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URL

Nutrient uptake capacity of three riparian species under intermittent water conditions

May-July 2024

1. Introduction

1.1 Introduction

In the Mediterranean region, intermittent rivers are especially vulnerable to pollution from wastewater treatment plant (WWTP) effluents, which, despite complying with the regulation, significantly disrupt water quality and stream ecology due to water scarcity conditions (Martí, Riera and Sabater, 2009). These effluents introduce high levels of dissolved organic carbon (DOC), dissolved inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP), leading to nutrient saturation conditions for stream communities and further degrading water quality. Additionally, contaminants of emerging concern from effluents pose unknown risks to stream ecosystems, with fluctuating seasonal flows complicating the management of nutrient loads (Castelar et al., 2022).

The European Water Framework Directive (WFD) underscores the importance of achieving good ecological status in all watercourses across Europe, driving the need for innovative strategies to manage urban pollutants. Moreover, there is growing criticism of traditional sewer systems for their inflexibility in adapting to future climate and urbanization challenges (Zhou, 2014).

Nature-Based Solutions (NBS) have become central in addressing urban challenges related to climate resilience, human and ecosystem, and well-being. In urban areas, NBS are increasingly recognized for their potential to mitigate climate change impacts, enhance ecosystem, and improve life quality through Green Infrastructure projects like green roofs, rain gardens, and constructed wetlands (Eggermont et al., 2015; IUCN, 2016). In addition, NBS emphasize a multifunctional strategy that integrates natural processes into urban water management, improving water quality, enhancing biodiversity, and providing social and recreational benefits (Fletcher et al., 2015; Raymond et al., 2017).

We investigated the temporal patterns and controlling factors of nutrient uptake along a subsurface flow experimental system fed with WWTP effluent. More specifically, we assessed the capacity of 3 riparian plant species (*Iris pseudacorus*, *Cornus sanguinea*, and *Alnus glutinosa*) to remove nutrients, which were subjected to an intermittent hydrological regime. This research is part of the Riparian Ecorest project (PID2022-141330OB-I00), which explores sustainable management strategies using Nature-Based Solutions (NBS) to reduce solutes from WWTP effluents in receiving watercourses.

1.2 Material and methods

The experiment was conducted at the Urban River Lab (URL; www.urbanriverlab.com), an open-air laboratory consisting of 12 artificial flumes which allow the simulation of subsurface flow stream conditions. Each flume measures 12m long, 60cm wide and 40cm deep and it is fed by treated wastewater from the Montornès del Vallès WWTP. The flumes were filled with 2.88 m^3 of heterogeneous granitic granular medium. The substrate had a well-calibrated particle size range of 0.063 to 40mm, an estimated density of 1.59 gr m^{-3} and an estimated porosity of 35% to 45% that could influence water infiltration and hydraulic conductivity (Diaz-Curiel et al., 2022). Water from the WWTP effluent was fed through each flume at a constant flow rate of 3L m^{-1}, with at least one week without flow between sampling times to simulate intermittency. Each flume was randomly assigned one of the four treatments consisting of plants with different structure: herbaceous, shrub, and tree, and a control with no vegetation, which served to evaluate the nutrient uptake capacity without the influence of plants and associated microbial assemblages in the rhizosphere. The four treatment groups, with three replicates each, were as follows:

- Treatment 1: Control (no vegetation).
- Treatment 2: Herbaceous vegetation (*Iris pseudacorus*; yellow iris).
- Treatment 3: Shrub vegetation (*Cornus sanguinea*; dogwood).
- Treatment 4: Tree vegetation (*Alnus glutinosa*; black alder).

Sampling was conducted monthly from May to July 2024 to capture temporal variation in ammonium (NH_4^+), nitrate (NO_3^-) soluble reactive phosphate (SRP), DOC and total dissolved nitrogen (TDN) (mg L^{-1}) concentration and physicochemical parameters such as conductivity (mS cm^{-1}), water temperature (ºC), dissolved oxygen concentration and its percentage (mg L^{-1} and %), and pH. Climatological conditions were within historical averages, though June experienced lower-than-average precipitation, and July temperatures approached historical highs (Meteoblue, 2024). Early biofilm growth in the flume inlets required modification of the inlet design to ensure proper subsurface flow. To assess nutrient retention capacity, water samples were taken at the inlet and outlet of the flume. Samples from the outlet of the flume were taken later than samples from the inlet of the flume. The time difference in sample collection between the inlet and the outlet corresponded to the average water travel time between these two sites (Nominal Travel Time, NTT).

