28.5 $^{\circ}\text{C}$. The concentration of NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$, SRP, and TDN of the inflow water differed among dates when samplings and solute injections were conducted at the different treatments (Kruskal–Wallis, $p < 0.0001$; Table 2). Flumes with *Iris* received the highest NH_4^+ concentration, while flumes with *Phragmites* the lowest (10% of the concentration that *Iris* flumes received). Inflow water of Control flumes and flumes with *Schoenoplectus* had higher $\text{NO}_3^- + \text{NO}_2^-$ concentration than water of flumes with *Iris* and with *Phragmites*. Concentration of SRP in inflow water was the highest in Control flumes and flumes with *Schoenoplectus*. TDN inputs were highest in the Control and in flumes with *Schoenoplectus*.

and lowest in flumes with *Phragmites*, however the proportion of DON in TDN was similar across treatments (ANOVA, $p > 0.05$). No statistically significant differences were found across treatments in inflow DON, and in DO concentration in the flumes' inflow or outflow (ANOVA, linear mixed model, $p > 0.05$). DO concentration averaged 6.02 and 0.57 mg L⁻¹ in the inflow and outflow respectively, revealing substantial DO consumption as subsurface water moved along the flumes. No significant relationships were found between the nutrient uptake rate coefficients and the nutrient concentrations of the inflow water (Spearman correlations, $p > 0.05$). Chemical properties of outflow are presented in Table S1.

Table 2. Chemical properties of inflow water and outflow DO concentration in the flumes for each treatment during the experiment ^a

	Variable	Control	<i>Iris</i>	<i>Phragmites</i>	<i>Schoenoplectus</i>
Inflow	NH ₄ ⁺ (mg N L ⁻¹)	0.46 ± 0.03 a	1.59 ± 0.02 b	0.16 ± 0.00 a	0.63 ± 0.04 a
	NO ₃ ⁻ +NO ₂ ⁻ (mg N L ⁻¹)	3.57 ± 0.08 a	2.02 ± 0.01 b	1.77 ± 0.04 b	3.78 ± 0.10 a
	SRP (mg P L ⁻¹)	0.68 ± 0.02 a	0.18 ± 0.00 b	0.44 ± 0.01 ab	0.77 ± 0.03 a
	DON (mg N L ⁻¹)	3.81 ± 0.98	4.45 ± 0.81	1.67 ± 0.16	3.93 ± 0.49
	TDN (mg N L ⁻¹)	8.22 ± 1.03 a	8.94 ± 0.08 ab	4.34 ± 0.09 b	9.28 ± 0.90 a
	DO (mg L ⁻¹)	6.15 ± 0.04	5.93 ± 0.02	6.11 ± 0.02	5.89 ± 0.04
Outflow	DO (mg L ⁻¹)	0.45 ± 0.11	0.58 ± 0.06	0.57 ± 0.01	0.67 ± 0.03

^a Values are means ± standard error for the different days that each treatment (Control, *Iris*, *Phragmites*, *Schoenoplectus*) was sampled. Data of NH₄⁺, NO₃⁻+NO₂⁻, SRP, and DO was obtained during 7 h of monitoring at each flume inlet. Data of TDN was averaged from two inflow water

measurements per flume ($n = 6$ per treatment) during the longitudinal sampling. DON was estimated as TDN – DIN (as measured from the longitudinal sampling). Values of NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$ presented here are not to be compared with TDN and DON values, since they derive from different samplings. For each variable, lowercase letters denote statistically significant differences across treatments, whereas absence of letters denotes no statistically significant differences ($p > 0.05$).

