Wastewater treatment plant effluent inputs influence the temporal variability of nutrient uptake in an intermittent stream

Sara Castelar1 · Susana Bernal1 · Miquel Ribot1 · Stephanie N. Merbt1,2 · Marta Tobella3 · Francesc Sabater3 · José L. J. Ledesma1,4 · Helena Guasch1 · Anna Lupon1 · Esperança Gacia1 · Jennifer D. Drummond5 · Eugènia Martí1

Accepted: 28 March 2022 / Published online: 20 April 2022 © The Author(s) 2022

Abstract
Wastewater treatment plant (WWTP) effluents alter water chemistry and in-stream nutrient uptake rates of receiving freshwaters, thus changing the magnitude and fate of the nutrients exported. In Mediterranean regions, the dilution capacity of receiving streams can vary strongly over time due to the seasonal occurrence of floods and droughts, causing temporal variability of nutrient uptake. We assessed the temporal patterns and the controlling factors of net nutrient uptake in an intermittent Mediterranean stream receiving WWTP effluent inputs. We compiled the longitudinal concentration profiles of ambient dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) along a 800 m reach on 47 sampling dates between 2001 and 2017, encompassing a wide range of hydrological conditions. We estimated net nutrient uptake in the receiving stream. In 72% of the dates, high rates of net ammonium uptake co-occurred with net releases of either nitrate or nitrite. This pattern suggests that the receiving stream has a high nitrification capacity. Conversely, 75% of the dates did not show any longitudinal pattern in SRP concentration, suggesting that uptake and release processes for this element were either counterbalanced or both occurred at very low rates. Finally, net ammonium uptake was low when the stream had a low dilution capacity (< 40%) and ammonium concentration was high. Overall, we demonstrate that consideration of the receiving stream’s dilution capacity is imperative to the management of freshwaters to guarantee an adequate dilution of WWTP effluent inputs and avoid saturation of in-stream nutrient uptake capacity under low flow conditions in urban landscapes.

Keywords Wastewater treatment plant effluents · Hydrologic regime · Nitrogen · Phosphorus · Nitrification · Nutrient uptake · Intermittent streams
Introduction

Urban wastewater treatment plant (WWTP) effluents are point sources of nutrients, organic matter, emergent pollutants, microbes, and pathogens to receiving streams (Marti et al. 2004; Meng et al. 2013; Mußmann et al. 2013; Kay et al. 2017; Pascual-Benito et al. 2020). These point sources generate abrupt physical and chemical discontinuities along the river continuum by increasing discharge, temperature, and nutrient availability that can consequently deteriorate water quality (Brion and Billen 2000; Gray 2004; Jin et al. 2007; Martí et al. 2010). Enrichment of nutrients and organic matter from WWTPs inputs enhance both the growth of primary producers and the activity of heterotrophic microbial assemblages associated with organic matter breakdown (Gücker et al. 2006; Wakelin et al. 2008). These changes can induce shifts in the composition, functioning and trophic state of the microbial communities in receiving streams (Miltner and Rankin 1998; Jin et al. 2007; Beyene et al. 2009; Aristi et al. 2015).

Streams and rivers have an important capacity to store, transform and retain dissolved inorganic nitrogen (DIN) and reactive solute phosphorous (SRP) because in-stream biota relies on these nutrients to grow (Peterson et al. 2001; Mulholland et al. 2008). In-stream biota can remove DIN and SRP from the water column via different biogeochemical pathways, which include both assimilatory and dissimilatory processes (Peterson et al. 2001; Bernot and Dodds 2005; Ribot et al. 2017). Assimilatory processes refer to the transitory uptake of DIN and SRP into biomass during biosynthetic processes (Tank et al. 2018; Abril et al. 2019). On the other hand, dissimilatory processes are those biological pathways that transform DIN from one form to another (Kuyper et al. 2018). The most relevant dissimilatory processes for DIN are nitrification, the aerobic oxidation of ammonium (NH$_4^{+}$-N) to nitrate (NO$_3^{-}$-N) (Prosser 1989; Lin et al. 2009), and denitrification, the reduction of NO$_3^{-}$-N to N gas under sub-oxic conditions during organic matter mineralization (Seitzinger et al. 2006). Besides biological processes, SRP can also be removed by sediment adsorption and co-precipitation (Trentman et al. 2020). These processes taken together influence the in-stream bioreactive capacity to help alleviate the excess of nutrients downstream from WWTP effluents. Uptake efficiencies of stream biota depend on nutrient availability, but excess nutrient loads can lead to a saturation of nutrient uptake (Newbold et al. 2006; Bernal et al. 2018). In fact, streams receiving WWTP effluent inputs have been shown to have low nutrient uptake efficiency (Marti et al. 2004; Grimm et al. 2005; Gücker et al. 2006; Merseburger et al. 2011). Still, longitudinal decreases in ambient nutrient concentration have been observed in WWTP receiving streams (Haggard et al. 2005; Ribot et al. 2012; Bernal et al. 2020). Therefore, even when nutrient retention efficiency is low, the stream biota can reduce to some extent the high concentrations of nutrients in reaches located downstream from WWTP. In particular, these streams show a high capacity of nitrification due to the combination of streambed biofilm colonization by ammonium oxidizing bacteria and high NH$_4^{+}$-N concentrations sourced from the WWTP effluent (Mußmann et al. 2013; Merbt et al. 2015).

Besides nutrient concentrations, the hydrological condition is a key factor for in-stream nutrient uptake, influencing the extent of interaction between stream biota and the water column (Peterson et al. 2001). Under high flows, low water residence times limit the contact time between nutrients dissolved in the water column and stream biota; therefore, nutrient downstream transport dominates over-stream biogeochemical processing (Martí et al. 1997; Argerich et al. 2008). Conversely, during low flows, receiving streams can act as net sinks of DIN and SRP because higher water residence time favors the interaction between stream biota and nutrients; and thus, biogeochemical processing (Ribot et al. 2012; Rahm et al. 2016; Bernal et al. 2020). Moreover, stream hydrology also affects water chemistry, especially in reaches located downstream of WWTP inputs. For instance, during low flows, the dilution capacity of the receiving stream decreases and water chemistry is basically driven by the WWTP inputs (Keller et al. 2014; Bernal et al. 2020). Given that nutrient uptake velocity (i.e., V$_f$ sensu Stream Solute Workshop 1990) is inversely related to nutrient concentrations (Earl et al. 2006; Newbold et al. 2006), it is also expected that, during extremely low flows, the capacity of stream biota to take up nutrients can be overwhelmed by the high nutrient concentrations. Nevertheless, the extent to which stream hydrology interacts with water chemistry to control the temporal variation of nutrient uptake in receiving streams is not fully understood.

