

Implications of global change for stream nitrogen and phosphorus retention in a Mediterranean catchment

Implicaciones del cambio global sobre la retención de nitrógeno y fósforo en ríos de una cuenca mediterránea



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A mi madre

*Nuestras vidas son los ríos
que van a dar en la mar,
qu'es el morir;
allí van los señoríos
derechos a se acabar
e consumir;
allí los ríos caudales,
allí los otros medianos
e más chicos,
allegados, son iguales
los que viven por sus manos
e los ricos.*

Jorge Manrique
Coplas a la muerte de su padre

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1. GENERAL INTRODUCTION

1.1 Fluvial ecosystems and global change

Global change, the array of changes in the environment derived from human activities, is substantially transforming the Earth's landscape and climate. Today, about 50% of the Earth's land has been altered by human activity (Vitousek *et al.*, 1997), and the effects of climate change are already being observed (IPCC, 2007). Together these changes are having profound consequences on the Earth's ecosystems.

Although only about 0.01% of the Earth's freshwater is present in streams and rivers (Giller & Malmqvist, 1998), fluvial ecosystems are of great significance to the biosphere and provide a plethora of goods and services to humans (Table 1.1). Because of strong linkages to their catchments, streams and rivers are highly susceptible to land transformation (Hynes, 1975, Likens & Borman, 1995). Moreover, fluvial ecosystems are characterized by unidirectional flow, so that any pollutant reaching these ecosystems may have effects for a long distance downstream (Giller & Malmqvist, 1998). As a consequence, global change has severely altered the Earth's freshwater ecosystems, even to a greater extent than terrestrial ecosystems have been altered (Vitousek *et al.*, 1997). Urbanization, agricultural and industrial development, mining, and watercourse alterations directly affect natural forcing factors in fluvial ecosystems, including hydrology, water chemistry, habitat structure, and biotic interactions (Table 1.2). Multiple disturbances are contributing to local ecological degradation, which may lead to irreversible changes and a significant loss of the goods and services that fluvial ecosystems provide to humans (Malmqvist & Rundle, 2002).

Together with land transformation, climate change is the most evident human alteration of the Earth's ecosystems (Vitousek *et al.*, 1997). Recent scientific findings confirm that climate change, caused by greenhouse gas emissions from human activities, may exert an additional pressure on ecosystems (EEA, 2007; IPCC, 2007). The global average temperature is now 0.8 °C higher than in pre-industrial times (CRU, 2006; GISS/NASA, 2006), and it is projected to increase by 1.8–4.0 °C during this century, with some studies suggesting a wider possible range of 1.1–6.4 °C (IPCC, 2007). Changes in the precipitation regime have also been registered and are projected to accelerate in the future (EEA, 2007).

Generally, climate change is expected to influence fluvial ecosystems through alterations of the hydrological and thermal regime, but it may also affect them indirectly through the alteration of terrestrial ecosystems (Carpenter *et al.*, 1992). Increases in air temperature are expected to cause concomitant increases in water temperature and to

increase evapotranspiration from the catchment. These changes together with modifications in the magnitude and temporal distribution of precipitation may cause changes in the amount and variability of flow (Carpenter *et al.*, 1992). Alterations in temperature and hydrology may have large implications for many organisms living in the stream or the riparian zone, which have narrow thermal and hydrological tolerances (Malmqvist & Rundle, 2002). This is expected to cause profound shifts in the structure and function of fluvial ecosystems. However, the effects of climate change on fluvial ecosystems are difficult to predict because they may interact with the effects of other anthropogenic activities such as land transformation (Dale, 1997). In addition, there are important regional differences in the degree and direction of climate change (IPCC, 2007).

Table 1.1 Goods and services provided by fluvial ecosystems (adapted from Poff *et al.*, 2002).

Water supply

Domestic uses (drinking, washing, gardening)
Industrial uses (manufacturing, power generation)
Agricultural uses (irrigation of crops)
Recreational uses (Irrigation of parks and golf courses)
Aquaculture

Supply of goods other than water

Fish
Waterfowl
Clams, mussels, other shellfish, crayfish
Timber products

Non-extractive benefits

Biodiversity
Wildlife habitat
Recreation (swimming, boating, fishing)
Aesthetic values
Transportation
Flood control
Waste disposal
Pollution dilution and water quality protection

1.2 Vulnerability of Mediterranean fluvial ecosystems to global change

Climate in the Mediterranean region is characterized by warm, dry summers and humid, wet winters. In addition, high inter-annual variability in the precipitation regime is typical (Strahler & Strahler, 1989). Annual potential evapotranspiration is large and may account for more than 80% of annual precipitation (Piñol *et al.*, 1991). This climatic setting results in a complex hydrology, characterized by frequent summer intermittency, high seasonality, and high inter-annual variability (Gasith & Resh, 1999). The scarcity of water during long periods of time makes Mediterranean fluvial ecosystems particularly vulnerable to global change (EEA, 2007).

