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Nutrient dynamics and metabolism in Mediterranean streams affected by nutrient inputs from human activities

Dinàmica de nutrients i metabolisme en rius Mediterranis afectats per entrades de nutrients procedents de l'activitat humana

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4. Net changes in nutrient concentrations

below a point source input

Summary

We examined net changes in ammonium (NH_4^+-N), nitrate (NO_3^--N), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC) chloride-corrected ambient concentrations along a reach located below a wastewater treatment plant (WWTP) input in a forested (12 dates) and an agricultural (6 dates) stream. Based on those net changes, we estimated processing length (Snet) and mass transfer coefficient (Vf) of the cited nutrients. Results from the agricultural stream suggest that diffuse nutrient inputs from adjacent agricultural fields had a greater effect on water chemistry than the WWTP input, and probably overwhelmed the stream capacity to retain and transform nutrients. In the forested stream, we observed consistent longitudinal trends below the WWTP input only for NH_4^+-N and NO_3^--N. The tight coupling between longitudinal NH_4^+-N decreases and NO_3^--N increases in the forested stream, and lack of longitudinal trends of DIN on most dates suggest that NH_4^+-N from the WWTP input was being nitrified along the reach. Hence, WWTP inputs are likely to favor conditions to support hot spots for chemoautotrophic activity.

Introduction

Urbanization and agriculture increase nutrient inputs to freshwater ecosystems (Casey *et al.* 1993, Heathwaite *et al.* 1996, Jordan and Weller 1996), and may thus affect the water quality of streams, rivers and lakes, as well as of downstream estuarine and coastal waters (Baker 2003). Nutrient inputs originate mostly in point sources (i.e., wastewater treatment plants, WWTP) in non-agricultural influenced streams, but are dominated by diffuse sources in agricultural streams. During the last 50 years many countries have reduced direct discharge of sewage to streams through the implementation of WWTPs (Tchobanoglous and Burton 1991). However, the use of existing technology to remove nutrients from wastewater is often limited because of financial constraints

(Paul and Meyer 2001), and non-point sources are difficult to control. In addition, human population and related activity are expected to increase in the future decades. This increase will result in an impairment of freshwater quality. Hence, it is important to increase our understanding on how high nutrient loads affect stream structure and function in order to improve management tools.

Streams are able to transform and retain nutrients during downstream transport (Stream Solute Workshop 1990). Pristine streams can retain or transform more than 50 % of nitrogen coming from their catchments within tens to hundreds of meters (Peterson *et al.* 2001). Over the past decades, high nutrient retention efficiency has been reported for a wide range of pristine streams (Mulholland *et al.* 1985, Triska *et al.* 1989, Munn and Meyer 1990, Martí and Sabater 1996, Valett *et al.* 1996, Martí *et al.* 1997, Peterson *et al.* 2001). Most recently, some studies have evidenced that stream nutrient retention efficiency can be significantly reduced in streams receiving WWTP inputs compared to less polluted streams (Haggard *et al.* 2001, Martí *et al.* 2004). Factors controlling stream nutrient retention efficiency have been intensely examined for pristine streams (Butturini and Sabater 1998, Webster *et al.* 2003), but additional research about nutrient dynamics is needed for polluted streams (Paul and Meyer 2001).

The overall goal of our study was to examine the stream capacity to modify dissolved nutrients in transport along a reach receiving nutrient inputs from a WWTP in two streams: one draining a forested catchment and one draining an agriculture-dominated catchment. In a previous study, Martí *et al.* (2004) mostly focused on comparison of nutrient responses across a number of different streams receiving WWTP inputs. This previous study was conducted during the summer season, and showed high variability of nutrient retention efficiency among streams but also showed that, overall, nutrient retention efficiency decreased compared to pristine streams. In this present study, we aim to examine within stream variability in two streams receiving WWTP inputs due to temporal variation in environmental conditions. Specifically, we sought to

compare the point source effect on water chemistry between the two study streams, characterize each stream in terms of net retention and transformation of ammonium (NH_4^+-N), nitrate (NO_3^--N), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP) and dissolved organic carbon (DOC) below the WWTP input and, finally, examine relationships between net changes and nutrient concentrations, temperature, and hydromorphological parameters. We hypothesized that the effect of the WWTP input on stream water chemistry would be greater in the forested stream than in the agricultural stream because of additional nutrient inputs from diffuse sources either upstream or downstream of the WWTP input in the agricultural stream. Also, we hypothesized that diffuse sources of nutrients in the agricultural stream may overwhelm in-stream capacity to process nutrients. Therefore, we expected higher nutrient transformation rates in the forested than in the agricultural stream.

Materials and methods

Field sampling

Along the two study reaches, we examined longitudinal variation in nutrients and chloride (Cl^-) concentrations at ambient level. There were 10 equidistant sampling sites along the two reaches. Additionally, we selected 1 sampling site located about 30 m upstream of the point source in the two study streams. In La Tordera, monthly samplings were conducted on 12 dates from August 2001 to September 2002, with the exception of March and July 2002. In Gurri stream, monthly samplings were done on 6 dates from February 2002 to September 2002, with the exception of June and August. On each sampling date, we collected 3 replicate water samples at all sampling points to analyze concentrations of NH_4^+-N, nitrite (NO_2^--N), NO_3^--N, SRP and DOC. Water samples were filtered *in situ* through pre-ashed fiberglass filters (Whatman$^®$ GF/F) and cold-stored for subsequent analysis. We also measured conductivity and water temperature (WTW$^®$ LF 340) at each sampling point, as well as

channel width, wetted perimeter, water depth and water velocity. Depth and velocity were measured using the velocity-area method at cross-sectional areas of the channel at each sampling point using a NEURTEK (MiniAir 2) current meter as described in Gordon *et al.* (1992), and then calculated average values of these hydromorphological parameters for the entire reaches. We measured discharge using slug additions of NaCl (Gordon *et al.* 1992) at the bottom of the reach. Each sampling was completed within 3 hours to avoid changes in effluent discharge and chemical composition. We also calculated the percentage of hydrological dilution along the reaches using longitudinal variation in concentration of Cl⁻ as proposed by Martí *et al.* (2004). Cl⁻ is a conservative solute and thus, a decrease in its concentration along a reach may be due to lateral or vertical groundwater diffuse inputs with lower Cl⁻ concentration relative to surface stream water. Percentage of dilution (D) along the reaches was calculated using the following equation:

$$D = 100 - \left[\left(\frac{Cl_x}{Cl_1} \right) \cdot 100 \right]$$

where Cl_x and Cl_1 are Cl⁻ concentrations at the sampling site x and at the head of the study reach, respectively.

Laboratory analyses

Water samples were transported on ice to the laboratory. Once there, samples for DOC were acidified to pH 2 (Kaplan 1994) using HCl for their adequate conservation until analyses (Norrman 1993, Butturini personal communication). Then, all water samples were cold-stored at 4 ºC for subsequent analysis of NH_4^+-N, NO_2^--N, NO_3^--N, SRP, DOC and Cl⁻. All chemical analyses were conducted within a week after sampling, except in the case of DOC samples, which were analyzed within 2 months. For up to this time, DOC concentration in acidified water samples remains invariable (Norrman 1993). Concentration of NH_4^+-N was analyzed on a Bran-Luebbe® Technicon

Autoanalyzer II (method 98-70W, Technicon method guide 1976). NO_2^--N, NO_3^--N and SRP concentrations were analyzed on a Bran-Luebbe® TRAACS 2000 Autoanalyzer (methods J-003-88E, J-002-88E, and G-033-92C, respectively), in the case of NO_3^--N using the cadmium-copper reduction method, and in the case of SRP using the molybdenum blue colorimetric method (Murphy and Riley 1962). Concentration of DOC was analyzed using a high-temperature catalytic oxidation (Shimadzu® TOC 5000 analyzer). To determine Cl^- concentration we used the capillary electrophoresis technique (Waters®, CIA-Quanta 5000, Romano and Krol 1993).

Calculation of processing length and mass transfer coefficient

We examined longitudinal variation in ambient concentrations of NH_4^+-N, NO_3^--N, DIN (obtained as the sum of NH_4^+-N, NO_2^--N, and NO_3^--N concentrations), SRP, DOC, and Cl^- along the study reaches in order to calculate processing length and mass transfer coefficient. Processing length refers to those values (in units of m) obtained from ambient longitudinal decline/increase in nutrient concentration, in our case downstream of a WWTP input. Longitudinal variation in nutrient ambient level reflects net result of all removal processes (e.g., uptake, denitrification, precipitation) minus all release processes (e.g., nitrification, mineralization, redissolution). Processing length is analogous to uptake length calculated from short-term additions (Fisher *et al.* 1998), and reflects whether the stream acts as a net sink or a net source of nutrients. The mass transfer coefficient is conceptualized as the velocity at which a nutrient moves from the water column to the sediment, and thus, represents the demand for nutrients relative to the concentration in the water column (Stream Solute Workshop 1990).

To calculate processing length, we assumed that the net variation in nutrient concentration along the reach can be described by the equation (Martí *et al.* 1997):

$$N_x = N_1 \cdot \left(\frac{Cl_x}{Cl_1} \right) \cdot e^{-bx}$$

where N is nutrient concentration and Cl is chloride concentration, both at site 1 (sampling point located at the top of the reach) and at each downstream sampling point (x). In our case, x is the distance from the point source input because we aimed to examine longitudinal net changes in nutrient concentrations from the WWTP input. When ambient nutrient concentrations are corrected by chloride concentrations, changes in nutrient concentration along the reach that are due only to dilution are excluded (Martí *et al.* 2004). The natural logarithm of the ratio between N_x/N_1 and Cl_x/Cl_1 at each sampling point was plotted versus downstream distance. The slope of this regression (b) is the downstream net nutrient change coefficient at ambient levels (in 1/m), and the inverse of the slope (-1/b) is the nutrient processing length (Snet, in m) from ambient nutrient variation. Similar to uptake length (Newbold *et al.* 1981), a short positive processing length indicates a higher nutrient retention efficiency than a longer processing length. In contrast, a negative processing length indicates a net export of nutrient from the reach, with lower absolute values indicating greater export. A lack of significant longitudinal pattern in corrected nutrient concentration along the reach would indicate that removal processes and release processes are approximately balanced, and thus, in this case the stream does not act as a net sink or a net source of the nutrient.

The mass transfer coefficient (Vf, m/s) was calculated using the following equation (Stream Solute Workshop 1990):

$$Vf = h \cdot \frac{v}{Snet}$$

where h is the average depth (m) of the study reach, v is the average water velocity in the reach (m/s), and Snet is the processing length (m). A positive Vf indicates that the nutrient moves from the water column to the stream bed sediment and represents nutrient biological demand (Stream Solute Workshop 1990). In contrast, a negative Vf indicates the velocity at which a nutrient moves from the streambed back to the water column.

Statistical analyses

All data was log-transformed prior to statistical analyses in order to stabilize variances and normalize the data sets. In the two study streams, we evaluated the effect of the point source on stream nutrient concentrations (NH_4^+-N, NO_3^--N, DIN, SRP and DOC), conductivity, and molar ratios between nutrients (NO_3^-:NH_4^+, DOC:DIN, DOC:SRP, DIN:SRP) by comparing these parameters between sampling sites located upstream of the WWTP input and the top of the reach using the paired *T*-test. Additionally, in those cases where net longitudinal decreases in nutrient concentrations were significant, we compared concentrations between the upstream site and the sampling site located at the end of the study reach using the paired *T*-test, in order to examine if nutrient concentrations returned to upstream conditions along the reach. We used the Student's *T*-test to compare nutrient concentrations and conductivity upstream of the WWTP input between La Tordera and Gurri streams. We used regression analyses to examine relationships between wetted perimeter, depth, velocity, discharge, water temperature, nutrient concentrations and molar ratios as independent variables and nutrient retention/release metrics (Snet and Vf) as dependent variables, as well as to examine relationships between retention/release metrics among nutrients (e.g., Vf-NO_3^--N versus Vf-NH_4^+-N). The significance level used for all statistical tests was *P* ≤ 0.05. All statistical analyses were performed with the SPSS® 11.0 for Windows statistical package.

Results

Physical and chemical parameters

Water temperature followed a seasonal pattern in the two streams, with the lowest temperature values during winter, the highest during summer, and intermediate values between these two seasons. The range of temperature was narrower in Gurri than in La Tordera stream (Table 4.1). Ranges of wet channel width, water depth, velocity and discharge were wider in La Tordera than in Gurri stream (Table 4.1). Percentage of point source contribution to the stream discharge increased as discharge above the point source decreased in the two streams. In La Tordera, the input represented 100 % of the stream discharge in August, September and October 2001 because the stream was completely dry upstream of the input. In contrast, the input only represented 3 % of the stream flow when the stream discharge was the highest. On average, the point source input represented 38 (±11) % in La Tordera. In Gurri, the contribution of the point source to the stream discharge ranged from 21 % in March to 66 % in July, with an average of 39 (±7) %. Therefore, variation in point source contribution to stream discharge was wider in La Tordera than in Gurri stream, but annual averages were similar.

Upstream of the WWTP input, concentration of NO_3^--N, DIN, SRP and DOC, as well as conductivity were higher in Gurri than in La Tordera

Table 4.1. Minimum, maximum, and mean ± standard error (SE) of water temperature and hydromorphological parameters in La Tordera (n = 12) and Gurri (n = 6) streams.

Parameter	La Tordera			Gurri		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Temperature (ºC)	8.3	25.7	16.5 ± 1.7	7.9	19.9	13.6 ± 2.1
Width (m)	3.7	8.6	5.7 ± 0.4	3.4	4.2	3.8 ± 0.1
Depth (m)	0.059	0.315	0.147 ± 0.021	0.077	0.179	0.116 ± 0.016
Velocity (m/s)	0.05	0.61	0.23 ± 0.05	0.08	0.53	0.28 ± 0.06
Discharge (L/s)	12	1450	267 ± 115	19	148	79 ± 24

(respectively: t = -3.022, -2.747, -3.025, -5.134, -5.152; df = 13; P (two tail) = 0.025, 0.033, 0.010, 0.002, 0.003) (Table 4.2). In the two streams, NO_3^--N was the predominant form of DIN relative to NH_4^+-N upstream of the point source, accounting for the 92 (±3) % of the total DIN (n = 9) in La Tordera, and for a 98.4 (±0.5) % of DIN (n = 6) in Gurri.

In La Tordera (the forested stream) there were significant increases of NH_4^+-N, DIN and DOC concentrations and conductivity downstream of the outfall (P ≤ 0.05 in all cases; see Table 4.2). DIN concentration not only increased but also changed the proportion of the different N forms (NH_4-N and NO_3-N) transported in the stream water relative to the upstream site. The NO_3^-:NH_4^+ ratio significantly decreased downstream of the point source (P = 0.004). Downstream of the point source NO_3^--N represented 54 (±8) % (n = 12) of total DIN. No significant differences were found for DOC:DIN, DOC:SRP or DIN:SRP molar ratios between the upstream and the downstream sites (P > 0.05; Table 4.2). In Gurri (the agricultural stream), the WWTP input only increased conductivity (P = 0.002), but no other significant effects were measured (Table 4.2).

Dilution along the study reaches averaged 12 (±4) % for La Tordera and Gurri streams (Table 4.3). No clear patterns were observed between discharge and dilution, or between Snet and dilution in the two streams. Highest percentages of dilution occurred after some important rainfall events in the study streams. Also, previous soil moisture conditions may influence the degree of soil water saturation, and thus, diffuse lateral inputs along the study reaches after particular rainfall events.

Net changes in nutrient concentrations along the study reach: the agricultural stream

In Gurri stream, longitudinal patterns of SRP concentration tended to increase along the reach, and thus, in these cases we obtained negative Snet and Vf (Table 4.3). We found significant net increases in DOC concentration along the study reach on most dates, as indicated by negative Snet and Vf (Table

Table 4.2. Mean ± standard error of nutrient concentrations, conductivity, and molar ratios for La Tordera and Gurri streams. In the case of La Tordera, we show results for sampling dates when the stream flowed upstream and downstream of the WWTP input (n = 9), and for all sampling dates including the three dates when the stream was dry upstream of the WWTP input (n = 12). For La Tordera and Gurri, we present significance from the paired T-test (df = 8 and 5, respectively) comparing the variables considered (Log_{10} transformed values) between sampling sites located upstream of the WWTP input (i.e., ~ 30 m above) and the top of the gradient reach (downstream).

Parameter	n	La Tordera Upstream	La Tordera Downstream	t	P	n	Gurri Upstream	Gurri Downstream	t	P
NH_4^+-N (mg N/L)	9	**0.28 ± 0.14**	**1.59 ± 0.40**	-3.99	**	6	0.02 ± 0.01	0.05 ± 0.03	-0.96	ns
	12		2.09 ± 0.43							
NO_3^--N (mg N/L)	9	2.71 ± 0.27	2.57 ± 0.30	0.78	ns	6	5.92 ± 1.03	6.33 ± 0.89	-0.91	ns
	12		2.21 ± 0.31							
DIN (mg N/L)	9	**3.02 ± 0.34**	**4.23 ± 0.42**	-3.29	*	6	5.99 ± 1.02	6.43 ± 0.93	-0.92	ns
	12		4.38 ± 0.40							
SRP (mg P/L)	9	0.08 ± 0.02	0.33 ± 0.12	-2.14	ns	6	0.18 ± 0.03	0.27 ± 0.10	-1.42	ns
	12		0.59 ± 0.20							
DOC (mg/L)	9	**1.63 ± 0.21**	**2.31 ± 0.14**	-2.71	*	6	5.37 ± 0.70	5.20 ± 0.50	0.27	ns
	12		3.50 ± 0.68							
Conductivity (μS/cm)	9	**213.2 ± 14.1**	**279.8 ± 26.9**	-5.20	***	6	**909.7 ± 134.4**	**1354.5 ± 249.5**	-6.12	**
	12		403.8 ± 67.9							
NO_3^-:NH_4^+	9	**165.3 ± 98.8**	**4.0 ± 1.5**	3.95	**	6	643.4 ± 256.4	421.2 ± 201.7	1.00	ns
	12		3.1 ± 1.2							
DOC:DIN	9	0.69 ± 0.11	0.72 ± 0.12	-0.63	ns	6	1.45 ± 0.51	1.07 ± 0.21	1.07	ns
	12		1.03 ± 0.22							
DOC:SRP	9	448.7 ± 272.1	75.4 ± 37.8	1.53	ns	6	104.1 ± 29.7	124.0 ± 60.6	0.71	ns
	12		62.1 ± 28.9							
DIN:SRP	9	431.8 ± 232.5	87.6 ± 27.2	1.85	ns	6	84.3 ± 15.1	99.1 ± 36.4	0.12	ns
	12		68.2 ± 22.5							

ns, not significant (P > 0.05); *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001. Significant differences are depicted in bold.

4.3). In this stream we did not find any clear longitudinal patterns in NH_4^+-N concentration. Only at the time of the lowest discharge (July), we observed a net decrease in NH_4^+-N concentration along the reach. Similarly to SRP and DOC, we observed net increases of NO_3^--N along the reach on most dates, which resulted in negative Snet and Vf (Table 4.3). DIN was dominated by NO_3^--N, and we observed simultaneous net increases in DIN and NO_3^--N concentrations during the study. In addition, ranges, averages and standard errors of both Snet and Vf were very similar between NO_3^--N and DIN (Table 4.3).

Net changes in nutrient concentrations along the study reach: the forested stream

No significant changes in SRP concentration were observed along the reach in La Tordera (Table 4.3). DOC concentration decreased along the reach only during low flow conditions (September and October 2001), and increased during high flow (April 2002). In contrast to SRP and DOC, we observed consistent patterns for NH_4^+-N and NO_3^--N. There was a net decrease in NH_4^+-N concentration along the reach on all sampling dates, which resulted in positive Snet and Vf (Table 4.3). The greatest percentage (95 %) of NH_4^+-N concentration decrease from the head to the end of the reach occurred under lowest discharge, in September 2001. In contrast, the lowest percentage (25 %) of NH_4^+-N concentration decline along the reach was measured with the highest discharge, in May 2002. Despite the net decrease of NH_4^+-N along the reach on all dates, its concentration was still significantly higher at the end of the study reach than upstream of the WWTP input (P = 0.032). On average, Snet-NH_4^+-N was in the order of km, and Vf-NH_4^+-N in La Tordera was four times higher than in Gurri. Longitudinal patterns of NO_3^--N concentration tended to be the converse to those of NH_4^+-N. There were significant increases in NO_3^--N concentration along the reach in 8 of 12 dates, from which we obtained negative Snet and Vf (Table 4.3). The lowest longitudinal increase (4 %) in NO_3^--N concentration concurred with the lowest decrease of NH_4^+-N concentration. Despite clear patterns of NH_4^+-N and NO_3^--N in La Tordera, on most dates there were no significant changes in DIN concentration along the reach. There was a significant decrease of DIN concentration along the reach only in September and August 2001, coinciding with low flow conditions. In contrast, we found a significant increase of DIN in November 2001 and June 2002.

We examined relationships between retention/release metrics (Snet and Vf) and nutrient concentrations, temperature, and hydromorphological parameters only for NH_4^+-N and NO_3^--N in the case of La Tordera, due to consistency of NH_4^+-N and NO_3^--N longitudinal patterns and the high number of

available cases. Specifically, we found a negative relationship between Snet-NH_4^+-N and water temperature, and the DOC:DIN molar ratio (Fig. 4.1). In addition, there was a negative relationship between Vf-NH_4^+-N and DIN concentration (Fig. 4.2).

Table 4.3. Processing length (Snet) and mass transfer coefficient (Vf) of nutrients obtained for each sampling date in La Tordera and Gurri streams. The table also shows dilution along the study reaches for the two streams. For each parameter and each nutrient, we show minimum, maximum, mean and standard error (SE). In cases with positive and negative values of Snet and Vf, we only present minimum and maximum. Significance of the regression between net changes in ambient chloride-corrected nutrient concentrations versus downstream distance is shown.

Month	Year	Dilution %	SRP Snet m	SRP Vf cm/min	DOC Snet m	DOC Vf cm/min	NH_4^+-N Snet m	NH_4^+-N Vf cm/min	NO_3^--N Snet m	NO_3^--N Vf cm/min	DIN Snet m	DIN Vf cm/min
La Tordera												
August	2001	19	ns	ns	ns	ns	580**	0.033	-322	-0.060	8576*	0.002
September	2001	0	ns	ns	2311*	0.010	181***	0.122	ns	ns	498***	0.044
October	2001	12	ns	ns	1824*	0.040	448***	0.164	-493***	-0.149	ns	ns
November	2001	27	ns	ns	ns	ns	669***	0.378	-931***	-0.271	-1451**	-0.174
December	2001	4	ns	ns	ns	ns	1791*	0.059	-441*	-0.238	ns	ns
January	2002	0	487***	0.286	ns	ns	2872*	0.048	-1758***	-0.079	ns	ns
February	2002	10	ns	ns	ns	ns	1678*	0.185	-2296*	-0.135	ns	ns
March	2002	na	na	na	na	na	na	na	na	na	na	na
April	2002	39	ns	ns	-3889*	-0.146	1076*	0.529	-1082*	-0.526	ns	ns
May	2002	0	ns	ns	ns	ns	954*	1.217	ns	ns	ns	ns
June	2002	28	ns	ns	ns	ns	478***	0.287	-1044**	-0.131	-1287**	-0.106
July	2002	na	na	na	na	na	na	na	na	na	na	na
August	2002	7	ns	ns	ns	ns	443**	0.327	ns	ns	ns	ns
Setember	2002	0	ns	ns	ns	ns	979*	0.210	ns	ns	ns	ns
Minimum		0	-	-	-3889	-0.146	181	0.033	-2296	-0.526	-1451	-0.174
Maximum		39	-	-	2311	0.040	2872	1.217	-322	-0.060	8576	0.044
Mean		12	-	-	-	-	1012	0.297	-1046	-0.199	-	-
SE		4	-	-	-	-	221	0.094	242	0.053	-	-
Gurri												
February	2002	6	-340***	-0.333	-911***	-0.124	-336***	-0.340	-1828**	-0.062	-997***	-0.114
March	2002	22	-1852**	-0.073	-1335***	-0.101	ns	ns	ns	ns	ns	ns
April	2002	27	-205***	-1.607	-1828**	-0.180	ns	ns	-1684***	-0.196	-1704**	-0.193
May	2002	11	ns	ns	ns	ns	ns	ns	-7473*	-0.076	-7692*	-0.074
July	2002	3	ns	ns	ns	ns	499**	0.075	-3476**	-0.011	-3616**	-0.010
September	2002	3	1393*	0.113	-1799**	-0.088	ns	ns	ns	ns	ns	ns
Minimum		3	-1852	-1.607	-1828	-0.180	-336	-0.340	-7473	-0.196	-7692	-0.193
Maximum		27	1393	0.113	-911	-0.088	499	0.075	-1684	-0.011	-997	-0.010
Mean		12	-	-	-1468	-0.123	-	-	-3615	-0.086	-3502	-0.098
SE		4	-	-	217	0.020	-	-	1349	0.039	1502	0.038

na, not available; ns, not significant ($P > 0.05$); *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$
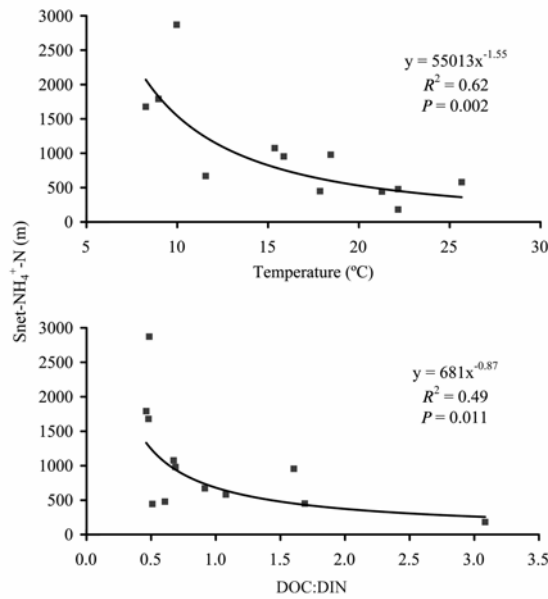
Fig. 4.1. NH_4^+-N processing length (Snet-NH_4^+-N) as a function of water temperature and DOC:DIN molar ratio for La Tordera (forested) stream. The solid lines show the relationship between dependent (Snet-NH_4^+-N) and independent (temperature and DOC:DIN) variables. For each relationship, the equation and statistics (R^2 and P) are shown (n = 12).
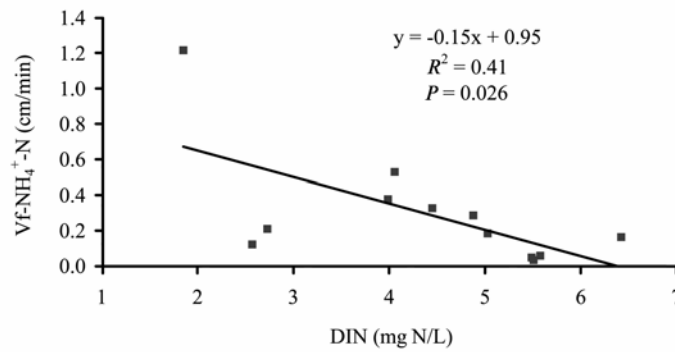

Fig. 4.2. NH_4^+-N mass transfer coefficient (Vf-NH_4^+-N) as a function of DIN concentration for La Tordera (forested) stream. The solid line shows the relationship between the dependent (Vf-NH_4^+-N) and the independent (DIN) variable. Equation and statistics (R^2 and P) of this relationship are shown (n = 12).