3.2 Net nutrient uptake

We observed statistically significant differences across treatments for $k_{\text{net}}\text{-NH}_4^+$ (Figure 2, Kruskal–Wallis; $p < 0.05$), whereas no significant differences were found for $k_{\text{net}}\text{-NO}_3^- + \text{NO}_2^-$ and $k_{\text{net}}\text{-SRP}$ (Figure 2, Kruskal–Wallis; $p > 0.05$). Regarding $k_{\text{net}}\text{-NH}_4^+$, all three vegetated treatments (*Iris*, *Phragmites*, and *Schoenoplectus*) showed a positive mean value indicating that uptake predominated over release. In contrast, in Control flumes k_{net} equaled to zero, indicating that uptake and release processes counterbalanced each other. Flumes with *Schoenoplectus* showed the highest $k_{\text{net}}\text{-NH}_4^+$, followed by flumes with *Iris*, both treatments were statistically different from the Control. Treatment with *Phragmites* showed the lowest $k_{\text{net}}\text{-NH}_4^+$ among the treatments with presence of vegetation, which was not statistically different from the Control. Anecdotally, mean $k_{\text{net}}\text{-NO}_3^- + \text{NO}_2^-$ was positive in flumes with *Phragmites*, while it was negative in the rest of treatments. For SRP, k_{net} was positive in flumes with *Phragmites* and *Schoenoplectus*, and negative in Control and *Iris* flumes.

Figure 2. Mean and standard error of net uptake rate coefficients (k_{net}) for NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$, and SRP among different flume treatments (Control, *Iris*, *Phragmites*, and *Schoenoplectus*; $n = 6$ per treatment). p -values result from a non-parametric Kruskal–Wallis test, and multiple comparisons from a Dunn’s post hoc test; $\alpha = 0.05$. Uppercase letters denote statistically different means across treatments, whereas absence of letters denotes no statistically significant differences ($p > 0.05$). Positive and negative values of k_{net} indicate whether the flumes acted as net nutrient sinks or sources, respectively. $k_{\text{net}} = 0$ indicates that uptake and release along the flumes counterbalance each other.

3.3 Gross nutrient uptake

Gross uptake rate coefficients statistically differed across treatments for both NH_4^+ and SRP (Figure 3a, linear mixed model, $p < 0.05$). For NH_4^+ , flumes with *Phragmites* and *Schoenoplectus* showed higher mean k_{gross} values than Control and *Iris* flumes. Particularly flumes with *Schoenoplectus* showed the highest $k_{\text{gross-NH}_4^+}$. $k_{\text{gross-SRP}}$ was similar in flumes with *Phragmites* and *Schoenoplectus* and higher than in the Control flumes, which showed the lowest k_{gross} . In *Iris*, $k_{\text{gross-SRP}}$ showed no statistically significant differences from the rest of treatments. Overall, $k_{\text{gross-NH}_4^+}$ seemed to be higher than $k_{\text{gross-SRP}}$ regardless of flume treatment.

Additionally, the ratio between $k_{\text{gross-NH}_4^+}$ and $k_{\text{gross-SRP}}$ was similar among all treatments (linear mixed model, $p > 0.05$).

Figure 3. Mean and standard error of gross uptake rate coefficients (k_{gross}) for NH_4^+ and SRP among different flume treatments (Control, *Iris*, *Phragmites*, and *Schoenoplectus*). p -values result from the log likelihood ratio test in a linear mixed model; fixed effects: treatment, random effects: flume; Tukey's post hoc; $\alpha = 0.05$. Uppercase and lowercase letters denote statistically different means for NH_4^+ and SRP, respectively ($n_{\text{Control}} = 6$, $n_{\text{Iris}} = 3$, $n_{\text{Phragmites}} = 6$, and $n_{\text{Schoenoplectus}} = 6$).

3.4 Relationships between nutrient uptake and hydrological characteristics

The relationship between subsurface hydrological characteristics and nutrient uptake considering data from all flume treatments together differed among the nutrients (NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$, SRP) and the hydrological and uptake metrics considered. Both k_{net} and k_{gross} of NH_4^+ and SRP yielded positive correlations with M_1/t_{peak} and RT_{max} , revealing monotonic associations between these variables (Figure 4, Spearman correlations, $p < 0.05$). However, k_{gross} of each nutrient had a stronger association with the hydrological metrics than k_{net} , as shown by Spearman's ρ ranging from 0.80 to 0.95 for k_{gross} , and from 0.60 to 0.63 for k_{net} . k_{gross} of both NH_4^+ and SRP

yielded negative correlations with the time-fractional exponent (γ), while only $k_{\text{net}}\text{-NH}_4^+$ was correlated with γ . Between uptake metrics of NH_4^+ , k_{gross} had a stronger association with γ than k_{net} , as indicated by higher absolute value of Spearman's ρ . No significant correlation was found between $k_{\text{net}}\text{-NO}_3^- + \text{NO}_2^-$ and any of the considered hydrological metrics.