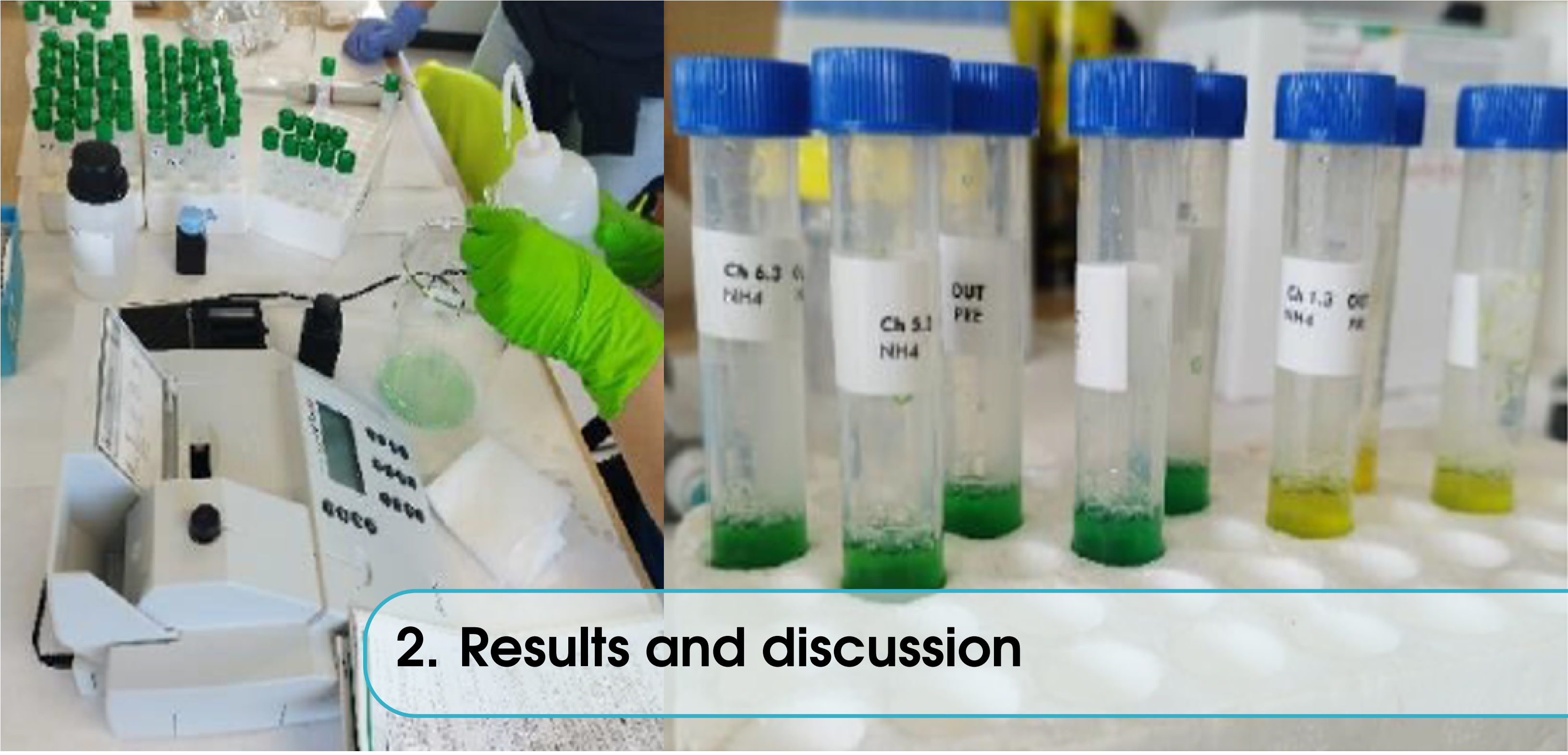
NTT was determined for the 12 flumes before each of the 3 samplings using slug additions of salt (NaCl) serving as a hydrological tracer. NaCl was added at the inlet of the flume and conductivity was continuously recorded at the outlet with a conductivity meter (WTW 3310). We used the chloride breakthrough curves to estimate NTT, which was the time needed to reach the peak of the curve. For each sampling and in each flume, the collection of water samples at the inlet and outlet of the flume was repeated for three consecutive hours. The samples were filtered with a 0.7 µm glass fiber filter in vials and the concentration of NH_4^+ and SRP was analyzed using a colorimetric method with a field spectrophotometer, according to the protocol established for each nutrient. The NO_3^- concentration was analyzed at the Department of Evolutionary Biology, Ecology and Environmental Sciences of the University of Barcelona using the standard colorimetric method with an autoanalyser. Water samples for DOC and TDN analyses were spiked with 4 drops of 10% chloridric acid and concentrations were analysed in the labqa service of the CEAB-CSIC using a total organic carbon (TOC) analyser. The rest of the physicochemical parameters were measured once on each sampling date, both at the inlet and outlet of the flume, using a WWT multiparametric sensor.