Due to the water deficit, many of the goods and services provided by fluvial ecosystems are particularly critical in the Mediterranean region. Streams and rivers provide the majority of fresh water supply in many areas (EEA, 2007, Mas-Pla, 2005). In addition, fluvial ecosystems represent *humid islands* within a *dry landscape*, thus maintaining a high diversity of endemic aquatic life and constituting wildlife corridors for multiple terrestrial species (Naiman *et al.*, 1993). It is worth noting that the Mediterranean region is considered one of the most endangered global biodiversity hotspots (Sala *et al.*, 2000). In addition, well-preserved fluvial ecosystems are crucial for the control of periodic floods and the dilution of pollutants (Mas-Pla, 2005). Due to its scarcity, freshwater ecosystems in the Mediterranean region are highly valued for their aesthetic, artistic and spiritual characteristics (Meyer *et al.*, 1997).

The Mediterranean region has been subjected to human development for millennia; however, during the last decades the impact of land transformation has substantially increased. Forested and traditional extensive agricultural land has been widely converted to either urban or intensive agricultural areas supported with irrigation. The abandonment of many rural regions is also leading to the afforestation of former agricultural land, which has caused increased fire risk in many areas (EEA, 2006a). Multiple factors such as local population growth, tourism, increased mobility, and commercial investment have spectacularly enhanced urbanization. Urban sprawl during the last decade has been particularly intensive in countries experiencing a rapid economic growth. For instance, in Spain up to 50% of the Mediterranean coastline is already covered by impervious surfaces (EEA, 2006b). The agricultural and urban expansion is often accompanied by intense watercourse alteration and abstraction (EEA, 2006a). All these land transformations exert strong effects on the fragile Mediterranean fluvial ecosystems.

Table 2.2 Main anthropogenic factors forcing change in fluvial ecosystems (adapted from Malmqvist & Rundle, 2002).

Forcing factor	Subfactor	Proximate causes	Abiotic alteration	Biotic implications
Ecosystem destruction		Urban and agricultural expansion, water abstraction	Complete ecosystem loss	Species and population extinctions
Habitat alteration	Hydrology	Damming, channelization, water abstraction, deforestation	Loss of natural flow periodicity, increased risk of drought, severing of upstream-downstream linkages	Altered habitat conditions, reduced dispersal
	Siltation	Deforestation, urban and agricultural development	Reduced substratum complexity	Altered habitat conditions
Water chemistry	Alteration of riparian corridor	Channelization, urban and agricultural development	Altered energy inputs (organic matter/light) and in-stream marginal habitat	Altered trophic dynamics and habitat conditions
	Acidification	Industrial emissions (SO ₂ and NO _x), exhaust emissions (NO _x)	Reduced pH, increased Al ³⁺	Direct physiological effects, indirect (food chain) effects
	Nutrient addition	Deforestation, point and non-point pollution, atmospheric deposition	Increased N and P	Increases primary production, algal blooms
	Toxic metals	Mining, industrial gaseous emissions, landfill/sewage works	Many trace metals (e.g., Cu, Hg, Zn, Al, Pb, Cd)	Direct physiological/toxic effects
Organochlorine toxins		Industry (atmospheric and water emissions), landfill/sewage works, waste incineration, agriculture	PCBs, organochlorine pesticides (e.g., DDT, dieldrin)	Toxic effects through biomagnification
	Organic pollution	Urbanization, sewage works, agriculture	Reduced O ₂ , increased suspended solids	Reduced habitat availability
Endocrine disruptors		Industry, agriculture, waste incineration	Organohalogen (e.g., dioxins, furans, PCBs), pesticides (e.g., DDT, dieldrin), pharmaceuticals (estrogens)	Interference with naturally produced hormones
	Species removal and addition	Fisheries, aquaculture/aquarium trade, sport fishing, horticulture (riparian plants)	Invasive fishes	Increased/reduced competition, altered energy inputs (riparian) and ecosystem dynamics

In addition to land transformation, climate change may affect the integrity of Mediterranean freshwater ecosystems. Europe has warmed more quickly than the global average, showing a temperature about 1.4 °C higher than pre-industrial levels (EEA, 2007). The whole continent is likely to warm 2.1–4.4 °C by 2080, or possibly 2.0–6.3 °C, and this warming is expected to be especially intense in the Mediterranean region (Schröter *et al.*, 2005). For instance, previous research in the same study area as the present dissertation has already shown evidence of altered life cycles for some Mediterranean plants and animals due to global warming (Peñuelas *et al.*, 2002, 2003). The amount of annual precipitation has decreased up to 20% in southern Europe during the 20th century and it is projected to continue to decrease in the future (Schröter *et al.*, 2005). In addition, in many areas a higher frequency of extreme precipitation events is expected (de Castro *et al.*, 2005).

Generally higher mean temperature and lower mean precipitation is expected to cause lower baseflow (Carpenter *et al.*, 1992). In fact, a decrease in flow has already been observed in various Mediterranean rivers during the last decades (EEA, 2007). Climate change is expected to turn many perennial streams and rivers into intermittent ones; some may even disappear (Alvarez-Cobelas *et al.*, 2005). The risk of floods may increase due to extreme precipitation events (Mas-Pla, 2005). Finally, the biodiversity of many of these ecosystems may decline and the rates of metabolic and biogeochemical processes may be altered (Carpenter *et al.*, 1992, Alvarez-Cobelas *et al.*, 2005).

