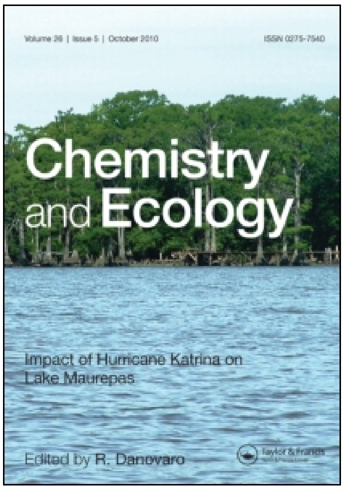

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Nitrogen and phosphorus retention in a human altered stream

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Nitrogen and phosphorus retention in a human altered stream

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A whole stream study was designed to determine the nutrient retention capacity of Fosso Bagnatore, a third-order stream in the Mediterranean region. Nitrogen (NH_4-N, NO_3-N) and phosphorus (PO_4-P) retentions were assessed using controlled nutrient addition experiments. The experimental reaches were selected with reference to a waste water treatment plant effluent and characterized from a physical, hydrological, chemical, and biological standpoint on each nutrient addition date. Nutrient retention was thus compared between control and impacted reaches to detect the possible effluent effects on the stream retention capacity. The impacted reach showed on average a higher concentration of NH_4-N, NO_2-N, DOC, a larger standing crop of benthic organic matter, a lower gross primary production, and a higher respiration rate. Nutrient retention of NH_4-N and PO_4-P was negatively correlated with discharge, but differences between reaches were significant only for PO_4-P retention, being higher in the impacted reach. No differences were observed for NO_3-N retention. We conclude that discharge is the main factor influencing nutrient retention of Fosso Bagnatore, but in summer months, at lower discharge levels, nutrient retention is also influenced by nutrient background concentrations and microbial activity associated with the benthic organic matter.

Keywords: Nutrient addition; Pollution; Waste water treatment plant; Mediterranean region

1. Introduction

Streams are important regulators of nutrient export from watersheds [1–3]. Therefore, the understanding of their functioning is particularly relevant for an effective management of water quality at the river basin level. The nutrient spiralling concept describes the combined process of nutrient cycling and downstream transport and makes possible quantification on a longitudinal scale, coupling of biotic utilization, and downstream transport of nutrients [4, 5]. The amount of nutrients removed from the water column by biotic or physical processes can be estimated with controlled nutrient addition experiments [6].

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The general design of nutrient addition experiments is that solutes are released (known concentration) at the top end of the experimental reach, while the variation of their concentrations along time is measured at the reach bottom end. Solutes can be classified as non-conservative (their concentration is changed by abiotic processes and possible biotic processes) or conservative (their concentration is changed by abiotic processes only). Accordingly, with the addition of a solution of non-conservative solutes (N and P) along with a conservative tracer (either Cl^- or Br) one can estimate: (1) hydrological properties and (2) the nutrient retention efficiency of the experimental reach [6].

Although several studies indicated the importance of hydrological and biological variables in influencing the nutrient retention efficiency in temperate streams [7], little information is available for streams in the Mediterranean region where human activities substantially influence watershed structure and functioning. The aim of this study was to address the nutrient retention of a Mediterranean stream (Fosso Bagnatore, Central Italy) receiving a waste water treatment plant effluent. Controlled nutrient additions were performed in experimental reaches which were selected with reference to this point source of pollution (i.e. control and impacted reaches).

2. Material and methods

2.1 *Study area*

The Fosso Bagnatore (12° 02′ E, 42° 02′ N) (Arsoli, Rome) is a third-order stream, located 50 km north-east of Rome. It drains a limestone catchment of 19 km^2 by a 10 km long and 2–4 m wide channel that is moderately incised in its last part. Headwaters are situated 828 a.s.l. (discharge: 2–10 $l\,s^{-1}$) while the stream ends into the Aniene river 320 m a.s.l. (average slope: 72 $m\,km^{-1}$; mean annual discharge: 300–400 $l\,s^{-1}$). The dominant substrates are rocks, cobbles, and coarse gravel with large pool areas behind dikes. Climate of the basin is Mediterranean with rainfall mainly from September to December and annual precipitation ranging from 1000 to 1600 $mm\,yr^{-1}$. Dominant land uses are arable and grassland (60%), forest and open land (36%), and urban (4%). The human population (2318 inhabitants) is mainly distributed in two villages (Riofreddo and Arsoli), and their wastewater treatment plants (WWTP) represent the main nutrient point sources of Fosso Bagnatore.

We selected the reaches for the experimental work (table 1) after the assessment of spatial and temporal variation in the hydrochemistry across the whole stream [8]. Three of these reaches were unaffected by the WWTP effluents (hereafter collectively referred to as control reach), while a fourth was just downstream the Arsoli WWTP effluent (hereafter referred to as impacted reach).