The nutrient retention capacity in each flume was calculated using the following equation:

$$\% \; Retention \; = \frac{(Conc \; IN) - (Conc \; OUT)}{Conc \; IN} \; x \; 100$$

where Conc IN and Conc OUT are the nutrient concentrations at the inlet and outlet of the flume, respectively.

Positive values indicate a decrease in nutrient concentration at the outlet of the flume, thus a net retention of the nutrient (retention > release). Negative values, on the other hand, indicate an increase in nutrient concentration at the end of the flume, thus a net release of nutrients (retention < release).



2. Results and discussion

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Data from NaCl additions indicated that average NTT gradually increased over time (from May to July). Average NTT for all flumes was lowest in May (2.50±0.14 hours), rising to 3.08±0.22 hours in June, and reaching its maximum in July (4.91±0.51 hours). These results could be explained by the development of both biofilm assemblages on the sediment surface and plant root system which may decrease free interstitial sediment space, thus making more difficult the water circulation along the flume. Among treatments, *Cornus sanguinea* exhibited the shortest mean NTT (2.50 hours); while *Alnus glutinosa* had the longest NTT (3.69 hours), slightly above the unvegetated flumes (3.50 hours) and *Iris pseudacorus* (3.43 hours). Differences in NTT among plant treatments may be related to their specific root architecture (Nikolakopoulou et al. 2018).

Average water temperature steadily increased over the sampling period, both at the inlet and outlet. Conductivity also consistently increased over time, peaking in July, particularly at the outlet, suggesting a potential accumulation of dissolved salts along the flumes due to an increase in evapotranspiration. Concentration of dissolved oxygen was consistently very low along the flumes indicating hypoxic conditions (< 2mg O_2 L^-). In addition, we observed an increase in hypoxia over the study period and along the flumes on each sampling date (Table 1). Decrease was more pronounced at the outlet, indicating a net dissolved oxygen consumption within the flumes. PH remained relatively stable throughout the experiment, with minor fluctuations (Table 1).

Month	IN/OUT	Conductivity ($mS\ cm^{-1}$)	T°C	O_2 ($mg\ L^{-1}$)	O_2 (%)	pH
May	IN	2.06±0.02	21.58±0.05	1.22±0.18	13.59±2.02	7.33±0.00
	OUT	2.02±0.00	21.97±0.20	1.05±0.26	12.71±2.74	7.39±0.01
June	IN	2.18±0.01	25.34±0.06	1.06±0.11	12.53±1.32	7.39±0.00
	OUT	2.18±0.00	24.87±0.19	0.52±0.08	6.05±0.96	7.44±0.01
July	IN	2.44±0.00	28.18±0.13	0.50±0.10	6.56±1.29	7.40±0.01
	OUT	2.54±0.06	27.58±0.07	0.19±0.02	2.43±0.25	7.34±0.00

Table 1. Monthly average ± standard error (SE) of inlet (IN) and outlet (OUT) physico-chemical parameters considering all 12 flumes together.