Figure 4. Relationships between uptake rate coefficients (k_{net} , k_{gross}) of NH_4^+ and SRP and different hydrological metrics, used as descriptors of subsurface solute transport along the flumes, considering data from all different flume treatments together (i.e., Control, Iris, Phragmites, and *Schoenoplectus*). Data from hydrological metrics for each flume come from 21. Values of M_1/t_{peak} reflect extent of water transient storage along the flumes, RT_{max} is the maximum water residence time, and γ describes the slope of the tail of the power law residence time distribution $n = 12$ in all cases (from the 12 flumes studied). The locally estimated scatterplot smoothing is showed with solid and dashed curves for NH_4^+ and SRP respectively, only when Spearman's correlation between hydrologic and nutrient uptake coefficients were statistically significant ($\alpha = 0.05$).

4. Discussion

4.1 Influence of macrophytes species on nutrient retention

Macrophytes have a species-specific influence on the capacity for NH_4^+ and SRP retention from subsurface water, demonstrated by differences in k_{gross} across flumes with *Iris*, *Phragmites* and *Schoenoplectus*. Additionally, net NH_4^+ uptake, which accounts for both gross uptake and release processes, varied among treatments suggesting that macrophyte species also influence decomposition and mineralization processes. Nevertheless, comparison of nutrient uptake metrics between vegetated and unvegetated flumes showed that the influence of macrophytes on nutrient retention varies among species, since not all vegetated treatments were statistically different from the Control. Possible negative influence of the NaCl additions on the plants' ability to retain nutrients should be excluded, since the average EC elevation induced by the additions (388 $\mu\text{S}/\text{cm}$) was within the range of the natural EC fluctuation of the inflow water (from 70 until 770 $\mu\text{S}/\text{cm}$) the dates of the additions. These findings are in line with other studies reporting differences in nutrient retention among macrophyte species^{24, 40-42} and suggest that selection of macrophyte species in water management or stream restoration planning is critical to enhance nutrient retention.

In the vegetated flumes, $k_{\text{net}}\text{-NH}_4^+$ was higher than $k_{\text{net}}\text{-NO}_3^- + \text{NO}_2^-$ uptake suggesting that the former N form is more actively retained than the latter supporting the idea that NH_4^+ is the most biologically active dissolved inorganic N species in freshwater ecosystems.^{43,44} Interestingly, flumes with *Iris* and *Schoenoplectus* showed higher net uptake capacity to retain NH_4^+ than the Control, and acted as a net sink of NH_4^+ ($k_{\text{net}} > 0$), while no treatment was remarkably efficient for $k_{\text{net}}\text{-NO}_3^- + \text{NO}_2^-$ (in all cases $k_{\text{net}} \sim 0$). Accumulation of N in plant biomass can be substantial in the studied macrophytes,²⁴ yet microbial processes (assimilation, nitrification, and denitrification)

associated with the rhizosphere are considered the major mechanism contributing to N uptake.⁴⁵

⁴⁶ The fact that relative gross uptake (i.e., $k_{\text{gross}} \text{NH}_4^+ / k_{\text{gross}} \text{SRP}$) was similar among all treatments, regardless the presence of macrophytes, supports the idea that gross nutrient retention is mostly attributed to microbial processes in the flumes. Hence, differences in $k_{\text{net}}\text{-NH}_4^+$ among vegetated treatments suggest differential extents of the relative contribution of either nitrification (NH_4^+ uptake) or denitrification ($\text{NO}_3^- + \text{NO}_2^-$ uptake) to N cycling. Macrophytes can influence the predominance of nitrification over denitrification and vice versa by root-mediated oxygen diffusion into sediments and root release of carbon exudates, respectively.^{4, 47} Our finding that in flumes with *Schoenoplectus* and *Iris*, positive net NH_4^+ uptake co-occurs with negative net $\text{NO}_3^- + \text{NO}_2^-$ uptake suggests that nitrification is a relevant N cycling process. This result coincides with studies showing higher root-mediated oxygen release by *Schoenoplectus* than by *Phragmites* roots,⁴⁸ conditions that favor nitrification since it is a predominantly aerobic process. However, oxygen release in the rhizosphere of *Iris* has been found lower than in *Phragmites*,⁴⁹ indicating that net NH_4^+ uptake might be ascribed to other processes than nitrification, such as microbial assimilation, or anaerobic NH_4^+ oxidation (anammox). In addition to high efficiency for NH_4^+ retention, *Schoenoplectus* flumes showed the highest gross uptake of NH_4^+ as well, indicating high retention capacity for this N form. Together, these results point at *Schoenoplectus* as a species appropriate for management of freshwater ecosystems receiving high N loads.