2.2 *Nutrient addition experiments*

Nutrient retention was estimated using the slug addition technique [9] from February to July 2002 in different stream reaches (table 1). At each reach and sampling date, we performed two subsequent additions, the first to estimate PO_4-P and NH_4-N retention and the second to estimate NO_3-N retention. We added a known volume of solution (e.g. nutrients + Cl^-, as conservative tracer) all at once from a carboy in the mid-channel at the top end of the experimental reach. For phosphate and ammonium additions, we released a solution containing NH_4-N as ammonium chloride (NH_4Cl) and PO_4-P as sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$). For nitrate (N-NO_3) additions, we released a solution containing NO_3-N

Table 1. Characteristics of study reaches and number of addition experiments performed.

Study reach	Distance from headwaters (km)	Length (m)	Width (m)	Depth (mm)	Slope (%)	Large substrata vs. small substrata	Manning roughness	Type	Number of addition experiments
1	5.3	50	1.53	80	1.14	0.51	1.06	Control	1
2	5.7	100	2.03	60	2.16	0.44	0.5	Control	7
3	6.6	100	1.52	50	1.63	0.34	0.2	Impacted	7
4	8.7	100	1.94	70	1.76	–	0.13	Control	2

Note: At each reach and sampling date, we performed two subsequent additions: the first to estimate PO_4-P and NH_4-N retention and the second to estimate NO_3-N retention. Reach length, width, depth, large substrata (%), small substrata (%), and slope were measured directly. Manning roughness was calculated from depth, slope, and water velocity. Values (except slope) are means of all measurements made on the dates of addition experiments. The type for each study reach (i.e. control = unaffected by WWTP effluent; impacted = affected by WWTP effluent) is indicated.

as sodium nitrate ($NaNO_3$) and PO_4-P as sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$). Common salt (NaCl) was added to the solutions, and Cl^- was used as a conservative tracer. Note that sodium dihydrogen phosphate was released along with sodium nitrate to keep resulting N:P rates constant, but P retention was calculated using data from ammonium + phosphate additions only.

All solutions of mixed salts were weighed in the laboratory and made up to volume (*c*. 10 L) on-site with stream water. Complete mixing across the stream channel width was reached within 2 m of the point of the release. Samples for the final concentrations of nutrients and Cl^- in the carboy were collected before the addition started. Once the addition started, a probe (Wissenschaftlich-Technische-Werkstatten 340i) was used to continuously record conductivity values at the bottom of the experimental reach. Water samples were collected at regular intervals in time, increasing the frequency of collection during the passage of the solution. Since the added chloride increased the stream background conductivity, the passage of the solution was clearly detected. The collection of samples stopped when stream conductivity returned to background values measured before the addition.

Water samples were kept on ice and transported to the laboratory. Samples for NH_4-N and PO_4-P were analysed within 24 h following standard methods (see below), while samples for NO_3-N and Cl^- were properly stored until the analysis [10].

2.3 *Nutrient retention estimation*

The mass (mg) of nutrient retained (M_{ret}) is given by:

$$M_{\mathrm{ret}} = M_{\mathrm{exp}} - M_{\mathrm{rec}},$$

where M_{exp} is the nutrient mass expected to be retrieved, if no nutrient uptake occurs, at the bottom end of the experimental reach, and M_{rec} is the nutrient mass retrieved which we measured at the end of the addition experiment.

M_{exp} is calculated from the product of stream discharge and the integral of nutrient expected concentration–time curve. M_{rec} is calculated from the product of stream discharge and the integral of the nutrient observed concentration–time curve. The nutrient expected concentration at time t ($[\mathrm{Nut}]_{\mathrm{exp}\,t}$) is given by:

$$[\mathrm{Nut}]_{\mathrm{exp}\,t} = [\mathrm{Cl}^-]_t \times \left(\frac{[\mathrm{Nut}]_{\mathrm{C}}}{[\mathrm{Cl}^-]_{\mathrm{C}}}\right),$$

where: $[\mathrm{Cl}^-]_t$ is the chloride concentration at time t in the stream water; $[\mathrm{Nut}]_\mathrm{C}$ is the nutrient concentration in the carboy, before the addition started; and $[\mathrm{Cl}^-]_\mathrm{C}$ is the chloride concentration in the carboy, before the addition started.

The amount of nutrient retained was expressed as percentage of the nutrient released divided by the stream bed area S (m^2, see below).