1.3 Nutrient retention processes in fluvial ecosystems

Fluvial ecosystems have been traditionally viewed as mere conduits that transport nutrients and other materials from the land to the ocean. However, streams and rivers are able to store, transform and remove nutrients during downstream transport, processes generally compiled under the term nutrient retention. Nutrients such as nitrogen (N) and phosphorus (P), which often limit primary production in fluvial ecosystems, are especially influenced by the retention ability of streams (e.g., Triska *et al.*, 1989; Martí *et al.*, 1996; Simon *et al.*, 2005).

The nutrient spiralling concept (Webster & Patten, 1979; Newbold *et al.*, 1981) revolutionized fluvial biogeochemistry by establishing a theoretical and mathematical framework for the study of nutrient retention in streams. The first spiralling studies used ³²P as a tracer to investigate stream nutrient retention processes (Newbold *et al.*, 1981, Newbold *et al.*, 1983; Mulholland *et al.*, 1985), but the use of this radioactive tracer was

rapidly abandoned because of its risks for ecosystem and human health. Since then, N and P retention in streams has been generally studied using nutrient enrichment experiments (Stream Solute Workshop, 1990; Webster & Valett, 2006). However, this technique does not allow differentiating retention pathways and it has been shown to underestimate uptake rates at ambient levels (Mulholland *et al.*, 2002; Dodds *et al.*, 2002). More recently, stream N retention has been explored using ^{15}N tracer addition techniques, which allow quantifying different N retention processes simultaneously occurring in a stream at ambient conditions, while avoiding the limitations of enrichment experiments (Peterson *et al.*, 1997; Peterson *et al.*, 2001; Mulholland *et al.*, 2008).

Many studies have emphasized the important role of pristine headwater streams in retaining dissolved N and P (Alexander *et al.*, 2000; Peterson *et al.*, 2001; Lowe & Likens, 2005). Global change has already altered forcing factors affecting the nutrient retention ability of these ecosystems. However, studies on the effects of land transformation are scarce, and only indirect projections of the effects of climate change have been made. Results from recent studies show that human activities reduce retention efficiency (i.e., retention relative to nutrient flux) through increased nutrient loading (e.g., Martí *et al.*, 2004, Newbold *et al.*, 2006; Ruggiero *et al.*, 2006; Mulholland *et al.*, 2008), decreased benthic organic matter (Meyer *et al.*, 2005), channel modification (Sweeney *et al.*, 2004; Grimm *et al.*, 2005; Bukaveckas, 2007) and other forms of water pollution that inhibit organisms responsible for nutrient uptake (Grimm *et al.*, 2005; Newbold *et al.*, 2006; Lottig *et al.*, 2007). Conversely, retention efficiency increases with other human activities, such as riparian vegetation removal, through increases in light for primary producers (Sabater *et al.*, 2000). Climate change may directly alter stream nutrient retention through changes in the hydrological and thermal regime, and indirectly through interactions with other components of global change. However, the direction of these changes is difficult to predict (Carpenter *et al.*, 1992; Meyer *et al.*, 1999).

Understanding how global change affects stream biogeochemical processes is important because these processes are crucial for the maintenance of many of the ecosystem services that streams provide to humans (Palmer *et al.*, 2004). Stream nutrient retention represents an ecosystem service *per se* because it can help mitigate problems associated with nutrient pollution by reducing nutrient delivery to downstream ecosystems (Peterson *et al.*, 2001). Moreover, ecosystem processes can be ideal integrative measures of the ecological condition of streams, even if their use is still limited (Bunn *et al.*, 1999). Nutrient retention may be a good candidate to fill this gap because it has a good theoretical background, it is easily measurable and comparable

across different types of ecosystems and it is sensitive to human impacts. Finally, the majority of studies on the effect of human activities on stream nutrient retention have targeted on streams from temperate regions of North America. However, studies in different bioclimatic regions are needed for a complete understanding of the influence of global change on stream biogeochemistry.

1.4 Objectives of the present dissertation

The general objective of the present dissertation was to explore the implications of global change for nutrient retention in streams from a Mediterranean catchment. A space for time approach was used to address the influence of two important aspects of global change: (i) land transformation and (ii) climate change.

This dissertation is articulated in four independent publications. The first three articles focus on the effects of land transformation on nutrient retention processes, whereas the fourth article explores the effects of climate change. The specific objectives addressed by each of these four articles are the following:

1. *Influence of land use on stream ecosystem function in a Mediterranean catchment.*

A multivariate approach was used to examine how physicochemical and functional (i.e., nutrient retention and metabolism) attributes of streams are related to catchment variables, including land use. The study was done in 13 streams located within a single catchment, but draining sub-catchments with contrasting land uses.

2. *Nitrate retention and removal in Mediterranean streams with contrasting land uses: a ^{15}N tracer study.*

^{15}N -nitrate tracer additions were used to explore nitrate retention (i.e., assimilatory uptake) and removal (i.e., denitrification) pathways in three headwater streams situated within the same catchment but subjected to contrasting land uses.