Discussion

Contrasting effects of a WWTP input on water chemistry between the forested and the agricultural stream

Inputs from WWTPs had different effects on the water chemistry of the two streams. Concentrations of NO_3^--N, DIN, SRP and DOC above the WWTP input were higher in Gurri (agricultural) stream than in La Tordera (forested) stream. The WWTP discharging to La Tordera and Gurri lack specific mechanisms to actively remove nitrogen or phosphorus. However, the effect of the WWTP input on water chemistry was greater in La Tordera than in Gurri (Table 4.2). In La Tordera, the input increased concentrations of DIN and DOC, whereas in Gurri it did not affect the concentrations of any nutrient. DIN concentration in La Tordera not only increased but also changed the relative proportion of NO_3^--N relative to NH_4^+-N. A recent study has also reported this effect on the $NO_3^-:NH_4^+$ ratio due to WWTP inputs at several streams from Catalonia (Martí *et al.* 2004). Our results suggest that in Gurri diffuse sources of nutrients from agricultural activity overwhelm the local point source effect on water chemistry. Diffuse sources to streams increase nutrient concentrations and cause the impairment of water quality. It is well known that diffuse pollution, predominantly from agricultural activity, is a major source of nitrogen and phosphorus to surface waters (Carpenter *et al.* 1998). Additionally, our results evidence that inputs from WWTPs can also decrease stream water quality, mainly increasing NH_4^+-N concentration.

Net changes in nutrient concentrations below a WWTP input in the forested and the agricultural stream

In this study we examined in-stream capacity to influence nutrient concentrations in La Tordera and Gurri streams based on the longitudinal variation in ambient concentrations of nutrients below a WWTP input. Based on an idea introduced by Bartsch (1948), Hynes (1978) proposed that in a stream

receiving sewage inputs the physical, chemical and biological (i.e., communities structure) attributes should change with increasing downstream distance from the input. Regarding the chemical characteristics, more recently studies have shown that in-stream uptake and release of nutrients strongly influence stream water nutrient concentrations (Mulholland and Hill 1997), and that the link between upstream and downstream water chemistry is controlled by biogeochemical in-stream processes (Mulholland *et al.* 1995).

Our study shows that the influence of in-stream processes on solutes in transport varies depending on the nutrient considered and differs between the two streams. Neither La Tordera nor Gurri showed any clear influence on SRP or DOC concentrations below the WWTP input. Longitudinal variation of SRP along a reach can decrease due to assimilation and co-precipitation with calcium carbonate (calcite), and can increase due to subsurface inputs or redissolution (Neal *et al.* 2000, Haggard *et al.* 2001). In La Tordera, the saturation index of calcite was negative during the study (STREAMES group, unpublished data), indicating that the water in La Tordera was undersaturated with respect to this mineral. Therefore, co-precipitation of SRP with calcite was unlikely to occur along the study reach. On the other hand, previous studies have shown that the percentage of phosphorus exported downstream tends to be larger in streams draining areas influenced by human activity (e.g., WWTP inputs) than in low influenced streams (Bowes *et al.* 2003). Altogether, these factors may explain the lack of a net decrease of SRP along the study reach in La Tordera. In Gurri, in contrast, co-precipitation was likely to occur due to positive saturation index of calcite during the study (STREAMES group, unpublished data). Also, biotic uptake of SRP was expectable because riparian canopy did not cover the stream channel, and thus, light availability enhanced algal growth. Algal activity plays an important role in controlling SRP retention efficiency (Sabater *et al.* 2000). Therefore, in this stream we predicted net decreases of SRP along the reach. However, generally there were net increases or no net changes of SRP along Gurri reach. In this stream, diffuse inputs from adjacent agricultural fields along

the reach, balance between SRP removal and release processes, or low vertical exchange due to bedrock dominance covering the streambed may explain why no clear longitudinal trends in SRP concentration were observed.

Regarding DOC, La Tordera did not show net changes in 75 % of all cases. In those few cases where longitudinal patterns were significant, the trends of DOC concentration along the reach were opposite depending on the stream flow. During late summer (low flow), DOC concentration decreased along the reach. Concentration of DOC can decrease along a reach as a consequence of heterotrophic demand, and can increase as a result of autotrophic production (Mulholland and Hill 1997) or, depending on biogeochemical processes other than antecedent moisture conditions, as a result of diffuse inputs during storm flow (Bernal *et al.* 2002). DOC tended to increase during spring storm flow in La Tordera, which suggested that under these conditions subsurface diffuse sources of DOC were greater than in-stream removal. In Gurri stream, similar to SRP, DOC tended generally to increase along the study reach. Additionally, this stream did not show capacity to process NH_4^+-N or NO_3^--N. In Gurri DIN was dominated by NO_3^--N, and net increases in both NO_3^--N and DIN concentrations along the reach were observed on most dates. The lack of inverse longitudinal patterns between NH_4^+-N and NO_3^--N, together with the predominant increase of DIN and NO_3^--N (as well as of the other nutrients) suggested that diffuse sources of NO_3^--N were relevant along the study reach in Gurri stream. This finding agrees with previous studies, which suggested that input of groundwater NO_3^--N in agriculturally-influenced streams exceed the stream uptake capacity (Kemp and Dodds 2001). Mass transfer coefficients in Gurri were 4 times lower than in La Tordera, and were between 4 and 17 times lower than those reported by Hall *et al.* (2002) from their own study and from other studies (Martí and Sabater 1996, Mulholland *et al.* 2001). As previous researchers have proposed, low mass transfer coefficients of NH_4^+-N estimated in Gurri may be explained because high NO_3^--N concentrations may reduce heterotrophic demand for NH_4^+-N, reducing competition for NH_4^+-N between heterotrophs and nitrifiers, and stimulating

nitrification rates (Bernhardt *et al.* 2002, Hall *et al.* 2002). In addition, the dominance of bedrock and lack of sediment in the streambed of Gurri stream reduces vertical exchange, constraining the transient storage zone to surface. This constraining may also explain low biological demand for NH_4^+-N in this stream. Previous studies have suggested that large transient storage zones may enhance nutrient uptake in streams (Valett *et al.* 1996, Martí *et al.* 1997, Mulholland *et al.* 1997), probably resulting in higher biological demand.

Net changes in N concentration below a WWTP input in the forested stream

In La Tordera, the WWTP input constituted a clear source of NH_4^+-N to the stream. In this case, NH_4^+-N concentration longitudinally decreased below the input on all dates. However, NH_4^+-N concentration did not return to upstream conditions in any case along the 550 m of the study reach. Processing lengths of NH_4^+-N from our study were in the order of km, as in other streams receiving WWTP inputs (Haggard *et al.* 2001, Martí *et al.* 2004). These previous studies suggested that nutrient retention efficiency is reduced in comparison with non-polluted streams where nutrient concentrations are much lower. On average, mass transfer coefficients of NH_4^+-N in La Tordera were very similar to those reported by Hall *et al.* (2002) from their own study and from another study (Mulholland *et al.* 2001), but were between 2 and 4 times lower than those obtained from Martí and Sabater (1996) in two pristine streams located near La Tordera. Higher NH_4^+-N biological demand in these pristine streams than in La Tordera may be due to lower nutrient availability, especially NH_4^+-N and NO_3^--N, and thus, higher NH_4^+-N heterotrophic demand.

Our results indicated that in La Tordera there was a significant relationship between NH_4^+-N processing length and water temperature. Earlier investigations have shown that water temperature plays an important role in regulating NH_4^+-N uptake in pristine streams (Martí and Sabater 1996, Butturini and Sabater 1998). Our results suggest that biotic uptake plays an important role

controlling NH_4^+-N dynamics in La Tordera because temperature regulates the activity of organisms. Additionally, processing length of NH_4^+-N decreased (i.e., NH_4^+-N retention efficiency increased) as DOC increased relative to DIN, indicating that heterotrophic uptake of NH_4^+-N was enhanced with increasing DOC concentration relative to DIN. Negative relationship between NH_4^+-N biological demand versus DIN concentration also suggested that biotic removal of NH_4^+-N from the water column was saturated by high DIN concentrations.

The net result of all processes involved in the nitrogen cycle modulates longitudinal patterns of DIN concentration. In La Tordera, simultaneous net decreases in DIN and DOC concentrations along the reach during low flow (<15 L/s) suggested that part of DIN was being lost via denitrification, as shown in previous studies (Steinhart *et al.* 2000). In contrast, there were net increases of DIN concentration along the reach during those dates with high discharge or after floods, suggesting that under these conditions, diffuse inputs overwhelmed in-stream nitrogen processing. Inverse patterns of NH_4^+-N and NO_3^--N concentrations were observed in 70 % of all cases, which may explain why on most dates there were no variations in DIN concentration along the reach (i.e., NH_4^+-N removal was being equilibrated with NO_3^--N release). Also, mass transfer coefficients of NH_4^+-N and NO_3^--N from our study were negatively related (Fig. 4.3). This relationship explained 68 % of variation during the study, and had a slope very close to -1 (b = -0.7), suggesting that most of NH_4^+-N removal could be attributed to nitrification. The tight coupling between NH_4^+-N and NO_3^--N dynamics found in La Tordera during the study had been suggested before across a number of streams receiving WWTP inputs (Martí *et al.* 2004). This study showed a correlation between declines in NH_4^+-N concentration and increase in NO_3^--N concentration in 40 % of cases, and also suggested that in these cases NH_4^+-N from the WWTP input was being nitrified. In addition, we expected uptake of SRP in La Tordera given the high DIN:SRP molar ratios measured there (i.e., potential P limitation). Thus, the lack of measurable SRP uptake supports our conclusion that nitrification is the main process going on in

this stream. Under experimental conditions, high concentrations of NH_4^+-N favor chemoautotrophic relative to heterotrophic activity (Butturini *et al.* 2000). These authors evaluated the nitrification efficiency of a Mediterranean stream to be between 77 and 96 %, and suggested that the chemoautotrophic community determined NH_4^+-N transformation and NO_3^--N availability in their study stream. Results from our study support this previous finding, and suggest that nitrification may be a common process in streams affected by WWTP inputs.
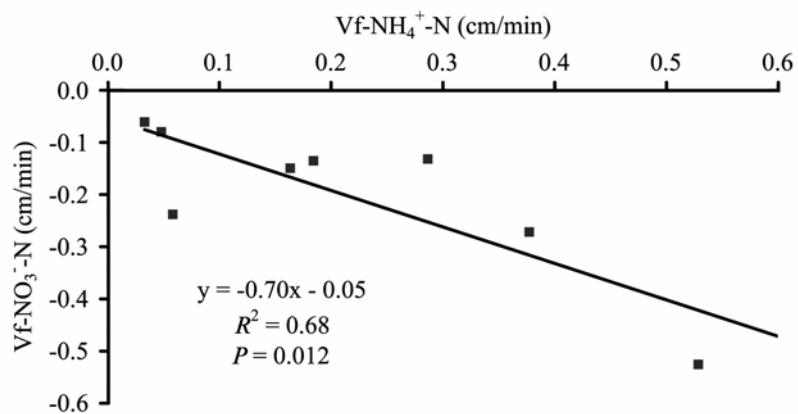
Fig. 4.3. Linear relationship between mass transfer coefficients of NH_4^+-N and NO_3^--N (Vf-NH_4^+-N and Vf-NO_3^--N, respectively) for La Tordera (forested) stream. The solid line shows the relationship between the dependent (Vf-NO_3^--N) and the independent (Vf-NH_4^+-N) variable. Equation and statistics (R^2 and P) of this relationship are shown ($n = 8$).

5. Influences of a point source on

N and P in-stream retention

Summary

We examined the effect of point source inputs on in-stream nutrient retention in two streams draining catchments with contrasting land use composition. The selected streams were La Tordera and Gurri (NE Spain) that drain a forest- and an agricultural-dominated catchment, respectively. In each stream, we compared nutrient retention metrics from nutrient additions conducted upstream and downstream of a wastewater treatment plant input (i.e., point source). Nutrient additions were conducted on 8 and 9 dates during 2001-2003 in La Tordera and Gurri streams, respectively, encompassing changes in environmental conditions.

The effect of point source on physical, chemical and biological parameters differed between the two streams. Water velocity increased downstream of the point source relative to upstream in the two streams, with an overall increase in discharge only in the agricultural stream. In the forested stream, the point source significantly increased concentrations of ammonium (NH_4^+-N), nitrate (NO_3^--N), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP) and dissolved organic carbon (DOC), whereas in the agricultural stream, the point source only increased concentration of SRP. Increases in nutrient concentrations downstream of the point source relative to upstream did not result in overall significant increases in habitat weighted patch specific (i.e., biofilm and filamentous green algae) chlorophyll *a* and biomass in the forested stream, but did in the agricultural. In the former, the point source effect on habitat weighted chlorophyll *a* and biomass was likely to be masked by greater effect of washout during flood disturbance occurring on few dates than by nutrients availability.

Point source effects on stream nutrient retention were also different between the two streams. In the forested stream, uptake lengths (S_w) of NH_4^+-N and phosphate (PO_4^{3-}-P) averaged hundreds of m upstream of the point source and they increased (i.e., decreased retention efficiency) about 4 and 5 times, respectively, below the point source. NO_3^--N S_w were ≥ 2 km regardless of the reach location. In the agricultural stream, S_w of NH_4^+-N, NO_3^--N and PO_4^{3-}-P were

mostly within the km range at the two reaches with only a marginal ($P < 0.10$) point source effect increasing S_w of NO_3^--N and $PO_4^{3-}-P$.   Mass transfer coefficients (V_f) of nutrients (NH_4^+-N, NO_3^--N and $PO_4^{3-}-P$) in the study streams were within the same range (10^{-4}-10^{-6} m/s) than those previously reported from a variety of streams, and were similar between the two reaches. Finally, the relative change in uptake rates (U) of NH_4^+-N between the two reaches (i.e., (U_{down}-U_{up})/U_{up}) were positively related with the relative point source contribution to downstream NH_4^+-N loads in La Tordera. In Gurri, the relative change in U of NH_4^+-N and NO_3^--N between the two reaches were positively related with the relative point source contribution to downstream loads of NH_4^+-N, NO_3^--N and DOC.

Nutrient retention responses to variation in stream nutrient concentrations due to point source inputs indicate that nitrification of NH_4^+-N from the point source and denitrification of NO_3^--N play important roles in the study streams. Demand for $PO_4^{3-}-P$ was likely to saturate at high SRP concentrations in the two study streams, a trend that remained significant when combining our results with those from literature. Hence, despite the variety of biological compartments involved in phosphorus uptake, biotic saturation under high SRP concentrations is likely to occur across streams from diverse regions.

Introduction

Availability and quality of freshwater has become a major international problem during the last decades (Pimentel *et al.* 1997). Point source inputs, such as those from wastewater treatment plants (WWTPs), are a major cause of impairment of streams draining urban areas (Walsh 2000, Walsh *et al.* 2005). These local inputs not only change the hydrological regime and increase ambient nutrient levels (House and Denison 1997, Kim *et al.* 2002, Albek 2003, Martí *et al.* 2004), but also affect the ecological integrity of the stream ecosystems (Paul and Meyer 2001). This is especially relevant in regions where freshwater is

scarcer, such as the Mediterranean region, because contribution of point sources can account for most of the stream nutrient loads (Gasith and Resh 1999). In addition to point sources, human-derived nutrients can also reach the stream through diffuse pathways (i.e., non-point sources). While point sources may cause an abrupt hydrological and chemical discontinuity along the stream, changes due to diffuse sources may not be as discrete at local scale. These contrasting patterns between human-derived sources of nutrients should have differentiated effects on the ecology of stream ecosystems. In particular, in this study we examined the relative effect of point sources on stream nutrient dynamics under two contrasted land use scenarios (i.e., forested dominated and agricultural-dominated) and for different nutrients (i.e., ammonium, NH_4^+-N; nitrate, NO_3^--N; and phosphate, PO_4^{3-}-P).

Streams have the capacity to transform and retain nutrients entering from adjacent terrestrial ecosystems, the so called self-purification capacity (McColl 1974, Elosegui *et al.* 1995), and thus, regulate to some extent the export of nutrients to downstream ecosystems (Alexander *et al.* 2000). Several studies conducted across a broad geographical and climatic range have evidenced the high stream efficiency to retain nutrients (Sebetich *et al.* 1984, Mulholland *et al.* 1985, Triska *et al.* 1989, Munn and Meyer 1990, D'Angelo and Webster 1991, Martí and Sabater 1996, Valett *et al.* 1996, Martí *et al.* 1997, Mulholland *et al.* 1997, Peterson *et al.* 2001). Most of these studies were conducted in streams draining catchments with low human activity pressure, and thus having relatively low nutrient loads. Human-derived nutrient inputs reaching streams through point and nonpoint sources may act as a subsidy or a stress to stream communities, this latter case being termed as nutrient saturation conditions (Odum *et al.* 1979, Lohman *et al.* 1991, Hart and Finelli 1999, Bernot and Dodds 2005). Such saturation may result in a decrease of the stream nutrient retention efficiency (i.e., stream nutrient retention relative to nutrient flux), with consequences for downstream ecosystems. In fact, some recent studies suggest that nitrogen and phosphorus retention efficiency is reduced in streams receiving WWTP inputs

relative to pristine streams (Haggard *et al.* 2001, Martí *et al.* 2004, Merseburger *et al.* 2005). However, these studies focused on longitudinal net changes in ambient nutrient concentrations below point sources and compared their results with literature values from pristine streams obtained using short-term additions at constant rate (e.g., Munn and Meyer 1990, Webster *et al.* 1991, Martí and Sabater 1996). Other recent studies have shown that urban streams are less retentive than forested streams by comparing among them retention metrics obtained by use of nutrient additions (Grimm *et al.* 2005, Meyer *et al.* 2005). However, they did not examine point source effects on nutrient retention metrics within streams.

The present study takes an experimental approach to examine the point source effects on in-stream nutrient retention by comparing nutrient retention metrics (i.e., nutrient uptake length, mass transfer coefficient and nutrient uptake rate; Stream Solute Workshop 1990) between reaches located upstream (i.e., as the reference site) and downstream (i.e., as the altered site) of a WWTP input under varying environmental conditions. This comparison was done in a stream where high nutrient loads were point source originated (i.e., forest-dominated stream), and in a stream receiving diffuse-sources from adjacent agricultural fields in addition to the point source (i.e., agricultural-dominated stream).

We hypothesized that increases in stream discharge and nutrient concentrations due to point source inputs would have a significant effect on nutrient retention efficiency. Higher discharge below the point source may reduce the surface-volume ratio and thus, may decrease nutrient exchange with active sediments, resulting in lower nutrient retention efficiencies (Butturini and Sabater 1998, Peterson *et al.* 2001, Hall *et al.* 2002). On the other hand, high nutrient concentrations may enhance growth of biofilm communities, fact that can result in an increase in stream nutrient retention capacity (i.e., higher nutrient uptake rates) or in a decrease in nutrient demand (i.e., decrease mass transfer coefficient). Both effects should also affect stream nutrient retention efficiency. Finally, we expected that point source effects will be conditioned/modulated to/by

the environmental setting above the local input that somehow may be influenced by the land use context of the stream. Also, we expected that point source effects will depend on the nutrient under consideration if dynamics of each element are controlled by different processes. Our study addresses actual gaps on the biogeochemistry of human-altered streams at ecosystem level.

Materials and methods

Short-term nutrient additions

To estimate in-stream retention metrics for NH_4^+-N, NO_3^--N and PO_4^{3-}-P, we conducted short-term nutrient additions on several dates though 2001-2003 in the two reaches of La Tordera (8 dates) and Gurri (9 dates) streams. The short-term nutrient addition at constant rate is the most commonly used methodology to estimate stream nutrient retention metrics (e.g., Munn and Meyer 1990, Webster *et al.* 1991, Martí and Sabater 1996). However, the high ambient nutrient concentrations of the two streams constrained the application of this methodology, especially in the reaches located downstream of the point source input. Instead, we conducted slug additions (McColl 1974, Meals *et al.* 1999, Wilcock *et al.* 2002), which were more suitable for this particular study and allowed comparison of results between the two reaches. Only upstream of the point source in La Tordera stream, which was the reach with lowest nutrient loads, we were able to conduct both slug and constant rate additions on some dates. Results from these nutrient additions were comparable especially at low discharge.

On each sampling date and at each reach, we conducted two nutrient additions—NH_4+PO_4 and NO_3+PO_4—on two consecutive days. NH_4^+, NO_3^- and PO_4^{3-} were added as NH_4Cl, $NaNO_3$ and $NaH_2PO_4.H_2O$, respectively. The added solution was set to result in low increases of ambient nutrient concentrations (c.a., 2-4 times ambient levels) and to vary N and P concentrations proportionally to stream ambient ratios. The solution also contained chloride (as NaCl) used as

conservative tracer to account for solute dilution and dispersion (Bencala *et al.* 1987) and to signal the passage of the added solution at the bottom of the reach. All experiments were conducted at noon on unclouded days and under baseflow conditions.

For each slug addition, reagents were mixed in 10-50 L (depending on the stream discharge) of stream water in a carboy. The solution was added as a single pulse to the stream at a point of concentrated flow (i.e. high turbulence) about 10 m above the head of the reach to ensure a fast mixture between the addition solution and the stream water along the selected reach. Once the solution was poured into the stream, we recorded conductivity and water temperature (WTW® LF 340 conductivity meter) at 5-seconds intervals with a data logger (Campbell CR 510) at the bottom of the reach. Data was recorded until conductivity returned again to ambient levels after the passage of the solution pulse. During this period, we simultaneously collected water samples with 30-ml plastic bottles at the bottom of the reach every 10-60 seconds following the frequency of conductivity changes over time. Water samples were filtered in situ through pre-ashed fiberglass filters (Whatman ® GF/F) and placed in a cooler for transportation to the laboratory where they were kept in the refrigerator until analysis.

Photosynthetic active radiation (PAR, μmol m^{-2} s^{-1}) was recorded every 10 min during the sampling period using a spherical quantum sensor (Li-Cor Li-193SA, Lincoln, NB, USA) connected to a data logger (Li-Cor, Li-1000). The spherical quantum sensor was placed 20 cm above the stream water surface at the middle of the reach. Temperature and PAR for each addition were estimated as the average of all data recorded during the sampling period. On each date and for each reach, we also measured wet channel width and water depth at 6 transects evenly distributed along the reach. At each transect, water depth, substrata type, and patches of biofilm and filamentous green algae were measured or characterized every 20 cm across the channel width. Wet channel width and water depth for each reach was computed as the average from

transect measurements. Composition of streambed substrata was computed from the number of transect measurements for a given substratum type relative to the total number of measurements. Percentage of coverage of biofilm and filamentous green algae along the reach was calculated from the number of transect measurements for a given patch relative to the total number of measurements. This percentage of coverage was also visually estimated in the field, and these values were then compared with those obtained from transect measurements. We also collected samples of biofilm and filamentous green algae for characterization of patch specific chlorophyll *a* and biomass. To determine patch specific chlorophyll *a* and biomass, we randomly collected 3 stones from each transect for duplicated (18 for chlorophyll *a* and 18 for biomass analyses) for each patch (i.e., biofilm and filamentous green algae). We broke the stones and selected flat surface pieces of about 4 cm^2. The exact area of each rock piece was latter measured using the computer program Scion Image (for Windows release Beta 4.0.2, Scion Corp., Frederick, Maryland) through high-resolution digital photographs of the stone pieces to determine pigment concentration per unit area. All samples for determination of chlorophyll *a* and biomass were frozen until analysis.

Laboratory analysis

Water samples collected to determine concentrations of NH_4^+-N, NO_3^--N, soluble reactive phosphorus (SRP), dissolved organic carbon (DOC) and chloride (Cl^-), were transported on ice to the laboratory and placed in the refrigerator until chemical analysis were done. Description of methods used to determine nutrient concentrations is given in Chapter 4. Pigment extraction to determine habitat weighted chlorophyll *a* was performed with acetone 90 % (4 ºC for 24 h) and processed according to Steinman and Lamberti (1996). Absorbance was measured with a Shimadzu double-beam spectrophotometer (UV-2100). Habitat weighted biomass was measured as mass loss of samples dried to constant mass (60 ºC, 48 h) then combusted (450 ºC, 4-5 h). For each date, we computed

the average chlorophyll *a* and biomass for the reach based on percentage of reach coverage of patch specific (i.e., biofilm and filamentous green algae) data and total reach surface area.

Calculation of nutrient retention metrics

Nutrient concentrations and conductivity data from the additions were used to estimate uptake length (S_w, in units of m), mass transfer coefficient (V_f, in m/s) and uptake rate (U, in mg m^{-2} s^{-1}) of nutrients. These metrics allow characterization of different aspects of stream nutrient retention, and thus, all three should be reported (Stream Solute Workshop 1990, Doyle *et al.* 2003, Grimm *et al.* 2005). S_w is defined as the average distance an element travels between its release to the water column and subsequent removal from the water column by the benthic zone (Newbold *et al.* 1981). This parameter reflects the in-stream nutrient retention efficiency in terms of nutrient removal relative to the nutrient flux (Hart *et al.* 1992, Martí and Sabater 1996). Shorter S_w indicates higher nutrient retention efficiency than longer values. V_f is a measure of the velocity at which a nutrient molecule moves from the water column to the sediments, and it is an index of biological demand (Stream Solute Workshop 1990, Hall *et al.* 2002). U is the mass of nutrient retained per unit area of the stream bottom and per unit time, and indicates the stream nutrient retention capacity (Stream Solute Workshop 1990).