2.4 *Experimental reaches characterization*

The selected experimental reaches were as homogeneous and representative as possible (e.g. dominant stream channel morphology) of the whole stream. They were similar in morphological features and in the degree of canopy cover. Accordingly, the differences in nutrient retention between the two reaches can mostly be attributed to the WWTP effluent effects. In addition, in the selection of the experimental reaches, we avoided including any lateral or vertical significant water input (e.g. from the parafluvial, the riparian or the hyporheic zones). We measured physical, chemical, hydraulic, and biological characteristics (including whole-stream metabolism) at each experimental reach. These measurements were repeated on each addition date.

2.4.1 Physical and chemical characteristics. We made the basic physical measurements of experimental reaches on each addition date. Stream width (w, wet channel only) was measured with a metre tape across the stream. Along six transects, we recorded the water depth (h), water velocity (with a General Oceanics flowmeter) and the type of substrate following the Wentworth scale [9] at 10 cm intervals. Cross-sectional area (A) was calculated as the product between width and depth in each transect and then averaged to obtain a whole reach estimate. The wetted perimeter was calculated as $P = A/h$. The stream bed area (S) was calculated as the product between reach length (L) and the wetted perimeter ($S = P \times L$).

We made on-site measurements for pH and conductivity using a multiparametric probe (Wissenschaftlich-Technische-Werkstatten 340i). Water samples for the determination of PO_4-P, NO_3-N, NO_2-N, NH_4-N, DOC, and Cl^- concentrations were collected in stream water primed dark polyethylene bottles, immediately stored in refrigerated boxes, and transported to the laboratory. On returning to the laboratory, the water samples were filtered through precombusted glass microfiber filters (Whatman GF/F, 0.7 μm). The subsamples for PO_4-P and NH_4-N were analysed within 24 h after having been collected. The subsamples for NO_3-N, NO_2-N, DOC, and Cl^- were properly stored until analysis [10]. NO_3-N plus NO_2-N were analysed automatically on a Technicon Model II auto-analyser using the cadmium–copper reduction method (Technicon Industrial System, Tarrytown, UK; method 94–7w); the NO_2-N was analysed by the same method (without the cadmium reduction), and the NO_3-N was then calculated by subtracting the NO_2-N concentration from the NO_3-N plus NO_2-N concentration. The NH_4-N was analysed by the Indophenol Blue colorimetric method [11]. The PO_4-P was analysed by the Molybdenum Blue colorimetric method [12]. The DOC was analysed using a high-temperature catalytic oxidation Shimadzu TOC analyser. Analyses of Cl^- were performed by the Capillary Electrophoresis technique (Waters, CIA-Quanta 5000, Milford, MA) [13].

2.4.2 Hydrological characteristics. The stream discharge (Q) was calculated by the integration of the Cl^- concentration over the addition time:

$$Q = \frac{([\mathrm{Cl}^-]_\mathrm{C} \times \mathrm{VOL}_\mathrm{C})}{[\mathrm{Cl}^-]_{ts}},$$

where: $[\mathrm{Cl}^-]_\mathrm{C}$ is the concentration of Cl^- in the carboy; $\mathrm{VOL_C}$ is the volume of the carboy solution; and $[\mathrm{Cl}^-]_{ts}$ is the concentration of Cl^- background corrected over the addition time t.

The values of discharge obtained were comparable with those of a monitoring station in the same stream stretch and with those obtained with the point transect methods.

2.4.3 Biological characteristics. We measured the epilithon standing crop by collecting six cobbles from each reach. On return to the laboratory, the cobbles were stored in the refrigerator and processed within 24 h. In the lab, we scraped the cobbles surfaces, washed the material into a container, and then filtered two subsamples of this slurry through precombusted glass microfibre filters (Whatman GF/F, 0.7 μm). One subsample was processed to determine the chlorophyll *a* (Chl *a*) of epilithon algal component, after extraction in 90% acetone overnight, by following the method of Lorenzen (as reported in [10]). The other subsample was processed to determine the ash-free dry mass (AFDM). Filters were dried (60 °C), weighed, combusted (500 °C) and reweighed. The AFDM was then calculated as the difference between precombusted and combusted weight. The area of each cobble was measured by wrapping it into aluminium foil and measuring the resulting area. For all the experimental reaches, whole-stream rates of gross primary production (GPP), respiration (R) and net ecosystem production (NEP) were determined within a day of the addition experiment using the upstream–downstream diurnal dissolved oxygen change technique [14]. The measurements of dissolved oxygen concentration and water temperature (by a multiparametric probe, Wissenschaftlich-Technische-Werkstatten 340i) were made at 15 min intervals over a 24 h period at the top end and bottom end of the experimental reach. The calculation of the exchange of dissolved oxygen with the atmosphere was based on the average oxygen saturation deficit or excess within the experimental reach and on reaeration rates. Reaeration coefficients in each sampling dates were calculated according to the equation of Owens *et al.*, as reported in [15].