Combined results from net and gross uptake evidence differential pathways of nutrient retention and cycling among the studied macrophyte species. For instance, flumes with *Phragmites* showed high $k_{\text{gross}}\text{-NH}_4^+$ and low $k_{\text{net}}\text{-NH}_4^+$, revealing high release of this N form back to the water column. Similar conditions of low net nutrient retention, but high retention capacity have been previously observed in headwater streams.²⁷ In contrast, flumes with *Iris* were net sinks of NH_4^+

despite showing low $k_{\text{gross-NH}_4^+}$, similar to those in Control flumes. This pattern suggests that release processes were relatively low in *Iris* flumes. Net uptake of SRP in flumes with *Iris* and *Phragmites* was close to zero, despite these two treatments showed differences in gross uptake. This result indicates that nutrient retention capacity associated with the two macrophytes species is different, yet their net influence on nutrient export is equally low. To our knowledge, there are no previous studies using nutrient spiraling metrics (e.g. k_{net} , k_{gross}) to assess the influence of macrophytes on nutrient transport along subsurface freshwater flowpaths. Therefore, we do not have previous data to compare our results with. However, some studies reporting the efficiency of these macrophyte species to retain nutrients, estimated by a mass balance approach between inflow – outflow nutrient loads, have contrasting results in relation to the relative differences in net and gross uptake among the treatments observed in our study.^{42, 50, 51} We attribute these differences to the temporal variability in nutrient uptake, due to the variability in plant and microbial activity, suggesting that an estimation of the temporal variability of net and gross uptake could contribute to better characterize the role of macrophytes species in nutrient retention in freshwater ecosystems.⁵² Yet, this study highlights that nutrient uptake processes in subsurface water associated with presence of macrophytes can be of equal importance to release processes, which would result in negligible influence of these plants on nutrient export. Therefore, it is important to consider both net and gross nutrient uptake for an accurate assessment of the contribution of macrophytes to the performance of water treatment systems.

4.2 Influence of hydrology on nutrient retention

Consistent to our hypothesis, root-architecture induced differences in subsurface solute transport¹⁸ had a significant influence on both net and gross nutrient retention of NH_4^+ and SRP. Nevertheless, solute transport had a stronger influence on gross than on net nutrient retention. This

could be explained by the different biogeochemical processes involved in net and gross uptake. While gross uptake of NH_4^+ and SRP includes both assimilatory and dissimilatory uptake process leading to removal of nutrients from water, net uptake includes additional processes that release nutrients to water (e.g. remineralization, desorption). Each biogeochemical process involved in net nutrient uptake is controlled by complex interactions among different factors that affect the bioreactivity of the system, such as the physiology of plants and microbial assemblages, the physical and chemical characteristics of the substrate, nutrient and organic matter availability, redox conditions, light, temperature, and hydrological retention.⁵³ Since gross uptake is not influenced by nutrient release processes that can take place over time scales beyond the duration of the solute injections,³⁴ the influence of hydrology on gross uptake is stronger than that observed for net uptake. However, our findings also suggest that hydrological retention associated with different macrophytes species is not a relevant factor controlling the interplay between uptake and release processes of $\text{NO}_3^- + \text{NO}_2^-$ along the flumes. In other words, the increase in $\text{NO}_3^- + \text{NO}_2^-$ uptake that hydrological retention may cause is not enough to determine the predominance of uptake over release processes; and thus, ultimately $\text{NO}_3^- + \text{NO}_2^-$ export. Overall, our results indicate that hydrological retention influence the NH_4^+ and SRP uptake in the study treatments, with higher uptake in flumes with higher hydrological retention. Given that species-specific root architecture can influence hydrological retention, this plant trait could be used as a surrogate to infer the potential of macrophytes to contribute to NH_4^+ and SRP retention from the water column.

