The role of helophyte species on nitrogen and phosphorus retention from wastewater treatment plant effluents

Esperança Gacia^{a*}, Susana Bernal^{a,b}, Myrto Nikolakopoulou^b, Ester Carreras^a, Laura Morgado^a,
Miquel Ribot^a, Manel Isnard^c, Albert Sorolla^d, Francesc Sabater^b, Eugènia Martí^a

*Corresponding address: gacia@ceab.csic.es

^a Integrative Freshwater Ecology Group, Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Ctra.
Accés Cala Sant Francesc 14, 17300 Blanes, Catalonia, Spain.

^b Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals (BEECA), Universitat de
Barcelona, Barcelona, Catalonia, Spain.

^c Consorci Besòs-Tordera, Granollers, Catalonia, Spain,

^d Naturalea S.L., Castellar del Vallés, Catalonia, Spain.

Keywords: helophytes, WWTP_effluent, nitrogen, phosphorus, biomass standing stock,
nutrient retention

Abstract

In the Mediterranean region, water scarcity compromises stream water quality particularly downstream of wastewater treatment plants (WWTP). We tested the potential of four helophyte species to reduce dissolved inorganic nitrogen (N) and phosphorus (P) from WWTP effluents. We conducted an 11-month mesocosm experiment to assess differences in N and P content among plant compartments and among species. Moreover, we quantified the relative contribution of above and belowground parts of the plants to N and P retention. The experiment was conducted at the Urban River Laboratory (www.urbanriverlab.com) in artificial channels (12 m long x 0.6 m wide x 0.4 m deep) planted with monospecific stands of *Iris pseudoacorus*, *Typha angustifolia*, *Phragmites australis* and *Scirpus lacustris*. Channels (three replicates per species) received water from the WWTP effluent, which flowed at a constant rate of 5 L min⁻¹ through the sub-surface. The helophytes were planted in November 2014 and biomass standing stocks of carbon (C), N and P were measured in October 2015 at the time of maximum plant biomass. Differences in the concentration of N and P were larger among plant compartments than among species. The highest N concentration was measured in leaves while rhizomes showed the highest P concentration. The total plant biomass varied greatly among species from 11.4 to 4.6 Kg DW m⁻² for *Iris* and *Scirpus*, respectively. *Iris* accumulated the highest amount of N (256 g N m⁻²) and P (27 g P m⁻²) in biomass. Plants retained from 8% (*Scirpus*) to 19% (*Iris*) of total dissolved inorganic N inputs to the channels (10.4 kg N) during the experiment, and from 6% (*Phragmites*) to 14% (*Iris*) of total dissolved inorganic P inputs (1.3 Kg P). This study provides quantitative evidence to water managers of the potential role of helophytes to improve water quality in freshwater ecosystems receiving water from WWTP effluents.

Keywords: helophytes, wastewater treatment plant effluent, nitrogen, phosphorus, biomass standing stock, nutrient retention.

1. Introduction

In the heavily populated areas of the Mediterranean region, high water demand and low water resources often results in water scarcity particularly during the summer period. Under these conditions, waste water treatment plant (WWTP) effluents can have a strong impact on the chemistry of the receiving streams, which may not achieve the ecological standards established by the Water Framework Directive (2000/60/EC; WFD), even though water quality of WWTP spillages is within the legal standards (Directive 91/271/CEE). Thus, the degradation of stream water quality in urbanized areas is an increasing environmental problem that needs to be urgently addressed in the Mediterranean region.

The capacity of WWTP depuration technologies to reduce nutrient inputs to receiving freshwater ecosystems can be improved for example with membrane bioreactors (MBR; Sepehri and Sarrafzadeh 2018) but at higher economic and environmental costs (Carey and Migliaccio, 2009; Martí et al., 2009). The implementation of nature-based solutions, such as green bioengineered infrastructures in streams impacted by WWTP effluents can promote their self-depuration capacity, and thus, contribute to improve water quality. Emergent aquatic macrophytes have long been used as secondary and tertiary treatment strategies in WWTP because they can improve the quality of outflow waste waters. This function relies on both macrophytes that take up and store elemental nutrients (Sistla et al., 2015) and microbiota at the plant-sediment interface which can also process nutrients (i.e. González-Alcaraz 2013). For macrophytes, the assimilation and storage of nutrients can differ between plant compartments depending on their physiology and growth rate, and also among species depending on plant architecture. For microbiota, nutrient uptake depends on a complex set of environmental conditions as well as on plant-microbial interactions (i.e. González-Alcaraz et al., 2013).