2.5 *Statistical analyses*

A one-way ANOVA was used to test differences between mean values of environmental variables among control and impacted reaches. A principal-components analysis (PCA) based on physical, chemical, and biological characteristics was used to order in the reduced space each site × date combination. Multiple regression was used to study the relationship between nutrient retention, discharge, and reach type (as dummy variable). Original variables were log-transformed to homogenize variances when necessary.

3. Results

3.1 *Experimental reaches characterization*

The physical, chemical, and biological characteristics of the control reach (CR) and the impacted reach (IR) are summarized in table 2. Significant differences were found between the control reach and the impacted reach for the $\mathrm{NH_4}$-N (ANOVA, $P < 0.001$, $n = 14$), the $\mathrm{NO_2}$-N (ANOVA, $P < 0.001$, $n = 12$) and the DOC (ANOVA, $P < 0.001$, $n = 12$) concentrations. For the biological characteristics, significant differences were found between the CR and the IR for respiration (ANOVA, $P < 0.01$, $n = 17$).

Table 2. Mean (standard errors) of physical, chemical, and biological variables measured in the control (reaches 1, 2, and 4 pooled) and impacted reaches at time of nutrient addition experiments.

Variable type	Variable	Unit	Control reach		Impacted reach	
Hydrological	Q	$\mathrm{l\,s^{-1}}$	10.80	(7.35)	6.9	(4.22)
	V	$\mathrm{m\,s^{-1}}$	0.09	(0.02)	0.09	(0.01)
	S	$\mathrm{m^2}$	192.2	(76.6)	135.89	(23.4)
Chemical	NH_4-N	ppm	0.055	(0.03)	10.959	(1.95)
	NO^{-3}-N	ppm	3.183	(0.619)	9.84	(2.411)
	NO_2-N	ppm	0.008	(0.001)	0.067	(0.017)
	PO_4-P	ppm	0.303	(0.068)	0.842	(0.226)
	DOC	ppm	2.71	(0.77)	12.243	(1.707)
Biological	EB	mg AFDM $\mathrm{m^{-2}}$	23947	(7521)	68577	(36148)
	Chl *a*	mg Chl *a* $\mathrm{m^{-2}}$	57.36	(11.02)	75.92	(18.59)
	GPP	$\mathrm{gO_2\ m^{-2}\ d^{-1}}$	2.93	(0.97)	0.31	(0.31)
	R	$\mathrm{gO_2\ m^{-2}\ d^{-1}}$	10.92	(3.16)	29.3	(6.09)
	NEP	$\mathrm{gO_2\ m^{-2}\ d^{-1}}$	−7.99	(3.37)	−28.99	(6.14)
	P:R		0.37	(0.12)	0.01	(0.01)

Note: Acronyms are as follows: Q = discharge; V = average velocity; S = stream bed area; DOC = dissolved organic carbon; EB = epilithic biomass; Chl *a* = epilithic Chl *a*; GPP = gross primary production; R = respiration; NEP = net ecosystem production; P:R = NEP:R ratio.

The PCA ordination based on physical, chemical, and biological parameters measured in each site × sampling date combination resulted in three groups dividing the IR sites from CR sites (figure 1). Additionally, a third group was formed by control reach site in the summer sampling dates. The first two axes explained nearly 55% of total variance.

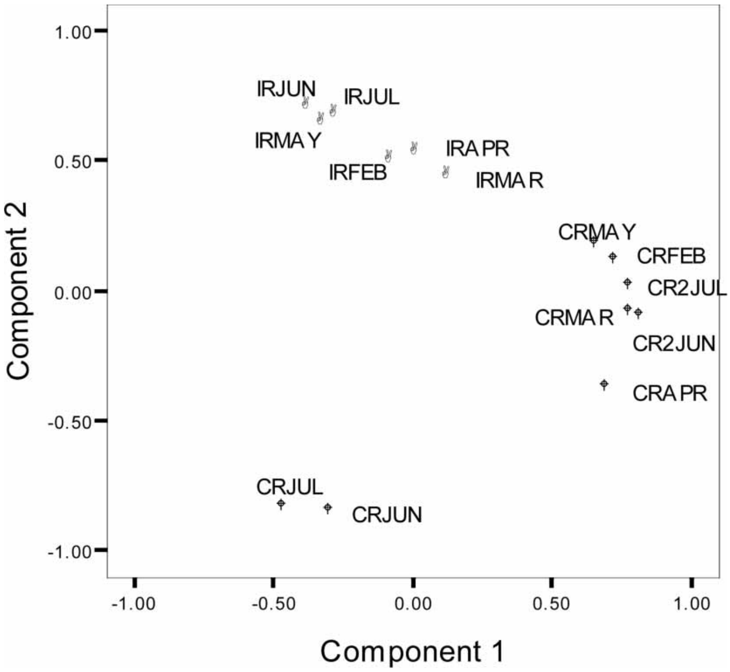

Figure 1. Principal-component analysis ordination based on physical, chemical, and biological characteristics. Each point is a reach-month combination (IR: impacted reach; CR: control reach).