Most existing studies in WWTP-receiving streams have focused on quantifying the effects of the WWTP inputs on nutrient concentration and in-stream nutrient uptake (Gücker et al. 2006; Merseburger et al. 2011; Arnon et al. 2015), but less information is available on assessing the temporal variation and controlling factors of net nutrient uptake (Bernal et al. 2020). However, understanding variation in in-stream nutrient processing over time in rivers that receive WWTP effluent inputs is essential for management of freshwater resources in urban landscapes. This is especially true for landscapes with water scarcity, such as the Mediterranean region, where the dilution capacity of receiving streams can vary from 0% to > 95% (Martí et al. 2010; Bicknell et al. 2020), encompassing large seasonal changes in both water mean residence time and stream nutrient concentrations.
downstream of WWTP effluent inputs. In this context, we aimed to assess the temporal variability and controlling factors of nutrient uptake in a Mediterranean stream with an intermittent hydrologic regime receiving WWTP effluent inputs. We conducted 47 longitudinal profiles of ambient nutrient concentrations along a 800 m reach downstream of a WWTP effluent input during the years 2001–2017. From this dataset, we calculated in-stream net nutrient uptake velocity (Vf) and assessed to which extent its seasonal variability related to stream hydrology and water chemistry.

**Material and methods**

**Study site**

This study was conducted in a third-order stream reach of La Tordera river (NE of Catalonia, Spain) located immediately downstream of the WWTP effluent input of the Sant Esteve and Santa Maria de Palautordera municipalities (lat. 41º41′34″N, long. 2º27′33″W). The reach is 800 m long, has no major lateral inputs (e.g., tributaries), a channel morphology characterized by low sinuosity, run–riffle sequences with a few shallow pools, and a shallow slope of approximately 1%. Streambed substrata consist of cobbles (34%), pebbles (22%), and boulders (22%) (Merseburger et al. 2005). The reach is flanked by a dense canopy of riparian trees, with some areas of sparse vegetation (Bernal et al. 2020). The mean annual stream discharge is 267 ± 115 L s⁻¹, varying by several orders of magnitude within and among hydrological years due to the intrinsic characteristics of the Mediterranean climate of the region (Merseburger et al. 2005). Specifically, in summer the stream commonly dries out upstream of the WWTP input.

The WWTP treats 17,900 population equivalents, where 1 population equivalent is the biodegradable organic-matter load corresponding to a biological O₂ demand (BOD₅) of 60 g O₂ d⁻¹. Despite meeting the quality standards established by European legislation (Council Directive 91/271/EEC), the WWTP effluent inputs substantially increase DIN and SRP concentrations of the receiving stream (Bernal et al. 2020). Nutrient concentrations in the WWTP effluent vary over time, ranging from 3.7 to 24.3 mg L⁻¹ for DIN and from 0.01 to 4.9 mg L⁻¹ for SRP (Merseburger et al. 2011; Bernal et al. 2020). Nevertheless, for DIN, > 90% in the WWTP effluent is in the form of NH₄⁺-N (Merseburger et al. 2005). The WWTP discharge is relatively constant over the year, with a mean ± SD of 23.1 ± 2.4 L s⁻¹ from daily measurements during 2000–2010 period provided by the municipality. The annual contribution of the WWTP effluent to stream discharge varies seasonally from 3 to 100% due to the stream hydrologic variability (Merseburger et al. 2005), with the maximum contribution during summer low flow.

**Field sampling and laboratory analysis**

We selected eight sampling sites 100 m distant from each other along the 800 m reach. The first sampling site was located 100 m downstream of the WWTP input to allow a complete mixing of WWTP effluent and stream water. In addition, we selected a sampling site upstream of the WWTP that served to assess the relative contribution of the WWTP outflow to the receiving stream. We conducted a total of 47 sampling dates from August 2001 to October 2017. Despite the irregular sampling periodicity, the data set covers a wide range of hydrological conditions and encompasses the four climatic seasons of this Mediterranean region (winter n = 7, spring n = 16, summer n = 13, and autumn n = 11).

On each sampling date, we took surface water samples from each sampling site (3 replicates) using 100 mL acid-washed plastic syringes. Water samples were immediately filtered through a GF/F glass fiber filter (Whatman ®, 0.7 µm pore size) and kept refrigerated on ice in the field. Once in the laboratory, we stored samples at ~20 °C until subsequent analysis of NH₄⁺-N, NO₃⁻-N, NO₂⁻-N and SRP. At each sampling site, we also recorded electrical conductivity (EC, in μS cm⁻¹), water temperature (in °C), and dissolved oxygen concentration (DO, in mg L⁻¹) using WTW 340i portable sensors. Furthermore, on each sampling date, we estimated stream discharge (Q, in L s⁻¹) based the wetted width (w, in m), average water velocity (v, in m s⁻¹; measured with a Shiltknecht MiniAir 20 current meter), and average water column depth (h, in m) measured in a representative cross-sectional transect (Gordon et al. 2004) located 200 m below the WWTP effluent.

Concentrations of NH₄⁺-N, NO₃⁻-N, NO₂⁻-N and SRP were analyzed following standard colorimetric methods from the American Public Health Association (APHA 1995) on a continuous flow autoanalyzer FUTURA (Alliance Instruments). Concentration of DIN for the data set (n = 47) was estimated as the sum of NH₄⁺-N and NO₂⁻-N. Concentration of NO₂⁻-N was not included in DIN estimates because values were only available for half of the dates and on average represented ≤1% of DIN. We also calculated the molar ratios of DIN: SRP and NO₃⁻-N: NH₄⁺-N as proxies of relative availability and potential N or P limitation and relative proportion of oxidized and reduced DIN forms, respectively.

**Parameter calculations**

On each sampling date, we estimated the contribution of the WWTP effluent to stream discharge (in %) by using electrical conductivity (EC) of the stream water as a conservative tracer as follows:

**Contribution WWTP(%) = 100 * (EC₁ – ECₚ)/((ECWWTP – ECₚ))**  

(1)
where $EC_1$ is the electrical conductivity measured at 100 m downstream of the WWTP input (i.e., first sampling site along the study reach). Empirical measurements in 2001 indicated that this distance from the WWTP input is enough to ensure a complete mixing of the effluent water with stream water under different hydrological conditions. $EC_{up}$ is the electrical conductivity measured upstream of the WWTP input and $EC_{WWTP}$ is the electrical conductivity of the WWTP effluent. Then, we estimated the hydrologic dilution factor of the receiving stream (DF, in %) as the inverse of the WWTP contribution to discharge (i.e., DF = 100−Contribution WWTP). Thus, low DF values indicate that the stream flow is low relative to WWTP inputs (Gupta 2008). Some studies have indicated deleterious effects from WWTP inputs on receiving streams at DF < 40 (Keller et al. 2014; Romero et al. 2019). Therefore, in engineering practice, this threshold can be used to determine risk vs no risk conditions in terms of the vulnerability of the stream to WWTP inputs.