The efficiency of artificial wetlands on retaining nutrients is usually assessed by conducting mass balance calculations of the input-output flowing water (i.e. Sim et al., 2008; Ko et al., 2011; Ilyas and Masih 2017). Yet, there is little understanding of the relative contribution of macrophytes and microbiota to nutrient retention. Moreover, there is little information on how different the potential for nutrient retention is among macrophyte species, or between aboveground and belowground plant compartments. Although several studies have shown that macrophytes can retain substantial amounts of nitrogen (N), less is known about phosphorus (P) retention, and particularly, about the balance between P uptake and release in highly P loaded waters (Halbedel, 2018). In addition, WWTP effluent waters have a rather unbalanced nutrient stoichiometry which may also affect the nutrient processing capacity of biota (Carey and Migliaccio, 2009). These gaps of knowledge limit our ability to provide scientific criteria to water administration agencies and environmental engineers; and thus, hamper the use of this type of green infrastructure for improving stream water quality in urbanized landscapes. Therefore, further research is needed for assessing the potential for nutrient retention of different nature-based solutions such as helophytes which could be used as waste water treatments.

The aim of this study was to assess the contribution of four Mediterranean helophyte species to N and P retention in highly nutrient loaded waters such as WWTP effluents. To do so, we quantified the standing stocks of carbon (C), N and P in biomass for different plant compartments at the time of maximum biomass accumulation. Moreover, we explored differences in C: N: P stoichiometry among different plant compartments and helophyte species. Finally, we estimated the potential for N and P retention for each species by combining measurements of standing stocks with inputs of dissolved inorganic N and P from the WWTP effluent. We focused on autochthonous helophyte species typically used in stream restoration for landscape conditioning and riverbed stabilization in order to assess their potential for improving water quality in urban streams.

2. Materials and Methods:

2.1 Experimental set-up

The study was conducted at the Urban River Lab (URL; <https://urbanriverlab.com/es/>) an outdoor research facility built within the WWTP of Montornés del Vallès in Catalonia, Spain (41°32'31.6"N 2°14'09.2"E). The facility consists of 18 concrete channels measuring 12 m long, 0.6 m wide and 0.4 m deep (Fig. 1). The channels are flat (zero slope), contain a 25 cm thick end-to-end gravel bed of commercial coarse granitic sediment (average particle size = 40 mm), and are fed with water from the WWTP effluent (Ribot et al., 2017).

The four different helophyte species were planted monospecific in triplicated channels (12 experimental channels in total). Water from the WWTP effluent flowed sub-superficially through the gravel bed interstices maintaining the water level at approximately 2 cm below the surface (~23 cm deep), with an unsaturated surface gravel layer). Inflow rates were kept constant in all channels at $5.02 \pm 0.01 \text{ L min}^{-1}$. The helophyte species were: *Iris pseudacorus* L. (common yellow flag, hereafter as *Iris*), *Typha angustifolia* (common cattail, hereafter as *Typha*), *Phragmites australis* L. (common reed, hereafter as *Phragmites*), *Scirpus lacustris* L. (common bulrush, hereafter as *Scirpus*). The four species are perennial, and are commonly used for riverbank stabilization in constructed wetlands (Vymazal, 2011). The species differ in their root system architecture and the morphologic characteristics of roots and rhizomes (Nikolakopoulou et al., 2018). All channels were planted with sprouts, obtained from a nursery, on November 2014 at a density of $6.7 \text{ shoots m}^{-2}$ (50 shoots per channel).

2.2 Field sampling, sample processing, and laboratory analysis

In October 2015, eleven months after plantation, we collected a representative sample of the gravel bed with a size of 20 cm long x 60 cm wide x 25 cm depth (making a total volume of 30 L). The sample was collected from each flume at 6 m from the inlet. The sampling time

corresponded to the time of maximum aboveground biomass development. From each sample, we sorted the different plant compartments: leaves, stems (only for *Phragmites*), rhizomes and roots. Spikes were also sampled for all species but *Iris*. For each compartment and species, we measured (i) the total fresh biomass with a domestic scale, and (ii) the dry weight of three subsamples which were carried to the lab and dried at 60 °C until constant weight. The wet to dry ratio was used to infer the dry biomass of the sample taken. Samples were properly wiped before being dried except for spikes. The obtained values were scaled to m^{-2} and to the total surface area of each channel (7.2 m^2).