3.2 *Nutrient retention*

Not all nutrient addition experiments were successful, as on same occasions we could not raise the nutrient concentration over the background concentration. Representative curves for slug additions in the two reaches are shown in figures 2–4. Ammonium retention was negatively correlated with discharge (figure 5), but there were no differences between reaches (table 3) as the percentage of NH_4-N retained per m^2 of stream bed area was on average similar between CR (0.36 ± 0.46) and IR (0.09 ± 0.05). Also, the amount of NO_3-N retained (% m^{-2}) was neither significantly different between reaches ($CR = 0.12 \pm 0.15$; $IR = 0.2 \pm 0.16$) nor correlated with discharge (figure 6, table 3). On the other hand, the amount of phosphorus retained (% m^{-2}) was on average higher in the impacted reach (0.09 ± 0.03) than in the control reach (0.06 ± 0.09). Retention of PO_4-P was negatively correlated with discharge (figure 7), but the correlation holds only in the control reach (significant interaction between reach and discharge; table 3).

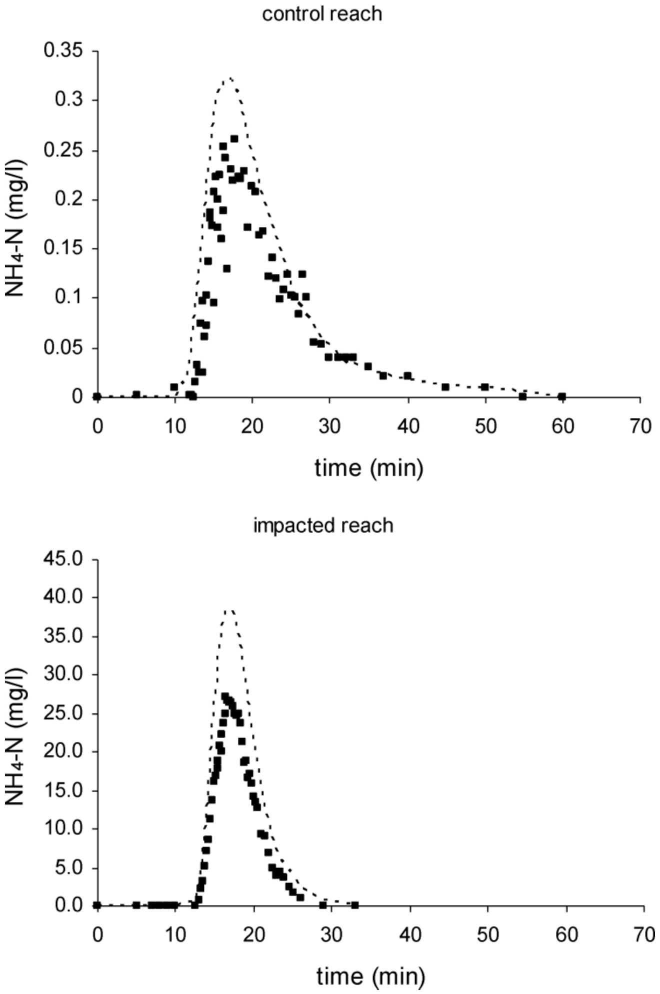

Figure 2. Typical slug addition curve for NH_4-N experimental release (March 2002). Closed boxes are the observed (corrected for the background concentration) concentrations of NH_4-N through the time of the addition, and the dashed line shows the concentrations (background-corrected) of Cl^-. Note the different y-axis scale.