This study highlights differential nutrient retention performance among systems with different macrophyte species and provides mechanistic understanding of differences in their performance. These findings can guide the implementation of best management practices for stormwater management aiming at water quality improvement as well as for tertiary treatment of effluent water

from WWTP. In particular, biofiltration systems (e.g. wet vegetated swales), a common stormwater management practice targeting pollution retention, are expected to address the episodic nutrient loading associated with stormwater runoff. In this context, the use of macrophyte species that effectively enhance nutrient retention can contribute to buffer excess nutrient inputs to freshwater ecosystems. Additionally, in recent years, the hyporheic zone (i.e. the biogeochemically active zone of the streambed beneath or next to the main channel, where surface water mixes with groundwater), has been identified as a critical zone to be restored to address stream impairment.⁵⁴ Yet, there are few restoration practices that aim at modifying the streambed to change solute transport along the hyporheic zone.^{55, 56} The present study complements our previous findings by showing that different macrophyte species do not only influence differently hydrological subsurface flows,¹⁸ but also that subsurface hydrological flowpaths, especially through rhizospheres from aquatic macrophytes, can influence NH_4^+ and SRP uptake. Therefore, the ability of macrophytes to harness the hyporheic zone should be considered in future restoration practices to select appropriate species that favor nutrient retention.

AUTHOR INFORMATION

Corresponding Author

*E-mail: mnikolakopo@gmail.com

ORCID: Myrto Nikolakopoulou: 0000-0002-4555-4053

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REFERENCES

1. Carpenter, S. R.; Caraco, N. F.; Correll, D. L.; Howarth, R. W.; Sharpley, A. N.; Smith, V. H., Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* **1998**, 8, (3), 559-568.
2. Paul, M. J.; Meyer, J. L., Streams in the urban landscape. *Annual Review of Ecology and Systematics* **2001**, 32, 333-365.
3. Vymazal, J., Plants used in constructed wetlands with horizontal subsurface flow: a review. *Hydrobiologia* **2011**, 674, (1), 133-156.
4. Brix, H., Do macrophytes play a role in constructed treatment wetlands? *Water Science and Technology* **1997**, 35, (5), 11-17.
5. Stottmeister, U.; Wiessner, A.; Kuschik, P.; Kappelmeyer, U.; Kastner, M.; Bederski, O.; Muller, R. A.; Moormann, H., Effects of plants and microorganisms in constructed wetlands for wastewater treatment. *Biotechnology Advances* **2003**, 22, (1-2), 93-117.
6. Brix, H., FUNCTIONS OF MACROPHYTES IN CONSTRUCTED WETLANDS. *Water Science and Technology* **1994**, 29, (4), 71-78.

- 472 7. Reddy, K. R.; Patrick, W. H.; Lindau, C. W., NITRIFICATION-DENITRIFICATION AT
473 THE PLANT ROOT-SEDIMENT INTERFACE IN WETLANDS. *Limnology and Oceanography*
474 **1989**, 34, (6), 1004-1013.
- 475 8. Bratieres, K.; Fletcher, T. D.; Deletic, A.; Zinger, Y., Nutrient and sediment removal by
476 stormwater biofilters: A large-scale design optimisation study. *Water Research* **2008**, 42, (14),
477 3930-3940.
- 478 9. Tanner, C. C., Plants for constructed wetland treatment systems - A comparison of the
479 growth and nutrient uptake of eight emergent species. *Ecological Engineering* **1996**, 7, (1), 59-83.
- 480 10. Dagenais, D.; Brisson, J.; Fletcher, T. D., The role of plants in bioretention systems; does
481 the science underpin current guidance? *Ecological Engineering* **2018**, 120, 532-545.
- 482 11. Brisson, J.; Chazarenc, F., Maximizing pollutant removal in constructed wetlands: Should
483 we pay more attention to macrophyte species selection? *Science of the Total Environment* **2009**,
484 407, (13), 3923-3930.
- 485 12. Shelef, O.; Gross, A.; Rachmilevitch, S., Role of Plants in a Constructed Wetland: Current
486 and New Perspectives. *Water* **2013**, 5, (2), 405-419.
- 487 13. Cheng, X. Y.; Chen, W. Y.; Gu, B. H.; Liu, X. C.; Chen, F.; Chen, Z. H.; Zhou, X. Y.; Li,
488 Y. X.; Huang, H.; Chen, Y. J., Morphology, ecology, and contaminant removal efficiency of eight
489 wetland plants with differing root systems. *Hydrobiologia* **2009**, 623, (1), 77-85.
- 490 14. Gagnon, V.; Chazarenc, F.; Comeau, Y.; Brisson, J., Influence of macrophyte species on
491 microbial density and activity in constructed wetlands. *Water Science and Technology* **2007**, 56,
492 (3), 249-254.