Subsamples of dry biomass were powdered with a machine grinder (Retsch RM200) and further processed for elemental analysis. For C and N content determination, 2.5 – 3.0 mg of sample were encapsulated in tin capsules and subsequently analyzed at the Serveis Científics Tècnics de la Univ. de Barcelona (SCT) with a Perking Elmer CHNS 2400 elemental analyzer. For P analysis, three replicated samples of 50 – 100 mg were wet digested in 60 ml Teflon vessels with 2 ml of a 3:1 acid mixture of nitric and perchloric acid (HNO_3 : HClO_4) under microwaves at high-pressure (17 min, 600W). After cooling, the obtained extracts were diluted with HNO_3 1% to 10ml. Phosphorus content was determined by inductively coupled plasma – mass spectrometry (ICP-MS) at the SCT. A total of ten blanks and four samples of certified reference material (Rye Grass ERM®-CD281) were included in the P digestions to check analytical accuracy. Reagents used were Merck EMSURE® ISO and EMSURE® ACS for HNO_3 and HClO_4 , respectively. The standing stock of N and P for each plant compartment and species was calculated by multiplying N or P concentrations by the dry weight, and referred to the area of the channel (i.e., g N or P per m^{-2}). For each plant compartment and species, we calculated the C:N:P molar ratios (elemental stoichiometry) in order to explore the potential for nutrient accumulation from a more functional perspective.

We collected water samples at the entrance of the channels by using a plastic syringe. All samples were immediately filtered through ashed Whatman GF/F glass fiber filters (0.7 μm pore size) and 10 mL aliquot was placed in a Falcon tube and stored frozen until analyzed. We measured nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and soluble reactive phosphorus (SRP) with standard colorimetric methods (Apha and WPCF, 1995) on an Automatic continuous Flow Futura-Alliance Analyzer at the Nutrient Analysis Service of the CEAB-CSIC. We estimated the total input of dissolved organic N and SRP received by each channel over the course of the experiment by multiplying monthly average concentrations times subsurface flow (5 L min^{-1}). We estimated the capacity for N and P retention of each plant species by comparing total nutrient inputs and estimated N and P standing stock in each channel.

2.3. Statistical analysis

Differences in N and P concentration and N:P elemental ratios among species and compartments (leaves, stems, spikes, rhizomes, and roots) were tested with a two-factorial ANOVA. Whenever the interaction among the two factors (species and compartment) was significant, each test was run separately with one-way ANOVA. Differences among groups were tested by using Tuckey multiple comparisons. Data were log transformed to fulfill ANOVA requirements when needed.

3. Results:

3.1. Nitrogen and phosphorous concentration in plant compartments of helophyte species

Concentration of N in the different plant compartments of the four helophytes ranged from 1.3 to 2.9 % of the dry weight (Table 1). The coefficient of variation (CV) of N concentration considering all data together was 32.7 %. Differences in N concentration among compartments were statistically significant; leaves had the highest N concentration while roots had the lowest (Table 2). Among species, average N concentration was

significantly higher in *Phragmites* than in the other species (Table 2). The P concentration in the four helophytes ranged from 2.1 to 3.3 mg P g DW⁻¹ and the CV of all samples was 37.5 %. Differences in P concentration were not consistent among plant compartments for the different species, though roots showed lower P concentration than rhizomes and spikes (Table 1 and Table 2). There were no statistically significant differences in the average P concentration among species (Table 2).

Considering data from all species together, the N:P ratio was higher in leaves than in the other compartments as a consequence of the higher N concentrations (Figure 2). The leaves of *Phragmites* and *Typha* showed the highest and lowest N:P ratios, respectively (Table 2).

3.2. Biomass and nutrient standing stocks of helophyte species

The total biomass of helophytes at the end of the experiment varied from 11.4 to 4.6 Kg DW m⁻² for *Iris* and *Scirpus*, respectively (Fig. 3). Given that the initial shoot size was similar among species, and densities were the same in all channels, this result indicates that *Iris* stored between 2- and 3-fold more biomass than the other three species. For all species, leaves contributed the most to total plant biomass (Fig. 3), accounting for 50 to 70 % of the total dry weight, followed by stems in the case of *Phragmites* (21%), and rhizomes which accounted for 11 to 38% of total plant biomass. Roots accounted for 9 to 28% of total plant biomass (Fig. 3).

Iris accumulated the highest amount of N (258 g N m⁻²) and P (27 g P m⁻²) compared to the other three species (Figures 4a and b) because it was the species that accumulated the largest biomass (Figure 3). The lowest N standing stock was measured in *Scirpus* (107 g N m⁻²) while the lowest P standing stock was measured in *Phragmites* (10.3 g P m⁻²). Leaves stored the highest amount of nutrients compared to the other plant compartments, and accounted for 55 to 75% of the total N standing stock and from 40 to 65% of the total P standing stock

depending on the species. Rhizomes accounted for up to 40% of the total P standing stock in *Typha* (Fig. 4a, b). Both, *Iris* and *Typha* showed the highest rhizome N and P standing stock.