Nutrient uptake length was estimated based on the comparison between measured nutrient concentrations (NH_4^+-N, NO_3^--N and SRP) and those predicted from Cl^- concentrations (Fig. 5.1). The predicted concentrations assumed that nutrients were conservative elements, and thus, variation over time was solely due to hydrological factors (i.e., advection, dispersion and dilution). Variation over time of measured nutrient concentrations was additionally influenced by retention/release processes. The predicted nutrient concentration at different times (t) during the experiment ($\partial N_{(pred)}/\partial t$) was calculated using the following equation:

$$N_{(pred)t} = \left(\frac{Cl_t - Cl_b}{Cl_I} \cdot N_I \right) + N_b$$

This equation assumes that changes in background-corrected nutrient concentration (N_t-N_b) over time relative to the nutrient concentration of the added solution (N_I) are equal to the changes in background-corrected chloride concentration (Cl_t-Cl_b) over time relative to the chloride concentration in the added solution (Cl_I). If nutrient are retained along the reach, predicted nutrient concentrations should be higher than measured nutrient concentrations (Fig. 5.1). The difference in the integrated area of the concentration-time curve between the predicted and measured values (in mg L^{-1} s) times the stream discharge (L/s) equals the amount of nutrient retained along the reach during the experiment (mg). We calculated the nutrient uptake rate at experimental concentration levels (U_{exp}, in mg m^{-2} s^{-1}) as the mass of nutrient retained per unit area of the stream
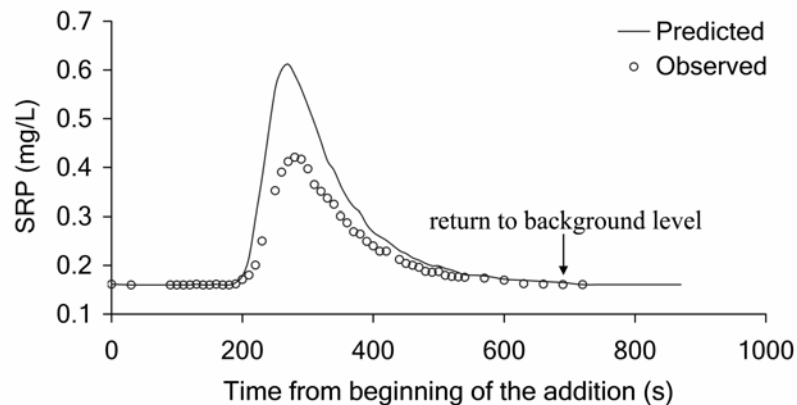

Fig. 5.1. Variation of predicted and observed nutrient concentrations at the bottom of an experimental reach over the course of a slug addition passage. The arrow indicates the time in which nutrient concentration returns to background level after the peak. The graph shows as an example data for SRP concentration from a slug addition conducted downstream of the point source in La Tordera on December 6, 2002.

bottom (i.e., reach area, in m^{-2}) and per unit time (i.e., time needed for the added solution to travel along the reach, in s; Fig 5.1). S_w was then calculated based on the following equation:

$$Sw = \frac{N_{peak} \cdot Q}{U_{exp} \cdot w}$$

where N_{peak} is the peak nutrient concentration (mg/L) measured during the addition, Q is the stream discharge (L/s) and w is the wet channel width (m). From S_w we estimated uptake rates at ambient level (U, in mg m^{-2} s^{-1}) based on the equation above and considering stream ambient nutrient concentrations. This calculation assumes that U is linearly related to nutrient concentration within the range of resulting concentrations from the slug addition. If this assumption is not met, the estimated U would underestimate actual rates. V_f was calculated as the product of water depth and velocity divided by S_w (Stream Solute Workshop, 1990).

Statistical analyses

For each stream, we used paired *T*-test analysis on log-transformed data from the two reaches to examine the effect of the WWTP input on physical, chemical and biological parameters. Independent samples *T*-test on log-transformed data from the upstream reaches of the two streams was used to compare chemical, biological and functional parameters between the two streams. All data was log-transformed to stabilize variances and normalize the data sets. The effect of the point source on the nutrient retention metrics was also examined using regression analysis between the relative change in nutrient retention metrics downstream of the WWTP (i.e., (downstream value – upstream value)/ upstream value) and the relative contribution of the point source to downstream nutrient loads (i.e., downstream loads – upstream loads)/upstream loads). Finally, to remove the hydrological effect on nutrient retention response associated to the point source input and focus only on the effect due to chemical

changes, we examined relationships between V_f of NH_4^+-N, NO_3^--N and PO_4^{3-}-P and stream nutrient concentrations and molar ratios (NH_4^+-N:NO_3^--N, DIN:SRP and DOC:DIN). Linear, power, exponential and logarithmic regression analyses were done with data from each stream separately and also considering data from all sites together. This latter approach was done to examine existence of general patterns among different human-altered stream scenarios and to compare responses among different nutrients. The significance level used for all statistical tests was $P \leq 0.05$. All statistical analyses were performed with the SPSS® statistical package (for Windows, version 11.0.1, SPSS Inc., Chicago, Illinois).

Results

Physical, chemical and biological characterization

Average water temperature and PAR values during the additions were comparable between reaches in the two study streams (Table 5.1). Of the measured hydromorphological parameters, only water velocity increased downstream of the point source relative to upstream in the forested stream (Table 5.1). In contrast, discharge significantly increased downstream of the point source in the agricultural stream, with the consequent increases in water velocity, depth and channel width (Table 5.1). The point source contributed to 20-90 % and 22-100 % of the downstream discharge in La Tordera (mean ± SE, 42 ± 9 %) and Gurri (mean ± SE, 61 ± 9 %) streams, respectively.

Ambient nutrient concentrations upstream of the WWTP were higher in the agricultural than in the forested stream (independent samples *T*-test, *df* = 14: NO_3^--N, *t* = 8.122, *P* < 0.001; DIN, *t* = 8.199, *P* <0.001; SRP, *t* = 6.157, *P* < 0.001; DOC, *t* = 7.112, *P* < 0.001) except for NH_4^+-N concentration (independent samples *T*-test: *t* = 0.495, *df* = 14, *P* = 0.629; Table 5.1). In the forested stream, the WWTP input significantly increased concentrations of all studied nutrients and decreased the NO_3^-:NH_4^+ and DIN:SRP molar ratios (Table 5.1). The change in

Table 5.1. Range and mean ± standard error (SE) of water temperature, photosynthetic active radiation (PAR), morphohydraulical, chemical and biological parameters measured on the addition dates at the upstream and downstream reaches in La Tordera (n = 8) and Gurri (upstream, n = 8; downstream, n = 9) streams. The table shows the degree of significance from paired T-test on these parameters between the two reaches within each study stream. Benthic chlorophyll a and biomass values are habitat weighted considering patch especific values for biofilm and filamentous green algae and their percentage of reach coverage. Ratios between nutrients are molar ratios.

Parameter	Reach	La Tordera			Gurri		
		Range	Mean ± SE	P	Range	Mean ± SE	P
Temperature (ºC)	up	10.3-25.4	15.3 ± 1.9	ns	5.3-20.2	13.5 ± 2.0	ns
	down	9.4-27.2	16.0 ± 2.3		5.3-25.8	14.8 ± 2.5	
PAR (μmol m^{-2} s^{-1})	up	224-2183	893.1 ± 277.4	ns	142.8-2011.4	776.9 ± 254.8	ns
	down	124-1094	440.1 ± 127.3		277.2-2415.5	1525.8 ± 289.9	
Width (m)	up	2.1-8.8	6.8 ± 0.7	ns	2.2-3.1	2.6 ± 0.1	***
	down	4.0-6.7	5.8 ± 0.3		2.5-4.1	3.5 ± 0.2	
Depth (cm)	up	3.0-16.5	11.7 ± 1.7	ns	6.4-20.9	11.9 ± 1.8	*
	down	8.1-20.1	14.0 ± 1.3		3.1-16.1	9.0 ± 1.5	
Velocity (m/s)	up	0.03-0.47	0.27 ± 0.05	**	0.04-0.59	0.27 ± 0.07	*
	down	0.09-0.71	0.39 ± 0.07		0.17-0.85	0.39 ± 0.08	
Discharge (L/s)	up	1.5-405.9	171.1 ± 47.3	ns	5.8-117.0	48.6 ± 16.1	**
	down	15.0-504.7	227.9 ± 61.4		8.7-395.0	101.2 ± 40.5	
NH$_4^+$-N (mg N/L)	up	0.006-0.072	0.037 ± 0.009	***	0.011-0.150	0.050 ± 0.017	ns
	down	0.146-2.210	0.780 ± 0.309		0.014-0.210	0.078 ± 0.022	
NO$_3^-$-N (mg N/L)	up	0.66-2.20	1.11 ± 0.18	**	2.69-13.38	7.43 ± 1.18	ns
	down	1.59-6.40	2.99 ± 0.58		4.47-10.87	7.33 ± 0.78	
DIN (mg N/L)	up	0.72-2.21	1.15 ± 0.18	**	2.76-13.68	7.53 ± 1.20	ns
	down	1.80-9.33	3.90 ± 0.89		4.54-11.02	7.48 ± 0.79	
SRP (mg/L)	up	0.006-0.040	0.014 ± 0.004	***	0.02-0.38	0.21 ± 0.05	*
	down	0.05-3.78	0.68 ± 0.45		0.12-0.69	0.38 ± 0.07	
DOC (mg/L)	up	0.55-2.39	1.16 ± 0.25	**	3.22-7.75	5.33 ± 0.62	ns
	down	1.01-2.71	1.90 ± 0.18		3.86-7.86	5.30 ± 0.40	
NO$_3^-$:NH$_4^+$	up	10-244	71 ± 30	**	41-598	287 ± 81	ns
	down	2-28	8 ± 3		32-502	175 ± 51	
DIN:SRP	up	40-493	243 ± 50	**	19-901	188 ± 104	ns
	down	5-197	64 ± 25		22-156	59 ± 14	
DOC:DIN	up	0.5-2.8	1.3 ± 0.3	ns	0.4-3.3	1.2 ± 0.4	ns
	down	0.3-1.3	0.8 ± 0.1		0.5-2.0	1.0 ± 0.2	
Chl a (mg/m^2)	up	6-110	41 ± 12	ns	12-474	129 ± 51	**
	down	4-916	204 ± 106		33-776	384 ± 95	
Biomass (g/m^2)	up	7.2-41.7	22.0 ± 5.9	ns	11.8-93.5	35.8 ± 13.0	*
	down	13.7-69.8	30.3 ± 8.4		32.9-112.6	66.9 ± 12.8	

ns, not significant (P > 0.05); *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

the NO_3^-:NH_4^+ ratio represented an increase in the average (±SE) percentage of DIN as NH_4^+-N from 5 ± 1 % in the upstream reach to 20 ± 4 % in the downstream reach. The point source did not affect the DOC:DIN molar ratio (Table 5.1). In contrast, the point source only caused a significant increase in PO_4^{3-}-P concentration in the agricultural stream. In this stream, the nutrient molar ratios did not significantly vary between the two reaches (Table 5.1). In the agricultural stream, DIN was dominated by NO_3^--N (≥ 98 %) in the two reaches.

In the forested stream, range and mean of habitat weighted chlorophyll *a* were wider and higher, respectively, downstream than upstream of the point source, but high variability through the study resulted in lack of statistical differences between the two reaches (Table 5.1). Significant differences in habitat weighted biomass between the two reaches of the forested stream were neither found (Table 5.1). In contrast, in the agricultural stream, both chlorophyll *a* and biomass were significantly higher downstream than upstream of the point source (Table 5.1).

Point source effects on nutrient retention metrics in the two streams

In the forested stream, S_w of NH_4^+-N and PO_4^{3-}-P averaged <1 km upstream of the point source and, on average, increased 4 and 5 times, respectively, below the point source (Table 5.2, Fig. 5.2a). In this stream, NO_3^--N S_w was in the range of km and did not significantly differ between the two reaches (Table 5.2, Fig. 5.2a). V_f of NH_4^+-N, NO_3^--N and PO_4^{3-}-P ranged between 10^{-4} and 10^{-6} m/s, and were not affected by the point source (Table 5.2, Fig. 5.2b). The point source effect on U was marginally significant for NO_3^--N, but no significant effect was observed for PO_4^{3-}-P or NH_4^+-N (Fig. 5.2c). However, the relative change between the two reaches in U (i.e., (U_{down}-U_{up})/U_{up}) for NH_4^+-N increased as the contribution of the point source to stream NH_4^+-N load (i.e., ($load_{down}$-$load_{up}$)/$load_{up}$) increased (linear regression, R^2 = 0.912, P = 0.003). This relationship was not observed for NO_3^--N U and PO_4^{3-}-P U or for the rest of the nutrient retention metrics.

Table 5.2. Retention parameters for NH_4^+-N, NO_3^--N, and PO_4^{3-}-P measured conducting experimental nutrient additions (NH_4^++PO_4^{3-} and NO_3^-+PO_4^{3-}) upstream and downstream of the point source in La Tordera stream. The table shows these parameters for all sampling dates, the number of available cases (n), mean and standard error (SE). Retention metrics of PO_4^{3-}-P have been calculated as the average of measurements conducting NH_4^++PO_4^{3-} and NO_3^-+PO_4^{3-} additions. Sw = uptake length. Vf = mass transfer coefficient. U = uptake rate. NA denote not available cases due to lack of increase in background nutrient concentration during the experiment.

| Date	NH_4^+-N Sw (m) up	down	NH_4^+-N Vf (m/s) up	down	NH_4^+-N U (mg N m^{-2} min^{-1}) up	down	NO_3^--N Sw (m) up	down	NO_3^--N Vf (m/s) up	down	NO_3^--N U (mg N m^{-2} min^{-1}) up	down	PO_4^{3-}-P Sw (m) up	down	PO_4^{3-}-P Vf (m/s) up	down	PO_4^{3-}-P U (mg P m^{-2} min^{-1}) up	down
Nov. 2001	NA	NA	NA	NA	NA	NA	783	1769	5.8E-05	4.4E-05	2.77	3.96	911	888	5.0E-05	1.4E-04	0.022	0.050
Jan. 2002	176	NA	1.2E-04	NA	0.177	1.031	5555	7726	3.7E-06	5.0E-06	0.17	0.56	109	4471	2.0E-04	7.3E-05	0.050	0.024
Mar. 2002	277	2283	2.3E-04	4.1E-05	0.464	2.824	NA	2564	NA	3.6E-05	NA	3.99	428	3265	1.5E-04	2.9E-05	0.043	0.066
Apr. 2002	407	771	1.9E-04	1.8E-04	0.041	0.056	7064	2024	1.1E-05	7.0E-05	0.35	3.85	3350	2847	3.9E-05	5.0E-05	0.002	0.369
Jun. 2002	130	3569	1.4E-04	8.4E-06	0.543	0.093	2279	NA	7.7E-06	NA	0.32	NA	56	7181	3.1E-04	4.2E-06	0.103	0.107
Sep. 2002	65	3822	3.6E-04	1.0E-05	0.265	0.137	NA	3350	NA	1.2E-05	NA	0.99	79	10457	2.9E-04	3.8E-06	0.095	0.111
Dec. 2002	1385	1663	3.3E-05	3.2E-05	0.031	0.145	1750	2340	2.6E-05	2.3E-05	1.22	1.03	1264	998	3.6E-05	5.4E-05	0.031	0.160
Jul. 2003	2248	3401	3.6E-07	2.2E-06	0.001	0.145	730	9332	1.1E-06	8.2E-07	0.04	0.15	1646	6808	4.9E-07	1.1E-06	0.011	0.142
n	7	6	7	6	7	6	6	7	6	7	6	7	8	8	8	8	8	8
mean	670	2585	1.5E-04	4.6E-05	0.217	0.714	3027	4158	1.8E-05	2.7E-05	0.81	2.08	980	4614	1.3E-04	4.5E-05	0.045	0.129
SE	313	496	4.6E-05	2.8E-05	0.082	0.448	1083	1157	8.7E-06	9.3E-06	0.43	0.67	398	1176	4.3E-05	1.7E-05	0.013	0.038
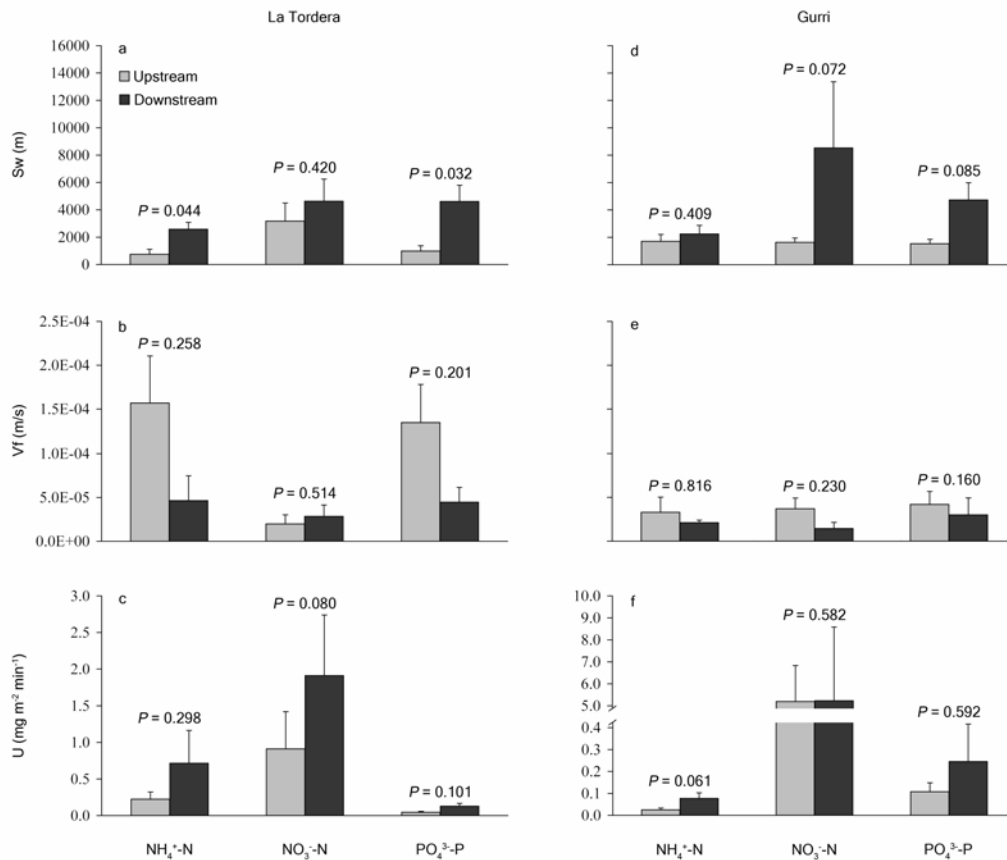
Fig. 5.2. Mean ± standard error of uptake length (S_w), mass transfer coefficient (V_f) and uptake rate (U) for NH_4^+-N, NO_3^--N and PO_4^{3-}-P measured upstream (grey columns) and downstream (black columns) of the point source in La Tordera (a, b, c) and Gurri (d, e, f) streams. Mean values come from those dates when data were available at the two experimental reaches. *P* values indicate results from paired *T*-test analysis to compare results between the two reaches within each stream. Note that y-axes are equal between La Tordera and Gurri streams for S_w and V_f, but are different in the case of U.

In the agricultural stream, average S_w of NH_4^+-N, NO_3^--N and PO_4^{3-}-P were >1 km upstream and downstream of the point source input (Table 5.3; Fig. 5.2d). The point source input tended to increase S_w of NO_3^--N and PO_4^{3-}-P, but this effect was only significant considering a 90 % significance level (Fig. 5.2d).

Table 5.3. Retention parameters for NH_4^+-N, NO_3^--N, and PO_4^{3-}-P measured conducting experimental nutrient additions (NH_4^++PO_4^{3-} and NO_3^-+PO_4^{3-}) upstream and downstream of the point source in Gurri stream. The table shows these parameters for all sampling dates, the number of available cases (n), mean and standard error (SE). Retention metrics of PO_4^{3-}-P have been calculated as the average of measurements conducting NH_4^++PO_4^{3-} and NO_3^-+PO_4^{3-} additions. Sw = uptake length. Vf = mass transfer coefficient. U = uptake rate. NA denote not available cases due to lack of increase in background nutrient concentration during the experiment. The upstream reach was dry in July 2003.

Date	NH_4^+-N Sw (m)		NH_4^+-N Vf (m/s)		NH_4^+-N U (mg N m^{-2} min^{-1})		NO_3^--N Sw (m)		NO_3^--N Vf (m/s)		NO_3^--N U (mg N m^{-2} min^{-1})		PO_4^{3-}-P Sw (m)		PO_4^{3-}-P Vf (m/s)		PO_4^{3-}-P U (mg P m^{-2} min^{-1})	
	up	down	up	down	up	down	up	down	up	down	up	down	up	down	up	down	up	down
Feb. 2002	386	428	1.3E-05	2.9E-05	0.006	0.081	3016	2853	1.7E-06	4.4E-06	0.4	1.9	2440	3738	2.8E-06	4.4E-06	0.026	0.064
Mar. 2002	2804	1694	1.3E-05	2.0E-05	0.024	0.044	1259	4940	2.9E-05	7.0E-06	2.3	1.0	1197	4298	3.1E-05	8.0E-06	0.275	0.094
Apr. 2002	581	3703	1.2E-04	2.2E-05	0.051	0.020	846	32569	7.9E-05	2.5E-06	7.9	0.3	674	2738	1.1E-04	3.0E-05	0.122	0.108
May. 2002	1411	NA	5.7E-05	NA	0.110	NA	1588	5409	5.1E-05	1.6E-05	8.1	2.8	2082	7457	4.0E-05	1.2E-05	0.159	0.073
Jul. 2002	NA	NA	NA	NA	NA	NA	3609	NA	1.0E-06	NA	0.7	NA	544	NA	6.9E-06	NA	0.115	NA
Sep. 2002	3103	2945	3.2E-06	9.1E-06	0.001	0.015	1183	3102	8.4E-06	8.6E-06	2.3	3.5	4599	NA	2.3E-06	NA	0.035	NA
Dec. 2002	2549	3958	4.0E-05	2.8E-05	0.036	0.147	1910	2302	5.4E-05	4.8E-05	10.2	21.8	2161	910	5.4E-05	1.2E-04	0.021	1.098
Jun. 2003	800	773	1.3E-05	1.8E-05	0.033	0.156	2161	NA	4.9E-06	NA	1.1	NA	609	9241	1.8E-05	1.5E-06	0.047	0.035
Jul. 2003	dry	609	dry	8.6E-06	dry	0.051	dry	1076	dry	4.9E-06	dry	0.9	dry	1485	dry	4.3E-06	dry	0.083
n	7	7	7	7	7	7	8	7	8	7	8	7	8	7	8	7	8	7
mean	1662	2016	3.6E-05	1.9E-05	0.037	0.073	1946	7464	2.9E-05	1.3E-05	4.1	4.6	1788	4267	3.3E-05	2.6E-05	0.100	0.222
SE	430	570	1.5E-05	3.1E-06	0.014	0.022	337	4222	1.0E-05	6.0E-06	1.4	2.9	483	1160	1.2E-05	1.7E-05	0.031	0.146

Average V_f of NH_4^+-N, NO_3^--N and PO_4^{3-}-P were within the range of 10^{-5} m/s, and did not differ between reaches (Table 5.3; Fig. 5.2e). The effect of the point source on U was marginally significant for NH_4^+-N and not significant for the other nutrients (Fig. 5.2f). In this stream, we found positive relationships between the relative downstream change in NH_4^+-N U and the relative point source contribution to the downstream loads of NH_4^+-N, NO_3^--N and DOC (Fig. 5.3a-c). Similarly, the relative increase in NO_3^--N U downstream of the point source was magnified as the point source contribution to downstream NH_4^+-N and DOC loads
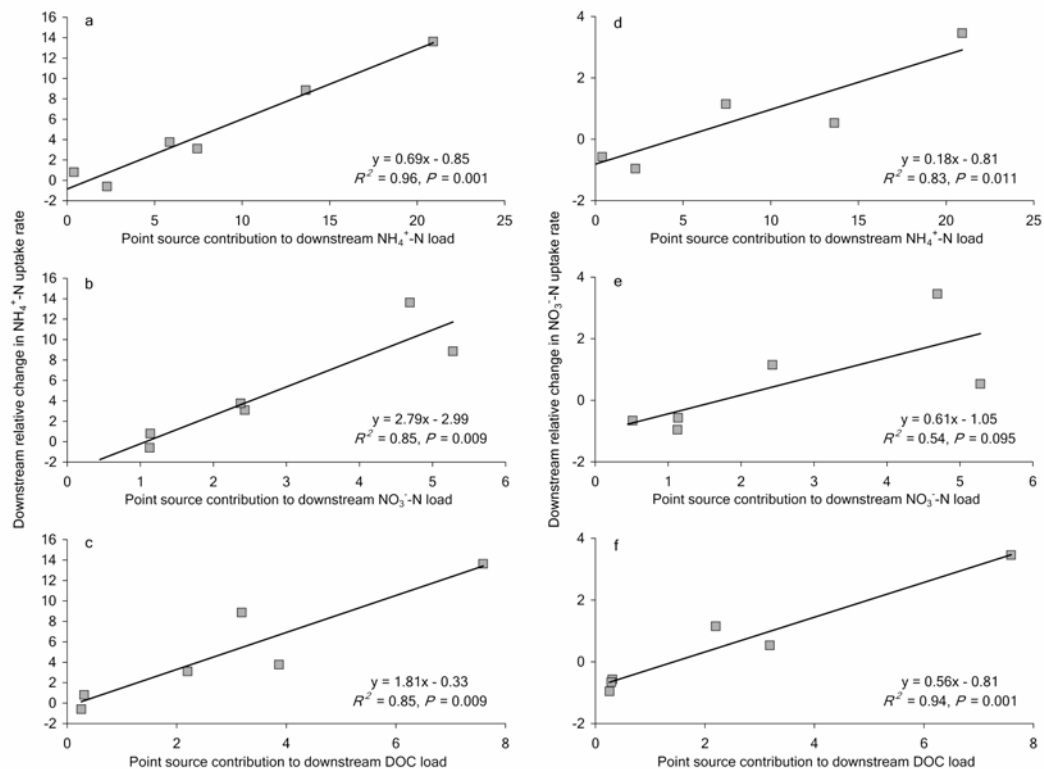
Fig. 5.3. Relationships between downstream relative change in U (i.e., (U_{down}-U_{up}/U_{up})) of NH_4^+-N (a, b, c) and NO_3^--N (d, e, f) and point source contribution to downstream loads ($load_{down}$-$load_{up}$/$load_{up}$) of (a, d) NH_4^+-N, (b, e) NO_3^--N and (c, f) DOC for Gurri stream. Functions and statistics (R^2 and P) of these relationships are shown.

increased (Fig. 5.3d-f). No significant relationships were found between the relative change in PO_4^{3-}-P U and the point source contribution to nutrient loads, nor for the rest of the nutrient retention metrics.