Considering the average nutrient uptake capacity of each experimental treatment over the entire experiment, *Cornus sanguinea* had the highest overall retention capacity, thus being the most effective treatment to reduce solutes from the WWTP effluent (Table 2) . S urprisingly, the unvegetated treatment (i.e., control), also had a general good performance in retaining the considered nutrients (Table 2). On the other hand, despite *Alnus glutinosa* and *Iris pseudacorus* also showed a high retention capacity for NO_3^- and a remarkable capacity to retain DOC and TDN, it had a very low or null capacity to retain SRP and NH_4 (Table 2).

Treatment	% Retention				
	NH_4^+	NO_3^-	SRP	DOC	TDN
Control	4.1±4.8	73.6±6.9	6.5±10.1	3.9±4.1	10.2±3.4
I. pseudacorus	-2.4±3.6	87.6±3.8	-0.7±8.2	9.2±2.5	18.1±2.6
C. sanguinea	7.7±9.2	89.0±4.6	10.6±13.3	6.2±2.9	11.9±4.1
A. glutinosa	-5.4±2.6	85.2±4.9	4.6±8.7	18.9±7.7	17.9±5.2

Table 2. Mean (± SE) of nutrient retention capacity (%) for the 4 experimental treatments over the experiment.

When considering the temporal variability of nutrient uptake capacity for each treatment, we observed remarkable differences over time. Mean NH_4^+ retention capacity was positive in May (15%), null in June (0) and negative in July (-10%) with no noticeable differences among species. Therefore, NH_4^+ release tended to prevail over uptake in the flumes (Fig.1.A) In contrast, the three species studied showed a high capacity to retain NO_3^-. In May, *Cornus sanguinea* retained the most (90% ± 6.5), while in July slightly decreased (80% ± 13.1) and *Alnus glutinosa* reached a retention capacity of 84% ± 7.9. Meanwhile, the unvegetated flumes showed a decline in retention capacity over time, being the lowest in July (57.96% ± 7.36) (Fig.1.B). Mean SRP retention was remarkable in May (30%) but dropped dramatically to negative values in June and July (-10 and -15%, respectively); thus SRP release tended also to prevail over retention. Regarding DOC, retention capacity was low and sustained over time in the unvegetated flumes. *Iris pseudacorus* had a higher retention capacity in May (18% ± 2.6) than in June and July (3.8% ± 6.3 y 6.3% ± 3.2) (Fig.1.D). In contrast, the DOC retention capacity of *Cornus sanguinea* and *Alnus glutinosa* decreased over time, being null at the end of the experiment (Fig.1.E). Finally, for TDN, a progressive decrease in retention capacity was observed in all treatments throughout the experiment with no remarkable monthly differences among treatments (Fig. 1.C).