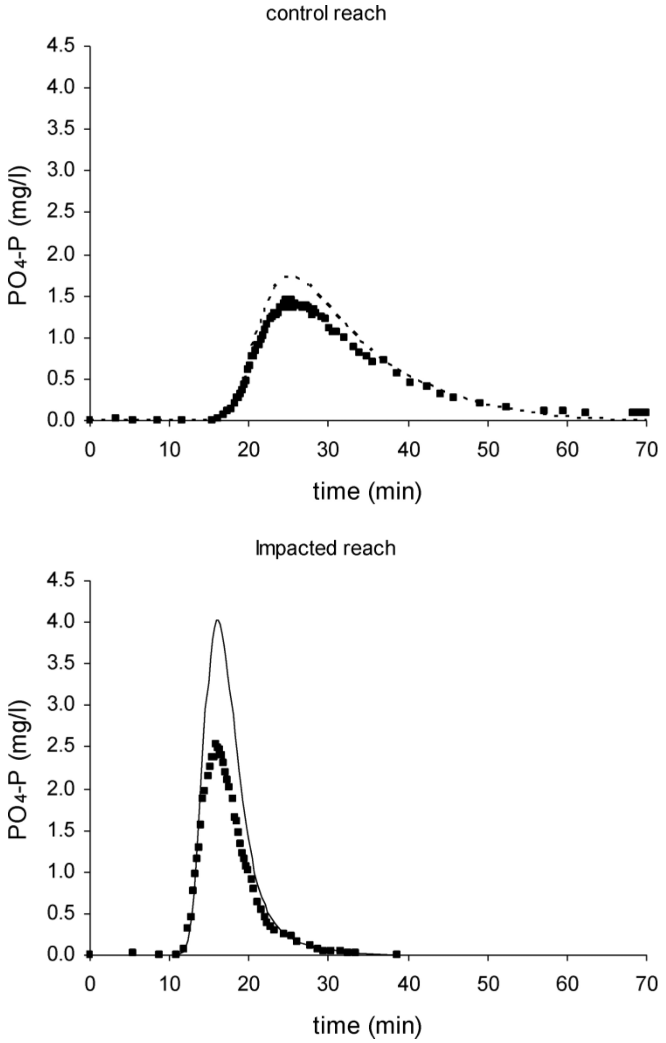

Figure 3. Typical slug addition curve for PO_4-P experimental release (April 2002). Closed boxes are the observed (corrected for the background concentration) concentrations of PO_4-P through the time of the addition, and the dashed line shows the concentrations (background corrected) of Cl^-.

4. Discussion and conclusion

4.1 *Nitrogen and phosphorus retention*

Earlier studies have shown that phosphorus and nitrogen retention are affected by both physical and biological factors [3, 16–19] and that the relative importance of physical and biological factors may vary spatially and temporally within a stream [17, 20]. In particular, discharge was previously reported to affect nutrient uptake efficiency both within and between streams [17]. Our results indicate that nutrient retention of Fosso Bagnatore is negatively correlated with stream discharge.

The fact that nutrient retention capacity decreased with discharge supports the idea that the degree of contact with the streambed increased the potential for abiotic or biotic uptake [21]. Several processes, occurring at the streambed water interface, remove nutrients from water

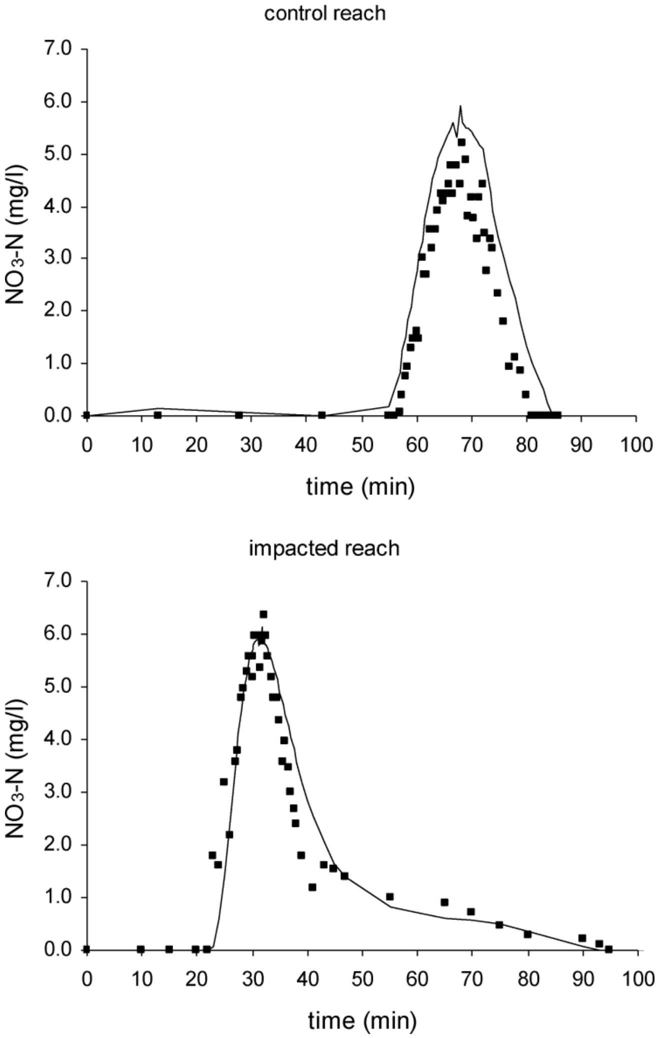

Figure 4. Typical slug addition curve for NO_3-N experimental release (June 2002). Closed boxes are the observed (corrected for the background concentration) concentrations of NO_3-N through the time of the addition, and the dashed line shows the concentrations (background corrected) of Cl^-.

column in streams. These processes include biotic uptake, denitrification, and abiotic sorption by sediments [22, 23]. Biotic uptake occurs when nutrients are removed from the water column by algae (including cyanophyte bacteria), heterotrophic microbes, macrophytes, bryophytes, and riparian plants [24]. For nutrients with a high affinity for surface complexation or adsorption, such as phosphorus and ammonium, the uptake may be controlled by abiotic processes, too [6]. Accordingly, some portions of ammonium and phosphorus spiralling involve adsorption and desorption on organic and inorganic surfaces [24].