Relationships between mass transfer coefficient and nutrient concentrations

V_f of NH_4^+-N, NO_3^--N and PO_4^{3-}-P were significantly related to their respective nutrient concentrations only in the case of PO_4^{3-}-P (Fig 5.4). V_f of PO_4^{3-}-P decreased as SRP concentration increased for data from the two streams separately and combined (Table 5.4; Fig. 5.4a). In addition, V_f of NH_4^+-N was negatively related to NO_3^--N and DOC concentrations taking data from the two streams together (Table 5.4). These concentrations similarly regulated variations in NH_4^+-N V_f (i.e., the two relationships were similar with an intercept = 0.0001 and a slope = $-5 \cdot 10^{-5}$). Positive relationships between nutrient V_f and nutrient molar ratios for data from the forested stream and from the two streams combined were found between V_f of NH_4^+-N and PO_4^{3-}-P and DIN:SRP, and between V_f of NO_3^--N and DOC:DIN (Table 5.4). However, the percentage of V_f variance explained by the ratios was lower when combining data from the two streams than when taking data only from the forested stream (Table 5.4).
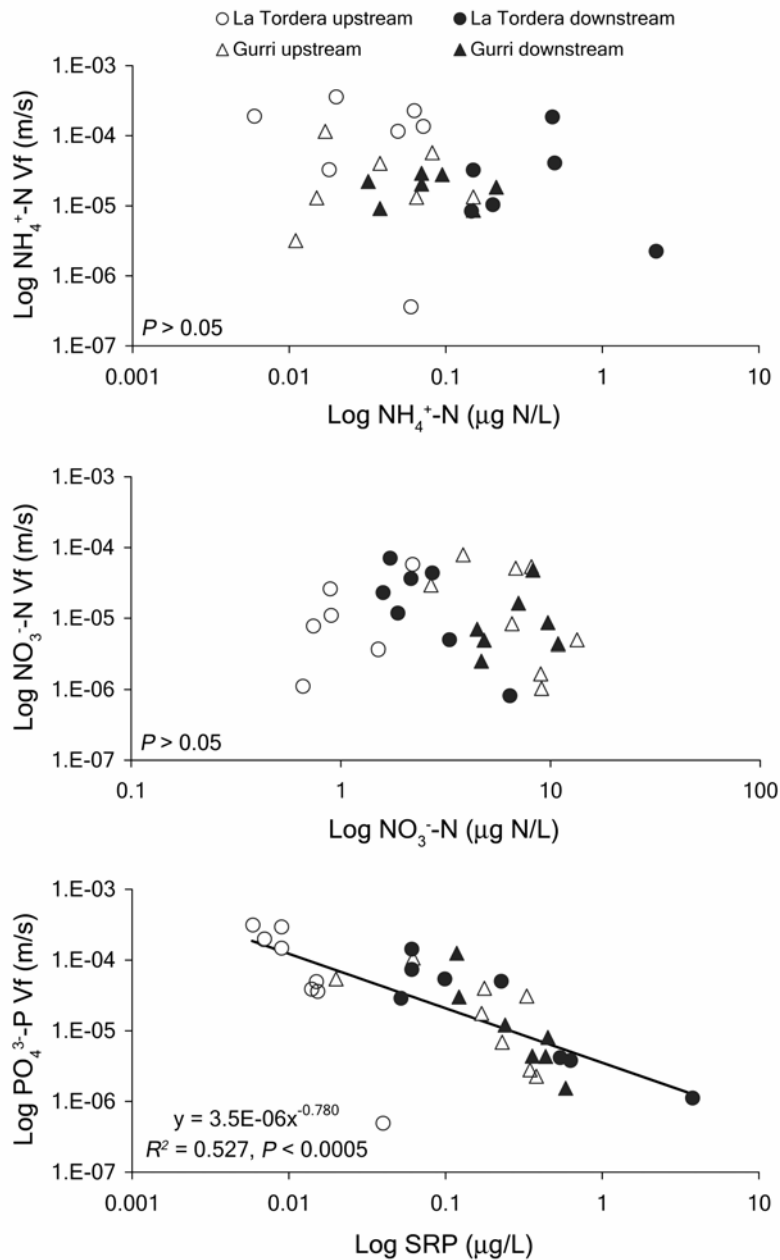
Fig. 5.4. Relationships between (a) NH_4^+-N V_f and NH_4^+-N concentration, (b) NO_3^--N V_f and NO_3^--N concentration, and (c) PO_4^{3-}-P V_f and SRP concentration for the upstream and downstream reaches of La Tordera and Gurri streams. Statistics and functions of significant relationships are given.

Table 5.4. Results from regression analyses between mass transer coefficients (Vf) of NH_4^+-N, NO_3^--N and PO_4^{3-}-P (as dependent variables) and nutrient concentrations and molar ratios (as independent variables). Regression analyses were conducted with data from the two reaches and the two streams together. For significant relationships, it is indicated whether they are positive (+) or negative (-) and linear, power, logarithmic or exponential, and R^2 and P are shown.

	NH_4^+-N Vf (m/s)			NO_3^--N Vf (m/s)			PO_4^{3-}-P Vf (m/s)		
	La Tordera	Gurri	All data	La Tordera	Gurri	All data	La Tordera	Gurri	All data
NH_4^+-N (mg N/L)	ns	ns	ns	ns	ns	ns	ns	ns	ns
NO_3^--N (mg N/L)	ns	ns	Log. - $R^2 = 0.297$ $P = 0.003$	ns	ns	ns	ns	nc	nc
SRP (mg/L)	ns	nc	nc	ns	nc	ns	Pow. - $R^2 = 0.488$ $P = 0.003$	Exp. - $R^2 = 0.719$ $P < 0.0005$	Pow. - $R^2 = 0.527$ $P < 0.0005$
DOC (mg/L)	ns	ns	Log. - $R^2 = 0.228$ $P = 0.012$	ns	ns	ns	ns	nc	nc
NO_3^-:NH_4^+	ns	ns	ns	ns	ns	ns	ns	ns	ns
DIN:SRP	Pow. + $R^2 = 0.424$ $P = 0.016$	ns	Pow. + $R^2 = 0.345$ $P = 0.001$	ns	ns	ns	Pow. + $R^2 = 0.613$ $P < 0.0005$	ns	Pow. + $R^2 = 0.486$ $P < 0.0005$
DOC:DIN	ns	ns	ns	Pow. + $R^2 = 0.437$ $P = 0.014$	ns	Pow. + $R^2 = 0.23$ $P = 0.010$	ns	ns	ns

ns, not significant ($P > 0.05$); nc, significant but not considered

Discussion

Point source effects on physical, chemical and biological stream attributes

The point source affected the hydrology of the two streams, resulting in a decrease of water residence time downstream of its input. However, this effect slightly differed between the two streams. In the agricultural stream, discharge significantly increased downstream of the point source, whereas in the forested stream, we only observed a significant increase in water velocity. The effect also varied over time due to the irregular hydrologic regime of these two Mediterranean streams. The contribution of the point source to downstream discharge was higher at lower discharges. This effect was exacerbated in summer when the point source accounted for 100 % of the downstream flow. Therefore, point source inputs not only altered the amount of stream flow, but also shifted the hydrologic regime of the study streams from being intermittent to permanent. These results support previous findings (Martí *et al.* 2004) and evidence the vulnerability of streams, especially those located in arid or semi-arid regions, to point source inputs (Gasith and Resh 1999).

The effect of the WWTP input on stream nutrient concentrations clearly differed between the two streams. In the forested stream, the point source increased concentrations of all nutrients considered, whereas in the agricultural stream, we only observed a significant increase in SRP concentration. This difference can be attributed to the contrast between dominant land use in the catchment upstream of the point source. Agricultural activity is a major source of N and P to surface waters through diffuse pathways (Carpenter *et al.* 1998, Kronvang *et al.* 1999, Kemp and Dodds 2001). In fact, DIN and SRP concentrations in the agricultural stream, upstream of the point source, were higher than those in the upstream reach of the forested site. Therefore, the local effect of the point source in the agricultural stream was overwhelmed by diffuse sources from adjacent agricultural fields. Our results suggest that the effect of the point sources on stream nutrient concentrations should be considered within the

landscape context of each particular site. Finally, it is interesting to notice that the point source in the forested site not only increased nutrient concentrations, but also changed the relative proportions among nutrients (i.e., DIN:SRP) and among N forms (i.e., NO_3^-:NH_4^+). This effect was not found in the agricultural stream. The stoichiometric shifts in nutrient availability may influence the development of biofilm communities, and ultimately affect how nutrients are cycled within the stream (Battin *et al.* 2003, Bernot and Dodds 2005).

The increases in nutrient concentrations due to the point source in the forested stream did not result in an overall increase of habitat weighted biomass or chlorophyll *a*. Nevertheless, biomass and chlorophyll *a* were clearly greater downstream than upstream of the point source on 6/8 sampling dates. On June 2002, after a spate that occurred in May, chlorophyll *a* and biomass were lowest than in the rest of dates, and similar between the two reaches. Washout during flood disturbance can play an important role affecting stream biofilm and filamentous algae, and thus, decreasing benthic chlorophyll *a* and biomass (Biggs 2000). Point source inputs of nutrients were likely to favor habitat weighted chlorophyll *a* and biomass, but overall point source effect was masked by greater effect of other factors such as washout than by nutrients availability on few dates. In the agricultural stream, habitat weighted chlorophyll *a* and biomass were two and three times higher, respectively, at the downstream than at the upstream reach. These contrasting effects between the two streams are consistent with the contradictory results from existing studies related to the effect of nutrients on stream biofilm. For instance, some studies show that biofilm chlorophyll decreased downstream of the input of treated sewage (Masseret *et al.* 1998), some that biofilm chlorophyll *a* increased along a gradient affected by urbanization and agricultural activities (Shieh *et al.* 1999), and others that biofilm communities were not closely regulated by nutrient availability (Bernhardt and Likens 2004). Lack of general patterns related to the effect of nutrient concentration on biofilm is likely to be due to the multiple factors affecting biofilm development, the interaction of which may mask the effect of a single factor.

Effects of point source inputs on in-stream nutrient retention

In the forested stream, S_w (i.e., uptake lengths) of NH_4^+-N and PO_4^{3-}-P averaged hundreds of meters above the point source. These S_w significantly increased downstream of the point source relative to upstream, being mostly in the km range and indicating that retention efficiency of NH_4^+-N and PO_4^{3-}-P was lower downstream than upstream of the point source. However, NO_3^--N retention efficiency was not affected by the point source. Indeed, NO_3^--N S_w ranged about 1-9 km ($\geq$ 2 km in 85 % of the cases) at the two reaches in the forested stream, indicating that NO_3^--N retention efficiency was already low above the point source. In contrast to the forested stream, the point source did not affect retention efficiency of NH_4^+-N, NO_3^--N or PO_4^{3-}-P in the agricultural stream. In this stream with diffuse nutrient sources from agricultural fields surrounding the two reaches, retention efficiency was very low upstream and downstream of the point source as indicated by longer Sw of NH_4^+-N, NO_3^--N and PO_4^{3-}-P than those measured at the upstream reach in the forested stream and than those previously reported for pristine streams for NH_4^+-N (Martí and Sabater 1996, Hall *et al.* 1998, Tank *et al.* 2000, Mulholland *et al.* 2000b, Bernhardt and Likens 2002, Dodds *et al.* 2002, Webster *et al.* 2003), for NO_3^--N (Munn and Meyer 1990, Webster *et al.* 1991) and for phosphorus (Hill 1982, Munn and Meyer 1990, D'Angelo and Webster 1991, D'Angelo *et al.* 1991, Webster *et al.* 1991, Hart *et al.* 1992, Maltchik *et al.* 1994, Martí and Sabater 1996, Mulholland *et al.* 1997, Valett *et al.* 2002). Previous studies showed that nutrient S_w increased (i.e., retention efficiency decreased) with increasing nutrient concentrations in pristine streams (Dodds *et al.* 2002). Others suggested that nutrient retention efficiency was lower in streams receiving point sources than in pristine streams (Haggard *et al.* 2001, Martí *et al.* 2004, Merseburger *et al.* 2005). Most recent studies have shown that urban streams are less retentive than pristine streams (Grimm *et al.* 2005, Meyer *et al.* 2005). Our results are in agreement with and support these previous studies, indicating that in-stream nutrient retention efficiency (uptake/supply)

gradually decreases under an increasing gradient of nutrient concentrations. Retention efficiency of N tended to be lower for NO_3^--N than for NH_4^+-N in the two study streams, a similar finding to that reported for pristine streams (Valett *et al.* 1996, Mulholland *et al.* 2000a, Dodds *et al.* 2002). Most benthic organisms (bacteria, fungi and algae) prefer to take up NH_4^+-N instead of NO_3^--N, despite availability of both, because the latter requires chemical reduction before it can be used as a source of nitrogen in biosynthesis (i.e., requires higher energetic cost).

The point source did not show significant effects on V_f (i.e., mass transfer coefficient) of NH_4^+-N, NO_3^--N and $PO_4^{3-}-P$ in the study streams. Regarding U (i.e., uptake rates), positive relationships between the relative contribution of the point source to stream nutrient loads and the difference in uptake rates of NH_4^+-N and NO_3^--N between upstream and downstream reaches demonstrate a clear effect of the point sources magnifying stream N uptake rates. In particular, the increase in NH_4^+-N uptake rates under increasing the relative contribution of the point source to downstream loads of NH_4^+-N in the forested stream, and to loads of NH_4^+-N, NO_3^--N and DOC in the agricultural stream, may be result of nitrifying activity. Nitrifiers are poor competitors for NH_4^+-N (Verhagen and Laanbroek 1991, Verhagen *et al.* 1992), and high NO_3^--N concentrations are likely to reduce competition for NH_4^+-N between heterotrophs and nitrifiers by reducing heterotrophic demand for NH_4^+-N (Bernhardt *et al.* 2002, Hall *et al.* 2002, Merseburger *et al.* 2005). Hence, increases in NO_3^--N and DOC availability may favor heterotrophic activity, in turn, stimulating nitrification. Other studies have shown that point sources are a source of nitrifying bacteria to streams (Brion and Billen 2000), and have suggested that nitrification plays an important role below WWTP inputs, under high NH_4^+-N concentrations (Martí *et al.* 2004, Merseburger *et al.* 2005). The relationship between downstream relative change in NO_3^--N uptake rates and point source contribution to downstream NH_4^+-N loads may indicate that increasing rates of nitrification of NH_4^+-N from the point source input result in increases in NO_3^--N concentration below the input, magnifying

downstream NO_3^--N uptake rates. These results are coherent with those from Grimm *et al.* (2005) showing that NO_3^--N U increased with increasing NO_3^--N concentration in urban streams. On the other hand, increase in NO_3^--N uptake rates downstream of the point source relative to upstream under higher contribution of the point source to stream DOC load in the agricultural stream could be attributed to denitrifying activity. Denitrification has been shown to be a significant sink for nitrogen in streams receiving point sources from treatment plants due to availability of NO_3^--N and DOC (Bradley *et al.* 1995).

Variability in stream retention responses due to anthropogenic nutrient inputs

Biological demand of NH_4^+-N was not related with NH_4^+-N concentration, but decreased under increasing NO_3^--N and DOC levels up to concentrations of 6 mg N/L and 4 mg/L, respectively, as indicated by relationships with data from the two streams together. Hence, variation in NO_3^--N rather than NH_4^+-N concentrations were likely to regulate NH_4^+-N demand. Positive relationships between NO_3^--N V_f and the DOC:DIN molar ratio for the forested stream and for the two streams combined suggest an enhancement of NO_3^--N demand with increasing DOC levels. Denitrification depends on availability of NO_3^--N and DOC downstream of point sources (Bradley *et al.* 1995). Hence, our results suggest that variations in NO_3^--N biological demand due to anthropogenic nutrient inputs mostly reflected denitrifying activity in the study streams. Finally, PO_4^{3-}-P V_f tended to decrease under increasing SRP concentration in the two streams separately and combined. In addition and accordingly, PO_4^{3-}-P V_f increased with DIN:SRP molar ratios increasing up to about 500 and then was likely to saturate. Support to such a saturation of phosphorus uptake was provided by the results of Mulholland *et al.* (1990), in which biological processes dominated at low stream phosphorus concentrations, but biological uptake became P-saturated at higher concentrations. A recent study also showed lower PO_4^{3-}-P V_f for urban than for

forest streams; the formers showing greater SRP concentrations than the latters (Meyer *et al.* 2005).

Mass transfer coefficients allow comparison of nutrient retention among streams (Davis and Minshall 1999). Hence, we compiled results from literature for a variety of streams to examine relationships between nutrient V_f and concentrations (Fig. 5.5). V_f of NH_4^+-N, NO_3^--N and PO_4^{3-}-P in the study streams were within the same range than those from literature. V_f of NH_4^+-N and NO_3^--N were not significantly related with NH_4^+-N and NO_3^--N concentrations, respectively (Fig. 5.5a-b). Grimm *et al.* (2005) predicted a negative relationship between NO_3^--N V_f and NO_3^--N concentration, but contrary to their expectations, the relationship was not significant. Our study agrees with lack of negative relationship between NO_3^--N V_f and NO_3^--N concentration (Grimm *et al.* 2005). In contrast, PO_4^{3-}-P V_f and SRP concentration were negatively related when including data from literature in addition to those from our study streams (Fig. 5.5c). Highest PO_4^{3-}-P V_f were in the order of 10^{-4} m/s under lowest SRP concentrations. With increasing SRP concentrations, PO_4^{3-}-P V_f progressively decreased up to SRP concentrations of 50 μ/L, above which PO_4^{3-}-P V_f remained between $4E10^{-5}$-$4E10^{-6}$ m/s. Despite the variety of biological compartments involved in phosphorus uptake in streams, thus, biotic saturation under high SRP concentrations is likely to be a general trend across streams at a broad geographical scale.

► **Fig. 5.5.** Relationships between (a) NH_4^+-N V_f and NH_4^+-N V_f concentration, (b) NO_3^--N V_f and NO_3^--N concentration and (c) PO_4^{3-}-P Vf and SRP concentration for a number of streams, including those from the present study and from literature. In particular, the figure shows results from: ○, our study; □, Mulholland *et al.* 1985; ●, Webster *et al.* 1991; ▫, Hart *et al.* 1992; ■, Kopáček and Blăzka 1994; Δ, Martí and Sabater 1996; ▲, Hall *et al.* 1998; ◊, Davis and Minshall 1999; ♦, Mulholland *et al.* 2000b; Χ, Sabater *et al.* 2000; +, Tank *et al.* 2000; Ж, Hamilton *et al.* 2001; ‒, Wollheim *et al.* 2001; ●, Bernhardt and Likens 2002; x, Bernhardt *et al.* 2002; ▪, Dodds *et al.* 2002; ▲, Hall *et al.* 2002; ♦, Merriam *et al.* 2002; ○, Simon and Benfield 2002; ■, Valett *et al.* 2002; Δ, Doyle *et al.* 2003; ◊, Hall and Tank 2003; ●, Grimm *et al.* 2005; □, Meyer *et al.* 2005, and ▲, Simon *et al.* 2005.
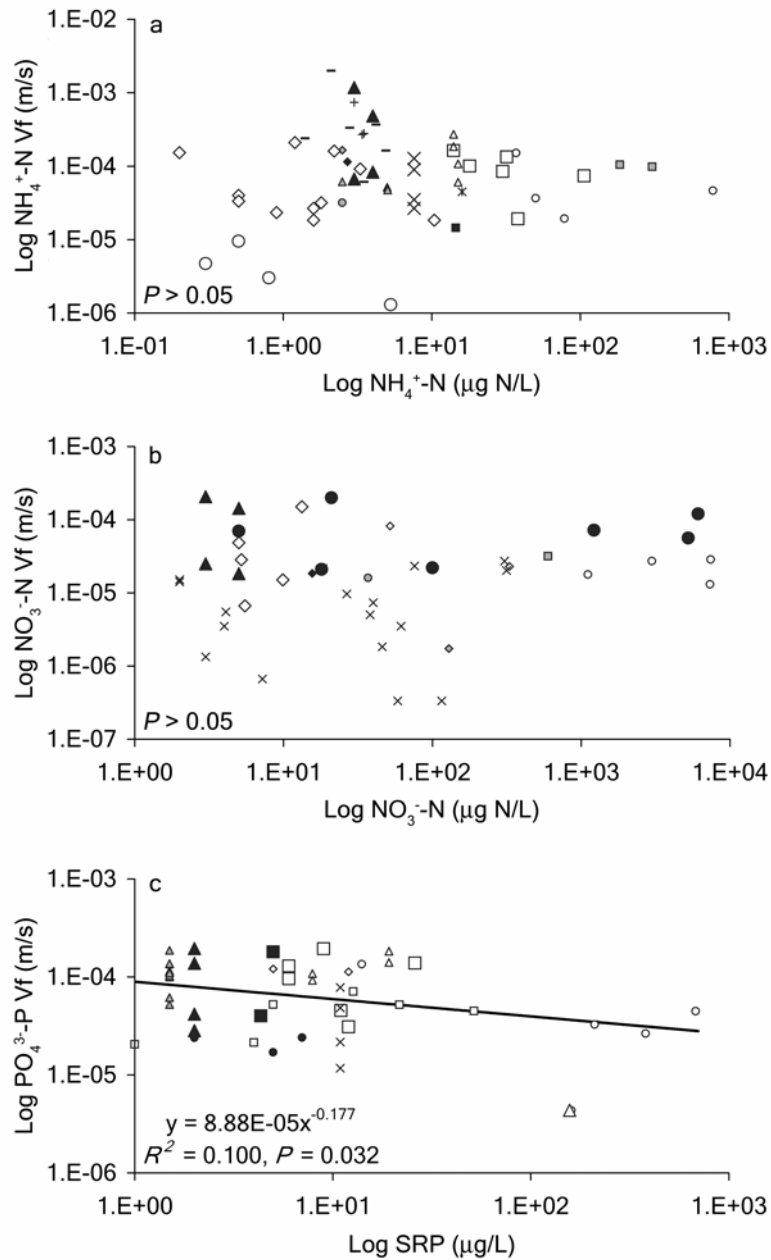
6. Influences of a point source on stream denitrification rates

Summary

We examined the influences of a point source on denitrification potential rates in La Tordera and Gurri streams, which drain a forest- and an agricultural-dominated catchment, respectively. Potential denitrification rates were estimated on several sampling dates through 2001-2003 at two reaches located upstream and downstream of a point source input in the two study streams. In addition, we examined factors limiting denitrification rates by conducting laboratory experiments based on different treatments (i.e., control with stream water, and amendments with N, C and N+C) on two additional sampling dates in 2004. We used the acetylene inhibition technique (Tiedje *et al.* 1989, Holmes *et al.* 1996) to measure denitrification rates.

Differences in dominant land uses between the two streams were reflected in different effects of the point source on those factors that may regulate stream denitrification rates. Deficit of dissolved oxygen (DO), and concentrations of nitrate (NO_3^--N) and dissolved organic carbon (DOC) increased downstream of the point source relative to upstream in La Tordera, whereas were similar between the two reaches in Gurri. These point source effects were likely to be masked by diffuse sources from adjacent agricultural fields in Gurri stream. Point source effects on denitrification rates were coherent with point source effects on the factors controlling these rates. Denitrification rates (expressed as mg N $m^{-2}\,h^{-1}$ and mg N g $AFDM^{-1}\,h^{-1}$) tended to increase downstream of the point source relative to upstream on all sampling dates in La Tordera. Nevertheless, the increases were statistically significant only on 50 % of dates, in the case of denitrification expressed /m^2 coinciding with those dates with lowest discharge, and in case of denitrification /g AFDM coinciding with greatest point source contribution to stream DOC loads. In contrast, denitrification rates expressed /m^2 or /g AFDM did not significantly differ between the two reaches in Gurri stream.

Point source inputs affected variation in potential denitrification rates in La Tordera stream through the study period. This variation was not significant at the

upstream reach, whereas denitrification rates (/m^2 and /g AFDM) significantly varied among sampling dates at the downstream reach. Downstream of the point source, these rates were positively related to DO deficit and NO_3^--N concentration, and negatively to stream discharge. In Gurri stream, where diffuse sources entered the stream channel in addition to the point source, denitrification rates (/m^2 and /g AFDM) significantly varied among sampling dates both at upstream and downstream reaches. In Gurri, denitrification rates expressed /m^2 were positively related to changes in NO_3^--N concentration with data from the two reaches combined. No relationship was found between denitrification expressed /g AFDM and other examined factors (e.g., DO deficit, stream discharge or NO_3^--N concentration). Hence, NO_3^--N availability was the common factor influencing denitrifying activity in the two study streams. Experiments on limiting factors showed that availability of both N and C determined the potential for denitrification regardless of flow conditions or reach location in La Tordera stream.

Introduction

Denitrification is a net sink for nitrate (NO_3^--N) from stream waters, and thus, may minimize the effects of nutrient inputs from human activities on stream water chemistry (Ostrom *et al.* 2002). Both assimilation of NO_3^--N and denitrification can reduce NO_3^--N from stream waters (Duff *et al.* 1984), but only denitrification results in a permanent removal of N from the ecosystem (Knowles 1982). Denitrification is carried out by heterotrophic bacteria that oxidize organic matter under anoxic conditions using NO_3^--N as the terminal electron acceptor, which is reduced via nitrite to N_2O and N_2 gases (Payne 1973, Knowles 1982). Human activities increase concentrations of NO_3^--N and dissolved organic carbon (DOC) in stream waters through point and diffuse sources (Sjodin *et al.* 1997, Smith *et al.* 1997, Kronvang *et al.* 1999). Point and diffuse sources may differently affect in-stream denitrification given that they not only increase N and

C availability in the receiving streams, but also modify nutrients content in different proportion. Point sources mostly release nutrients in form of ammonium (NH_4^+-N), soluble reactive phosphorus (SRP) and DOC, whereas diffuse sources represent an important input of NO_3^--N and DOC to receiving streams. In turn, high contents of DOC may favor microbial decomposition, increasing O_2 demand (Royer *et al.* 2004), and thus, promoting anoxic zones within stream sediments. Hence, point and diffuse sources may provide suitable conditions for denitrifiers, favoring their activity in receiving streams, but showing contrasting effects on denitrification rates. Overall, we aim to study existing gaps concerning denitrification in human-altered streams.