- 493 15. Kyambadde, J.; Kansiime, F.; Gumaelius, L.; Dalhammar, G., A comparative study of
494 *Cyperus papyrus* and *Miscanthidium violaceum*-based constructed wetlands for wastewater
495 treatment in a tropical climate. *Water Research* **2004**, *38*, (2), 475-485.
- 496 16. Lai, W. L.; Wang, S. Q.; Peng, C. L.; Chen, Z. H., Root features related to plant growth
497 and nutrient removal of 35 wetland plants. *Water Research* **2011**, *45*, (13), 3941-3950.
- 498 17. Read, J.; Fletcher, T. D.; Wevill, T.; Deletic, A., Plant Traits that Enhance Pollutant
499 Removal from Stormwater in Biofiltration Systems. *International Journal of Phytoremediation*
500 **2010**, *12*, (1), 34-53.
- 501 18. Nikolakopoulou, M.; Argerich, A.; Drummond, J. D.; Gacia, E.; Marti, E.; Sorolla, A.;
502 Sabater, F., Emergent Macrophyte Root Architecture Controls Subsurface Solute Transport. *Water*
503 *Resources Research* **2018**, *54*, (9), 5958-5972.
- 504 19. Zarnetske, J. P.; Haggerty, R.; Wondzell, S. M.; Baker, M. A., Dynamics of nitrate
505 production and removal as a function of residence time in the hyporheic zone. *Journal of*
506 *Geophysical Research-Biogeosciences* **2011**, *116*.
- 507 20. Valett, H. M.; Morrice, J. A.; Dahm, C. N.; Campana, M. E., Parent lithology, surface-
508 groundwater exchange, and nitrate retention in headwater streams. *Limnology and Oceanography*
509 **1996**, *41*, (2), 333-345.
- 510 21. Webster, J. R.; Mulholland, P. J.; Tank, J. L.; Valett, H. M.; Dodds, W. K.; Peterson, B. J.;
511 Bowden, W. B.; Dahm, C. N.; Findlay, S.; Gregory, S. V.; Grimm, N. B.; Hamilton, S. K.; Johnson,
512 S. L.; Marti, E.; McDowell, W. H.; Meyer, J. L.; Morrall, D. D.; Thomas, S. A.; Wollheim, W. M.,
513 Factors affecting ammonium uptake in streams - an inter-biome perspective. *Freshwater Biology*
514 **2003**, *48*, (8), 1329-1352.

- 515 22. Simon, K. S.; Townsend, C. R.; Biggs, B. J. F.; Bowden, W. B., Temporal variation of N
516 and P uptake in 2 New Zealand streams. *Journal of the North American Benthological Society*
517 **2005**, 24, (1), 1-18.
- 518 23. Argerich, A.; Haggerty, R.; Marti, E.; Sabater, F.; Zarnetske, J., Quantification of
519 metabolically active transient storage (MATS) in two reaches with contrasting transient storage
520 and ecosystem respiration. *Journal of Geophysical Research-Biogeosciences* **2011**, 116.
- 521 24. Gacia, E.; Bernal, S.; Nikolakopoulou, M.; Carreras, E.; Morgado, L.; Ribot, M.; Isnard,
522 M.; Sorolla, A.; Sabater, F.; Marti, E., The role of helophyte species on nitrogen and phosphorus
523 retention from wastewater treatment plant effluents. *Journal of Environmental Management* **2019**,
524 252.
- 525 25. Ribot, M.; Bernal, S.; Nikolakopoulou, M.; Vaessen, T. N.; Cocherro, J.; Gacia, E.; Sorolla,
526 A.; Argerich, A.; Sabater, F.; Isnard, M.; Marti, E., Enhancement of carbon and nitrogen removal
527 by helophytes along subsurface water flowpaths receiving treated wastewater. *Science of the Total*
528 *Environment* **2017**, 599, 1667-1676.
- 529 26. Day-Lewis, F. D.; Singha, K., Geoelectrical inference of mass transfer parameters using
530 temporal moments. *Water Resources Research* **2008**, 44, (5).
- 531 27. von Schiller, D.; Bernal, S.; Sabater, F.; Marti, E., A round-trip ticket: the importance of
532 release processes for in-stream nutrient spiraling. *Freshwater Science* **2015**, 34, (1), 20-30.
- 533 28. Aumen, N. G., CONCEPTS AND METHODS FOR ASSESSING SOLUTE DYNAMICS
534 IN STREAM ECOSYSTEMS. *Journal of the North American Benthological Society* **1990**, 9, (2),
535 95-119.
- 536 29. Keeney, D. R.; Nelson, D. W., *Nitrogen-Inorganic Forms*. Part 2 (2nd ed.) ed.; ASA,
537 SSSA: Madison, WI, 1982.