3.3. Nitrogen and phosphorus retention: balance across channels.

Input loads of dissolved inorganic N and SRP over the course of the experiment (Table 3) equaled 10.40 Kg N and 1.34 Kg P. This amount represented 7.7% and 18.5% of the N stored in *Scirpus* and *Iris*, respectively (Table 4). The P standing stock represented from 5.7% to 14.9 % of the input P load for *Phragmites* and *Iris*, respectively (Table 4).

4. Discussion

The mesocosm facility at the Urban River Lab allowed a synchronic comparison of nutrient concentrations and retention in four helophyte species (*Iris pseudacorus*, *Phragmites australis*, *Scirpus lacustris* and *Typha angustifolia*) grown with WWTP effluent water. Concentrations of N and P were more variable among plant compartments (i.e. leaves, rhizomes and roots) than among species even though they belong to different families. These results indicate that functional differences are more important than inter-specific differences among these plant species when it comes to nutrient concentration in tissues. The N concentration was high in leaves compared to the other compartments because of the higher proportion of proteins in the chloroplasts (Berendse et al. 2007). Rhizomes were enriched in P, which could be explained by the accumulation of P in vacuoles (Yang et al., 2017). In contrast, roots were poor in both nutrients which is in line with the idea that plant growth was slow at the time of collection (Sardans et al., 2012). Spikes were rich in P most likely because they contain seeds (Berendse et al. 2007) but also because they act as dust traps (unpublished data from Gacia). Leaves showed the highest variability among species because *Phragmites* was much more enriched in N than the rest of helophytes. Overall, N and P concentration in plant tissues was high and within the range found for plants growing in nutrient enriched waters at luxury consumption [i.e. nutrient absorption in excess of immediate growth requirements; Chapin (1980)], which

confirms that the four helophytes had a high capacity to take up nutrients from the effluent water.

In order to put the obtained results into a broader context, we gleaned data on nutrient concentration from the literature and found that the helophytes at URL were enriched in N compared to those from freshwater marshes, farms and urban runoff (Fansworth and Meyerson, 2003; Gottshall et al., 2007; among others). Moreover, we found that the experimental conditions were also favorable for P retention (i.e. Dierberg et al., 2002; Gottshall et al., 2007). The N/P ratios for *Typha* and *Scirpus* were around the 60th percentile of a global database (Supplementary Material 1) on N and P molar ratios of helophytes, but in favor for N reaching percentiles higher than 80th for *Phragmites* which sustains the low capacity to accumulate P in relation to N in this species. Overall, these results match with the fact that DIN concentrations were especially high [$4.87 \pm 1.94 \text{ mg L}^{-1}$] compared to P concentrations [$0.32 \pm 0.24 \text{ mg L}^{-1}$] in the effluent water entering to the channels.

After eleven month of growth, there were large differences in plant biomass among species: *Iris* reached 3-fold higher biomass than *Scirpus* that was the species with the lowest biomass. This result highlights that growth rates substantially differed between helophyte species, despite similar environmental conditions (also in González-Alcaraz et al., 2013) due in part to different resource acquisition by roots (Lai et al., 2011). The differential capacity of accumulating biomass under equal environmental conditions (i.e. differences in absolute growth rates; Pugnaire and Valladares, 1999) becomes relevant when designing green filters and artificial wetlands since biomass accrual may need to be either promoted (i.e. for gardening or biomass production) or reduced to minimize the maintenance cost of infrastructures such as in hydroponic reactors (Osem et al., 2007) or impervious covers in green roofs (Matos et al., 2019). The comparisons of growth rates and biomass allocation among species from different locations should be done with caution because it depends on

local environmental conditions (Vojtíšková et al., 2004). For instance, the values reported here fall within the medium-to-high range of biomass reported for *Iris* and *Phragmites* (Vymazal, 2011), and thus, could lead to an overestimation of total biomass if extrapolated to other sites with lower nutrient availability. Yet, our estimates are useful for understanding relative differences in biomass accrual among the species considered but further studies are needed to confirm the patterns encountered by considering different composition of waste water and or different geo-climatic conditions for the above mentioned species.