Denitrification rates have been deeply studied in forest soils, but less at the soil-water interface in streams (Ostrom *et al.* 2002). Existing research has shown that temporal variation in stream denitrification rates is mainly regulated by water temperature (Ventullo and Rowe 1982, Pfenning and McMahon 1996, Pattinson *et al.* 1998, García-Ruiz *et al.* 1998a), presence of anoxic zones (Nielsen *et al.* 1990, Rysgaard *et al.* 1994), discharge (Schaler *et al.* 2004), NO_3^--N concentration (Holmes *et al.* 1996, Pattinson *et al.* 1998, García-Ruiz *et al.* 1998b, Martin *et al.* 2001, Inwood *et al.* 2005) and availability of organic carbon (Ventullo and Rowe 1982, Pfenning and McMahon 1996, Grischek *et al.* 1998). Recent studies have shown that denitrification rates are higher in streams influenced by urban and agricultural activities than in pristine streams (Inwood *et al.* 2005). Other studies suggested that availability of NO_3^--N and DOC determined denitrifying activity downstream of a point source input (Bradley *et al.* 1995). However, these previous studies did not examine point source effects on stream denitrification rates, or factors controlling and limiting these rates upstream and downstream of point sources. Research on denitrification in human-altered streams should provide insight into the denitrification potential to remove N from the water column.

We aimed to examine if point sources favor in-stream denitrification rates in a stream with a point source as the major human influence, and a stream with

diffuse sources as the major human influence. Also, we aimed to examine factors controlling these rates in the two study streams, as well as factors limiting them above and below point sources. Differences in land use (i.e., forest- and agricultural-dominated) between the two study streams were reflected in contrasting concentrations of NO_3^--N and DOC (higher in the agricultural than in the forested stream upstream and downstream of the point source). Hence, we hypothesized higher denitrification rates in Gurri than in La Tordera stream. We also hypothesized that denitrification rates would increase downstream of the point source relative to upstream in La Tordera stream as a result of previously observed increases in NO_3^--N and DOC concentrations below the point source (Merseburger *et al.* 2005). Based on the fact that diffuse sources overwhelmed point source effects on NO_3^--N and DOC concentrations in Gurri stream (Merseburger *et al.* 2005), we hypothesized lack of point source effects on denitrification rates in this agricultural stream.

Materials and methods

Field sampling

To examine point source effects on denitrification, we collected six substrata samples along each study reach (i.e., upstream and downstream of the point source) in La Tordera and Gurri streams on six sampling dates through 2001-2003. In each stream, samples were collected according to the dominant substrata type. In La Tordera, where streambed consists of a mixture of cobbles, pebbles, gravels and fine-grained substrata upstream and downstream of the point source, we collected sediment samples. In Gurri, where bedrock mostly dominates streambed in the two reaches, we collected samples of biofilm. However, in this stream, the upstream reach also had patches of fine sediment, and thus, additional samples of this substratum were collected.

In addition, to examine limiting factors of denitrification above and below the point source, we collected six sediment samples at the two reaches of La

Tordera stream on two dates (2004) with contrasting discharge (Table 6.1). We focused on La Tordera rather than in Gurri due to clearer effects of the point source on denitrification rates in the former than in the latter stream.

All sediment samples were collected using a plastic cylinder (Ø = 26 cm) that was driven into the streambed to a depth of 5 cm. Biofilm samples were collected by scraping 100 cm^2 of colonized bedrock surface, as reported for measurement of denitrification potential of biofilm communities (Ventullo and Rowe 1982). All samples were transferred to plastic bags, transported in an icebox to the laboratory and stored in the refrigerator at ~4ºC until the denitrification incubations were initiated (within 24 h following samples collection). On each sampling date, we also collected samples of stream water from each reach. Water samples were used for the laboratory incubations and for chemical analysis. Samples were analyzed for concentration of NO_3^--N and DOC. Description of water sample collection and chemical analyses is described in Chapter 4. On each sampling date and at each reach, we also measured water

Table 6.1. Stream discharge and nutrient concentrations measured upstream and downstream of the point source in La Tordera on the two sampling dates in which experiments on factors limiting denitrification rates were conducted.

May 2004	Upstream	Downstream
Discharge (L/s)	493	768
NO_3^--N (mg N/L)	0.58	0.90
DOC (mg/L)	1.00	1.12
August 2004	Upstream	Downstream
Discharge (L/s)	21	37
NO_3^--N (mg N/L)	1.03	1.93
DOC (mg/L)	0.76	3.01

temperature (WTW® LF 340 conductivity meter), stream discharge (see Chapter 4) and deficit of dissolved oxygen (DO; see Chapter 7 regarding whole-stream metabolism). DO deficit for each sampling date was calculated as the average of DO deficits estimated every 10 min during 24 h from the difference between concentrations of DO at 100 % saturation for a given water temperature and DO measured using an oxygen meter (WTW® Oxi 340-A). Positive values indicate DO deficit in the water column.

Laboratory procedures

To estimate denitrification rates from all substrata samples, we used the acetylene (C_2H_2) inhibition technique (Tiedje *et al.* 1989, Holmes *et al.* 1996). Denitrification incubations were prepared by transferring a known weight of wet mass of the sample (approximately 350 g for sediment and 12 g for biofilm) into bottles of 300-mL (sediment) or 60-mL (biofilm), and by adding unfiltered stream water to bring the total volume to 250-mL (sediment incubations) or 30-mL (biofilm incubations). Incubation bottles were made anoxic by purging N_2 in the water for 10 min, time in which we had checked that O_2 concentration in water was < 1 mg/L. We then capped and sealed the bottles with septa-fitted screw-top lids. We added C_2H_2 (10 ml) with a syringe to each incubation bottle. Bottles were shacked to ensure C_2H_2 mixture within the substrata, water and headspace. Then, pressure was equalized by piercing for 10 s the bottle septa with a syringe needle. Bottles were incubated in the dark at ambient laboratory temperature. In preliminary experiments, gas samples were taken every 4 h during 24 h to determine appropriate sampling time. Subsequent incubations required only an initial and final (at 24 h) gas sampling for steady linear accumulation of nitrous oxide (N_2O). Gas samples were collected using a double needle with sealed, preevacuated vials. After collection of gas samples, an equal volume of N_2 (90 %) and C_2H_2 (10 %) was returned to each incubation bottle to avoid pressure changes. Vials containing gas samples were stored at ~4ºC until N_2O analysis. To express denitrification rates per unit of organic matter, we determined ash free

dry mass (AFDM) on substrata subsamples as mass loss of samples dried to constant mass (60 ºC, 48 h) then combusted (450 ºC, 4-5 h).

To examine factors limiting denitrification rates in La Tordera, we prepared a set of 24 incubation bottles (i.e., 6 replicates x 4 treatments) for each reach. Treatments used were: control with stream water, and amendments with NO_3^--N (as $NaNO_3$), with glucose and with NO_3^--N plus glucose. Concentrations of NO_3^--N and DOC in stream water used for the control treatment are shown in Table 6.1. Concentrations used in amendments of NO_3^--N (200 mg N/L) and/or glucose (200 mg C/L) were chosen for two reasons. First, NO_3^--N amendments with concentrations up to 200 mg N/L have been shown to be feasible to examine factors controlling denitrification rates in stream sediments (Holmes *et al.* 1996). Second, using DOC amendments of 200 mg/L, we maintained similar DOC:DIN molar ratios (DIN = dissolved inorganic nitrogen) than those measured under ambient conditions (upstream, 1.25 ± 0.26; downstream, 0.75 ± 0.13). Denitrification incubations were prepared as described to determine denitrification rates under ambient stream water conditions.

For N_2O analysis, we sampled 1 mL from the gas vials with a gastight syringe. We used a N_2O standard of 100 μg/mL. We determined N_2O using a gas chromatograph (Hewlett Packard 5890 A) equipped with a thermal conductivity detector (250 ºC). The components were separated on a Porapak Q column, increasing from 60 ºC (5 min) to 200 ºC (2 min) at a rate of 10 ºC/min, with helium as the carrier gas (60 ml/min).

Calculation of denitrification rates

Denitrification rates were calculated from the difference between final and initial headspace N_2O concentration. Total mass of N_2O in the headspace was calculated using the headspace N_2O concentrations and total microcosm volumes following correction for N_2O solubility in the liquid phase with an appropriate temperature-dependent Bunsen coefficient (Knowles 1979, Martin *et al.* 2001). In the case of Gurri, in which we measured denitrification rates from

two different substrata (i.e. biofilm and sediment), we calculated habitat weighted denitrification for the reach based on the percentage of coverage of the two substrata in the reach. All denitrification rates were expressed as mg N m^{-2} h^{-1} and as mg N g AFDM^{-1} h^{-1} to facilitate comparison with published denitrification rates. Denitrification rates in incubations with stream water could underestimate actual denitrification rates because C_2H_2 blocks nitrification, a potentially important source of NO_3^--N to denitrifiers (Tiedje *et al.* 1989). However, linear accumulation of N_2O during the 24 h of our denitrification assays indicated no NO_3^--N limitation. In addition, we measured similar NO_3^--N concentrations in stream water before and after denitrification incubations, fact that supported the assumption of no NO_3^--N limitation during the incubation period. Finally, denitrification rates measured using the described method are potential rates due to optimum conditions of anoxia in the sediments and interstitial NO_3^--N concentrations equal to those of overlying stream water.

Statistical analyses

Paired *T*-test was used to compare water temperature, DO deficit, stream discharge, and concentrations of NO_3^--N and DOC between the upstream and downstream reaches in the two study streams. We used two-way ANOVA to test the effect of reach and sampling date on denitrification rates in La Tordera and Gurri streams. Significant interactions between reach and date were explored by using independent samples *T*-test (point source effect for each date per separate). Univariate regression analyses were used to examine variation in denitrification rates among sampling dates as a function of parameters that may regulate these rates (i.e., temperature, DO deficit, discharge, and concentrations of NO_3^--N and DOC). For regression analyses, we included data from three sampling dates on which data from one of the two reaches of Gurri stream was not available because of methodological problems (April 2002 and June 2003) or drought (July 2003). Finally, one-way ANOVA was used to assess the effect of experimental treatment (i.e., control, N amendment, C amendment and N+C

amendment) on denitrification rates. Significant one-way ANOVAs were interpreted by pair wise comparisons among group means using Tukey's standardized range test (HSD). Data were log transformed when needed to meet assumptions of normality and homoscedasticity. The significance level used for all statistical tests was $P \leq 0.05$. These tests were performed with the statistical package SPSS® (for Windows, version 13.0, SPSS Inc., Chicago, Illinois).

Results

Potential denitrification rates

In the two study streams, water temperature was similar between the two reaches (see Table 7.1). Stream discharge was similar between the two reaches in La Tordera, but increased downstream of the point source relative to upstream in Gurri (see Table 5.1). Concentrations of NO_3^--N and DOC (see Table 5.1), as well as DO deficit, increased downstream of the point source relative to upstream in La Tordera, whereas did not change between the two reaches in Gurri stream. In particular, mean ± SE deficit of DO upstream and downstream of the point source was, respectively, 1.04 ± 0.47 and 1.98 ± 0.53 mg O_2/L in La Tordera (paired *T*-test, t = -2.74, df = 5, P = 0.041), and 0.96 ± 0.17 and 0.61 ± 0.24 mg O_2/L in Gurri (paired *T*-test, t = 0.67, df = 4, P = 0.541).

In La Tordera stream, potential denitrification rates expressed both /m² and /g AFDM varied between reaches and among dates, and the interactions between reach and date were significant (Fig. 6.1). Denitrification tended to be higher downstream than upstream of the point source on all sampling dates, but the difference was statistically significant only on 3/6 dates (Fig. 6.1a, b). Additionally, the point source effect was marginally significant for denitrification expressed /m² in November 2001 (P = 0.089) and for denitrification expressed /g AFDM in June 2002 (P = 0.058). Significant increases in denitrification rates /m² downstream of the point source relative to upstream coincided with sampling dates with lowest discharge at the downstream reach. In the case of
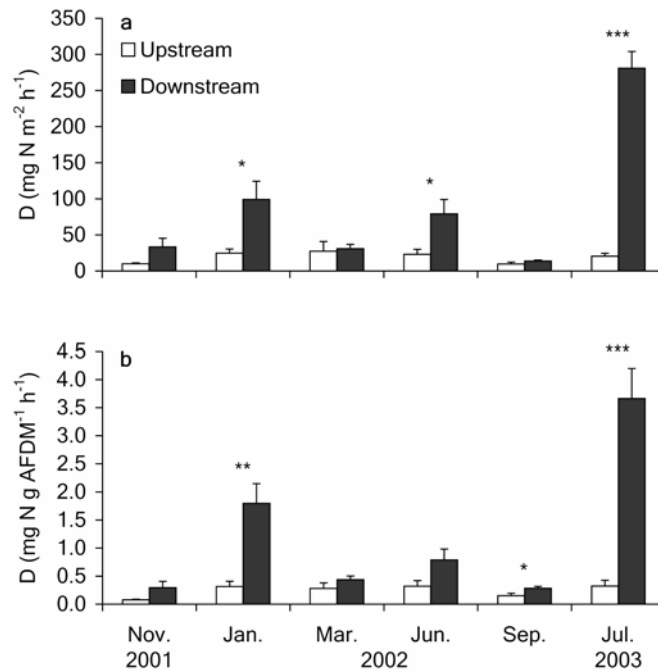
Fig. 6.1. Mean (± SE) denitrification rates expressed (a) /m^2 and (b) /g AFDM through the study period for the upstream reach (white bars) and the downstream reach (black bars) of La Tordera stream. Results of two-way ANOVA: for the two variables (/m^2 and /g AFDM), reach factor significant ($P < 0.0005$), date factor significant ($P < 0.0005$), reach x date interaction significant ($P < 0.0005$). Significant point source effect (independent samples T-test) considering each date separately is denoted as * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$).

denitrification rates /g AFDM, significant increases occurred with greatest relative contribution of the point source to downstream DOC loads. Greatest relative point source contribution to downstream DOC loads occurred in January 2002 (3.0 mg/s) and July 2003 (29.9 mg/s) relative to the rest of sampling dates (0.75-0.95 mg/s). Denitrification rates (both expressed /m^2 and /g AFDM) did not significantly vary between sampling dates upstream of the point source, whereas date effect was significant for the downstream reach. Variation in these rates among dates at the downstream reach was significantly related with variation in DO deficit, stream discharge and NO_3^--N concentration (Fig. 6.2).
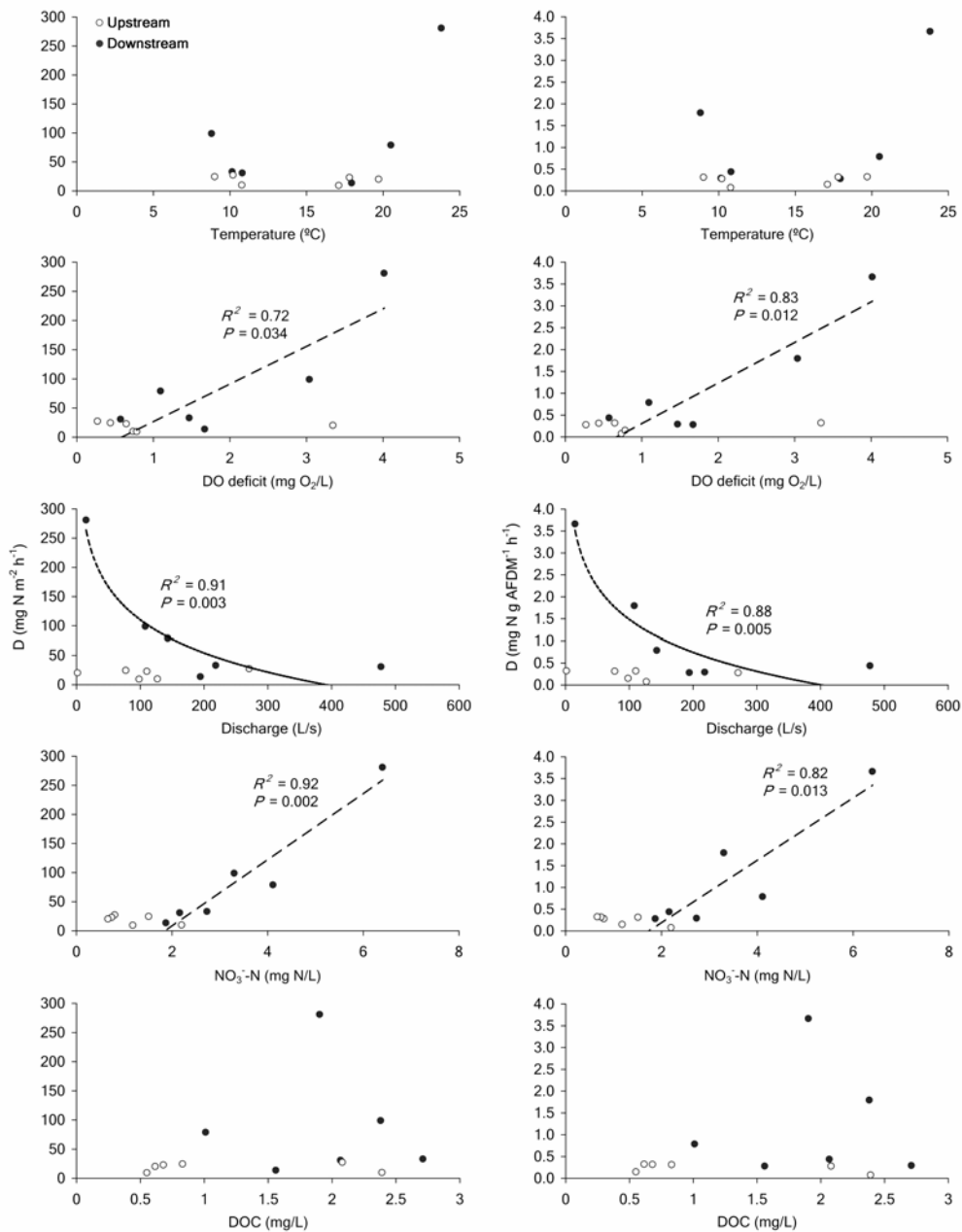
Fig. 6.2. Denitrification rates (left, /m^2; right, /g AFDM) in La Tordera stream as a function of water temperature, DO deficit, stream discharge, and NO$_3^-$-N and DOC concentrations. Open circles, upstream reach; solid circles, downstream reach. Dotted lines show significant relationships for the downstream reach. Statistics (R^2 and P) of these relationships are shown.

In Gurri stream, the point source effect on potential denitrification rates (expressed /m^2 or /g AFDM) was not significant, but these rates varied between sampling dates (Fig. 6.3). Variation between sampling dates in denitrification (expressed /m^2) was related to changes in stream discharge and NO_3^--N concentration upstream of the point source, but not downstream (Fig. 6.4). Nevertheless, when combining results from the two reaches, these relationships remained significant or marginally significant (with NO_3^--N, R^2 = 0.34, P = 0.038; with discharge, R^2 = 0.25, P = 0.081). No relationship between denitrification expressed /g AFDM and the parameters shown in Figure 6.4 was found.
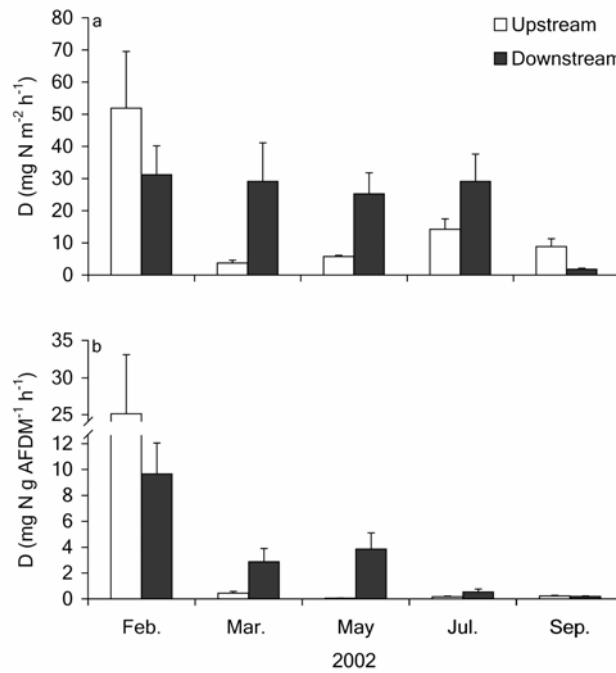
Fig. 6.3. Mean denitrification rates (± SE) through the study period for the upstream reach (white bars) and the downstream reach (black bars) of Gurri stream. Results of two-way ANOVA: (a) denitrification /m^2, reach factor not significant (P = 0.340), date factor significant (P = 0.011), reach x date interaction not significant (P = 0.095); (b) denitrification /g AFDM, reach factor not significant (P = 0.434), date factor significant (P < 0.0005), reach x date interaction significant (P = 0.027).
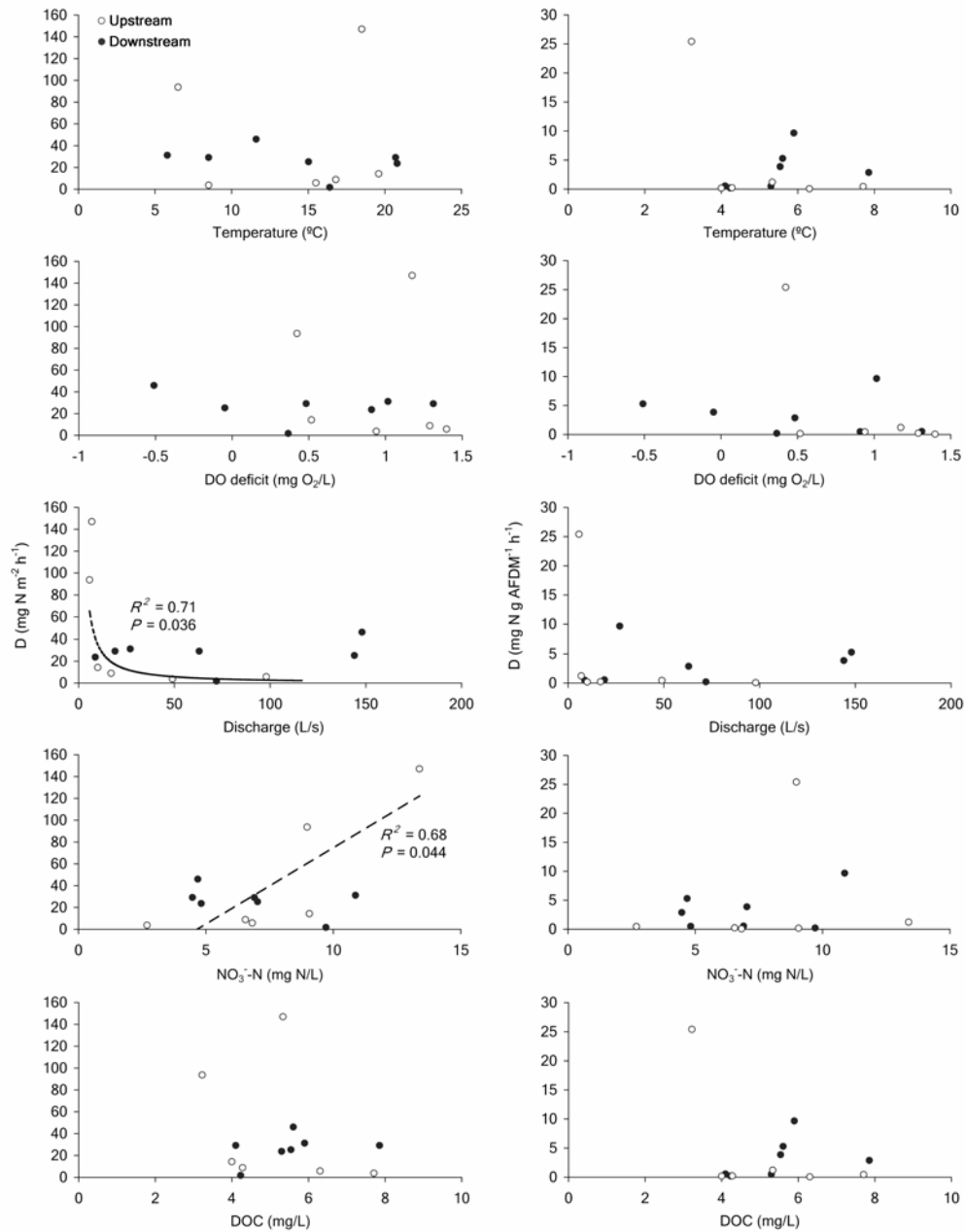
Fig. 6.4. Denitrification rates (left, /m^2; right, /g AFDM) in Gurri stream as a function of water temperature, DO deficit, stream discharge, and NO$_3^-$-N and DOC concentrations. Open circles, upstream reach; solid circles, downstream reach. Dotted lines show significant relationships for the upstream reach. Statistics (R^2 and P) of these relationships are shown.

Experiments on factors limiting denitrification rates

Stream discharge was higher in May than in August 2004, the two sampling dates on which experiments on factors limiting denitrification rates were conducted (Table 6.1). Differences between the two reaches in concentrations of NO_3^--N and DOC were clearer in August, under low flow conditions, than under high flow conditions (Table 6.1).