- 538 30. Baethgen, W. E.; Alley, M. M., A MANUAL COLORIMETRIC PROCEDURE FOR
539 MEASURING AMMONIUM NITROGEN IN SOIL AND PLANT KJELDAHL DIGESTS.
540 *Communications in Soil Science and Plant Analysis* **1989**, 20, (9-10), 961-969.
- 541 31. Murphy, J.; Riley, J. P., A modified single solution method for the determination of
542 phosphate in natural waters. *Analytica chimica acta* **1962**, 27, 31-36.
- 543 32. Mulholland, P. J.; Steinman, A. D.; Elwood, J. W., MEASUREMENT OF PHOSPHORUS
544 UPTAKE LENGTH IN STREAMS - COMPARISON OF RADIOTRACER AND STABLE PO₄
545 RELEASES. *Canadian Journal of Fisheries and Aquatic Sciences* **1990**, 47, (12), 2351-2357.
- 546 33. Marti, E.; Grimm, N. B.; Fisher, S. G., Pre- and post-flood retention efficiency of nitrogen
547 in a Sonoran Desert stream. *Journal of the North American Benthological Society* **1997**, 16, (4),
548 805-819.
- 549 34. Dodds, W. K.; Lopez, A. J.; Bowden, W. B.; Gregory, S.; Grimm, N. B.; Hamilton, S. K.;
550 Hershey, A. E.; Marti, E.; McDowell, W. H.; Meyer, J. L.; Morrall, D.; Mulholland, P. J.; Peterson,
551 B. J.; Tank, J. L.; Valett, H. M.; Webster, J. R.; Wollheim, W., N uptake as a function of
552 concentration in streams. *Journal of the North American Benthological Society* **2002**, 21, (2), 206-
553 220.
- 554 35. Merseburger, G. C.; Marti, E.; Sabater, F., Net changes in nutrient concentrations below a
555 point source input in two streams draining catchments with contrasting land uses. *Science of the*
556 *Total Environment* **2005**, 347, (1-3), 217-229.
- 557 36. Wilcock, R. J.; Scarsbrook, M. R.; Costley, K. J.; Nagels, J. W., Controlled release
558 experiments to determine the effects of shade and plants on nutrient retention in a lowland stream.
559 *Hydrobiologia* **2002**, 485, (1-3), 153-162.

560 37. Fraley-McNeal, L.; Schueler, T.; Winer, R., National pollutant removal performance
561 database- version 3, Center for Watershed Protection. In Center for Watershed Protection: Ellicott
562 City, MD, 2007; pp 1–10.

563 38. Kilpatrick, F. A.; Wilson, J. F., *Measurement*
564 *of time of travel in streams by dye tracing*. United
565 States Government Publishing Office: Washington,
566 DC, 1989.

567 39. Team, R. C., R: A language and environment for statistical computing. In R Foundation
568 for Statistical Computing: Vienna, Austria, 2016.

569 40. Akrotos, C. S.; Tsihrintzis, V. A., Effect of temperature, HRT, vegetation and porous media
570 on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. *Ecological*
571 *Engineering* **2007**, *29*, (2), 173-191.

572 41. Lai, W. L.; Zhang, Y.; Chen, Z. H., Radial oxygen loss, photosynthesis, and nutrient
573 removal of 35 wetland plants. *Ecological Engineering* **2012**, *39*, 24-30.