3. *Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses.*

Nutrient diffusing substrata were used in conjunction with the ^{15}N -nitrate tracer additions to determine the relative importance of nutrients and light as potential limiting factors of periphyton biomass and nitrogen uptake in three headwater

streams situated within the same catchment but subjected to contrasting land uses.

4. *Inter-annual, annual and seasonal variation of P and N retention in a perennial and an intermittent stream.*

Monthly nutrient enrichment measurements over two years were used to examine variation in retention of soluble reactive phosphorus and ammonium in two forested headwater streams with contrasting hydrological regimes (i.e., perennial vs. intermittent).

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2. STUDY SITES

2.1 Geographical, climatic and land use characteristics of the study catchment

Research was conducted in selected headwater streams located in the catchment of the river La Tordera (Catalonia, NE Spain; Fig. 2.1). This catchment has an area of 868.5 km² dominated by siliceous geology, covering an altitudinal gradient of approximately 1600 m in less than 30 km horizontal distance from the highest peaks (Montseny mountain range) to the river mouth at the Mediterranean Sea.

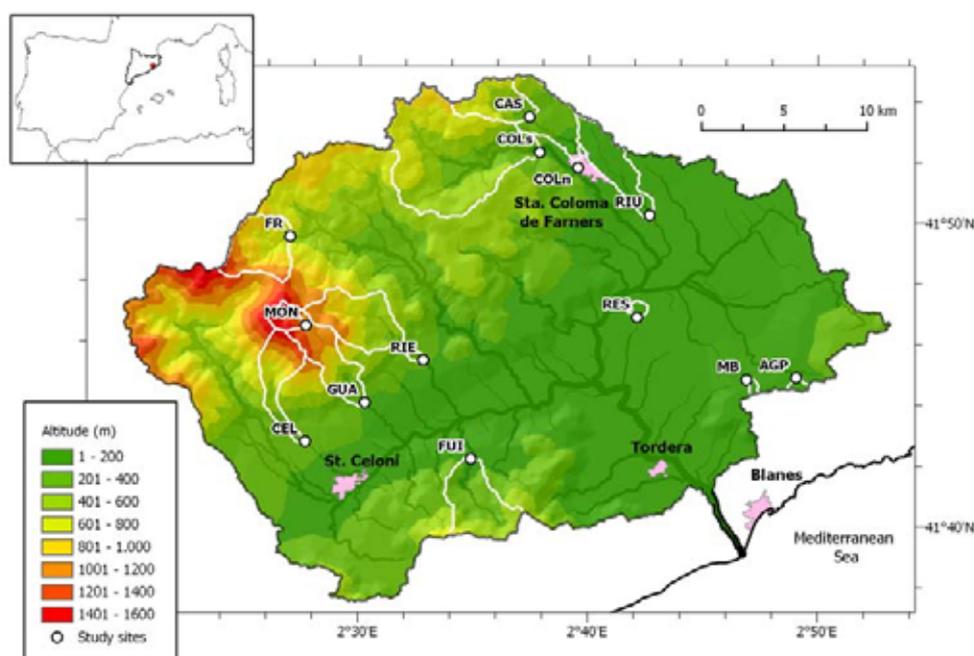


Figure 2.1 Geographical location of La Tordera catchment (Catalonia, NE Spain) and the study streams with their respective catchments (see table 2.1 for stream names). The largest towns in the area are shown in pink color.

The climate in La Tordera catchment is typically Mediterranean, with minimum air temperatures during winter and maximum during summer. Precipitation mostly occurs in spring and fall, but high inter-annual variability in the precipitation regime is typical (Piñol *et al.*, 1991). The pronounced altitudinal gradient of the catchment results in a mosaic of microclimates with contrasting local temperature (Fig. 2.2) and precipitation (Fig. 2.3) regimes. For instance, a strong contrast in the temperature and precipitation regime was found between two sites located at the extremes of the altitudinal range during the study period (Fig. 2.4). The climatic setting leads to high among-stream