Butturini and Sabater [21] stated that a significant relationship between discharge and nutrient uptake length suggests that changes in nutrient retention efficiency can be explained mainly by those biotic processes that are affected by changes in flow rate through season such as detritus accumulation and algae development. The fact that retention was higher at a very low flow in summer months when also whole reach respiration is at maximum levels supports this hypothesis. Respiration by microbial component of stream bed is an important

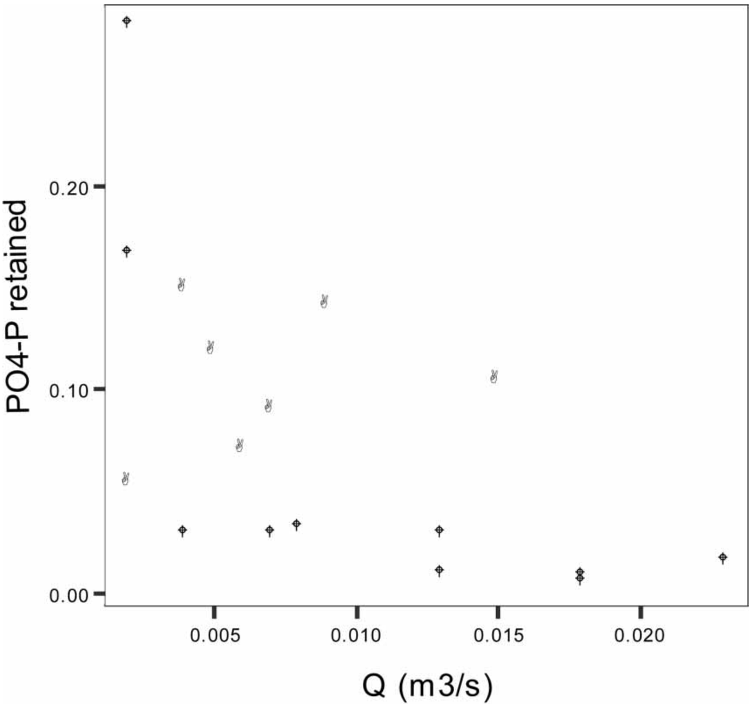

Figure 5. Retention of PO_4-P (as % m^{-2}) in Fosso Bagnatore. Closed circles represent the control reach, and open circles the impacted reach.

Table 3. Results of regression models with nutrient retention (percentage standardized per stream bed area) as dependent variable and discharge (*Q*) and stream reaches as predictor variables.

Nutrient	Parameter	Df	Ms	*F*	*P*
PO_4-P	Intercept	1	1.568	29.418	0.000
	Q	1	0.371	6.965	0.020
	Reach	1	1.067	20.026	0.001
	Q × reach	1	0.849	15.930	0.002
	Error	13	0.053		
NH_4-N	Intercept	1	1.323	18.936	0.002
	Q	1	0.655	9.369	0.014
	Reach	1	0.006	0.081	0.783
	Q × reach	1	0.032	0.459	0.515
	Error	9	0.070		
NO_3-N	Intercept	1	0.164	0.519	0.685
	Q	1	0.140	0.443	0.530
	Reach	1	0.004	0.013	0.913
	Q × reach	1	0.404	1.275	0.302
	Error	6	0.361	1.140	0.327

Note: Stream reach was entered as a dummy variable. Interaction between reach and discharge was also tested. Retention and discharge values were log-transformed to stabilize variances. Degree of freedom (Df); mean square errors (Ms); *F* and *P* values are reported in the table. The number of samples and R^2 of the full models were as follows: PO_4-P: $n = 17$, $R^2 = 0.85$; NH_4-N: $n = 13$, $R^2 = 0.75$; NO_3-N: $n = 10$, $R^2 = 0.21$.