574 42. Wu, H. M.; Zhang, J. A.; Li, P. Z.; Zhang, J. Y.; Xie, H. J.; Zhang, B., Nutrient removal in
575 constructed microcosm wetlands for treating polluted river water in northern China. *Ecological*
576 *Engineering* **2011**, *37*, (4), 560-568.

577 43. Ribot, M.; von Schiller, D.; Marti, E., Understanding pathways of dissimilatory and
578 assimilatory dissolved inorganic nitrogen uptake in streams. *Limnology and Oceanography* **2017**,
579 *62*, (3), 1166-1183.

580 44. Kemp, M. J.; Dodds, W. K., The influence of ammonium, nitrate, and dissolved oxygen
581 concentrations on uptake, nitrification, and denitrification rates associated with prairie stream
582 substrata. *Limnology and Oceanography* **2002**, *47*, (5), 1380-1393.

- 583 45. Gersberg, R. M.; Elkins, B. V.; Goldman, C. R., NITROGEN REMOVAL IN
584 ARTIFICIAL WETLANDS. *Water Research* **1983**, *17*, (9), 1009-1014.
- 585 46. Matheson, F. E.; Nguyen, M. L.; Cooper, A. B.; Burt, T. P.; Bull, D. C., Fate of N-15-
586 nitrate in unplanted, planted and harvested riparian wetland soil microcosms. *Ecological*
587 *Engineering* **2002**, *19*, (4), 249-264.
- 588 47. Clarke, S. J., Vegetation growth in rivers: influences upon sediment and nutrient dynamics.
589 *Progress in Physical Geography* **2002**, *26*, (2), 159-172.
- 590 48. van der Nat, F.; Middelburg, J. J., Seasonal variation in methane oxidation by the
591 rhizosphere of *Phragmites australis* and *Scirpus lacustris*. *Aquatic Botany* **1998**, *61*, (2), 95-110.
- 592 49. Liu, J.; Yi, N. K.; Wang, S.; Lu, L. J.; Huang, X. F., Impact of plant species on spatial
593 distribution of metabolic potential and functional diversity of microbial communities in a
594 constructed wetland treating aquaculture wastewater. *Ecological Engineering* **2016**, *94*, 564-573.
- 595 50. Korboulewsky, N.; Wang, R. Y.; Baldy, V., Purification processes involved in sludge
596 treatment by a vertical flow wetland system: Focus on the role of the substrate and plants on N and
597 P removal. *Bioresource Technology* **2012**, *105*, 9-14.
- 598 51. Ryciewicz-Borecki, M.; McLean, J. E.; Dupont, R. R., Nitrogen and phosphorus mass
599 balance, retention and uptake in six plant species grown in stormwater bioretention microcosms.
600 *Ecological Engineering* **2017**, *99*, 409-416.
- 601 52. Feijoo, C.; Giorgi, A.; Ferreira, N., Phosphate uptake in a macrophyte-rich Pampean
602 stream. *Limnologia* **2011**, *41*, (4), 285-289.
- 603 53. Marce, R.; von Schiller, D.; Aguilera, R.; Marti, E.; Bernal, S., Contribution of Hydrologic
604 Opportunity and Biogeochemical Reactivity to the Variability of Nutrient Retention in River
605 Networks. *Global Biogeochemical Cycles* **2018**, *32*, (3), 376-388.

- 606 54. Hester, E. T.; Gooseff, M. N., Moving Beyond the Banks: Hyporheic Restoration Is
607 Fundamental to Restoring Ecological Services and Functions of Streams. *Environmental Science*
608 *& Technology* **2010**, *44*, (5), 1521-1525.
- 609 55. Herzog, S. P.; Higgins, C. P.; McCray, J. E., Engineered Streambeds for Induced
610 Hyporheic Flow: Enhanced Removal of Nutrients, Pathogens, and Metals from Urban Streams.
611 *Journal of Environmental Engineering* **2016**, *142*, (1), 10.
- 612 56. Koryto, K. M.; Hunt, W. F.; Page, J. L., Hydrologic and water quality performance of
613 regenerative stormwater conveyance installed to stabilize an eroded outfall. *Ecological*
614 *Engineering* **2017**, *108*, 263-276.

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616