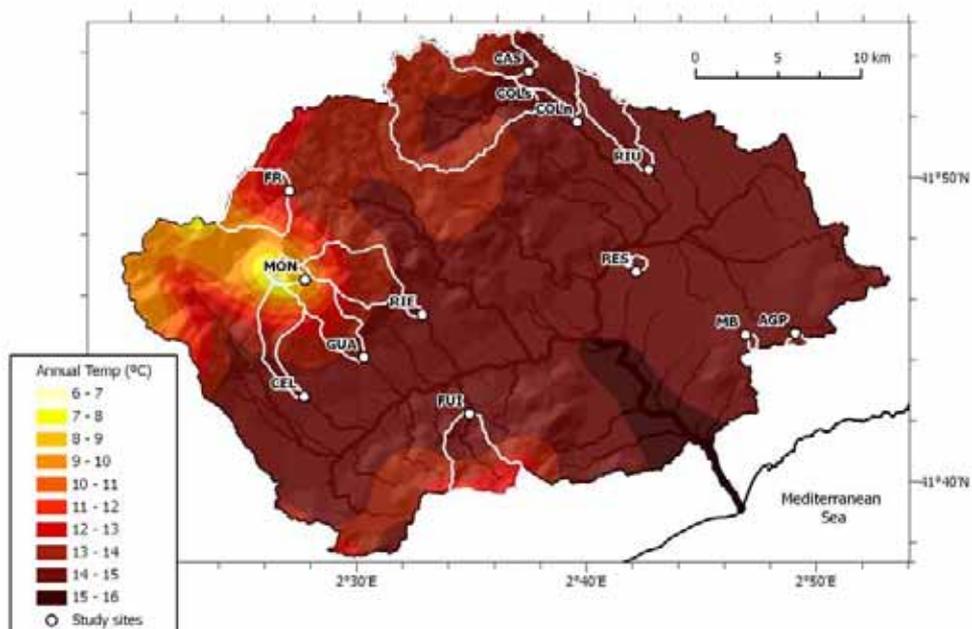


Figure 2.2 Spatial distribution of the mean annual temperature in La Tordera catchment. Data are from the period 1961-1990 and were obtained from the Department of Environment and Housing of Catalonia (<http://www.gencat.net>). See table 2.1 for stream names.

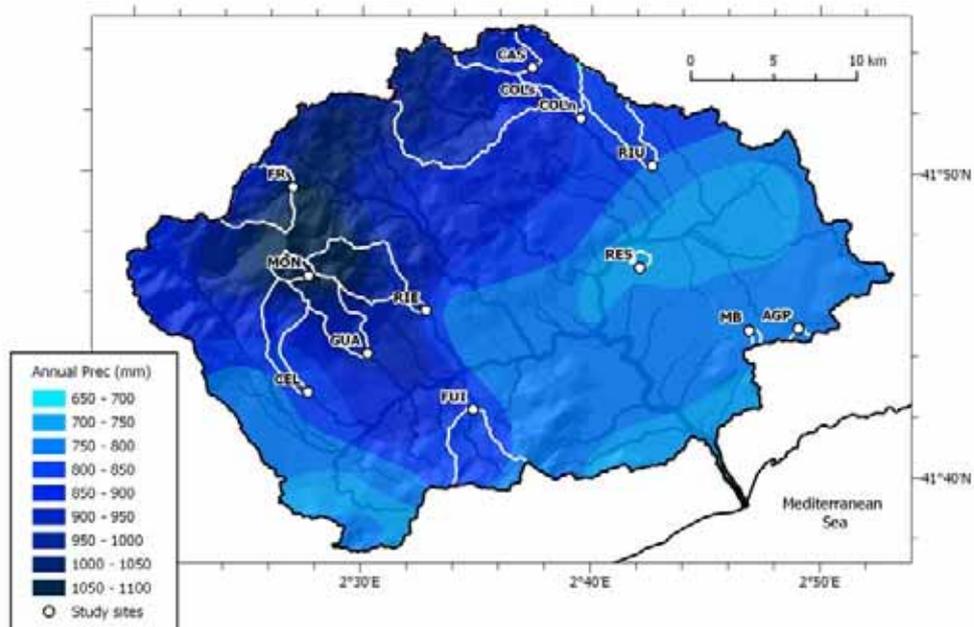


Figure 2.3 Spatial distribution of the mean annual precipitation in La Tordera catchment. Data are from the period 1961-1990 and were obtained from the Department of Environment and Housing of Catalonia (<http://www.gencat.net>). See table 2.1 for stream names.

variability in the hydrological regime. Most streams located at low altitudes are intermittent, showing a characteristic seasonal pattern with three distinct hydrological periods (Bernal *et al.*, 2005): (i) from late autumn to early spring (the cool-wet and dormant period, or *wet period*), (ii) from late spring to late summer (the warm-dry and vegetative period, or *dry period*), and (iii) in early autumn (the transition from dry to wet conditions, or *transition period*). Conversely, most streams located at high altitudes are perennial, with no *transition period*. The extent of each hydrological period may change substantially among years depending on the amount and distribution of precipitation.