We used the longitudinal profiles of nutrient concentrations along the reach to estimate net nutrient uptake following Von Schiller et al. (2011). Specifically, for each nutrient, we used the following first-order equation to estimate the net nutrient uptake coefficient per unit length of the reach ($k$, in $m^{-1}$):

$$C_x = C_1(EC_x/EC_1)e^{kx} \quad (2)$$

where $C_1$ is the nutrient concentration at the first sampling site of the reach below the WWTP effluent (in mg N L$^{-1}$ or mg P L$^{-1}$); and $C_x$ and $EC_x$ are the nutrient concentrations and electrical conductivities measured at each sampling site located $x$ meters from the WWTP effluent input. We estimate $k$ as the slope of the regression between the natural logarithm of nutrient concentration (corrected by EC) measured at each sampling site and the distance of each sampling site from the WWTP effluent input. Our calculations characterize in-stream net nutrient uptake as the balance between uptake and release processes. Therefore, $k$ values can be positive (uptake > release), negative (uptake < release), or $\approx 0$ (uptake = release) (Merseburger et al. 2005).

We used $k$ to estimate net uptake metrics in the study reach, including net uptake length ($S_k$), uptake velocity ($V_f$), and areal net uptake rates ($U$) following equations in Stream Solute Workshop (1990). All net uptake metrics are included in Table S1 SI, but here we focus on data from net uptake velocity ($V_f$ in mm min$^{-1}$) because this metric weighs nutrient uptake efficiency by hydraulic conditions; and thus, values can be compared among sampling dates. $V_f$ represents the hypothetical vertical velocity at which a nutrient molecule is removed from the water column and it is a proxy for in-stream nutrient demand (Bernhardt et al. 2002). Values of net $V_f$ can be positive, negative, or $\approx 0$ depending on the value of $k$; and are calculated following Bernal et al. (2020):

$$V_f = Q * k/w \quad (3)$$

where $Q$ is stream discharge, $k$ is the net uptake coefficient and $w$ is the mean stream width.

**Data analysis**

We inferred potential seasonal differences in stream physical and chemical characteristics based on the variables measured on each sampling date (see Table 1) by using a non-parametric Kruskal–Wallis test (season as a factor) because data sets were not normally distributed and showed heteroscedasticity.

We also examined how measured physical and chemical variables contributed to the observed temporal variability among sampling dates by using a principal component analysis (PCA). Data were transformed, if needed, scaled and centered prior to PCA analysis. The weight of a variable on a PCA component was considered significant for loadings $> 0.7$. Unfortunately, some of the variables were not measured in all sampling dates. Therefore, to avoid a significant reduction of available sampling dates in the PCA analysis, we excluded from the analysis those variables with a high number of missing values (i.e., concentrations of DO, NO$_2^-$-N, and SRP; and the DIN:SRP molar ratio; Table 1). We also excluded the NO$_3^-$:NH$_4^+$-N ratio from the PCA analysis to avoid autocorrelation with NO$_3^-$-N and NH$_4^+$-N concentrations; and thus, variance inflation. In addition, we used a non-parametric Kruskal–Wallis test on the PC1 and PC2 scores to examine differences among seasons.

For each nutrient, we estimated the frequency of cases when $V_f < 0$ (release > uptake), $V_f > 0$ (release < uptake) and $V_f \approx 0$ (release $\approx$ uptake) considering all data together to compare in-stream uptake among nutrients, and separated by seasons to identify temporal patterns in dominant pathways of nutrient cycling. Further, we used simple regression analysis to examine potential relationships between $V_f$ for NH$_4^+$-N and $V_f$ for NO$_3^-$-N or NO$_2^-$-N that infer in-stream nitrification as a dissimilatory N uptake pathway in the study reach (Merseburger et al. 2005; Ribot et al. 2012; Bernal et al. 2020).

Finally, we examined potential factors contributing to the temporal variability of net nutrient uptake of the receiving stream. We first explored the relationship between $V_f$ for each nutrient and the scores from PC1 and PC2 used as variables integrating stream physical and chemical characteristics measured on the different sampling dates. Given the high co-linearity between some of the physicochemical variables as shown by PCA results, we then ran a Partial Least Square
Table 1  Physical and chemical characteristics of the study stream, which receives inputs from a wastewater treatment plant effluent, measured on different seasons over the study period. For each variable, we report the range of values (minimum and maximum) and the number of data available (n) corresponding to all sampling dates. We also show the average ± Standard Error values of each variable considering data from each season separately. For each season, n indicates the number of sampling dates with available data. The results from Kruskal–Wallis non-parametric test to examine differences among seasons for each variable are shown; where n.s. indicates no significant differences (p-value > 0.05). Different letters indicate significant differences among seasons from post-hoc tests. Variables are: Temperature (T), discharge (Q), dilution factor of receiving stream (DF), electrical conductivity (EC), dissolved oxygen concentration (DO), concentrations of ammonium (NH₄⁺-N), nitrite (NO₂⁻-N), nitrate (NO₃⁻-N), dissolved inorganic nitrogen (DIN) and soluble phosphorus (SRP), and the molar ratios between nutrients (NO₃⁻-N:NH₄⁺-N and DIN:SRP).