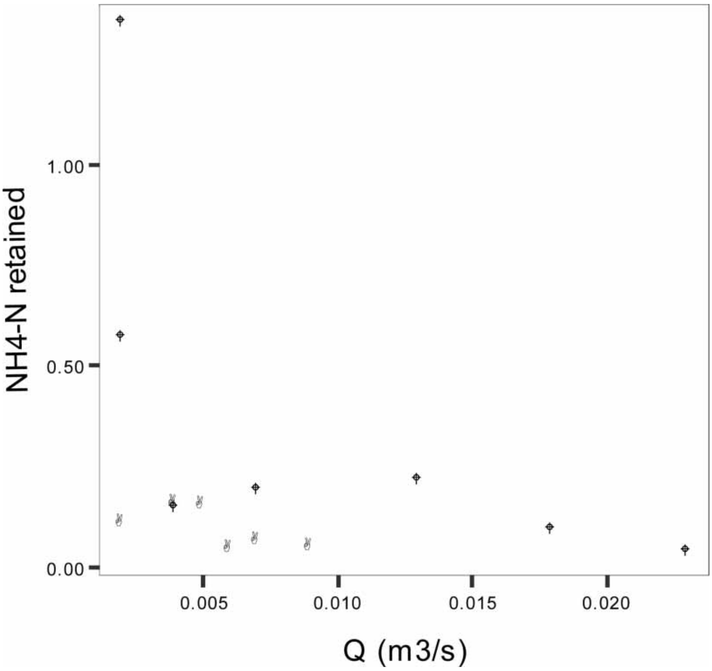

Figure 6. Retention of NH_4-N (as % m^{-2}) in Fosso Bagnatore. Closed circles represent the control reach, and open circles the impacted reach.

driver of nutrient cycling [18, 25] and has already been used in estimating nutrient demand in streams [19, 26].

4.2 *Sewage effluent and nutrient retention*

The amount of nutrient retention in Fosso Bagnatore was lower than previous studies conducted into pristine streams [3]. However, it is comparable to values calculated in a human impacted stream (Spavinaw Creek, Arkansas, USA) by Haggard and colleagues [27]. Therefore, our finding support earlier evidences that input from waste water treatment plant represents pollution point sources that decrease the nutrient retention capacity of receiving streams [28, 29].

In Fosso Bagnatore, the sewage affluent altered water chemistry, benthic organic matter, and whole reach respiration. Its effects can be summarized as follows: (1) it increased the nutrient availability; (2) it increased the bulk of living matter; (3) it increased the heterotrophic microbial activity; and (4) it increased the potential for exchanges between the water column and the streambed [30]. We expected these effects to be reflected in significantly different percentage of nutrient retained in CR and IR. On the other hand, retention of nitrogen forms was similar between CR and IR. High background of nitrate and ammonia concentrations might lead to the saturation of uptake capacity of organisms involved [19, 31]. In our case, even if CR and IR were significantly different from a chemical standpoint, the nutrient concentrations were high enough to saturate the uptake capacity of organisms living in CR, too. This fact would explain the lack of difference in the retained amount of nitrogen forms in CR and IR. Only the percentage of P-PO_4 retained was different among the experimental reaches, being higher in IR. This higher phosphorus retention downstream from the WWTP effluent is probably because of an increased microbial activity related to the more abundant benthic organic matter in the impacted reach.

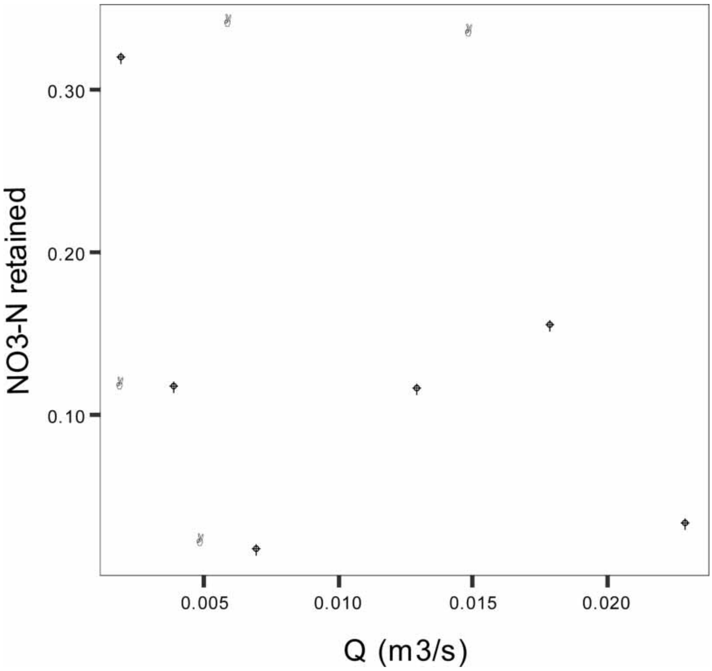

Figure 7. Retention of NO_3-N (as % m^{-2}) in Fosso Bagnatore. Closed circles represent the control reach, and open circles the impacted reach.

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