We found that nutrient were mostly stored in leaves because (i) this compartment accounted for a large portion of the total biomass, and (ii) the nutrient content in leaves was relatively high compared to the other compartments. However, leaves from helophytes are easily exported, particularly during storms and floods. Moreover, leaves decompose fast compared to other plant compartments (Březinová and Vymazal, 2018), and thus nutrients in leaves are rapidly released to the water column, usually in less than a year. In contrast, nutrients accumulated in belowground compartments, particularly in rhizomes, represent a long term storage because they are less exposed to export during flood events, this biomass has a slow turnover, and roots decompose slowly (Jonasson and Shaver, 1999). These differences between aboveground and belowground compartments can be essential when choosing the most appropriate helophyte species, depending on how exposed a particular site is to abrupt changes in hydrological conditions.

The N retention in plant biomass at the URL channels (from 8 to 19 % of total dissolved inorganic N inputs) was within the upper range of values reported in the literature (e.g., Lyas and Masih, 2017). Helophytes at URL also retained a relatively high amount of P (from 8 to 14 % of total SRP inputs) compared to other available studies (i.e. Maine et al., 2007; Halbedel, 2018). Our rates are conservative because the mass balances were based on nutrient standing stock and thus, did not account for any potential positive feedback between helophytes and

associated microbiota. Plant-microbes interactions can play a fundamental role on microbial nutrient transformation and retention, as previously shown in waste water treatments with green infrastructures (i.e. González-Alcaraz et al., 2013). Future work should consider these microbial-helophyte interactions, which could influence denitrification and nutrient excretion, and ultimately affect the overall efficiency of green infrastructures on improving the quality of effluent waste water.

Our results are coherent with patterns of N and P retention described in previous studies for some of the considered species. For instance, *Typha* sp. retains higher amounts of N than *Scirpus* sp. (Coleman et al., 2001), although this does not hold when only considering NH_4^+ removal (Finlayson and Chick, 1983). These results confirm the idea that NO_3^- is a relevant component of N uptake by aquatic plants (Peipoch et al., 2014). *Phragmites* is efficient retaining N, but inefficient retaining P compared to *Typha* or *Scirpus* (Tanner, 1996); (Greenway and Woolley, 1999). *Iris* is rarely used in artificial wetlands; however, we have shown that this species has a high capacity to retain N and P compared to other helophyte species because of its high nutrient standing stock.

Nutrient storage and retention reported in this study was based on almost one year of observations, and thus integrate climate variability as well as different periods of the vegetative cycle. In this sense, our estimates are robust and useful for environmental engineers and managers designing green filters and artificial wetlands for waste water treatment. *Iris* is the specie to use if we are to maximize overall nutrient retention while *Phragmites* may be more appropriate if the priority is to remove N from waste water minimizing harvesting costs. *Typha* would be the most appropriate species to promote intermediate levels of both N and P retention, while *Scirpus*, that grows slowly, would be particularly good for retaining P. On the other hand, *Iris* and *Scirpus* would be the best solution

if belowground biomass needs to be promoted, as would happen in locations experiencing abrupt changes in stream discharge.

Conclusions

This study quantifies the amount of N and P inputs from the WWTP effluent retained by four species of helophytes nursed with water from an urban WWTP effluent. *Iris* stored the highest amount of nutrients in both the above and belowground compartments, and thus was the species with the largest capacity to retain nutrients. During the course of the study, *Iris* contributed, to decrease dissolved inorganic N and P inputs by 18.5 % and 14.3%, respectively. The other three species had lower biomass and nutrient standing stocks. Nevertheless, *Phragmites* was efficient in retaining N, while *Scirpus* was capable of retaining substantial amounts of P. We have shown that biomass accrual in above and belowground compartments, nutrient standing stocks, and nutrient stoichiometry can differ among helophyte species growing the same environmental conditions. We have illustrated that these differences are essential when choosing the most appropriate helophyte species for stream and wetland restoration.

Acknowledgements

We acknowledge the help of Adriana Peña, Joaquin Cocherro and Sara Castelar as well as all the students who have joined fieldwork at URL. SB work was funded by NICUS (CGL-2014-55234-JIN) and a Ramon y Cajal fellowship (RYC-2017-22643). This work was funded by MEDSOUL ref. CGL2014-59977-C3-2-R and Eco_REACTOR ref: PGC2018-101975-B-C22 projects and URL facilities maintained under INTERFACES Initial Training Network (FP7-PEOPLE-2013-ITN, grant 607150), Consorci Besòs-Tordera, Ajuntament Montornès del Vallès, and Agència Catalana de l'Aigua. EG, SB, MR and EM are members of Grup de Recerca de Canvis Ambientals GECA (SGR-

DGR), Generalitat de Catalunya, SGR 2017SGR910. 2018-2021 group of excellence. We declare no competing conflicts of interest.