| Parameters | All seasons | Winter | Spring | Summer | Autumn | p-value seasons |
|------------|-------------|--------|--------|--------|--------|-----------------|
|            | n | Minimum | Maximum | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE | n | Average ± SE |
| T (°C)     | 49 | 8 | 26 | 7 | 10 ± 0.2a | 16 | 16 ± 0.4b | 12 | 22 ± 0.5c | 11 | 15 ± 0.8b | 0.000 |
| Q (L/s)    | 47 | 6 | 1639 | 7 | 351 ± 104b | 16 | 442 ± 59b | 13 | 55 ± 8c | 11 | 136 ± 28d | 0.001 |
| Dilution factor (%) | 47 | 0 | 100 | 7 | 56 ± 4a | 16 | 74 ± 4bc | 13 | 34 ± 6d | 11 | 41 ± 6e | 0.015 |
| EC (µS cm⁻¹) | 50 | 123 | 786 | 7 | 263 ± 18bc | 16 | 249 ± 21b | 13 | 506 ± 34c | 11 | 429 ± 33e | 0.000 |
| O₂ (mg L⁻¹) | 28 | 3.4 | 11.6 | 5 | 10.8 ± 0.2a | 7 | 7.6 ± 0.7bc | 6 | 6.1 ± 0.5cd | 7 | 8.7 ± 0.5de | 0.026 |
| NH₄⁺-N (mg N L⁻¹) | 46 | 0.03 | 6.74 | 7 | 1.66 ± 0.34a | 16 | 1.38 ± 0.24b | 13 | 1.44 ± 0.19c | 10 | 1.49 ± 0.24d | n.s |
| NO₂⁻-N (mg N L⁻¹) | 24 | 0.004 | 0.024 | 4 | 0.01 ± 0.002a | 8 | 0.05 ± 0.020a | 5 | 0.13 ± 0.030b | 7 | 0.03 ± 0.006c | n.s |
| NO₃⁻-N (mg N L⁻¹) | 46 | 0.4 | 4.4 | 7 | 2.0 ± 0.1a | 16 | 1.2 ± 0.1bc | 13 | 1.9 ± 0.2ac | 10 | 2.2 ± 0.2b | 0.026 |
| DIN (mg N L⁻¹) | 46 | 0.9 | 8.3 | 7 | 3.7 ± 0.4a | 16 | 2.6 ± 0.3a | 13 | 3.4 ± 0.2b | 10 | 3.7 ± 0.3c | n.s |
| SRP (mg P L⁻¹) | 35 | 0.02 | 0.25 | 6 | 0.10 ± 0.02a | 10 | 0.18 ± 0.04a | 10 | 0.88 ± 0.14b | 9 | 0.54 ± 0.15c | 0.009 |
| NO₃⁻-N:NH₄⁺-N | 46 | 0.04 | 28.96 | 7 | 1.35 ± 0.44a | 16 | 0.87 ± 0.2a | 13 | 3.12 ± 1.23b | 10 | 1.97 ± 0.76c | n.s |
| DIN:SRP | 35 | 1 | 157 | 6 | 35 ± 5a | 10 | 29 ± 4a | 10 | 6 ± 1b | 9 | 38 ± 9c | 0.009 |
Regression (PLSR) to select the most explanatory variables of $V_f$ for each nutrient. We used a mixed generalized linear model (GLM) with both categorical (i.e., season) and continuous (i.e., selected variables from PLSR) explanatory variables to assess which of them had a significant influence (i.e., $p$-value < 0.05) on $V_f$ for each nutrient, as the dependent variable. Each mixed GLM was run separately with and without including “year” as a random factor and then we assessed if this random factor significantly provided a better data fit using a likelihood ratio test. For these analyses, we selected the sampling dates when $V_f$ was greater or less than zero, when the net balance of in-stream processes had a significant influence on the downstream fate of nutrients.

We ran the PCA, the PLSR and the GLM analyses with R software (version 3.2.2; R project for Statistical Computing, Vienna, Austria). The rest of statistical analyses were performed with the SPSS 11.0 statistical package. We considered statistical results to be significant at a $p$-value < 0.05.

**Results**

**Physical and chemical stream characterization and seasonal variation**

Most of the physical and chemical variables in the receiving stream differed among seasons (Table 1). Average stream water temperature was relatively warm, with the lowest values in winter and the highest in summer (Table 1). Stream Q varied up to 3 orders of magnitude among seasons, being the lowest in summer and the highest in spring (Table 1). Similarly, the DF ranged from 0 to 100%, with higher values in winter and spring than in summer and autumn, when Q upstream of the WWTP was low or even nil. During summer and autumn, average DF values were ≤40%. Water EC varied seasonally following the inverse pattern of Q (Table 1). Regarding nutrient concentrations, $NO_3^-$ and SRP were consistently high and differed among seasons (Table 1). On average, $NO_3^-$ concentration was low in spring compared to the rest of the year, while SRP concentration was the highest in summer and the lowest in winter. In contrast, concentrations of $NH_4^+$, $NO_3^-$ and DIN did not show any significant seasonal differences (Table 1). The $NO_3^-$:$NH_4^+$ molar ratio varied widely and showed that, on some dates, $NH_4^+$ concentration was higher than $NO_3^-$ concentration (ratios < 1) (Table 1). Finally, the DIN:SRP molar ratio significantly differed among seasons with remarkably low values in summer (Table 1).

The first two PCA components, considering the physical and chemical variables measured in the study reach, accounted for 71.5% of the variance among sampling dates (Fig. 1). The first component of the PCA (PC1) explained 54.2% of the total variance, with a positive loading of EC, temperature and $NH_4^+$ concentration, and a negative loading of the DF and Q (Fig. 1). Thus, this axis shows the importance of the stream dilution capacity to counteract the physical and chemical influence of the WWTP effluent on the stream. The second component of the PCA (PC2) explained 17.3% of the total variance, with a negative loading of $NO_3^-$ concentration (Fig. 1). Given that DIN in the WWTP effluent is relatively enriched in $NH_4^+$ compared to upstream water (Bernal et al. 2020), this axis shows the relative influence of the point-source inputs on water chemistry of the receiving stream. Finally, a Kruskal–Wallis test indicated that there were significant differences among seasons for the PCA component scores. In relation to PC1 scores, summer cases showed significantly higher values than those in spring and winter (Kruskal–Wallis test, $p$-value < 0.05). In relation to PC2 scores, there were significant differences between autumn and spring (Kruskal–Wallis test, $p$-value < 0.05).

**Temporal variation of stream net nutrient uptake**

Both the magnitude and the temporal variability of net $V_f$ differed among nutrients (Table 2). We found that $NH_4^+$ concentration significantly decreased along the reach (i.e., net uptake) on 72% of all sampling dates; yet, this proportion slightly differed among seasons (Fig. 2A; Table S1 SI). In particular, the highest and lowest proportion of sampling dates with net uptake were observed in summer (85%) and
Fig. 2 Frequency of the different trends (i.e., significant decreases or increases, and no significant changes) in the longitudinal profiles of ambient nutrient concentrations of \( \text{NH}_4^+ \)-N (A), \( \text{NO}_2^- \)-N (B) and \( \text{NO}_3^- \)-N (C) along the study reach of the WWTP receiving stream. The longitudinal trends, when significant, were used to estimate net nutrient uptake (estimated as uptake velocity, \( V_f \) which could be either positive, negative or nil). Different colors in the column indicate the frequency of the three types of trends. A longitudinal decrease in ambient concentration indicates net nutrient uptake and it is labeled in black; a longitudinal increase indicates net nutrient release and it is labeled in gray; no significant trend indicates balance between nutrient uptake and release processes and it is labeled in white.