In May 2004, denitrification rates significantly varied between treatments for the sediments collected in the two reaches of La Tordera stream (Fig. 6.5). At the upstream reach, denitrification rates increased in response to added N, and even more in response to amendment with N+C together. Downstream of the point source, denitrification rates only increased in response to N+C amendment.
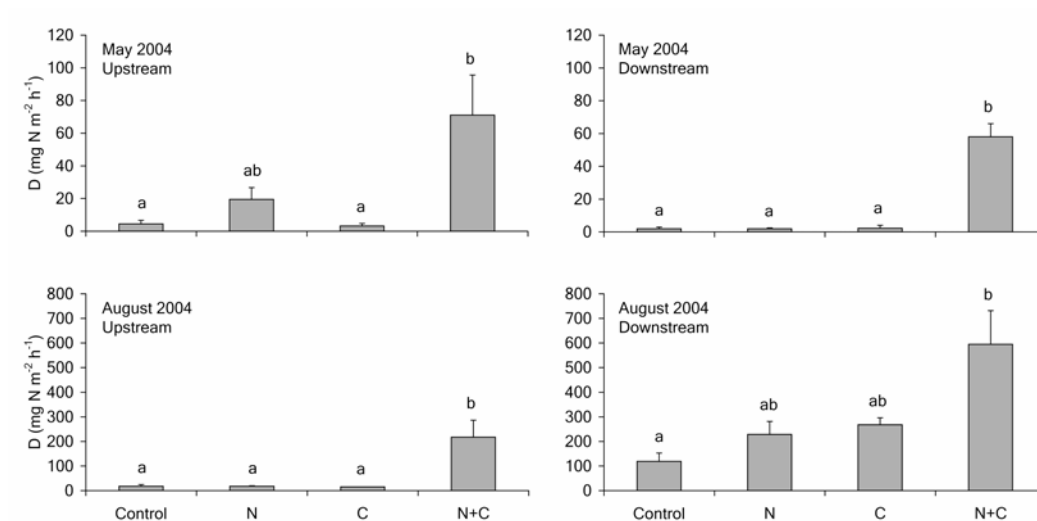

Fig. 6.5. Mean denitrification rates (± SE) under four treatments (control with stream water, N amendment, C amendment and N+C amendment) for the upstream and the downstream reaches from La Tordera stream in May and August 2004. Treatment factor significant in the four cases (one-way ANOVA): May, upstream, P = 0.007; May, downstream, P < 0.0005; August, upstream, P = 0.016; August, downstream, P = 0.018. Different lower-case letters above bars indicate significant differences among treatments (Tukey's HSD) for each reach and date.

In May 2004, stream discharge was higher, and denitrification rates measured in the control treatment were lower, than those measured for the 2001-2003 period. On this date, under high flow conditions, denitrification rates for the control treatments were comparable between the two reaches (Fig. 6.5; one-way ANOVA, $P > 0.05$).

Similar to May, the effect of nutrient amendment on denitrification was significant in August 2004 (Fig. 6.5). Upstream of the point source, only the amendment with N+C together resulted in higher denitrification rates than in the rest of treatments. Downstream of the point source, amendment with N and C separately resulted in higher denitrification rates than control treatment, but the response was greatest with amendment of N+C together. In August, discharge and denitrification rates measured in the control treatment were within the same range than those measured on previous sampling dates through 2001-2003. Under these conditions of low flow, differences between the two reaches in denitrification rates measured in the control treatment were clearer than in May, but the increase was not statistically significant (Fig. 6.5; one-way ANOVA, $P = 0.081$).

Discussion

Comparison of denitrification rates between pristine and human-altered streams

Based on denitrification rates (expressed both /m^2 and /g AFDM) from the literature and from our study, availability of NO_3^--N and DOC is unlikely to explain differences in these rates across stream ecosystems (Fig. 6.6). Mean denitrification rates published for a number of streams with NO_3^--N concentrations from 0.005 to 32 mg N/L (Duff *et al.* 1984, García-Ruiz *et al.* 1998a, Pattinson *et al.* 1998, Kemp and Dodds 2002, Royer *et al.* 2004) were within the order of mg N m^{-2} h^{-1} like in La Tordera and Gurri, but between 7 and 30 times lower than those from the two reaches of the two study streams (Fig.

6.6a). Between these studies, only Royer *et al.* (2004) reported DOC concentrations (Fig. 6.6b). Similarly, we examined relationships between denitrification rates expressed /g AFDM for a variety of pristine, urban and agricultural streams with a wide range of NO_3^--N (0.01-4.40 mg N/L) and DOC (0.11-9.80 mg/L) concentrations (Martin *et al.* 2001, Inwood *et al.* 2005; Fig. 6.6c, d). These rates were lower (in the order of μ N g AFDM^{-1} h^{-1}) than in the two reaches of La Tordera and Gurri streams (Fig. 6.6c, d). These comparisons support previous research indicating that variation in denitrification rates between different streams from North America was not predicted based only on stream water NO_3^--N concentrations, but also on other factors such as water temperature and availability of organic matter (Steinhart *et al.* 2000). Difficulty to find a single
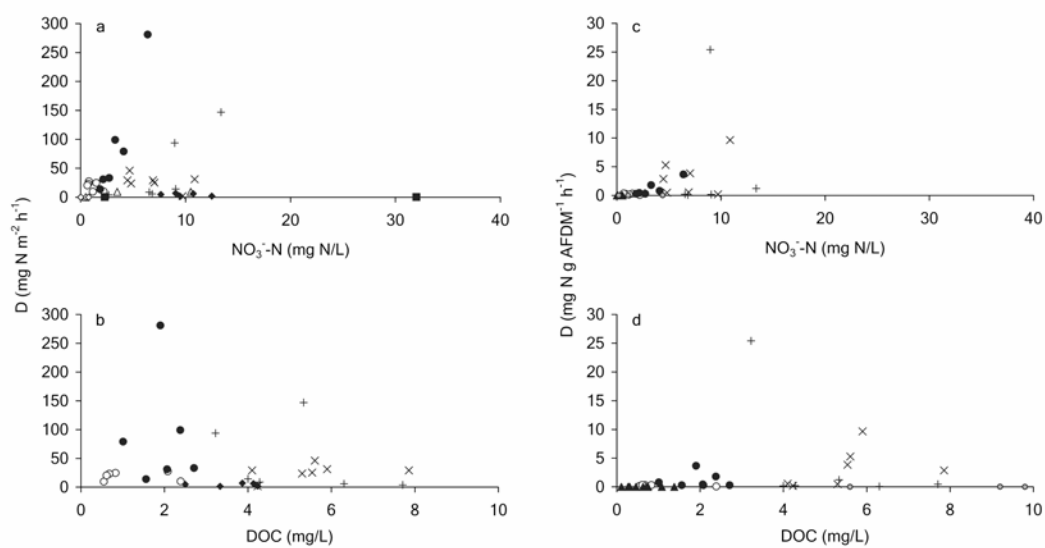

Fig. 6.6. Denitrification rates expressed /m^2 (a, b) and /g AFDM (c, d) as a function of NO_3^--N and DOC concentrations, including results from the literature and from our study. In particular, the figure shows results from: ○, La Tordera upstream; ●, La Tordera downstream; +, Gurri upstream; x, Gurri downstream; □, Duff *et al.* (1984); ■, García-Ruiz *et al.* (1998); Δ, Pattinson *et al.* (1998); ▲, Martin *et al.* (2001); ◊, Kemp and Dodds (2002); ♦, Royer *et al.* (2004), and •, Inwood *et al.* (2005).

factor explaining variation in denitrification rates (expressed both /m^2 and /g AFDM) across streams is likely to be due to a complex interaction of factors governing spatial variation in these rates.

We estimated the proportion of NO$_3^-$-N removed via denitrification (g N reach^{-1} d^{-1}) relative to influx of NO$_3^-$-N (g N reach^{-1} d^{-1}) for each sampling date and reach. Net removal of NO$_3^-$-N was higher in La Tordera (upstream, 8.6 ± 5.7 %; downstream, 5.9 ± 4.2 %) than in Gurri (upstream, 2.4 ± 1.4 %; downstream, 0.8 ± 0.2 %). In La Tordera, highest percentages of net NO$_3^-$-N removal (upstream, 37.1 %; downstream, 26.8 %) occurred under lowest stream discharge. Denitrification as a sink for N has been shown to be significant in pristine (Swank and Caskey 1982, Holmes *et al.* 1996, Steinhart *et al.* 2000) and human-altered streams (Seitzinger 1988, Bradley *et al.* 1995, Sjodin *et al.* 1997). In contrast, this role of denitrification was likely to be insignificant in other pristine (Martin *et al.* 2001) and human-altered streams (Inwood *et al.* 2005). Large NO$_3^-$-N export reported for an agricultural river (David *et al.* 1997) also suggest that denitrification did not significantly contribute to N loss from the water under high N inputs from diffuse sources. Overall, denitrification in La Tordera stream plays an important role in removing N from the water column below the point source, especially during low flow conditions. While denitrification rates are high above and below the point source in Gurri stream, denitrifying activity is unlikely to be able to compensate the amount of N entering the stream channel via diffuse sources.

Point source influence on stream denitrification potentials

In La Tordera stream, denitrification rates (both expressed /m^2 and /g AFDM) tended to be higher below the point source on all sampling dates, although the increases were statistically significant only in 50 % of studied cases. This agrees with what we expected given that DO deficit, and concentrations of NO$_3^-$-N and DOC significantly increased below the point source. Significant increases in denitrification expressed /m^2 coincided with sampling dates with

lowest discharge. Our results suggest that denitrifying activity is favored under lowest stream discharge, when both point source contribution to stream nutrient loads and surface-volume ratios are highest. Previous studies have shown that high surface-volume ratios favor in-stream nutrient retention (Butturini and Sabater 1998, Peterson *et al.* 2001, Hall *et al.* 2002). Our results show that high surface-volume ratios also favor in-stream denitrification. The present study supports our previous suggestion on the importance of denitrifying activity below the point source under low discharge conditions (Chapter 4). Other recent studies also suggested that highest denitrification rates occurred during lowest discharges in agricultural streams from North America (Schaler *et al.* 2004). On the other hand, significant increases in denitrification rates expressed /g AFDM downstream of the point source relative to upstream coincided with greatest relative contribution of the point source to stream DOC loads. Efficiency of denitrifiers, thus, was likely to be enhanced below the point source under greatest relative point source input of DOC as a C source to reduce NO_3^--N to N_2O and N_2.

Increases in stream water in NO_3^--N concentrations can result from its direct input from point and diffuse sources, and indirectly from nitrification of NH_4^+-N from point sources (Bradley *et al.* 1995, Martí *et al.* 2004, Merseburger *et al.* 2005). In a previous study, we evidenced that NH_4^+-N from the point source in La Tordera was oxidized to NO_3^--N along the downstream reach via nitrification (Merseburger *et al.* 2005). That study suggested that this NO_3^--N produced via nitrification was used for denitrification. Hence, in-stream microbial processes are likely to minimize effects of anthropogenic nutrient inputs on stream water chemistry, including concentrations of NH_4^+-N and NO_3^--N. Previous studies already suggested a coupling between nitrification of NH_4^+-N in the oxic zone and denitrification of NO_3^--N in the anoxic zone of estuarine (Nishio *et al.* 1983, Jenkins and Kemp 1984) and stream sediments (Bradley *et al.* 1995, Laursen and Seitzinger 2004).

Contrary to La Tordera, denitrification rates expressed /m^2 and /g AFDM did not significantly increase downstream of the point source relative to upstream in Gurri stream. These results were coherent with lack of point source effect on DO deficit or NO$_3^-$-N and DOC concentrations, which were already high upstream of the point source due to diffuse sources from agriculture (Merseburger *et al.* 2005). We expected higher denitrification rates above the point source in Gurri than in La Tordera due to higher NO$_3^-$-N and DOC concentrations in the former than in the latter stream as a result of diffuse sources. On average, potential denitrification rates were two times higher in the two reaches of Gurri stream than in the upstream reach of La Tordera. Nevertheless, variability of these rates was high in Gurri stream, where most rates were similar to those from the upstream reach of La Tordera. Below the point source input, regardless of higher NO$_3^-$-N and DOC concentrations in Gurri than in La Tordera, denitrification rates were lower in the former than in the later stream. Denitrification was mostly associated to biofilm with abundance of filamentous green algae (i.e., *Cladophora* sp.) in Gurri stream, whereas was associated to sediments in La Tordera. Denitrification in sediments has been shown to be greater than plant-associated denitrification in streams (Kemp and Dodds 2002, Schaler *et al.* 2004). Mats of the filamentous algae *Cladophora* sp. look like favorable sites to support high rates of denitrification, but they have been suggested to play a minor role as net sinks for N by this process (Schaler *et al.* 2004). In addition, denitrification efficiency increases as a function of rising stream hyporheic exchange (Sjodin *et al.* 1997). The dominance of bedrock and lack of sediment in the streambed of Gurri may reduce vertical water exchange in this stream, fact that may also contribute to counterbalance denitrification rates between the two streams in spite of water chemistry differences.

Factors controlling denitrification rates

Point source inputs affected variation in potential denitrification rates in La Tordera stream through the study period. There, denitrification rates did not

significantly vary through the sampling period upstream of the point source input. Downstream of the point source, in contrast, variation in denitrification rates (expressed /m^2 and /g AFDM) through the study period was significant. In Gurri, where nutrient inputs entered the stream channel via diffuse sources from adjacent agricultural fields in addition to the point source, denitrification rates (expressed /m^2 and /g AFDM) significantly varied among sampling dates both at upstream and downstream of the point source. These variations were explained by changes in DO deficit, stream discharge and NO_3^--N concentration in La Tordera stream, whereas only by changes in stream discharge and NO_3^--N concentration (only for denitrification /m^2) in Gurri stream. Previous studies have shown a response of stream denitrification rates to increases in water temperature (Ventullo and Rowe 1982, Pfenning and McMahon 1996, García-Ruiz *et al.* 1998a). We expected ranges in water temperature at the two reaches of the two study streams (La Tordera: upstream, 9.0-19.7 ºC, and downstream, 8.8-23.8 ºC; Gurri: upstream, 6.5-19.6 ºC, and downstream, 5.8-20.8 ºC) to be broad enough to result in different responses in denitrifying activity. However, we conducted denitrification assays under laboratory room temperature, fact that may explain lack of relationship between stream water temperature and measured denitrification rates. Similar to our findings, DOC concentration was unlikely to control denitrification rates in other pristine and human-altered streams (Martin *et al.* 2001, Bernhardt and Likens 2002, Inwood *et al.* 2005). The lack of explanatory power of DOC concentration as a predictor of denitrification rates might reflect that denitrifiers may use particulate organic C in stream sediments (Martin *et al.* 2001). Anoxic habitats may be denitrification hotspots when other factors are not limiting (McClain *et al.* 2003). However, it is difficult to measure O_2 concentration within the stream sediments in the field, and thus, address the role of anoxia in controlling denitrification activity in stream sediments (Clément *et al.* 2002). Other studies examining the effects of anoxia on denitrification used O_2 concentrations in the water column and did not find any relationship with denitrification rates (Kemp and Dodds 2002). Despite we did not directly measure

O_2 concentration within benthic substrata, we estimated DO deficit from *in situ*, dial O_2 curves. Our results show that increasing DO deficit and probably, thus, anoxic conditions within the stream sediments, resulted in enhanced denitrification rates downstream of the point source in La Tordera. Negative relationships between denitrification rates and stream discharge in the two study streams agree with previous studies suggesting that highest stream denitrification rates occur under lowest discharges (Schaler *et al.* 2004). Finally, NO_3^--N availability was a common factor between the two study streams regulating rates of denitrification potential. Previous studies also concluded that NO_3^--N concentration plays a major role in controlling denitrification rates in pristine and human-altered streams (García-Ruiz *et al.* 1998b, Martin *et al.* 2001, Inwood *et al.* 2005). Hence, it is likely that NO_3^--N concentration is an important regulator of denitrification activity within diverse streams. Experiments on limiting factors showed that availability of both N and C determined the potential for denitrification in La Tordera regardless of hydrological conditions and reach location. Results from these experiments agree with those relationships indicating the importance on NO_3^--N availability in controlling potential denitrification rates. These results also indicate the importance of C availability, which was not reflected by relationships between denitrification rates and DOC concentration in stream water. Denitrifiers may obtain most C from particulate organic matter in stream sediments. Availability of C plays an important role controlling denitrification rates, but the fact that denitrifiers can obtain most C from particulate organic matter in stream sediments (Martin *et al.* 2001) enables to find significant relationships between these rates and ambient levels of DOC concentration. Our results support previous studies suggesting that dissolved NO_3^--N and organic C significantly determined potential denitrification rates in sediments of a pristine stream (Holmes *et al.* 1996), a stream receiving a point source (Bradley *et al.* 1995), and also in riparian soils of streams (Pinay *et al.* 2003).

7. Influences of a point source on whole-stream metabolism

Summary

We examined effects of wastewater treatment plant (WWTP) inputs (i.e., point source) on the metabolism of La Tordera (8 samplings) and Gurri (9 samplings) streams by using the upstream-downstream dissolved oxygen change technique. Significant increases in inorganic nutrient concentrations downstream of the point source relative to upstream in the study streams resulted in higher daily rates of gross primary production (GPP) downstream of the point source relative to upstream on most sampling dates, but the increases were not consistent among all dates. Therefore, the overall point source effect on GPP was marginally significant in La Tordera, and was not statistically significant in Gurri. Photoinhibition was not observed in any sampling date in La Tordera or Gurri, supporting previous studies suggesting that photoinhibition may occur at the surface of biofilm mats, but not at the whole community level.

Daily rates of respiration (R) significantly increased downstream of the point source relative to upstream in La Tordera. In Gurri, R rates tended to be higher downstream than upstream of the point source on most dates, but the overall point source effect was not significant because the increases were not consistent among all dates. As expected, based on higher nutrient concentrations in Gurri than in La Tordera, R rates measured above the point source were higher in the former stream. Hyporheic respiration highly contributes to whole-stream R. Daily rates of R were high in Gurri stream regardless of being constrained to surface due to dominance of bedrock in the streambed. Nevertheless, R rates measured below the point source were similar between the two streams regardless of higher concentrations of nitrate (NO_3^--N) and dissolved organic carbon (DOC) in Gurri than in La Tordera. Lower vertical water exchange in Gurri than in La Tordera may counterbalance the potential effect of nutrient availability in the former stream. Specific attributes of each stream may reduce effective generalizations on R variation across streams as a function of dominant land uses within catchments. On the other hand, negative rates of net ecosystem

production (NEP) and GPP:R ratios lower than 1 in La Tordera and Gurri indicate that the metabolism of the two streams was predominantly heterotrophic.

In La Tordera, water temperature and DOC were the main control on R rates upstream of the point source, but not downstream. A positive relationship was found between R and ammonium (NH_4^+-N) concentration only when combining results from the two reaches. This latter relationship, together with the fact that R and GPP were more decoupled downstream than upstream of the point source, suggested that nitrification of NH_4^+-N from the point source input greatly contributed to ecosystem R. Nitrification is part of autotrophic productivity that is not accounted with the upstream-downstream dissolved oxygen change technique, but that contributes to whole-stream R. Daily rates of GPP were also positively related to NH_4^+-N concentration, but NH_4^+-N availability had a greater effect on daily rates of R than on those of GPP. Daily rates of NEP were negatively related to NH_4^+-N concentration when combining data from the two reaches, and thus, point source inputs of NH_4^+-N were also affecting the balance between GPP and R. These results suggest that the autotrophic and the heterotrophic components of the biofilm responded differently to additional nutrients from the WWTP in this stream.

In Gurri, the only effect of the point source on water chemistry (i.e., concentration of soluble reactive phosphorus—thereafter SRP) was likely to enhance daily rates of GPP, as indicated by the positive relationship between GPP and SRP concentration. Rates of R were not related to nutrients availability, and thus, the GPP:R ratio was also positively related to SRP concentration. Combining data from our study and from the literature showed a positive, linear relationship between GPP rates and SRP concentrations up to ~ 0.1 mg/L, above which GPP became saturated. This relationship indicates the important role of SRP availability regulating whole-stream rates of GPP across large geographic areas. This relationship also suggests that above a certain threshold of SRP availability, other factors may become critical in limiting GPP rates.

Introduction

Rates of whole-stream primary production and respiration characterize stream metabolism at ecosystem level, and are useful descriptors of its trophic status. Primary production represents the supply of organic matter produced within the ecosystem, whereas ecosystem respiration represents total consumption of autochthonous and allochthonous sources of organic matter. The relation between primary production and respiration determines whether the stream ecosystem acts as a net source or a net sink of organic matter. In human-altered streams (i.e., receiving nutrient inputs from human activities), metabolism has been less studied than in pristine streams. Wastewater treatment plant (WWTP) inputs (i.e., point sources) may alter metabolism of receiving streams because they represent an abrupt discontinuity on factors that may regulate metabolism rates (i.e., increase stream discharge and nutrient concentrations).

Responses of stream metabolism to nutrient inputs are complex because they do not only depend on nutrient availability, but are also controlled by other factors (Peterson *et al.* 1983, Grimm and Fisher 1986, Meyer *et al.* 1988, Hart and Robinson 1990, Peterson *et al.* 1993, Rosemond 1993, Rosemond *et al.* 1993). Rates of gross primary production (GPP) in pristine streams have been shown to be negatively affected by increases in water velocity (Fisher *et al.* 1982, Finlay and Bouden 1994, Uehlinger and Naegeli 1998, Biggs *et al.* 1999) and favored by increases in phosphorus concentration (Guasch *et al.* 1995, Mulholland *et al.* 2001). Rates of stream ecosystem respiration (R) in pristine streams are likely to be favored by supply of nutrients and organic matter (Tank and Winterbourn 1995, Young and Huryn 1999, Mulholland *et al.* 2001, Acuña *et al.* 2004), as well as by increases in water temperature (Bott *et al.* 1985, Edwards and Meyer 1987, Sinsabaugh 1997, Uehlinger *et al.* 2000). A recent review by Slavik *et al.* (2004) showed that N and P enrichments during few years (c.a., ≤ 4 y) increased productivity of some species from different trophic levels in streams from North America and Canada. Studies conducted in a tundra river in Alaska suggested that long-term (c.a., > 4 y) enrichments not only were likely to affect

the community structure of this river ecosystem, but also its biogeochemical dynamics (Miller *et al.* 1992, Peterson *et al.* 1993, Bowden *et al.* 1994, Arscott *et al.* 2000, Slavik *et al.* 2004, Benstead *et al.* 2005). Point source inputs from WWTPs should be analogous to those long-term nutrient enrichment experiments, and allow evaluation of human activity effects on stream metabolism.

We aimed to examine point source effects on the metabolism of a forested and an agricultural stream under different environmental conditions using a multiparametric approach in two contrasted stream scenarios, with and without diffuse sources. Conducting the study in these contrasted scenarios is of special interest given the need of improving existing knowledge about potential effects of different land uses on the ecology of running waters (Nilsson *et al.* 2003). In particular, we compared photosynthesis-irradiance (*P-I*) responses between two reaches located upstream and downstream of a point source, and examined differences between the two reaches in daily rates of GPP, R, net ecosystem production (NEP) and the GPP:R ratio. We also examined factors driving variability in metabolism rates in the two study streams. Point sources increase stream discharge, and are a source of inorganic nutrients and dissolved organic carbon to receiving streams. We hypothesized that, depending on the relative effect of the point source on stream discharge and inorganic nutrients, GPP would be disfavored (i.e., greater point source effect for discharge than for inorganic nutrients) or enhanced (i.e., greater point source effect for inorganic nutrients than for discharge) below the point source relative to above. We also hypothesized significant increases in DOC concentration downstream of the point source relative to upstream to favor whole-stream rates of R.

Materials and methods

Field sampling

Whole-ecosystem metabolism was measured upstream and downstream of a point source in La Tordera (8 samplings) and Gurri (9 samplings) streams on cloudless days through 2001-2003. To measure stream metabolism, we used the upstream-downstream dissolved oxygen change technique, introduced by Odum (1956) and refined by Marzolf *et al.* (1994). Measurements using this open system method include an entire section of a stream, and thus, results reflect metabolism rates at the ecosystem level. The basis of this method is that dissolved oxygen changes (Q_{DO}) between the two stations depend on GPP, R and the oxygen exchange (E) with the atmosphere (i.e., Q_{DO} = GPP – R ± E). Measurements of dissolved oxygen (DO) concentration and water temperature were recorded at 10-min intervals over a 24-h period using two oxygen meters (WTW$^{®}$ Oxi 340-A) placed at the top and bottom of the reach. The distance between the two stations depended on stream water velocity; it was commonly about 100 m, but was reduced to 30 m in the downstream reach of Gurri stream in July 2003, when the upstream reach was dry and discharge at the downstream reach was very low (9 L/s).

Oxygen exchange with the atmosphere was estimated based on reaeration rates, which were measured 1 day after the oxygen measurements using *in situ* gas (butane) addition coupled with a hydrological tracer (Marzolf *et al.* 1994). Reaeration rates were calculated from the decline in dissolved butane concentration during steady-state corrected by hydrological dilution. Reaeration rates of oxygen have been commonly calculated from those of propane using a conversion factor of 1.39 (Rathbun *et al.* 1978). This factor is the quotient between propane and oxygen molecular mass, and thus, we calculated oxygen reaeration rates from those of butane using a correction factor of 1.82. Because butane additions were successful only on those sampling dates with lowest discharge, reaeration rates for the rest of dates were estimated using a number

of indirect methods. In particular, we used the empirical equation of Owens (1974) and those compiled from literature by Genereux and Hemond (1992), based on physical attributes (i.e., channel slope, water velocity and depth) of streams. We also used the approach of Young and Huryn (1998) to calculate reaeration rates based on the night-time variation of mean DO deficits at two stations (i.e., upstream-downstream) and net DO changes. Results from indirect methods were compared with those available from *in situ* butane additions. Reaeration rates estimated with the equation of Owens (1974) were more similar to those from butane additions and the approach of Young and Huryn (1998) than those estimated with the rest of indirect methods for the two study streams, and thus, this equation was selected to estimate unavailable reaeration rates.

On each sampling date and for each reach, we also measured a number of hydromorphological, chemical and biological parameters to examine factors driving variability in metabolism rates in the two study streams. Hydromorphological parameters (i.e., channel width, water depth, velocity and stream discharge) were measured as described in Chapter 4. Photosynthetic active radiation (PAR, μmol m^{-2} s^{-1}) was recorded every 10 min during the 24-h period of metabolism measurements using a spherical quantum sensor (Li-Cor, Li-193SA, Lincoln, NB, USA) connected to a data logger (Li-Cor, Li-1000). The spherical quantum sensor was placed 20 cm above the stream water level at the middle of the reach. Instantaneous values of PAR were integrated over the 24-h period to estimate daily PAR (mol m^{-2} d^{-1}). We collected water samples for concentrations of ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), soluble reactive phosphorus (SRP) and dissolved organic carbon (DOC). Concentration of dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4^+-N, NO_2^--N and NO_3^--N concentrations. We also collected samples of biofilm and filamentous green algae for characterization of patch specific chlorophyll *a*, carotenoids and biomass. Chlorophyll *a* and biomass were also estimated at the reach scale by calculating the habitat weighted patch specific values based on the percentage of coverage of biofilm and filamentous green algae in the reach.