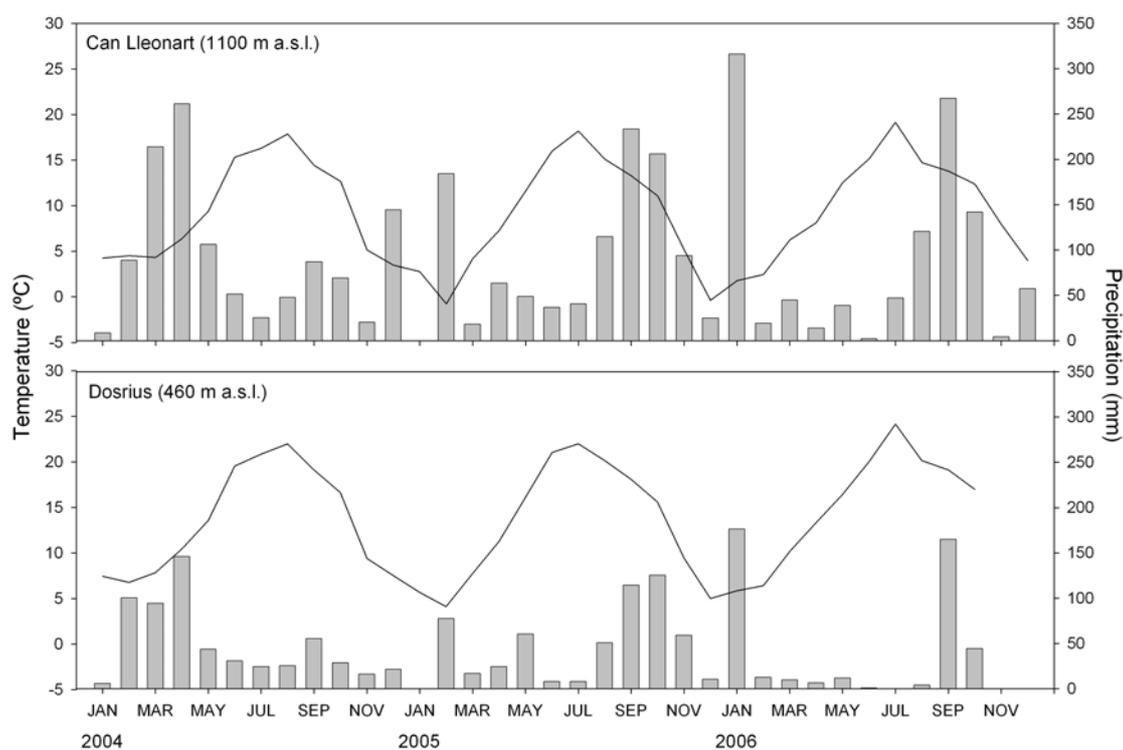


Figure 2.4 Mean monthly temperature (black lines) and precipitation (gray boxes) registered in the meteorological stations of Can Lleonart (Montseny Natural Protected Area; <1 km away from the study site MON) and Dosrius (Montnegre-Corredor Natural Protected Area; 5 km away from the study site FUJ) during the study period (2004-2006). Mean annual temperature was 9.3°C and 13.2°C in Can Lleonart and Dosrius, respectively. Mean annual precipitation was 1086 mm and 526 mm in Can Lleonart and Dosrius, respectively. Data were obtained from the Parc Natural del Montseny (Diputació de Barcelona) and the meteorological service of the Generalitat de Catalunya (<http://www.meteocat.com>).

La Tordera catchment contains a highly heterogeneous mixture of landscapes due to the combined influence of altitudinal changes and human land transformation (Fig. 2.5). Of the total catchment area, 78% is forested (including all types of forests), 13% agricultural (including irrigated and dry land crops) and 7% urban (including towns, residential areas, industrial zones, commercial zones and roads). Other types of

land use (including water impoundments, grasslands and areas without vegetation) represent <2% of the total catchment area.

Most forested areas are located within natural protected areas in the valley heads. Deciduous forests of silver fir (*Abies alba* Mill.) and beech (*Fagus sylvatica* L.) dominate at the higher altitudes, whereas dry sclerophyllous forests of evergreen oaks (*Quercus ilex* L. and *Quercus suber* L.) and pine (*Pinus halepensis* Mill. and *Pinus pinea* L.) dominate at the lower altitudes. At the valley heads the riparian forest is generally well preserved and dominated by beech at the higher altitudes and alder (*Alnus glutinosa* L.) at the lower altitudes. In the valley plains many forested areas have been transformed by urban and agricultural development, and the native riparian vegetation has been mostly eliminated or substituted by poplar (*Populus* sp.) and sycamore (*Platanus hispanica* Mill ex Muench) plantations. Very few well preserved riparian forests remain, and invasive species, such as black locust (*Robinia pseudoacacia* L.) and giant reed (*Arundo donax* L.), are abundant (Observatori de la Tordera, 2006).