For each dissolved inorganic nitrogen form, the frequency of the different longitudinal trends is reported for all data together and for data collected in each season separately.
spring (56%), respectively (Fig. 2A). Average $V_f$ for NH$_4^+$+N was consistently $>0$ in all seasons (Table 2). In contrast, longitudinal profiles of NO$_2^-$-N and NO$_3^-$-N concentrations either significantly increased along the reach (i.e., net release) or did not show a significant longitudinal trend (i.e., uptake = release) (Fig. 2B, C; Table S1 SI). Nevertheless, there were differences between NO$_2^-$-N and NO$_3^-$-N in the seasonal patterns of the longitudinal profiles. For NO$_2^-$-N, concentration increased along the reach in $>50\%$ of the sampling dates in each season, except in summer when concentration decreased longitudinally (Fig. 2B). For NO$_3^-$-N, autumn showed the highest proportion of sampling dates with longitudinal concentration increases ($>70\%$ of dates; Fig. 2C). Average $V_f$ for NO$_2^-$-N and NO$_3^-$-N were consistently $<0$ in most seasons (Table 2). Further, there was a strong negative relationship between $V_f$ for NH$_4^+$+N and $V_f$ for NO$_3^-$-N (linear regression, $R^2=0.54, df=22, p<0.001$, Fig. 3). These results indicate that NH$_4^+$+N uptake ($V_f>0$) was associated with NO$_3^-$-N release, an indication of nitrification occurring along the reach. There was a similar relationship between $V_f$ for NH$_4^+$+N and $V_f$ for NO$_2^-$-N, though it was not statistically significant because the number of cases was small ($n=5$).

Finally, concentrations of both DIN and SRP only showed significant longitudinal trends in 25% of the sampling dates with available data for DIN ($n=46$) and SRP ($n=35$) (Tables 2 and S1 SI). On these dates, average $V_f$ for DIN was $<0$ in summer and autumn and $>0$ in spring (Table 2). For SRP, average $V_f$ was $>0$ in all seasons except in spring (Table 2).

### Table 2: Net nutrient uptake velocity ($V_f$) measured in the study stream, which receives inputs from a wastewater treatment plant effluent, on different seasons over the study period. Values of $V_f$ were estimated from profiles of ambient nutrient concentrations that showed significant trends along the stream reach. For each nutrient, we report the range of $V_f$ values (minimum and maximum) and the total number of data available (n), which in all cases is a fraction of all sampling dates (Table 1). We also show the average $\pm$ Standard Error values of $V_f$ for each nutrient considering data from each season separately. For each season, n indicates the number of available data. Nutrients are: ammonium (NH$_4^+$+N), nitrite (NO$_2^-$-N), nitrate (NO$_3^-$-N), dissolved inorganic nitrogen (DIN) and soluble phosphorus (SRP).

| Nutrient | All seasons | Winter | Spring | Summer | Autumn |
|----------|-------------|--------|--------|--------|--------|
|          | n | Minimum | Maximum | n | Average $\pm$ SE | n | Average $\pm$ SE | n | Average $\pm$ SE | n | Average $\pm$ SE |
| NH$_4^+$+N | 33 | -0.1 | 11.9 | 5 | 1.4 $\pm$ 0.4 | 9 | 3.7 $\pm$ 0.7 | 11 | 1.3 $\pm$ 0.2 | 8 | 1.2 $\pm$ 0.3 |
| NO$_2^-$-N | 15 | -27.7 | 0.5 | 2 | -14.5 $\pm$ 5.8 | 6 | -3.4 $\pm$ 0.8 | 3 | 0.3 $\pm$ 0.1 | 4 | -4.7 $\pm$ 1.3 |
| NO$_3^-$-N | 26 | -4.7 | 3.1 | 3 | -0.6 $\pm$ 0.2 | 9 | -1.5 $\pm$ 0.3 | 6 | -0.6 $\pm$ 0.2 | 8 | -0.8 $\pm$ 0.2 |
| DIN | 12 | -2.4 | 5.2 | 0 | N.A | 6 | 0.2 $\pm$ 0.1 | 3 | -0.2 $\pm$ 0.1 | 3 | -0.7 $\pm$ 0.2 |
| SRP | 9 | -1.4 | 2.4 | 2 | 1.4 $\pm$ 0.6 | 2 | -0.7 $\pm$ 0.3 | 3 | 0.001 $\pm$ 0.001 | 2 | 0.1 $\pm$ 0.1 |
There was a negative relationship between $V_f$ for $NH_4^+$-N and the scores of PC1 and a positive relationship between $V_f$ for $NO_2^-$-N and the scores of PC1 (Fig. 4). The $V_f$ for the remaining nutrients showed no relationship with the scores from PC1. For any of the studied nutrients, $V_f$ showed no relationship with the scores of PC2. Results from the mixed GLM indicated that season was a significant explanatory variable of $V_f$ variability only for $NO_2^-$-N (p-value = 0.025, Fig. S1 SI). Values of $V_f$ for $NO_2^-$-N were more negative (i.e., higher release) in winter (Table 2). In addition, GLM outputs indicated that $V_f$ for $NH_4^+$-N was positively influenced by discharge and negatively by $NH_4^+$-N concentration (p-value = <0.0001 and 0.019, respectively; Fig. S2 SI). Variability of $V_f$ for $NO_3^-$-N was negatively influenced by discharge and positively by temperature (p-value = <0.001 and 0.049, respectively; Fig. S3 SI). Likewise, variability of $V_f$ for $NO_2^-$-N was negatively influenced by discharge (p-value = <0.0001 and 0.025; Fig. S1 SI). Outputs from mixed GLM for $V_f$ for SRP indicated a lack of significant explanatory variables (p-value > 0.05). For each nutrient, the comparison between the mixed GLM with and without the random factor “year” was no significant (likelihood test, p-value > 0.05) indicating that the simplest model (i.e., without the random factor) was the most parsimonious.

Discussion

The impact of the WWTP effluent in the study stream was dramatic for both physical and chemical variables, especially during summer and autumn when the dilution factor was well below the 40% threshold. WWTP effluent contribution to the receiving stream highly varied (from 0 to 100%) during the year, which it is common for intermittent streams across arid and semiarid regions (Arnon et al. 2015; Martí et al. 2010; Bicknell et al. 2020), because extreme events (i.e., floods and droughts) characterize the hydrologic regime. During high flow conditions (i.e., winter and spring), landscape features and upstream conditions strongly influence stream physical and chemical characteristics, whereas these characteristics become increasingly determined by WWTP effluent inputs as the dilution capacity of the receiving stream decreases due to upstream flow reduction (i.e., summer) (Keller et al. 2014). Under summer conditions, when upstream was dry for some weeks, water temperature and electrical conductivity were higher and oxygen concentration was lower compared to when the stream dilution factor was >40%. Low dissolved oxygen concentration could be explained by increases in ecosystem respiration commonly observed downstream of WWTP inputs (Gücker et al. 2006; Bernal et al. 2020). In addition, mean SRP concentration increased between 4- and eightfold.