Field sampling and laboratory analyses of these chemical and biological parameters are described in Chapters 4 and 5, respectively.

Calculation of whole-stream metabolism

We calculated instantaneous rates of net oxygen change (mg O_2 m^{-2} min^{-1}) along the reach as the change at 10-min intervals in DO between the two stations, and corrected them for air-water oxygen exchange to estimate metabolism rates. The daily rate of ecosystem R (g O_2 m^{-2} d^{-1}) was estimated by extrapolating average night-time respiration through the daylight hours (Edwards and Owens 1962, Bott 1996). The daily rate of GPP (g O_2 m^{-2} d^{-1}) was determined by summing the differences between measured corrected net oxygen change rate and the extrapolated value of R during the daylight period. The daily rate of NEP (g O_2 m^{-2} d^{-1}) was calculated as the difference between daily rates of GPP and R. Positive values of NEP characterize ecosystems that act as net sources of organic matter, whereas negative values of NEP are associated with ecosystems that act as net sinks of organic matter. We also calculated the GPP:R ratio. The simple balance between GPP and R does not necessarily indicate which source of carbon (autochthonous or allochthonous) is most important to ecosystem respiration (Rosenfeld and Mackay 1987, Meyer 1989). However, as originally proposed by Odum (1956), it is generally accepted that the GPP:R ratio indicates whether an ecosystem is dominated by autotrophic (GPP:R > 1) or heterotrophic (GPP:R < 1) metabolism (e.g., Vannote *et al.* 1980, Mulholland *et al.* 2001, McTammany *et al.* 2003).

Estimation of photosynthesis-irradiance curves

The *P-I* curves integrate the response of local communities to local environmental conditions, and describe the short-term response of photosynthesis to changes in light intensity (Uehlinger *et al.* 2000). The shape of the *P-I* curves is mainly affected by light (Falkowski and LaRoche 1991, Arscott *et al.* 2000, Uehlinger *et al.* 2000), water temperature (Davison 1991, Maxwell *et*

al. 1994, Arscott *et al.* 2000, Uehlinger *et al.* 2000), and stage of community development (Hill and Boston 1991).

We used non-linear regression analysis (STATISTICA, version 5.5, StatSoft Inc., Tulsa, OK, USA) to fit instantaneous rates of corrected net DO changes to photosynthetic active radiation (PAR) instantaneous values for each reach and sampling date to a hyperbolic tangent model (Jassby and Platt 1976) modified to include respiration (Guasch and Sabater 1995, Arscott *et al.* 2000). The model used is described by the following equation:

$$NEP = GPP_{max} \tanh (\alpha\, PAR / GPP_{max}) + R$$

where NEP is the net ecosystem production instantaneous rate, GPP_{max} is the maximum rate of gross primary production, α is the initial rate of increase of photosynthesis as light increases from darkness (estimated as GPP_{max}/K, where K is the half-saturation light intensity), and R is the ecosystem respiration. The curves fitted were used to estimate GPP_{max}, α (that represents photosynthetic efficiency), R, light at saturation (I_k) and light at which compensation (i.e., gross primary production = respiration) occurs (I_{comp}). The parameters I_k and I_{comp} were calculated as GPP_{max}/α and R/α, respectively (Henley 1993).

Statistical analyses

We used paired *T*-test to compare water temperature, daily PAR, concentration of carotenoids, parameters predicted using the hyperbolic tangent function (GPP_{max}, α, R, I_k and I_{comp}), and daily rates of ecosystem metabolism (GPP, R, NEP) and the GPP:R ratio between the two reaches within each study stream. Paired *T*-test was also used to compare within each reach GPP_{max} with GPP, and R predicted using the equation cited above with R measured from the average of instantaneous rates of corrected net DO changes measured at nighttime. Paired *T*-tests were conducted on log-transformed values when needed in order to stabilize variances and normalize the data sets. To assess factors driving variability in metabolism rates, we used univariate linear,

logarithmic, exponential and power regressions between stream metabolism rates and hydromorphological, chemical and biological parameters with data from the two reaches separately and combined. To assess factors driving this variability we also used stepwise multiple regressions ($P \leq 0.05$ as the criterion for entry into the model). To examine relationships between metabolism rates and nutrient concentrations from a number of streams with data from literature, we also used univariate regressions. The significance level used for all statistical tests was $P \leq 0.05$. All the statistical analyses were performed with the SPSS® statistical package (for Windows, version 12.0, SPSS Inc., Chicago, Illinois).

Results

Physical, chemical and biological characterization

Daily PAR was similar between the two reaches in La Tordera stream, but was 2.5 times higher downstream than upstream of the point source in Gurri stream (Table 7.1). The point source input did not significantly affect daily variation in water temperature, which showed slightly wider ranges below than above the input and similar means for the two reaches within each study stream (Table 7.1). The point source increased water velocity in the two study streams, and also affected width of the wet channel, water depth and stream discharge in

Table 7.1. Range and mean ± standard error (SE) of water temperature and photosynthetic active radiation (PAR) during the 24-h of metabolism measurements at upstream and downstream of the point source in La Tordera (n = 7) and Gurri (n =8) streams. The table shows the degree of significance from paired T-test comparing these parameters between the experimental reaches within each study stream. See also Table 5.1.

Parameter	Reach	La Tordera			Gurri		
		Range	Mean ± SE	P	Range	Mean ± SE	P
Temperature (ºC)	upstream	9.0-19.7	13.7 ± 1.7	ns	6.5-19.6	13.0 ± 1.9	ns
	downstream	8.8-23.8	13.9 ± 2.2		5.8-20.8	13.6 ± 2.3	
PAR (mol m^{-2} day^{-1})	upstream	11.0-45.8	24.7 ± 5.9	ns	2.9-28.6	15.7 ± 3.2	***
	downstream	7.7-37.5	21.4 ± 4.8		11.7-53.2	38.6 ± 4.8	

ns, not significant (P > 0.05); *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

Gurri (see Table 5.1). The point source significantly increased concentration of all studied nutrients (NH_4^+-N, NO_3^--N, DIN, SRP and DOC) in La Tordera, but only concentration of SRP in Gurri (see Table 5.1).

On average, habitat weighted chlorophyll *a* was five times greater downstream than upstream of the point source in La Tordera (see Table 5.1). However, high variability in chlorophyll *a* through the study (indicated by high SE of the mean) resulted in lack of statistical effect of the point source on this parameter. Mean concentration of carotenoids also tended to be higher downstream (74.4 ± 36.5 mg/m^2) than upstream (18.2 ± 4.1 mg/m^2) of the point source, despite the difference between the two reaches was not statistically significant (paired *T*-test: t = -1.654, df = 7, P = 0.142). Habitat weighted biomass did neither differ between the two reaches in La Tordera (see Table 5.1). In contrast, habitat weighted chlorophyll *a* and biomass significantly increased three- and twofold, respectively, below the point source in Gurri (see Table 5.1). Carotenoids also were three times higher downstream (173.9 ± 42.9 mg/m^2) than upstream (60.6 ± 23.5 mg/m^2) of the point source in Gurri (paired *T*-test: t = -3.917, df = 7, P = 0.006). The ratio between carotenoids and chlorophyll *a* (car/chl) upstream and downstream of the point source averaged (mean ± SE), respectively, 0.48 ± 0.05 and 0.42 ± 0.03 in La Tordera, and 0.41 ± 0.02 and 0.46 ± 0.01 in Gurri. This ratio was used to assess adaptation of primary producers to high light levels (Falkowski and LaRoche 1991).

Photosynthesis-irradiance parameters

Fitting the results from the instantaneous rates of corrected net DO changes to PAR instantaneous values to the hyperbolic tangent function (i.e., *P-I* curves) explained (mean ± SE) 89 ± 6 % (upstream) and 69 ± 6 % (downstream) of the variance in La Tordera, and 67 ± 10 % (upstream) and 84 ± 5 % (downstream) in Gurri (Table 7.2). We did not observe significant differences in any of the parameters estimated from the *P-I* curves between the two reaches in La Tordera. However, these comparisons had low degrees of freedom (see Table

Table 7.2. Parameters estimated by using the hyperbolic tangent relationship (see text for explanation) for each sampling date at upstream and downstream of the point source in the study streams: GPP_{max} and R (g O_2 m^{-2} day^{-1}), α (mg O_2 m^{-2} min^{-1}/μmol fotons m^{-2} s^{-1}), I_k and I_{comp} (μmol m^{-2} s^{-1}). The table also shows percentage of cases where instantaneous PAR > I_k during daily light hours, and percentage of variance explained by fitting net P-I data to the hyperbolic tangent function. Mean ± standard error (SE) of these parameters are given, except for GPP_{max} due to alternation of positive and negative values within a reach. NA denote not available cases; ns, cases in which the hyperbolic tangent function was not significant, and - cases in which I_k would result negative. The upstream reach of Gurri stream was dry in July 2003.

	GPP_{max}		α		R		I_k		PAR > I_k (%)		I_{comp}		Variance (%)	
a. La Tordera	up	down	up	down	up	down	up	down	up	down	up	down	up	down
Nov. 01	NA	NA	NA	0.065	NA	2.8	NA	143.8	NA	65	NA	30.4	NA	75.0
Jan. 02	13.5	30.2	0.029	0.066	2.3	32.6	324.2	317.3	61	52	55.3	342.9	96.9	86.9
Mar.	7.6	-16.2	0.012	0.032	10.6	29.4	442.5	-	55	-	615.9	637.1	94.3	71.9
Apr.	ns	13.5	ns	0.020	ns	0.6	ns	470.0	ns	63	ns	21.1	ns	55.1
Jun.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sep.	-9.9	NA	0.032	NA	9.2	NA	-	NA	-	NA	199.4	NA	90.4	NA
Dec.	11.2	-6.3	0.037	0.026	6.5	12.8	210.7	-	47	-	122.8	342.6	66.4	58.1
Jul. 03	-13.8	ns	0.025	ns	8.8	ns	-	ns	-	ns	244.5	ns	94.4	ns
Mean			0.027	0.042	7.5	15.6	325.8	310.3	54	60	247.6	274.8	88.5	69.4
SE			0.004	0.010	1.5	6.6	66.9	94.2	4	4	97.6	115.0	5.6	5.8
b. Gurri	up	down	up	down	up	down	up	down	up	down	up	down	up	down
Feb. 02	11.1	28.3	0.141	0.067	3.3	11.3	54.8	293.1	74	51	16.3	117.5	75.9	94.4
Mar.	14.8	38.1	0.055	0.016	5.1	12.4	186.4	1653.1	63	39	64.7	537.9	84.0	89.6
Apr.	ns	13.1	ns	0.013	ns	6.6	ns	699.1	ns	51	ns	354.3	ns	70.5
May	-3.2	NA	1.468	NA	35.6	NA	-	NA	-	NA	16.8	NA	17.4	NA
Jul.	70.2	40.6	0.163	0.068	16.4	23.6	299.0	414.1	27	41	70.0	240.7	96.9	94.7
Sep.	14.6	24.4	0.132	0.053	7.7	5.9	76.9	320.3	67	41	40.4	76.8	67.1	93.1
Dec.	-17.2	-23.1	7.912	0.23	23.7	54.9	-	-	-	-	2.1	165.7	45.8	57.9
Jun. 03	41.5	91.1	0.132	0.108	20.9	55.3	218.6	585.6	28	39	110.0	355.8	80.4	86.7
Jul.	dry	24.0	dry	0.055	dry	7.1	dry	303.7	dry	38	dry	90.2	dry	87.7
Mean			1.429	0.076	16.1	22.1	167.1	609.9	52	43	45.7	242.4	66.8	84.3
SE			1.097	0.024	4.4	7.5	45.4	183.4	10	2	14.4	57.4	10.2	4.7

7.2; paired T-test, df = 2, P > 0.05). In Gurri, GPP_{max}, α, R and I_k predicted using the hyperbolic tangent function were similar between the two reaches (Table 7.2; paired T-test, df = 5, P > 0.05). Average I_k was five times greater downstream than upstream of the point source, but its variability among dates was too high. I_{comp} was five times higher downstream than upstream of the point source (paired T-test: t = -3.200, df = 5, P = 0.024). GPP_{max} was negative at all light levels on few sampling dates in La Tordera and Gurri (Table 7.2). Negative values of GPP_{max} coincided with lowest GPP daily rates (< 7 g O_2 m^{-2} day^{-1}) in the two streams (Fig. 7.1a, b). In La Tordera, measured rates of GPP were close to the 1:1 line relating these rates with GPP_{max} (Fig. 7.1a). In Gurri, a similar trend than
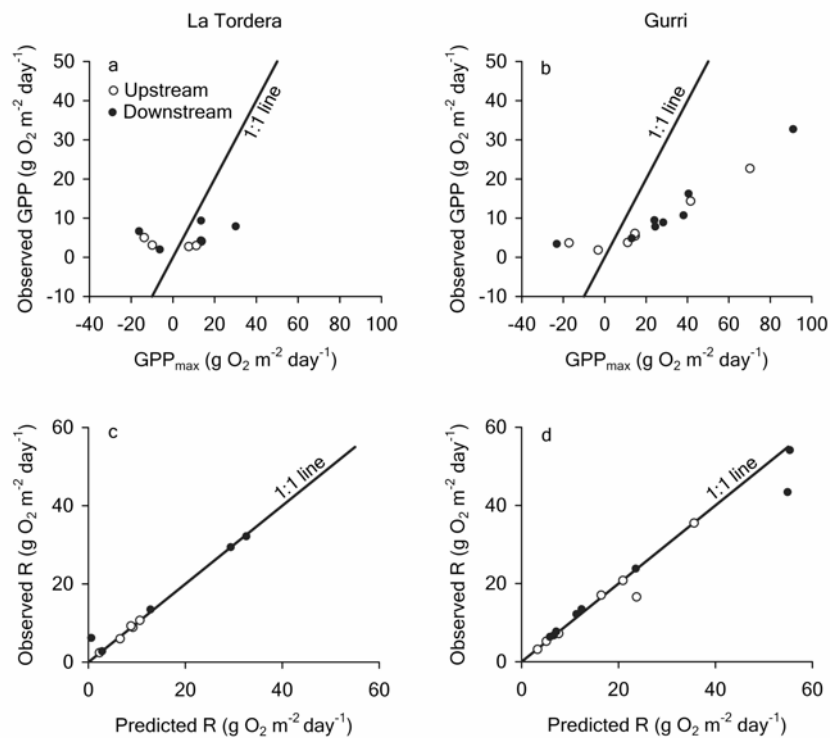
Fig. 7.1. Relationships between measured rates of GPP and predicted GPP_{max}, and between measured and predicted rates of R for La Tordera (a and c) and Gurri (b and d) streams. Solid lines represent 1:1 relationships. Open and solid circles symbolize upstream and downstream reaches, respectively.

in La Tordera was observed for lower rates of GPP, but further increases in GPP$_{max}$ did not proportionally result in GPP increases (Fig. 7.1b). Highest values of GPP$_{max}$ coincided with summer samplings in Gurri stream. Instantaneous measures of ambient PAR (μmol m^{-2} s^{-1}) were higher than I$_k$ during great part of daily light hours on each sampling date in the two reaches of the two study streams (Table 7.2). We did not observe photoinhibition on any sampling date in any of the stream reaches.

Point source effects on daily rates of whole-stream metabolism

Daily rates of GPP tended to be higher downstream than upstream of the point source on most sampling dates in the two study streams. Nevertheless, when considering all dates to perform the paired *T*-test, differences between the two reaches were only marginally significant in La Tordera and were not statistically significant in Gurri (Fig. 7.2a, b). GPP ranged 0.3-5.0 g O$_2$ m^{-2} day^{-1} above the point source input and 2.0-9.4 g O$_2$ m^{-2} day^{-1} below the input in La Tordera, and 0.5-22.7 g O$_2$ m^{-2} day^{-1} upstream and 3.4-32.7 g O$_2$ m^{-2} day^{-1} downstream of the point source in Gurri. Temporal variability of GPP was greater in Gurri (CV: upstream, 103%; downstream, 79%) than in La Tordera (CV: upstream, 51%; downstream, 43%). Mean rates of GPP were twofold in Gurri than in La Tordera among the upstream reaches (mean ± SE in La Tordera: 2.9 ± 0.6 g O$_2$ m^{-2} day^{-1}; in Gurri: 7.3 ± 2.7 g O$_2$ m^{-2} day^{-1}) and among the downstream reaches (mean ± SE in La Tordera: 5.7 ± 0.9 g O$_2$ m^{-2} day^{-1}; in Gurri: 11.8 ± 3.3 g O$_2$ m^{-2} day^{-1}).

Measured rates of ecosystem R were similar to those predicted from the *P-I* curves at the upstream and downstream reaches in the two study streams (Fig. 7.1c, d; paired *T*-test: La Tordera, upstream, *t* = 0.256, *df* = 4, *P* = 0.810, downstream, *t* = -1.059, *df* = 4, *P* = 0.349; Gurri, upstream, *t* = 1.002, *df* = 6, *P* = 0.355, downstream, *t* = 774, *df* = 7, *P* = 0.464). On average, the point source doubled daily rates of R in La Tordera (Fig. 7.2c). There, the range of R (mean ± SE within brackets) was 2.4-10.7 g O$_2$ m^{-2} day^{-1} (7.7 ± 1.1 g O$_2$ m^{-2} day^{-1}) upstream

of the point source, and 2.8-32.2 g O_2 m^{-2} day^{-1} (16.9 ± 4.2 g O_2 m^{-2} day^{-1}) downstream. In contrast, the daily rate of R was not significantly affected by the point source in Gurri stream (Fig. 7.2d). There, R ranged 3.2-59.6 g O_2 m^{-2} d^{-1} (20.6 ± 6.7 g O_2 m^{-2} day^{-1}) and 6.4-54.1 g O_2 m^{-2} d^{-1} (21.0 ± 6.5 g O_2 m^{-2} day^{-1}) upstream and downstream of the point source, respectively. Mean rates of R at the downstream reach of La Tordera were similar to those from upstream and downstream reaches in Gurri. Similar to GPP, R temporal variability was higher in
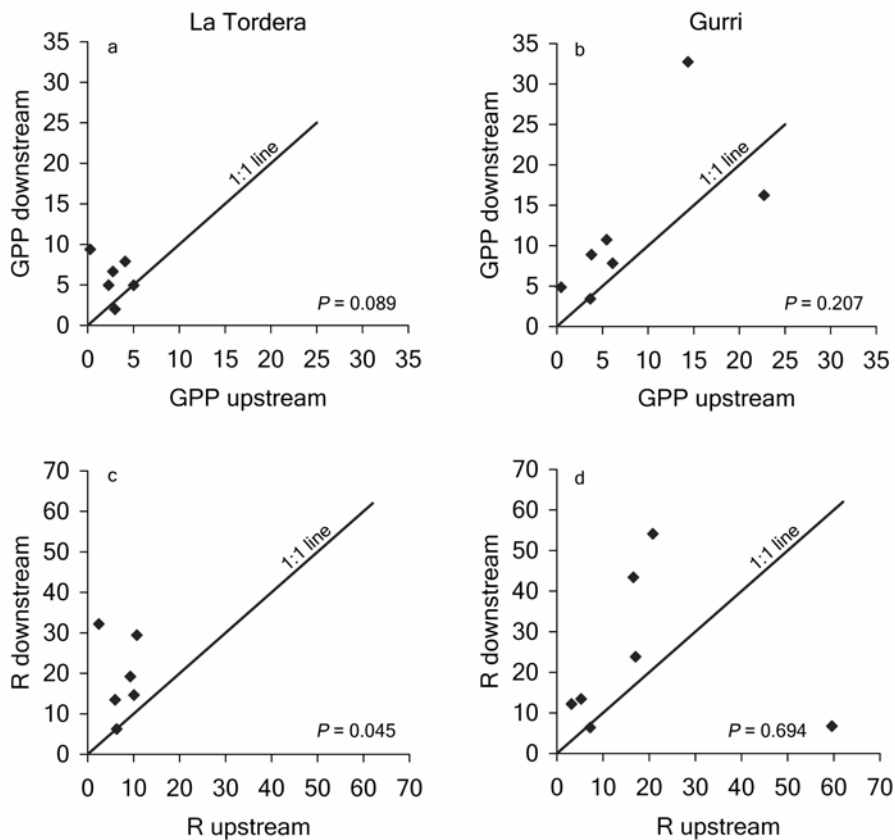
Fig. 7.2. Metabolism rates (GPP and R) measured downstream of the point source as a function of those rates measured upstream for La Tordera (a and c) and Gurri (b and d) streams. Solid lines represent 1:1 relationships. Significance from the paired *T*-test performed to compare metabolism rates between the two reaches is given.

Gurri (CV: upstream, 91%; downstream, 87%) than in La Tordera (CV: upstream, 38%; downstream, 65%). In the case of La Tordera, this variability increased almost two times downstream of the point source relative to upstream.

Daily rates of NEP were negative on most dates upstream and downstream of the point source in the two study streams (Fig. 7.3). Through the study period, NEP was more variable downstream (ranging from -24.3 to 3.2 g O_2 m^{-2} day^{-1}) than upstream (ranging from -7.9 to 1.7 g O_2 m^{-2} day^{-1}) of the point
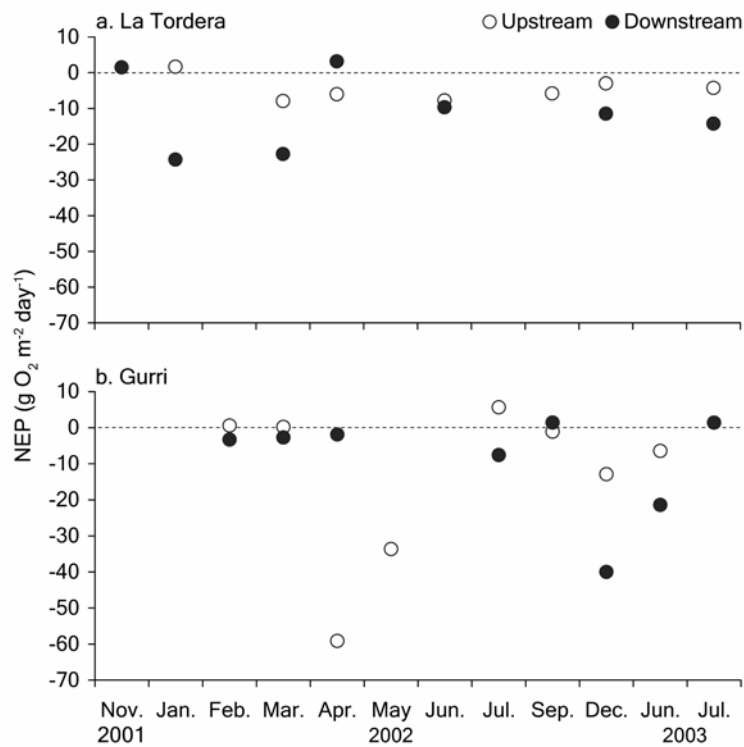
Fig. 7.3. Daily rates of NEP for the two reaches of La Tordera (a) and Gurri (b) for each sampling date. Open and solid circles symbolize upstream and downstream reaches, respectively. In the case of La Tordera, NEP data from November 2001 and September 2002 are not available for the upstream and the downstream reaches, respectively. In the case of Gurri, we lack NEP data from May 2002 for the downstream reach; the upstream reach was dry in July 2003.

source in La Tordera (Fig. 7.3a). In contrast, in Gurri, NEP showed broader ranges upstream (-59.1 to 5.7 g O_2 m^{-2} day^{-1}) than downstream (-40.0 to 1.8 g O_2 m^{-2} day^{-1}) of the point source (Fig. 7.3b). The GPP:R ratio was lower than 1 on most dates in the two reaches of the two study streams (Table 7.3). The point source did not show significant effects on NEP (paired *T*-test: La Tordera, $t = 1.787$, $df = 5$, $P = 0.134$; Gurri, $t = 0.033$, $df = 6$, $P = 0.975$) or the GPP:R ratio (paired *T*-test: La Tordera, $t = 0.226$, $df = 5$, $P = 0.830$; Gurri, $t = 0.402$, $df = 6$, $P = 0.701$) in any of the two study streams.

Table 7.3. GPP:R ratio upstream and downstream of the point source in La Tordera and Gurri streams for those dates on which metabolism was measured in each of the two streams. The table shows mean and standard error (SE) of this ratio. NA denote not available cases. The upstream reach of Gurri was dry in July 2003.