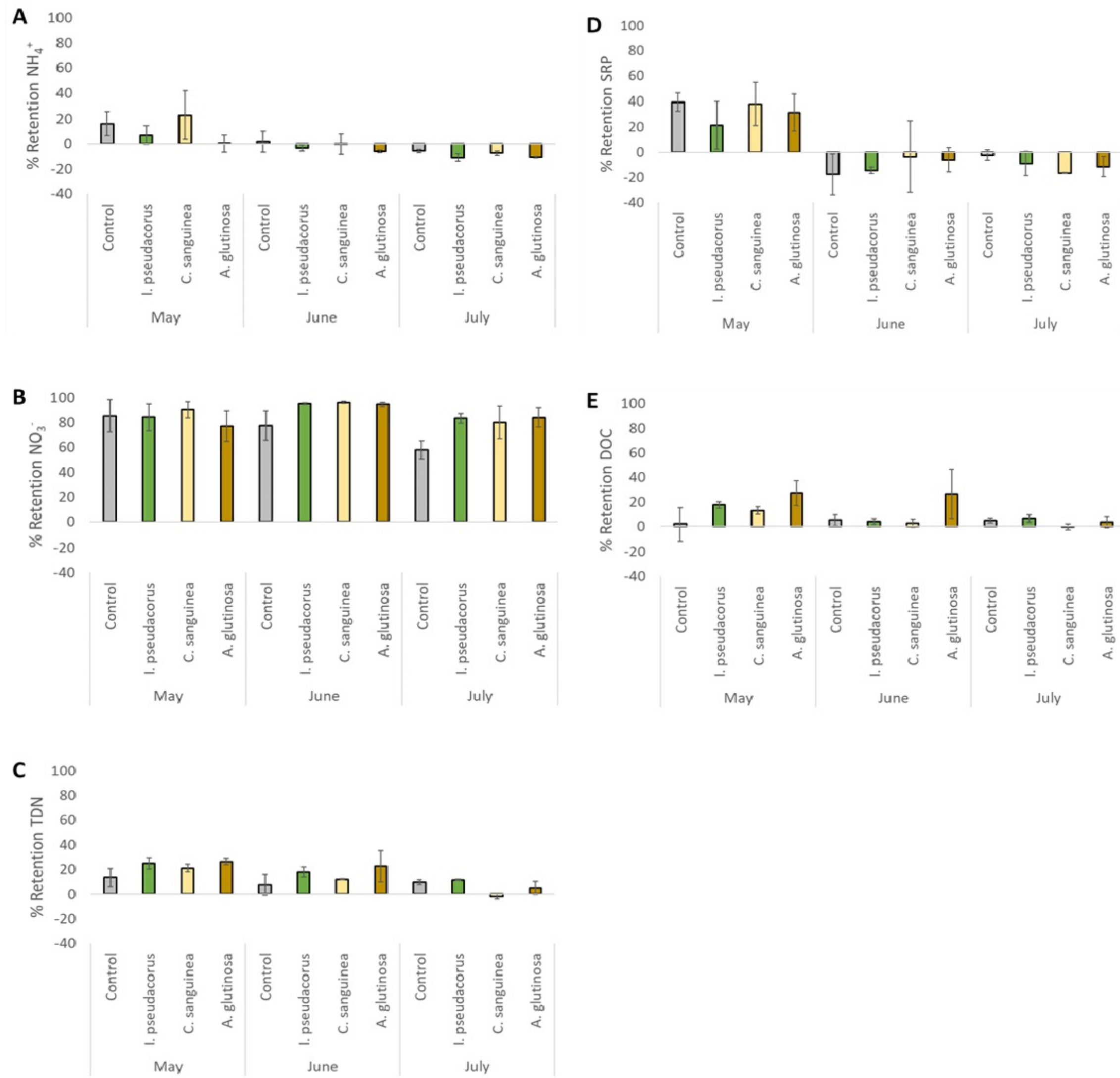

Figure 1. Monthly average nutrient retention capacity for NH_4^+ (A) , NO_3^- (B), TDN (C), SRP (D) and DOC (E) for the 4 experimental treatments.

Overall, the experimental flumes had a very high capacity to remove NO_3^-, suggesting that denitrification could be a relevant biogeochemical uptake pathway in the fl umes. The extreme hypoxic conditions (<1.2 mg L^-) and the relatively high concentration of DOC (<10 mg L^-), could favor anaerobic heterotrophic activity resulting in the reduction of NO_3^- to N gas as the respiratory pathway. Furthermore, assimilatory uptake of NO_3^- into biomass by plants and microbes could also contribute to reduce NO_3^- concentrations along the flumes. In addition, results suggest that dissimilatory nitrate reduction to ammonium (DNRA) could also occurs in the flumes. This anaerobic NO_3^- uptake pathway renders NH_4^+ which is in line with the general increases in NH_4^+ concentration along the flume (i.e., negative values of net retention). The fact that the NO_3^- uptake capacity was higher in the vegetated flumes than those unvegetated, suggest that these species may promote microbial denitrification in the rhizosphere. Regarding to SRP and NH_4^+, the observed temporal trends indicates a general net release of these nutrients along the flumes indicating that neither the physicochemical conditions nor the selected plants are effective to reduce concentrations of these solutes. On the contrary, all experimental treatments had a low but sustained capacity to reduce DOC and TDN concentrations along the flumes, being *Alnus glutinosa* which performed the best. These results indicated that the biogeochemical conditions in the flumes and/or the selected plant species may contribute to some extend to reduce both solutes.