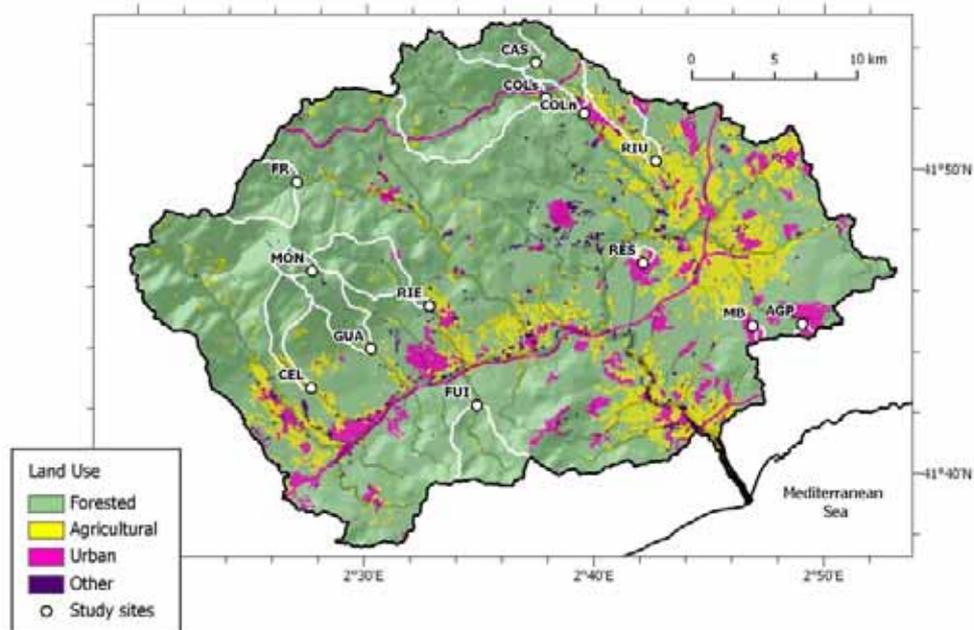


Figure 2.5 Spatial distribution of land uses in La Tordera catchment. Land uses were grouped into forested (including all types of forests), agricultural (including irrigated and dry land crops), urban (including towns, residential areas, industrial zones, commercial zones and roads) and other (including water impoundments, grasslands and areas without vegetation). Data are from the year 2002 and were obtained from the Department of Environment and Housing of Catalonia (<http://www.gencat.net>). See table 2.1 for stream names.

La Tordera catchment has a permanent population of approximately 299,000 inhabitants, but this number doubles during the summer months due to holiday homes and tourism, especially near the coast line (ACA, 2006). Water demand is mainly for domestic (41%) and industrial use (32%), whereas agricultural use (27%) is less important (ACA, 2006). The main source of water is groundwater (71%), whereas surface waters represent only 2% of water supply (Observatori de la Tordera, 2006). The rest of water demand is covered by desalinization (14%) and water extractions from other catchments (13%). The implementation of various waste water treatment plants during the last decade has ameliorated the impact of many urban point-sources on fluvial ecosystems, but the impact of fertilizers from agricultural non-point sources remains an unresolved problem (ACA, 2006).

2.2 Characteristics of the study streams

Research was conducted in 13 headwater streams (Fig. 2.6) located within La Tordera catchment that were sampled on a bi-weekly basis during the study period (2004-2006). The streams were selected to cover a variety of catchment microclimatic and land use characteristics (Table 2.1). Mean catchment altitude (122–1419 m a.s.l.) and size (0.7-45 km²) showed a wide range. Mean catchment slope was relatively similar (c. 20%) among most of the streams, except for four streams with slopes $\leq 11\%$. In nine of the 13 catchments over 90% of the land was forested. Urban land use accounted for >50% of the land area in three catchments, and agricultural land use accounted for 15% and 32% of the land area in two catchments.

Catchment characteristics were reflected in the physicochemical characteristics of the study streams (Table 2.2). Mean water temperature (9.3-13.9 °C) was similar and >11 °C in all streams except in MON, the stream located at the highest altitude. Mean discharge (0.9-180.8 L s⁻¹) varied over two orders of magnitude among the study streams and was lowest in the more urbanized streams. Seven out of the 13 selected streams were intermittent, with a dry period of variable length in summer. Mean conductivity (53-1117 $\mu\text{S cm}^{-1}$) and mean concentrations of nitrate + nitrite (129-1093 $\mu\text{g N L}^{-1}$), ammonium (10-604 $\mu\text{g N L}^{-1}$) and soluble reactive phosphorus (3-99 $\mu\text{g N L}^{-1}$) increased from the more forested streams located at the higher altitudes to the more urbanized streams located at the lower altitudes. A tendency to higher nitrate + nitrite concentration in the more agriculturally influenced streams was also observed. Temporal variability in stream physicochemical characteristics was high during the study period, especially in the most human-altered streams.

The channel morphology of the experimental reaches was relatively unmodified. Sand dominated the substratum in most of the streams, but in four streams (MON, RIE, GUA and CEL) cobbles and boulders were more abundant. The streams showed a relatively well developed riparian canopy, except for some of the more human-altered streams (RES, MB, RIU, COLn).



Figure 2.6 Selected study streams in which the experiments were conducted. See table 2.1 for stream names.

Table 2.1 Physiographic and land use characteristics of the catchments drained by the study streams. Geographical coordinates correspond to the lowermost station of the experimental reaches. Streams are listed in order of decreasing forested area.

Stream	Code	Longitude 2°E	Latitude 41°N	Mean altitude (m)	Mean slope (%)	Total area (km ²)	Forested area (%)	Urban area (%)	Agricultural area (%)
Font del Regás	FR	27° 00"	49° 32"	929	24.5	12.7	99.7	0.0	0.2
Castanyet	CAS	37° 25"	53° 28"	572	21.7	8.6	99.6	0.0	0.4
Santa Fe	MON	27° 42"	46° 37"	1419	24.1	2.6	99.4	0.0	0.0
Fuirosos	FUI	34° 54"	42° 14"	361	21.5	14.4	98.1	0.1	1.3
Riells	RIE	32° 50"	45° 27"	716	19.9	15.3	96.1	0.6	3.1
Gualba	GUA	30° 17"	44° 02"	940	22.6	13.5	96.0	0.6	2.1
Santa Coloma Sur	COLs	39° 32"	51° 48"	554	19.9	45.0	93.7	3.6	2.6
Santa Coloma Norte	COLn	37° 52"	52° 18"	425	18.7	19.1	92.6	3.7	3.4
Sant Celoni	CEL	27° 41"	42° 44"	845	21.2	9.3	90.4	0.0	8.9
Riudarenes	RIU	42° 40"	50° 15"	140	6.2	10.3	61.2	3.7	31.6
Residential Park	RES	42° 08"	46° 53"	122	6.9	0.8	27.7	57.2	15.0
Aiguaviva Park	AGP	49° 04"	44° 54"	200	11.2	0.7	23.2	69.0	0.0
Montbarbat	MB	46° 54"	44° 50"	182	10.8	0.4	8.7	91.0	0.0