Fig. 4 Relationships between net uptake velocity ($V_f$) for $NH_4^+$-N (A) and for $NO_2^-$-N (B) and the scores from the principal component 1 (PC1) of the PCA conducted with physical and chemical characteristics of the receiving stream. The arrow above the graphs indicates the significant variables (loading > 0.7) associated with the PC1 and their respective positive (+) or negative (-) weight. Different symbols distinguish data by seasons, with crosses = winter; triangles = spring; squares = summer; and circles = autumn.
during summer, and the DIN-SRP molar ratios reached the lowest seasonal values. Therefore, in-stream biogeochemical processing in an intermittent hydrological regime with consistent low summer flow conditions, is essential to regulating nutrient concentrations in the water column since dilution from either upstream or groundwater sources is almost negligible (Bernal et al. 2020).

The longitudinal profiles of nutrient concentrations in the receiving stream showed a differential capacity of the stream to process excess N and P from the WWTP over time. While DIN concentrations did not show any significant longitudinal trend on most sampling dates, our results indicate a higher in-stream capacity to process the different DIN forms, suggesting that the stream was acting to transform rather than sink N. Moreover, the processing capacity of the receiving stream was higher for NH$_4^+$-N, NO$_3^-$-N, and NO$_3^-$-N than for SRP because longitudinal profiles for the different DIN forms exhibited more significant trends (i.e., net uptake or release) compared to SRP. This pattern is relatively common among WWTP-receiving streams (Martí et al. 2010). The general lack of significant longitudinal trends in SRP concentrations (> 75% of the sampling dates with available data) suggests that uptake and release processes were counterbalanced, or alternatively, that uptake and release rates were low. This was likely because microbial demand of SRP was saturated downstream of the WWTP effluent input (House and Denison 1998; Haggard et al. 2005).

Regardless of the season, declines in NH$_4^+$-N concentration were accompanied by increases in NO$_3^-$-N and NO$_3^-$-N concentrations; supporting the idea that nitrification is the prevailing process in the receiving stream over time (Merseburger et al. 2005; Bernal et al. 2020). This pattern is consistent with a previous study conducted in the same stream, showing that nitrification can represent up to 90% of the uptake of NH$_4^+$-N (Bernal et al. 2017) and with other WWTP-receiving urban streams (Cébron et al. 2003; Marti et al. 2004; Gammons et al. 2011). High nitrification rates in WWTP receiving stream reaches can be explained by the high loads of both NH$_4^+$-N and nitrifying bacteria from active sludge via WWTP effluents (Merbt et al. 2015), but we also found evidence of other simultaneously occurring biogeochemical processes associated with N cycling. For instance, the slope between $V_f$ for NO$_3^-$-N and $V_f$ for NH$_4^+$-N was below 1 (Fig. 3), indicating that the NH$_4^+$-N uptake demand was higher than expected solely by nitrification. This result suggests that assimilatory NH$_4^+$-N uptake by photoautotrophs and heterotrophs additionally contributed to the observed declines in NH$_4^+$-N concentration. In contrast, net NO$_3^-$-N uptake only occurred on a few sampling dates, mostly during spring. This pattern suggests that, except for these unique dates, denitrification and assimilation of NO$_3^-$-N were generally led by NH$_4^+$-N nitrification. These findings are supported by the fact that uptake efficiency is generally lower for NO$_3^-$-N than for NH$_4^+$-N (Ribot et al. 2017), and that denitrification can be limited by the availability of dissolved organic matter in receiving streams (Ribot et al. 2019).

Based on the seasonal differences in physicochemical variables, we expected seasonal changes in stream net uptake because temperature, oxygen availability, and nutrient availability can strongly influence metabolic activity and nutrient demand of in-stream microbial communities (Butturini and Sabater 1998; Dodds et al. 2002; Von Schiller et al. 2008; Ribot et al. 2017), but this was not always the case in our study. While our results support the idea that WWTP effluents can support high rates of nitrification in receiving streams, $V_f$ for NH$_4^+$-N varied widely over time. In fact, the lack of significant differences in $V_f$ for NH$_4^+$-N among seasons, suggests that the environmental factors that clearly fluctuate seasonally (i.e., temperature or light availability) do not drive the temporal dynamics of in-stream NH$_4^+$-N uptake. Instead, the strong negative relationship between $V_f$ for NH$_4^+$-N and the PC1 scores suggests that the temporal variability of hydrological conditions and associated changes in water chemistry are the controlling factors of in-stream net NH$_4^+$-N uptake. This is further supported by results from the mixed GLM which indicate discharge and NH$_4^+$-N concentration as the most influential variables of $V_f$ for NH$_4^+$-N. We found lower $V_f$ for NH$_4^+$-N during relatively low flow conditions (Figs. 4 and S2 SI), contrasting the idea that stream nutrient uptake efficiency increases with decreasing stream discharge and increasing water residence time (e.g., Peterson et al. 2001; Drummond et al. 2016).

Our study demonstrates high nutrient concentrations likely contribute to saturate in-stream NH$_4^+$-N uptake during these low flow periods when the receiving stream had a small dilution capacity. Furthermore, the decrease in stream dissolved oxygen concentrations during low flows, which dropped down to as low as < 4 mg O$_2$ L$^{-1}$, can inhibit the activity of nitrifiers. This idea is supported by the concomitant reduction in NO$_3^-$-N net release (i.e., less negative values of $V_f$ for NO$_3^-$-N) with increasing PC1 scores. Additional inhibitory mechanisms of nitrification, such as a decrease in pH during low flow conditions, cannot be ruled out. Yet, pH averages 7.5 ± 0.1 in the study stream, and shows small seasonal variation (Bernal, personal communication). Moreover, large shifts in bacterial assemblages and associated microbial activity could also happen when the dilution factor in WWTP impacted streams is < 50% (Romero et al. 2019). To better contextualize our results, we examined how $V_f$ for NH$_4^+$-N varies with NH$_4^+$-N concentration considering a wider set of pristine and human impacted streams, using the data set generated by Marcé et al. (2018). We found that variability in $V_f$ for NH$_4^+$-N from the study stream was relatively low and values fall within the lower range of all the data set (Fig. 5; Table S2).
This comparison needs to be done with caution because the data set includes values of both gross and net uptake and there was no significant regression between $V_f$ for NH$_4^+$-N and NH$_4^+$-N concentration (Fig. 5). However, the funnel-type pattern suggests that in-stream NH$_4^+$-N uptake velocity decreases with increasing ambient NH$_4^+$-N concentrations, even under hydrological conditions favoring the interaction between nutrients and biota.