It is worth noting that results from this study contrast with results from past studies conducted in the same flumes (Table 3). In past studies, we observed a general high capacity to uptake NH_4^+, being nitrification and biological assimilation the dominant N uptake pathway in the flumes (Ribot et al. 2017, URL Technical Report vol. 2). On the other hand, both NO_3^- and SRP uptake capacity were lower with respect to NH_4^+ and even negative, indicating that release usually predominate over retention for these 2 solutes (Technical reports vol. 2). Regarding to DOC, we observed a low but a sustained uptake capacity across experiments. There are several factors that may explain to some extend those contrasting results. Firstly, flow regime in the past experiments was permanent in contrast to the flow intermittence in the present study. Flow intermittency may affect the development of both, microbial communities and plants as well as may reduce the accumulation of organic matter within the sediments due to chemical oxidation during the dry phase. Secondly, sediment size was more heterogeneous and finer in the present study than those carried out in the past, thus substrate porosity in the present study was much lower than in former experiments. Last but not least, the configuration of flumes inlet also varied between experiments. In the former experiments, inlet water dropped into the flumes in a 0.5m free-sediment zone (i.e., open water) and then flew into the sediments, whereas in the present study water directly dropped into the sediments trough a piezometer to avoid excess of algal development. This modification affected the O_2 concentration at the inlet of the flumes, which in the present study was consistently lower than in previous studies, even below hypoxia levels.

Dates	Treatment	% Retention				
		NH_4^+	NO_3^-	SRP	DOC	TDN
Present study (2024)	Control	4.1	73.6	6.5	3.9	10.2
	I. pseudacorus	-2.4	87.6	-0.7	9.2	18.1
	C. sanguinea	7.7	89.0	10.6	6.2	11.9
	A. glutinosa	-5.4	85.2	4.6	18.9	17.9
Former studies (2015 and 2019)	*I. pseudacorus*	81.5	12.3	-21.3	5.3	-
	Scirpus lacustris	88.9	-7.0	39.9	6.1	-
	Phragmites australis	93.2	25.5	35.3	5.8	-
	Apium nodiflorum	47.9	-19.2	-31.4	3.7	-
	Sparganium erectum	63.2	31.6	-7.2	7.9	-
	Lemna minor	11.6	7.5	-50.5	5.4	-

Table 3. Summary of nutrient uptake retention (in %) of the different plant species evaluated at the URL experimental flumes.

The combination of fine sediment with the entrance of inlet water directly into the sediments seems to create the optimal redox conditions in subsurface flow paths that favored anaerobic metabolism; and thus, anaerobic dissimilatory pathways of N uptake (i.e., denitrification and DNRA).

3. Conclusions

Results from the present study shows that the system was extraordinarily effective in retaining NO_3^- across all treatments under conditions of flow intermittency, likely due to enhanced DNRA and denitrification occurring simultaneously in the system, driven by the exceptional experimental conditions hipoxic created. The retention of NH_4^+ and SRP was significantly less effective, with some treatments leading to nutrient release.

Furthermore, the nutrient uptake capacity of the experimental treatments declined over time, with an overall good performance in May followed by a decrease in the subsequent months. This suggests that changes in meteorological conditions, such temperature, in combination with patterns of plant growth (i.e., higher growth rates in early spring than in mid summer), may also play a role in nutrient retention. A prematurely senescent *Cornus sanguinea* was the most effective overall, particularly for NO_3^- retention. Unexpectedly, the unvegetated flumes were also significantly effective in retaining solutes. These results suggest that microbial communities within the sediments played a relevant role in nutrient uptake in these systems.

4. References

4. References

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5. Personnel involved

5.1 Personnel

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- Audrey H. Sawyer, The Ohio State University.
- Toni Mas, Consorci Besòs-Tordera.

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5.3 Promoting entities