References:

- Berendse, F., de Kroon, H., Braakhekke, W.G., 1999. Acquisition, use, and loss of nutrients. *Handbook of functional plant ecology*, 315-345.
- Březinová, T.D., Vymazal, J., 2018. Evaluation of macrophytes suitable for agriculture drainage treatment with respect to their carbon sequestration potential. *Ecol. Eng.* 124, 31-37.
- Carey, R.O., Migliaccio, K.W., 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: a review. *Environ. Manag.* 44, 205-217.
- Coleman, J., Hench, K., Garbutt, K., Sextone, A., Bissonnette, G., Skousen, J., 2001. Treatment of domestic wastewater by three plant species in constructed wetlands. *Water, Air, and Soil Pollut.* 128, 283-295.
- Dierberg, F., DeBusk, T., Jackson, S., Chimney, M., Pietro, K., 2002. Submerged aquatic vegetation-based treatment wetlands for removing phosphorus from agricultural runoff: response to hydraulic and nutrient loading. *Water Res.* 36, 1409-1422.
- Farnsworth, E.J., Meyerson, L.A., 2003. Comparative ecophysiology of four wetland plant species along a continuum of invasiveness. *Wetlands* 23, 750-762.
- Finlayson, C.M., Chick, A.J., 1983. Testing the potential of aquatic plants to treat abattoir effluent. *Water Res.* 17, 415-422.
- González-Alcaraz, M.N., Conesa, H.M., Alvarez-Rogel, J. 2013. Nitrate removal from eutrophic wetlands polluted by metal-mine wastes: Effects of liming and plant growth. *J. Environ. Manag.* 128: 964-972.
- Gottschall, N., Boutin, C., Crolla, A., Kinsley, C., Champagne, P., 2007. The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater, Ontario, Canada. *Ecol. Eng.* 29, 154-163.
- Greenway, M., Woolley, A., 1999. Constructed wetlands in Queensland: performance efficiency and nutrient bioaccumulation. *Ecol. Eng.* 12, 39-55.
- Halbedel, S., 2018. Secretion of phosphorus by aquatic plants has an impact on their phosphorus uptake budget. The concept of phosphorus recycling. *Front. Env. Sci.* 6, 23.
- Ilyas, H., Masih, I., 2017. The performance of the intensified constructed wetlands for organic matter and nitrogen removal: A review. *J. Env. Manag.* 198, 372-383.
- Jonasson, S., Shaver, G.R., 1999. Within-stand nutrient cycling in arctic and boreal wetlands. *Ecology.* 80, 2139-2150.
- Ko, C.H., Lee, T.M., Chang, F.C., Liao, S.P., 2011. The correlations between system treatment efficiencies and aboveground emergent macrophyte nutrient removal for the Hsin-Hai Bridge phase II constructed wetland. *Bioresour. Technol.* 102, 5431-5437.
- Lai, W.L., Wang, S.Q., Peng, C.L., Chen, Z.H., 2011. Root features related to plant growth and nutrient removal of 35 wetland plants. *Water Res.* 45, 3941-3950.
- Maine, M.A., Sune, N., Hadad, H., Sánchez, G., Bonetto, C., 2007. Removal efficiency of a constructed wetland for wastewater treatment according to vegetation dominance. *Chemosphere* 68, 1105-1113.
- Martí, E., Riera, J.L., Sabater, F., 2009. Effects of wastewater treatment plants on stream nutrient dynamics under water scarcity conditions, in: Sabaté, S., Barceló, D. (Eds), *Water scarcity in the mediterranean*. Springer, Heidelberg, Dordrecht, London, New York, pp. 173-195.