The hydrologic regime and the capacity for biogeochemical processing are both controlling factors of the temporal variability of nutrient uptake in streams and the role of these ecosystems in the regulation of nutrient cycling and export along the river network (Battin et al. 2008; Acuña et al. 2019). Our results are concordant with the idea that biological processes can be equal or even more important than hydrological conditions to drive nutrient cycling in stream ecosystems (Marcé et al. 2018), especially in highly perturbed streams receiving chronic nutrient inputs. In particular, this study shows the relative importance of biological controls (i.e., nutrient saturation) over hydrologic controls (i.e., discharge and associated water residence time) on the temporal variation of nutrient uptake in WWTP-receiving streams. Moreover, the study suggests that temporal nutrient uptake dynamics are influenced by the interplay between the hydrologic regime and the WWTP influence (i.e., the dilution capacity). Overall, this study contributes to emphasize the distinct biogeochemical temporal dynamics of streams under human pressure (Grimm et al. 2005). Therefore, a better understanding of the temporal variability in nutrient uptake capacity of the receiving streams, and especially of the biogeochemical processes prevailing during low flow conditions, is important to improve the management practices of urban WWTP-impacted streams (i.e., Bicknell et al. 2020). In particular, our study suggests that it is critical to design WWTP operation procedures to consider not only the dilution factor, but also how nutrient inputs affect the bioreactive capacity of receiving streams. For instance, major efforts in WWTP to reduce nutrient content of the effluent should be placed during low dilution conditions (e.g., in summer) to favor in-stream nutrient uptake. The study suggests that an integrated management of both WWTP facility and the receiving stream is critical towards protecting water resources and water quality in urban landscapes, especially under water scarcity conditions.

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1007/s11252-022-01228-5.

**Acknowledgements** We thank M. Bolívar, A. Luque, M. Soler and R. Ventosa and the LabQA Service at the Centre for Advanced Studies of Blanes (CEAB-CSIC) for excellent assistance in field work and laboratory chemical analyses. This study was funded by the projects “EcoReactors” (PGC2018-101975-B-C22), “CANTERA” (RTI2018-094521-B-100) and “Fluvial P-removal” (PID2019-111803RB-H-100) from the I+D program of the Spanish Ministry of Science, Innovation, and Universities. AL was supported by the program Beatriz de Pinós (BP-2018-00082) from the Government of Catalonia and the European Commission. JLJL was supported by a Marie Skłodowska-Curie Individual Fellowship (H2020-MSCA-IF-2018, RIPARIONS ref: 834363). SB was supported by a Ramon y Cajal fellowship (RYC-2017-22643) from the Spanish Ministry of Science, Innovation, and Universities.
Authors’ contributions EM, FS, SB, MR participated in the design of the study. All co-authors contributed to the fieldwork on different sampling dates. SC, SB and EM conducted the compilation of the data set, the calculation of nutrient uptake metrics, and the data analyses. SC, EM, SB and MR wrote the manuscript with contributions from the rest of the co-authors.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This study was funded by the projects “EcoReactors” (PGC2018-101975-B-C22), “CANTERA” (RTI2018-094521-B-I00) and “Fluvial P-removal” (PID2019-111803RB-I00) from I+D program of the Spanish Ministry of Science, Innovation, and Universities. AL was supported by the program Beatriz de Pinós (BP-2018–00082) from the Government of Catalonia and the European Commission. JLJL was supported by a Marie Skłodowska-Curie Individual Fellowship (H2020-MSCA-IF-2018, RIPARIONS ref: 834363). SB was supported by a Ramon y Cajal fellowship (RYC-2017–22643) from the Spanish Ministry of Science, Innovation, and Universities.

Availability of data and material After manuscript acceptance, all data will be archived and available at Zenodo repository.

Declarations

Ethics approval No studies involving animal or human subjects are presented in this manuscript.

Consent to participate All authors participating in this study can withdraw their consent and stop participating at any time without prejudice.

Conflicts of interest/competing interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Abril M, Bastias E, von Schiller D et al (2019) Uptake and trophic transfer of nitrogen and carbon in a temperate forested headwater stream. Aquat Sci 81:1–15. https://doi.org/10.1007/s00027-019-0672-x

Acuña V, Casellas M, Font C et al (2019) Nutrient attenuation dynamics in effluent dominated watercourses. Water Res 160:330–338. https://doi.org/10.1016/j.watres.2019.05.093

American Public Health Association (APHA) (1995) Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association

Argérich A, Martí E, Sabater F et al (2008) Combined effects of leaf litter inputs and a flood on nutrient retention in a Mediterranean mountain stream during fall. Limnol Oceanogr 53:631–641. https://doi.org/10.4393/l jo.2008.53.2.0631

Aristi I, von Schiller D, Arroita M et al (2015) Mixed effects of effluents from a wastewater treatment plant on river ecosystem metabolism: Subsidy or stress? Freshw Biol 60:1398–1410. https://doi.org/10.1111/fwb.12576

Arnon S, Avni N, Gafny S (2015) Nutrient uptake and macroinvertebrate community structure in a highly regulated Mediterranean stream receiving treated wastewater. Aquat Sci 77:623–637. https://doi.org/10.1007/s00204-015-0407-6

Battin T, Kaplan L, Findlay S, Hopkinson C, Martí E, Packman A, Newbold JD, Sabater F (2008) Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geosci 1:95–100. https://doi.org/10.1038/ngeo101

Bernal S, Drummond J, Castelar S et al (2020) Wastewater treatment plant effluent inputs induce large biogeochemical changes during low flows in an intermittent stream but small changes in day-night patterns. Sci Total Environ 714:136733. https://doi.org/10.1016/j.scitotenv.2020.136733

Bernal S, Lupon A, Catalán N et al (2018) Decoupling of dissolved organic matter patterns between stream and riparian groundwater in a headwater forested catchment. Hydrol Earth Syst Sci 22:1897–1910. https://doi.org/10.5194/hess-22-1897-2018

Bernal S, Merbi SN, Ribot M et al (2017) Day-night ammonium oxidation in an urban stream: The influence of irradiance on ammonia oxidizers. Freshw Sci 36:272–283. https://doi.org/10.1086/691797