Date	La Tordera		Gurri	
	up	down	up	down
Nov. 2001	NA	1.53		
Jan. 2002	1.69	0.25		
Feb.			1.19	0.73
Mar.	0.26	0.23	1.04	0.80
Apr.	0.04	1.51	0.01	0.72
May			0.05	NA
Jun.	0.23	0.34		
Jul.			1.33	0.68
Sep.	0.35	NA	0.85	1.22
Dec.	0.50	0.15	0.22	0.08
Jun. 2003			0.69	0.60
Jul.	0.54	0.26	dry	1.23
mean	0.52	0.61	0.67	0.76
SE	0.21	0.24	0.18	0.13

Relationships between morphohydraulical, chemical and biological parameters and rates of whole-stream metabolism

In La Tordera, we observed a negative relationship between GPP and water velocity being significant only with data from the upstream reach (Fig. 7.4). In contrast, these rates were positively related with NH_4^+-N concentration for the upstream reach and for the two reaches combined (Fig. 7.5a). Increases in NH_4^+-N concentration were positively and negatively related with R and NEP, respectively, only when data from the two reaches were combined (Fig. 7.5b, c). The relationship between R and NH_4^+-N concentration had a greater slope (b = 8.08) than that between GPP and NH_4^+-N concentration (b = 1.02). Multiple regression analysis indicated that 93 % of the variance in R rates above the point source in La Tordera could be explained by a model that included water temperature and DOC (Table 7.4). Daily rates of GPP and R were not significantly correlated upstream (r^2 = -0.04, P = 0.930) or downstream (r^2 = 0.238, P = 0.608) of the point source in La Tordera stream (Fig. 7.6 a).
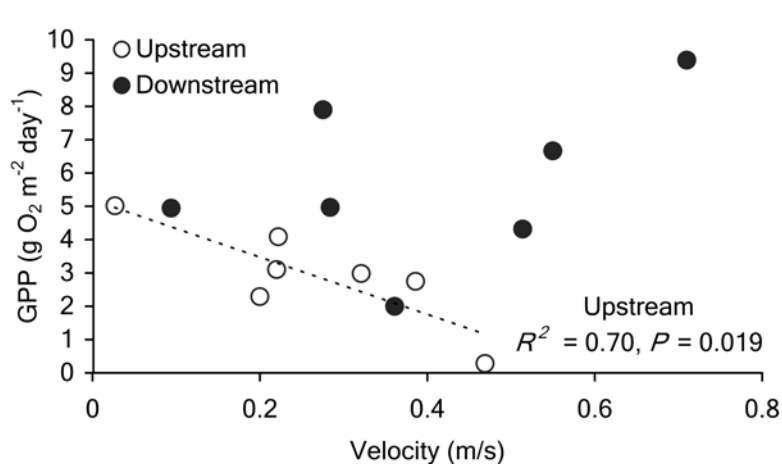

Fig. 7.4. Daily rates of GPP as a function of water velocity for the upstream (open circles) and the downstream (solid circles) reach in La Tordera. For the upstream reach, statistics (R^2 and P) of this relationship are shown (n = 7). The relationship was not significant for the downstream reach.
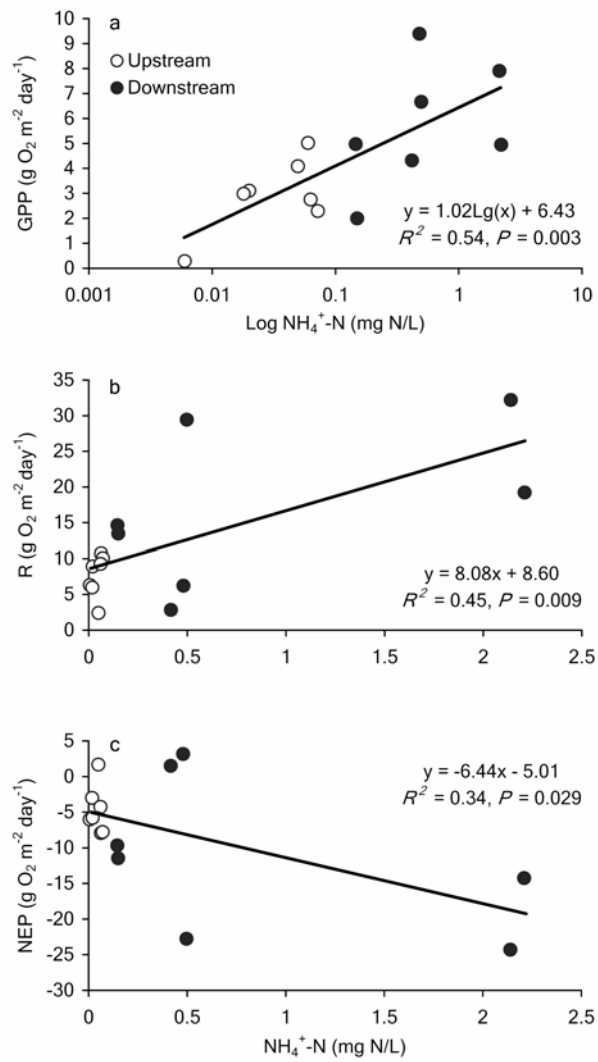
Fig. 7.5. Daily rates of GPP, R and NEP as a function of NH_4^+-N concentration for the upstream (open circles) and the downstream (solid circles) reach in La Tordera. Statistics (R^2 and P) of these relationships with data from the two reaches combined are shown (n = 14).
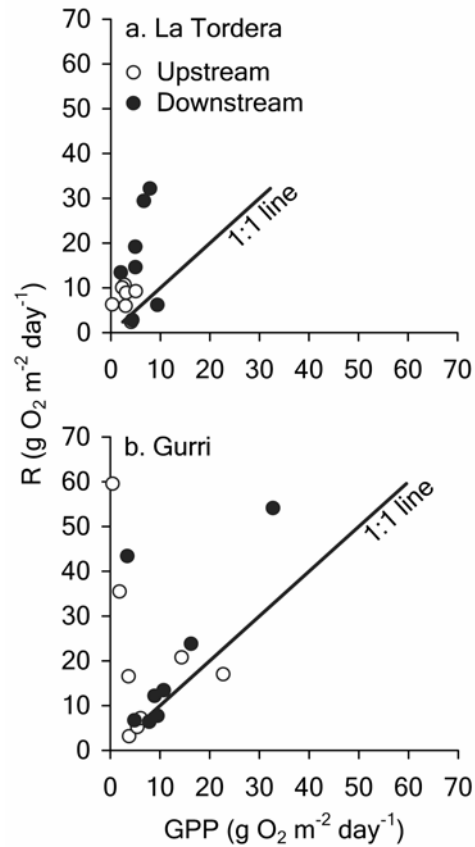
Fig. 7.6. Daily rates of R as a function of daily rates of GPP for the two reaches of La Tordera (a) and Gurri (b) streams. Solid lines represent 1:1 relationships. Open and solid circles symbolize upstream and downstream reaches, respectively.

Table 7.4. Results of stepwise multiple regression analysis for daily rates of respiration (R) upstream of the point source in La Tordera (n = 7, R^2 = 0.934). No other multiple regressions were found.

Dependent variable	Independent variable	Parameter estimate (SE)	Prob > F
R	Intercept	-8.770 (2.233)	0.017
	Temperature	0.817 (0.111)	0.002
	DOC	5.311 (0.905)	0.004
	Full model		0.004

In Gurri, daily rates of GPP were negatively related with water velocity (Fig. 7.7a), and positively with SRP concentration and chlorophyll *a* in biofilm (Fig. 7.7b, c) when combining results from the two reaches together. Daily rates of R were not related with any of the studied parameters in Gurri stream for each reach alone or the two reaches combined. Thus, similar relationships than those for GPP were found between the GPP:R ratio and water velocity, SRP concentration and chlorophyll *a*. In the case of NEP, a negative relationship with water velocity was observed. Like in La Tordera, rates of GPP and R from Gurri were not correlated in any of the reaches (upstream: r^2 = -0.284, P = 0.496; downstream: r^2 = 0.621, P = 0.100; Fig. 7.6 b).
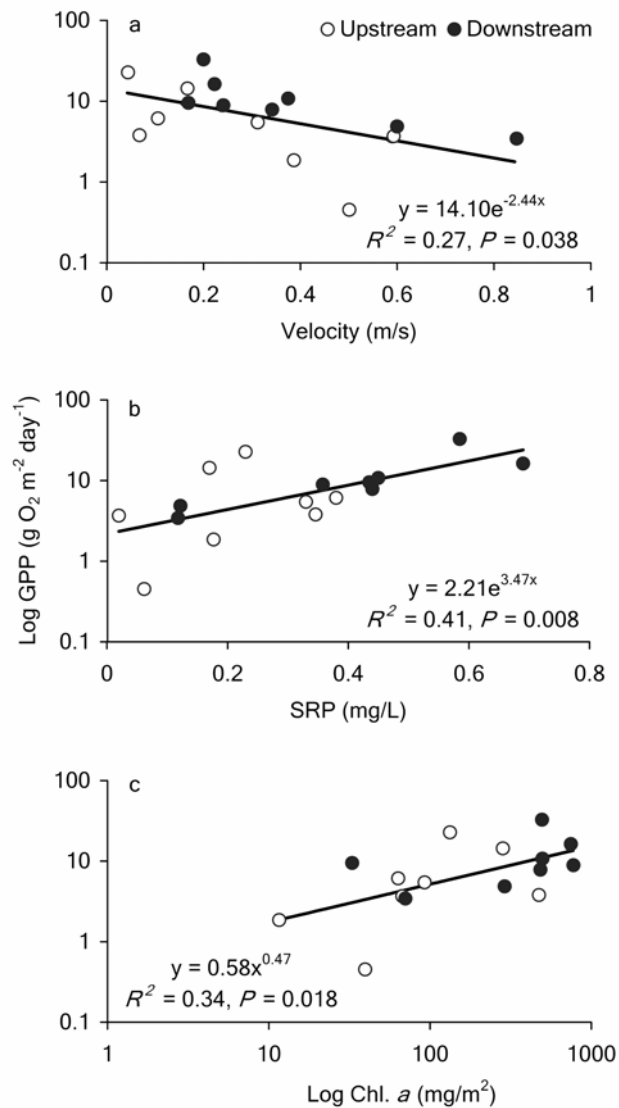
Fig. 7.7. Daily rates of GPP as a function of water velocity (a), SRP concentration (b) and chlorophyll *a* (c) for the upstream (open circles) and the downstream (solid circles) reach in Gurri. Statistics (R^2 and P) of these relationships with data from the two reaches combined are shown (n = 16).

Discussion

Comparison of gross primary production and photosynthetic attributes between pristine and human-altered streams

On average, daily rates of GPP in the two reaches of the two study streams were about 3-10 times higher than those reported for Fuirosos (0.05-1.9 mg O_2 m^{-2} day^{-1}), a forested Mediterranean stream located in the same region than our study streams (Acuña *et al.* 2004). Concentrations of NH_4^+-N, NO_3^--N and SRP reported for Fuirosos are lower (order of $\mu g/L$) than those from our study streams. Daily rates of GPP in the two reaches of Gurri were 3-4 times higher than those reported for La Solana (2.7 ± 0.3 g O_2 m^{-2} day^{-1}), a pristine stream located nearby to our study streams and comparable to Gurri in terms of streambed substrata and catchment geology (Guasch 1995). In contrast, GPP rates in Gurri were similar to those from other agricultural streams such as Great Ouse (England) and Mühlibach (Swiss Plateau), where GPP was 10.9 ± 1.7 g O_2 m^{-2} day^{-1} (Edwards and Owens 1962) and 12.5 ± 4.5 g O_2 m^{-2} day^{-1} (Kaenel *et al.* 2000), respectively. In other studies conducted in near-pristine streams from a wide range of bioclimatic regions from North America (Mulholland *et al.* 2001), reported GPP rates (2.7 ± 1.8 g O_2 m^{-2} day^{-1}) were similar to those from the upstream reach of La Tordera, but much lower than those from the downstream reach and the two reaches of Gurri. All the studies mentioned above except that of Guasch (1995) used, like us, the open-system oxygen change method. Comparing metabolism rates among studies using the same method is advisable because other methods (i.e., chambers) may underestimate these rates at the ecosystem level (Marzolf *et al.* 1994, Mulholland *et al.* 2001). In particular, Marzolf *et al.* (1994) showed that chamber measurements underestimated GPP by about 20 %. Hence, lower rates of GPP by about 65-75 % in La Solana than in Gurri would not be due only to methodology used, but also to other factors such as greater canopy cover and lower nutrient concentrations in the former than in the latter. These results agree with findings from previous studies (Young and

Huryn 1999), which suggest that variation in GPP among streams is not linear with the proportion of different land uses in the catchment because availability of light to sustain photosynthesis can be limited by other factors specific of each site and not necessarily related to land use.

Mean values of I_{comp} in the two reaches of La Tordera and Gurri were high compared to values from lotic and lentic biofilm communities compiled by Hill (1996) from several studies (10-23 μmol m^{-2} s^{-1}). Dodds $et\ al.$ (1999) related high values of I_{comp} to biofilm communities where respiration dominate over gross primary production for much of the diurnal cycle, and thus, to stream ecosystems mostly acting as net sinks of organic matter. These authors termed this condition as $net\ heterotrophic\ state$, attributed to respiration of algae and heterotrophic organisms in biofilm mats. Guasch and Sabater (1995) hypothesized that net heterotrophy could be achieved during winter in La Solana stream, when the heterotrophic metabolism was based on allochthonous inputs of organic matter rather than autochthonous primary production. These authors highlighted that, although not having data on this allochthonous preeminence, high DOC concentrations (2-3.5 mg/L) in their study stream reinforced their hypothesis. High I_{comp} values and DOC concentrations in La Tordera and Gurri support this hypothesis of Guasch and Sabater (1995).

Photoinhibition was not observed in any sampling date in La Tordera or Gurri streams, similarly to another study using the open-system oxygen change method in a New Zealand stream (Young and Huryn 1996). The car/chl ratio increases as does the adaptation of primary producers in biofilm to high light levels (Falkowski and LaRoche 1991). High values of the car/chl ratio in the study streams may indicate that the biofilm communities were protected against photoinhibition, as suggested in previous studies (Guasch and Sabater 1995). Dodds $et\ al.$ (1999) suggested that photoinhibition was likely to occur at the surface of biofilm mats, whereas it should be rare for whole communities due to compensation by deeper parts of the biofilm assemblage mats. Our results on

ecosystem metabolism support lack of photoinhibition at the level of whole communities.

Point source effects on whole-stream gross primary production

Lack of significant differences in GPP_{max}, α, I_k and I_{comp} between the upstream and the downstream reaches in La Tordera suggested that responses of biofilm communities to light were comparable between the two reaches. Light saturation of photosynthesis commonly occur at irradiances above 200-500 μmol m^{-2} s^{-1} in stream biofilm communities (Hill and Boston 1991, Hill *et al.* 1995, Hill 1996, Young and Huryn 1996), and rarely below 200 μmol m^{-2} s^{-1} (Hill 1996). In La Tordera, light saturation values were within this common range on most dates. Only during late autumn, after leaves fall, light saturation occurred at irradiances below 200 μmol m^{-2} s^{-1}, suggesting lack of adaptation of biofilm communities to increases in light availability in this close canopy stream. In Gurri, daily PAR was greater downstream than upstream of the point source. These differences between the two reaches in terms of daily PAR may explain higher values of I_k downstream than upstream of the point source in Gurri. Accordingly, concentration of carotenoids, the nature of which is protective against high levels of irradiance (Falkowski and LaRoche 1991), were lower upstream than downstream of the point source in Gurri stream.

Over saturating ambient light levels (i.e., PAR > I_k) during great part of the day on each sampling date in the two reaches of La Tordera and Gurri suggest that primary production was not light limited in these streams. Hence, increases in concentrations of inorganic nutrients (only that of SRP in the case of Gurri) should enhance primary production in the two study streams. Daily rates of GPP were higher downstream than upstream of the point source in the two study streams on most dates, but the increase was not consistent among all dates (see Fig. 7.2). For instance, the only case in which daily rate of GPP was clearly lower downstream than upstream of the point source in Gurri stream (see Fig. 7.2b) occurred in July 2002, coinciding with lowest percentage (27 %) of cases where

instantaneous PAR > I_k during daily light hours. This low percentage indicates under saturating ambient light levels (i.e., PAR < I_k) during great part (73 %) of daily light hours. Hence, daily rate of GPP at the downstream reach of Gurri stream in July 2002 was likely to be light limited, and thus, this rate could no increase regardless of nutrient inputs (Winterbourn 1990). As a result of these few inconsistent cases, the overall point source effect on GPP was only marginally significant in La Tordera, and was not statistically significant in Gurri.

Point source effects on whole-stream respiration

Data from direct measurement of the whole-stream R were coherent with those from *P-I* model predictions. Daily rates of R measured upstream of the point source in La Tordera stream were within the same range than R rates reported in previous studies (in the order of 1-10 g O_2 m^{-2} day^{-1}) that also used open-system oxygen change methods in near-pristine streams (Uehlinger and Naegeli 1998, Young and Huryn 1999, Mulholland *et al.* 2001). However, they were lower than R rates reported for a pristine, forested stream located nearby and with similar characteristics (e.g., substrate type) to La Tordera (Acuña *et al.* 2004). Highest R (32.1 g O_2 m^{-2} day^{-1}) in this forested stream coincided with highest supplies of organic matter during the study period. Acuña *et al.* (2004) related larger accumulations of benthic organic matter (BOM) with lower flows, and emphasized the influence of BOM accumulation enhancing R rates. BOM concentrations measured upstream of the point source in La Tordera during the same sampling dates than those from this dissertation were 25.6 g C/m^2 in autumn, 13.3 g C/m^2 in winter, 10.1 g C/m^2 in spring, and 2.0 g C/m^2 in summer (Ortiz 2005). Hence, lower BOM concentrations (twofold in autumn, winter and spring, and threefold in summer) in the upstream reach of La Tordera than in Fuirosos may explain differences in R rates between these two streams. Daily rates of R significantly increased downstream of the point source relative to upstream in La Tordera. In particular, mean daily rates of R were twofold higher below than above the point source input. Daily rates of R in Gurri stream tended

to be higher downstream than upstream of the point source on most sampling dates, but these increases were not consistent among all dates. Therefore, overall point source effect on R rates was not statistically significant in Gurri. In previous chapters, we suggested that high nutrient inputs from diffuse sources in Gurri overwhelmed point source effects on stream water chemistry (Chapter 4) and nutrient retention (Chapter 5). High concentrations of inorganic nutrients and DOC above and below the point source were likely to result in similar whole-stream R rates regardless of reach location, fact that made unlikely a clear point source effect on these rates in Gurri.

Based on results from previous studies (Wang *et al.*2003), we expected higher rates of R in Gurri than in La Tordera due to higher concentrations of NO_3^- -N, SRP and DOC above the point source in the former stream than in the latter. As expected, daily rates of R were higher in Gurri than in La Tordera above the point source input. A previous study showed that daily rates of whole-stream R were between three and seven times higher in a stream with greater groundwater-surface water exchange relative to a stream with lower exchange (Fellows *et al.* 2001). Greater R associated with higher vertical exchange was attributed to hyporheic R, which accounted between 40-58 % of the total whole-stream R in the stream with the lowest vertical exchange and between 85-93 % in the stream with the highest exchange. Dominance of bedrock covering the streambed in Gurri should result in lower vertical exchange than in La Tordera, where cobbles, pebbles and boulders dominated the streambed substratum. Thus, biofilm and filamentous green algae were likely to highly contribute to whole-stream R in Gurri stream. Nevertheless, daily rates of R measured below the point source were similar between the two study streams regardless of higher concentrations of NO_3^--N and DOC in Gurri than in La Tordera. Thus, despite nutrient concentrations were greater in Gurri than in La Tordera below the point source input, lower vertical exchange in the former may counterbalance daily rates of R between the two streams. Our results support previous research highlighting that generalizations on R variation across streams draining

catchments with contrasting land uses can be attainable, but that specific attributes of each stream may reduce the effective application of such generalizations (Young and Huryn 1999).

Point source effects on the balance between GPP and R

The point source did not significantly affect daily rates of NEP or GPP:R ratios in La Tordera and Gurri streams. Negative rates of NEP and GPP:R ratios < 1 on most dates in the study streams indicated that respiration dominated whole-stream metabolism both upstream and downstream of the point source. Daily rates of NEP and the GPP:R ratio upstream of the point source in La Tordera were within the same range than those reported for a number of forested, near-pristine streams with closed or semi-closed canopies (Mulholland *et al.* 2001). These authors related highly negative NEP and very low GPP:R ratios in their study streams to high inputs of particulate organic matter that were likely to fuel heterotrophic metabolism. Downstream of the point source in La Tordera and in the two reaches in Gurri, minimum NEP rates were much lower than those reported for these near-pristine streams (minimum NEP about -8 g O_2 m^{-2} day^{-1}). Inputs of dissolved organic matter to streams also fuel heterotrophic metabolism (Bott *et al.* 1985, Edwards and Meyer 1987). Hence, highly negative NEP in our study streams probably underlined the special importance of heterotrophic activity associated with DOC inputs from point and diffuse sources.

Factors driving variability in whole-stream metabolism

Only the response of daily rates of GPP to changes in water velocity was common between the two study streams. In Gurri, daily rates of GPP were negatively related to water velocity, which increased downstream of the point source input relative to upstream. This relationship was observed only when combining data from the two reaches. In La Tordera, this negative relationship was observed only upstream of the point source. The negative effects of high stream flows on benthic primary producers observed in our study agree with

previous findings (Hynes 1979, Fisher *et al.* 1982, Finlay and Bowden 1994, Uehlinger and Naegeli 1998, Biggs *et al.* 1999).

In La Tordera, increases in daily rates of R downstream of the point source relative to upstream may be controlled by observed increases in nutrient (N and P) and/or DOC concentrations induced by the point source. Most research has related increases in rates of R with autochthonous and allochthonous inputs of particulate organic matter. Nevertheless, other studies have related higher respiration rates to higher water temperature and greater DOC concentrations (Bott *et al.* 1985, Edwards and Meyer 1987). Similarly, water temperature and DOC were the dominant control on whole-stream R upstream of the point source in La Tordera. In contrast, relationships between R and chemical parameters suggested that increases in NH_4^+-N concentration downstream of the point source relative to upstream were the responsible of increases in R rates. The relationship between daily rates of R and NH_4^+-N concentration was not significant upstream of the point source, but became significant when combining results from the two reaches. Hence, the point source increased background concentration of NH_4^+-N enough to modify the response of heterotrophic activity in La Tordera. In a previous study, we suggested that nitrification played an important role on N dynamics downstream of the point source in La Tordera, under high NH_4^+-N concentrations (Merseburger *et al.* 2005). Results from the present study show lack of correlation between GPP and R in this stream, with a greater decoupling between GPP and R downstream than upstream of the point source (see Fig. 7.6 a). Nitrification is likely to be the responsible of high decoupling below the point source because it is part of autotrophic productivity that is not accounted with the technique used to measure metabolism, but that contribute to whole-stream R (i.e., there is a oxygen use by nitrification to oxidize NH_4-N). Present results support our previous finding regarding relevance of nitrification below the point source input, and suggest that chemoautotrophic activity is enhanced downstream of this input relative to upstream in La Tordera. Increases in NH_4^+-N concentration also resulted in increases in daily rates of

GPP, despite the increase in GPP rates downstream of the point source relative to upstream was only marginally significant. This latter relationship was significant for the upstream reach and for the two reaches combined. Hence, increases in NH_4^+-N concentration downstream of the point source relative to upstream in La Tordera stream enhanced daily rates of GPP. Previous studies have suggested that heterotrophic activity is highest with the addition of N and P (Tank and Winterbourn, 1995). Increases in NH_4^+-N concentration below the point source resulted in a greater increase in daily rates of R than those of GPP, and thus, daily rates of NEP changed from positive to negative with NH_4^+-N increases. Finally, NEP decreases as a function of NH_4^+-N concentration was significant only when combining data from the two reaches, and thus, increases in NH_4^+-N below the point source were also affecting the balance between GPP and R. A possible explanation is that the autotrophic and heterotrophic components of the biofilm responded differently to nutrient availability even though being under equal stream nutrient conditions, as suggested by Tank and Dodds (2003).

In Gurri, daily rates of GPP were positively related to SRP concentration and chlorophyll *a* in biofilm when compiling results from the two reaches. SRP availability is likely to affect primary production in pristine and near-pristine streams (Guasch *et al.* 1995, Mulholland *et al.* 2001). Daily rates of R were not related to any of the hydrological, chemical or biological parameters considered (e.g., water velocity, SRP and chlorophyll *a*). Similar to GPP, thus, the GPP:R ratio and daily rates of NEP decreased with increases in water velocity with data from the two reaches together. The GPP:R ratio was also positively related to SRP concentration and chlorophyll *a* when combining data from the upstream and downstream reaches in Gurri. Increases in SRP may be the main factor controlling increases in chlorophyll *a* downstream of the point source relative to upstream in this stream well lighted and with excess of NO_3^--N (DIN:SRP range, mean ± SE and median, respectively: upstream, 19-901, 188 ± 104, 87; downstream, 22-156, 59 ± 14, 49). Our results strongly suggested that the only effect of the point source on water chemistry in Gurri (i.e., the increase in SRP)

was likely to enhance primary production. These results agree with those from Mulholland *et al.* (2001), suggesting that increases in SRP concentration could predict increases in daily rates of GPP. These authors highlighted the low power of their tests due to low number of streams. Moreover, they manifested that the relevance of SRP as a predictor of GPP at a broad geographical scale was unclear. Here, we have compiled results from literature to examine this relationship between SRP and GPP (Fig. 7.8). A positive, linear relationship was found between SRP and GPP when combining results from diverse pristine and near-pristine streams, with a range of SRP concentration between 0.02 and 80.8 μg/L (R^2 = 0.41, *df* = 16, *P* = 0.004). This relationship was also significant when we included results from the upstream reach of La Tordera stream (R^2 = 0.41, *df*
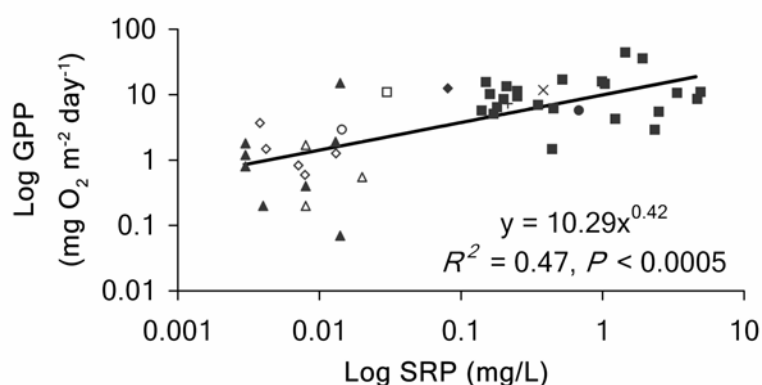

Fig. 7.8. Daily rates of GPP as a function of SRP concentration with results from the present study and from literature. In particular, the figure shows results from: ○, La Tordera upstream; ●, La Tordera downstream; +, Gurri upstream; x, Gurri downstream; □, Edwards and Owens (1962); ■, Wiley *et al.* (1990); ◊, Young and Huryn (1999); ♦, Kaenel *et al.* (2000); Δ, Fellows *et al.* (2001), and ▲, Mulholland *et al.* (2001). All these studies measured metabolism rates based on open-system oxygen change methods. Solid line represents the relationship between GPP and SRP with all the data combined. Statistics (R^2 and *P*) of this relationship are shown (*n* = 45).

= 17, P = 0.003). These two linear relationships were very similar (without data from our reach: intercept = 0.844, slope = 163.12; with this data: intercept = 0.830, slope = 163.09). However, the linear regression between GPP and SRP became a power regression (R^2 = 0.47, df = 43, P < 0.0005) when we included data from the downstream reach of La Tordera, from the two reaches of Gurri and from other streams with high SRP concentrations, in the order of mg/L (Fig. 7.8). This latter relationship, which explained almost 50 % of the variance, indicated that GPP was likely to be saturated under SRP concentrations above 0.1 mg/L. Mulholland et $al.$ (2001) suggested that nutrient limitation may play an important role in controlling stream GPP across large geographic areas. Our results support this hypothesis, but also suggest that above a certain threshold of SRP availability, other factors may become critical in limiting GPP rates.