- Matos, C., Sá, A.B., Bentes, I., Pereira, S., Bento, R., 2019. An approach to the implementation of Low Impact Development measures towards an EcoCampus classification. *J. Environ. Manag.*, 232: 654-659.
- Nikolakopoulou, M., Argerich, A., Drummond, J.D., Gacia, E., Martí, E., Sorolla, A., Sabater, F., 2018. Emergent Macrophyte Root Architecture Controls Subsurface Solute Transport. *Water Resour. Res.* 54, 5958-5972.
- Osem, Y., Chen, Y., Levinson, D., Hadar, Y., 2007. The effects of plant roots on microbial community structure in aerated wastewater-treatment reactors. *Ecol. Eng.* 29, 133-142.
- Peipoch, M., Gacia, E., Pastor, A., Ribot, M., Riera, J.L., Sabater, F., Martí, E., 2014. Intrinsic and extrinsic drivers of autotrophic nitrogen cycling in stream ecosystems: Results from a translocation experiment. *Limnol. and Oceanogr.* 59, 1973-1986.
- Pugnaire, F., Valladares, F., 2007. *Functional plant ecology*, second ed. CRC press, Boca Raton, FL.
- Ribot, M., Bernal, S., Nikolakopoulou, M., Vaessen, T.N., Cocherio, J., Gacia, E., Sorolla, A., Argerich, A., Sabater, F., Isnard, M., 2017. Enhancement of carbon and nitrogen removal by helophytes along subsurface water flowpaths receiving treated wastewater. *Sci. Total Environ.* 599, 1667-1676.
- Sardans, J., Rivas-Ubach, A., Penuelas, J., 2012. The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review and perspectives. *Biogeochemistry* 111, 1-39.
- Sepehri, A., Sarrafzadeh, M.-H., 2018. Effect of nitrifiers community on fouling mitigation and nitrification efficiency in a membrane bioreactor. *Chemical Engineering and Processing-Process Intensification* 128, 10-18.
- Sim, C.H., Yusoff, M.K., Shutes, B., Ho, S.C., Mansor, M., 2008. Nutrient removal in a pilot and full scale constructed wetland, Putrajaya city, Malaysia. *J. Env. Manag.* 88, 307-317.
- Sistla, S.A., Appling, A.P., Lewandowska, A.M., Taylor, B.N., Wolf, A.A., 2015. Stoichiometric flexibility in response to fertilization along gradients of environmental and organismal nutrient richness. *Oikos* 124, 949-959.
- Tanner, C.C., 1996. Plants for constructed wetland treatment systems—a comparison of the growth and nutrient uptake of eight emergent species. *Ecol. Eng.* 7, 59-83.
- Vojtíšková, L., Munzarová, E., Votrubová, O., Řihová, A., Juřicová, B., 2004. Growth and biomass allocation of sweet flag (*Acorus calamus* L.) under different nutrient conditions. *Hydrobiol.* 518, 9-22.
- Vymazal, J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. *Hydrobiol.* 674, 133-156.
- Vymazal, J., 2013. Emergent plants used in free water surface constructed wetlands: a review. *Ecol. Eng.* 61, 582-592.
- Yang, S.Y., Huang, T.K., Kuo, H.F., Chiou, T.J., 2017. Role of vacuoles in phosphorus storage and remobilization. *Journ. Exp. Bot.* 68, 3045-3055.

% N	<i>Iris pseudacorus</i>	<i>Typha angustifolia</i>	<i>Phragmites australis</i>	<i>Scirpus lacustris</i>
Leaves	2.44 ± 0.27	2.87 ± 0.71	3.55 ± 0.42	2.63 ± 0.35
Rhizomes	2.19 ± 0.47	1.57 ± 0.03	2.83 ± 0.25	2.53 ± 0.47
Roots	1.42 ± 0.02	1.12 ± 0.03	2.03 ± 0.11	1.44 ± 0.23
Spikes	-	2.42 ± 0.63	2.49 ± 0.10	1.85 ± 0.57
Stems	-	-	1.34 ± 0.37	-
% P				
Leaves	0.22 ± 0.03	0.36 0.05	0.19 ± 0.01	0.25 ± 0.02
Rhizomes	0.31 ± 0.05	0.30 0.06	0.32 ± 0.00	0.41 ± 0.03
Roots	0.17 ± 0.03	0.17 0.02	0.27 ± 0.05	0.17 ± 0.15
Spikes	-	0.46 0.06	0.36 ± 0.06	0.22 ± 0.03
Stems	-		0.22 ± 0.05	-
N/P	24.51 ± 1.41	17.69 ± 4.97	40.78 ± 6.56	23.46 ± 1.73
Leaves	15.83 ± 0.84	20.86 ± 0.26	19.29 ± 1.63	13.61 ± 2.70
Rhizomes	19.06 ± 2.83	15.24 ± 2.16	17.28 ± 2.97	60.09 ± 81.12
Roots	-	20.20 ± 0.62	18.99 ± 0.33	21.60 ± 0.28
Spikes	-	-	14.60 ± 6.21	-
Stems				

Table 1. Average and Standard deviation of the N and P concentration expressed as the percentage of dry weight and N/P molar ratios for the different plant compartments of the helophyte species studied. “-” means data not available.