Bernhardt ES, Hall RO, Likens GE (2002) Whole-system estimates of nitrification and nitrate uptake in streams of the Hubbard Brook Experimental Forest. Ecosystems 5:419–430. https://doi.org/10.1007/s10021-002-0179-4

Bernet MJ, Dodds WK (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8:442–453. https://doi.org/10.1007/s10021-003-0143-y

Beyene A, Legesse W, Triest L, Kloos H (2009) Urban impact on ecological integrity of nearby rivers in developing countries: The Borkena River in highland Ethiopia. Environ Monit Assess 153:461–476. https://doi.org/10.1007/s10661-008-0371-x

Bicknell K, Regier P, Van Horn DJ et al (2020) Linking hydrobiogeochemical processes and management techniques to close nutrient loops in an arid river. Front Water 2:1–12. https://doi.org/10.3389/frwa.2020.00022

Brion N, Billen G (2000) Wastewater as a source of nitrifying bacteria in river systems: The case of the River Seine downstream from Paris. Water Res 34:3213–3221. https://doi.org/10.1016/S0043-1354(00)00770-7

Butturini A, Sabater F (1998) Ammonium and phosphate retention in a Mediterranean stream: hydrological versus temperature control. Can J Fish Aquat Sci 55:1938–1945. https://doi.org/10.1139/cjfas-55-8-1938

Cébron A, Berthe T, Garnier J (2003) Nitrification and nitrifying bacteria in the lower seine river and estuary (France). Appl Environ Microbiol 69:7091–7100. https://doi.org/10.1128/AEM.69.12.7091-7100.2003

Dodds WK, López AJ, Bowden WB et al (2002) N uptake as a function of concentration in streams. J North Am Benthol Soc 21:206–220. https://doi.org/10.2307/1468410

Drummond J, Bernal S, von Schiller D, Martí E (2016) Linking instream nutrient uptake to hydrologic retention in two headwater streams. Freshwater Science 35:4. https://doi.org/10.1086/688599

Earl SR, Valett HM, Webster JR (2006) Nitrogen saturation in stream ecosystems. Ecology 87:3140–3151. https://doi.org/10.1890/0012-9658(2006)7[3140:NSISE]2.0.CO;2
Gammons CH, Babcock JN, Parker SR, Poulson SR (2011) Diel cycling and stable isotopes of dissolved oxygen, dissolved inorganic carbon, and nitrogenous species in a stream receiving treated municipal sewage. Chem Geol 283:44–55. https://doi.org/10.1016/j.chemgeo.2010.07.006

Gordon ND, McMahon TA, Finlayson BL, Gippel CJ, Mathan RJ (eds) (2004) Stream hydrology: An introduction for ecologists. Wiley, West Sussex, UK

Gray NF (2004) Biology of wastewater treatment, 2nd edn. Imperial College Press, London, UK

Grimm NB, Sheibley RW, Crenshaw CL et al (2005) N retention and transformation in urban streams. J North Am Benthol Soc 24:626–642. https://doi.org/10.1899/04-027.1

Gücker B, Brauns M, Pusch MT (2006) Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. J North Am Benthol Soc 25:313–329. https://doi.org/10.1899/0887-3593(2006)25[313:EOWTPD]CO;2

Gupta RS (2008) Hydrology and Hydraulic Systems, 3rd ed. Waveland Press, Long Grove, IL, USA

Haggard BE, Stanley EH, Storm DE (2005) Nutrient retention in a point-source-enriched stream. J North Am Benthol Soc 24:29–47. https://doi.org/10.1899/0887-3593(2005)024%3c:0029:NRAIPS%3eCO;2

House WA, Denison FH (1998) Phosphorus dynamics in a lowland river. Water Res 32:1819–1830. https://doi.org/10.1016/S0043-1354(97)00407-7

Jin SR, Sang DK, Nam IC, An KG (2007) Ecological health assessment of rivers and streams. Freshw Biol 40:145–158. https://doi.org/10.1002/jfb.4046

Keller VDJ, Williams RJ, Lofthouse C, Johnson AC (2014) Worldwide occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters. Environ Pollut 213:144–155. https://doi.org/10.1016/j.envpol.2016.10.087

Keller VDJ, Williams RJ, Lothhouse C, Johnson AC (2014) Worldwide estimation of river concentrations of any chemical originating from sewage-treatment plants using dilution factors. Environ Sci Technol 48:125–181. https://doi.org/10.1021/es404699x

Kuyers MMM, Marchant HK, Kartal B (2018) The microbial nitrogen-cycling network. Nat Rev Microbiol 16:263–276. https://doi.org/10.1038/s41579-018-0009-9

Lin Y-M, Täy J-H, Liu Y, Hung Y-T (2009) Biological Nitrification and Denitrification Processes. Biot Treat Process 8:539–588. https://doi.org/10.1007/978-1-60122-020-6_1

Marcé R, von Schiller D, Aguilera R et al (2018) Contribution of hydrologic opportunity and biogeochemical reactivity to the variability of nutrient retention in river networks. Global Biogeochem Cycles 32:376–388. https://doi.org/10.1002/2018GB005677

Martin E, Riera J, Sabater F (2010) Effects of wastewater treatment plants on stream nutrient dynamics under water scarcity conditions. Pages 173–195 in S. Sabater and D. Barceló (editors). Water quality modeling, monitoring and assessment of water bodies in the Mediterranean: Concepts and methods for assessing solute dynamics in stream ecosystems. JN Am Benthol Soc 9:95–119

Tank JL, Marti E, Riis T et al (2018) Partitioning assimilatory nitrogen uptake in streams: an analysis of stable isotope tracer additions across continents. Ecol Monogr 88:120–138. https://doi.org/10.1002/ecm.1280

```
Trentman MT, Tank JL, Jones SE et al (2020) Seasonal evaluation of biotic and abiotic factors suggests phosphorus retention in constructed floodplains in three agricultural streams. Sci Total Environ 729:138744. https://doi.org/10.1016/j.scitotenv.2020.138744
Von Schiller D, Bernal S, Martí E (2011) Technical Note: A comparison of two empirical approaches to estimate in-stream net nutrient uptake. Biogeosciences 8:875–882. https://doi.org/10.5194/bg-8-875-2011
Von Schiller D, Martí E, Riera JL et al (2008) Influence of land use on stream ecosystem function in a Mediterranean catchment. Freshw Biol 53:2600–2612. https://doi.org/10.1111/j.1365-2427.2008.02059.x
Wakelin SA, Colloff MJ, Kookana RS (2008) Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. Appl Environ Microbiol 74:2659–2668. https://doi.org/10.1128/AEM.02348-07