Variable		dF	MS	F	p	
%N	Intercept	0				
	Sps	2	1.8	12.6	p<0.0001	Phrag > Iris, Scir, Typh
	compart	2	5.6	38.9	p<0.0001	Leav > Steam,Root; Rhiz,Spik > Roots
	Sps*compart	8	0.3	2.3	0.0504	
	Error	30	0.1			
%P		dF	MS	F	p	
	Intercept	1	3.8	466.0	p<0.0001	
	compart	3	0.03	4.1	p<0.0001	Root < Rhiz, Spik
	Error	41	0.01			
		dF	MS	F	p	
N/P	Intercept	1	3.6	382.0	p<0.0001	
	Sps	3	0.010	1.7	0.19	
	Error	41	0.001			
		dF	MS	F	p	
	Intercept	1	17593.0	532.0	p<0.0001	
	compart	3	288.6	8.7	p<0.0001	Leav > Rhi, Root
	Error	41	33.0			
		dF	MS	F	p	
	Intercept	1	17518.0	411	p<0.0001	
	Species	3	158.4	3.7	p<0.05	Phrag > Scir, Typh
	Error	41	42.5			

Table 2. Summary of the Two way ANOVA to assess for differences in the nitrogen (%N) and phosphorous (%P) concentration, and in N/P molar ratios among species: *I. pseudoacorus* (Iris), *T. angustifolia* (Typh), *P. australis* (Phrag) and *S. lacustris* (Scir), and compartments; Leaves (Leav), Rhizomes (Rhi), Roots (Root) and Spikes (Spik). For the variables %P and N/P we present independent one-way ANOVA for each factor because the interaction among species and compartments was significant.

	NH ₄ mg N L ⁻¹	NO ₃ mg N L ⁻¹	DIN mg N L ⁻¹	PO ₄ mg P L ⁻¹	ALK meq L ⁻¹
average	0.78	3.27	4.16	0.55	339.1
SD	0.50	1.03	1.25	0.64	157.5
max	1.87	6.59	7.30	1.71	645
min	0.21	1.37	1.71	0.07	0

Table 3. Chemical composition of the water feeding the experimental channels. Values correspond to the monthly average, standard deviation (SD), and range of concentrations of dissolved inorganic nitrogen (DIN) forms (mg N L⁻¹) and phosphate (mg P L⁻¹) of WWTP effluent water during the study period. Values of alkalinity (ALK; meq CaCO₃ L⁻¹) correspond to data from the river just before the reception of the WWTP effluent.

	N retention (% input)	P retention (% input)
<i>Iris pseudacorus</i>	18.5 ± 1.2	14.9 ± 1.7
<i>Typha angustifolia</i>	10.5 ± 2.3	12.4 ± 2.7
<i>Phragmites australis</i>	10.0 ± 6.8	5.7 ± 2.6
<i>Scirpus lacustris</i>	7.7 ± 3.9	7.9 ± 3.3

Table 4. Average ± standard deviation as percentage of dissolved nutrient loads from the WWTP effluent retained by each helophyte species in relation to input integrated for the overall duration of the experiment.

Figure 1. Aerial view of the Urban River Laboratory (www.urbanriverlab.com) in Montornès del Vallès (Barcelona; Spain), which shows the experimental channels planted with the different studied helophyte species.

Figure 2. Variation in nitrogen vs. phosphorous molar ratio (N: P) among the different plant compartments considering data from the four helophytes species together. Boxes encompass 25 and 75% of the data variability and the line represents the median. Whiskers represent the 95% confidence interval. * Indicates significant differences at $p < 0.01$.

Figure 3. Biomass standing stocks (in kg DW m⁻²) of the four helophyte species at the end of the experiment (October 2016). The graph also shows the relative contribution of each compartment to the overall plant biomass, indicated by different colors in each stacked bar and expressed as percentage of total plant biomass. *Iris*= *Iris pseudoachurus*, *Typha*= *Typha angustifolia*, *Phragmites*= *Phragmites australis*, and *Scirpus*= *Scirpus lacustris*.

Figure 4. Average standing stocks for (A) nitrogen (N, in gN m^{-2}) and (B) phosphorous (P, in gP m^{-2}) for the four helophyte species at the end of the experiment (October 2016). The graphs also show the relative contribution of each compartment to the total N and P in plants, indicated by different colors in each stacked bar. Numbers show the percentage of total N and P associated with above and below ground biomass. *Iris*= *Iris pseudoachurus*, *Typha*= *Typha angustifolia*, *Phragmites*= *Phragmites australis*, and *Scirpus*= *Scirpus lacustris*.