Table 2.2 Physicochemical characteristics of the study streams. Data reported are the mean, standard error (SE), minimum (Min) and maximum (Max) from regular bi-weekly samplings conducted from September 2004 to September 2006. Streams are listed in order of decreasing forested area. See table 2.1 for stream names. Abbreviations: $\text{NO}_3^- + \text{NO}_2^-$, nitrate + nitrite; NH_4^+ , ammonium; SRP, soluble reactive phosphorus.

Code	Temperature ($^{\circ}\text{C}$)			Discharge (L s^{-1})			Conductivity ($\mu\text{S cm}^{-1}$)			NO_3^- ($\mu\text{g N L}^{-1}$)			NH_4^+ ($\mu\text{g N L}^{-1}$)			SRP ($\mu\text{g P L}^{-1}$)		
	Mean	SE	Min - Max	Mean	SE	Min - Max	Mean	SE	Min - Max	Mean	SE	Min - Max	Mean	SE	Min - Max	Mean	SE	Min - Max
FR	11.2	0.6	2.8 - 17.3	62.5	16.3	6.5 - 630.3	189	3	126 - 209	197	13	47 - 402	13	1	2 - 38	3	0.4	0 - 9
CAS	12.3	0.8	4.2 - 21.0	34.5	7.8	2.5 - 341.3	194	3	110 - 246	294	46	29 - 1429	17	1	4 - 44	4	1	0 - 24
MON	9.3	0.5	3.1 - 15.9	15.9	3.6	1.6 - 148.8	53	1	34 - 64	129	11	12 - 321	10	1	2 - 35	13	1	1 - 34
FUJ	13.1	0.9	2.5 - 21.4	20.0	6.4	0.0 - 194.9	206	6	132 - 282	452	78	14 - 2143	20	5	5 - 201	5	1	0 - 21
RIE	12.6	0.7	4.0 - 20.2	81.4	18.6	0.0 - 764.9	134	4	87 - 194	424	38	85 - 1249	22	2	4 - 59	20	2	1 - 54
GUA	13.4	0.7	3.6 - 21.4	85.9	27.4	1.7 - 1227.1	128	3	86 - 183	293	18	42 - 610	19	2	3 - 99	10	1	0 - 38
COLS	13.3	0.8	4.4 - 23.3	180.8	46.9	3.0 - 1849.1	277	6	132 - 342	844	45	209 - 1959	20	2	4 - 82	20	7	0 - 288
COLn	13.1	0.9	5.1 - 25.0	59.8	18.7	0.0 - 717.6	282	8	151 - 429	960	110	450 - 4357	25	4	5 - 117	15	3	0 - 113
CEL	12.2	0.7	3.2 - 19.4	43.4	12.1	0.0 - 504.2	113	3	80 - 167	734	59	142 - 1906	23	2	4 - 61	28	3	2 - 67
RIU	12.2	1.2	2.2 - 23.5	7.1	3.5	0.0 - 154.7	681	23	285 - 818	1093	141	149 - 2498	66	24	5 - 522	29	10	1 - 184
RES	12.7	0.7	3.8 - 19.8	0.9	0.1	0.0 - 4.5	770	40	126 - 1258	1047	112	363 - 3815	46	5	14 - 123	37	7	2 - 250
AGP	13.0	0.8	3.3 - 20.4	2.4	1.0	0.0 - 43.7	937	80	150 - 4030	397	66	15 - 6961	604	245	15 - 6961	99	34	1 - 996
MB	13.9	0.8	5.0 - 23.4	1.5	0.2	0.2 - 5.5	1117	56	200 - 1896	563	91	78 - 2370	197	37	52 - 1084	12	2	0 - 90

2.3 Previous studies on stream biogeochemistry in La Tordera catchment

Studies on stream biogeochemistry in La Tordera Catchment were started by researchers from the Universitat Autònoma de Barcelona in the late 1980's. These studies followed the approach of Likens *et al.* (1977) to explore water and solute budgets in the experimental catchment of the stream La Castanya, located in the Montseny mountain range (Àvila & Rodà, 1988; Àvila *et al.*, 1992; Piñol & Àvila, 1992; Piñol *et al.*, 1992). More recent studies on stream biogeochemistry have been conducted by researchers from the Universities of Barcelona and Girona in the catchment of the stream Fuirosos, located in the Montnegre-Corredor mountain range and also included in the present dissertation. These studies have focused on the interaction between stream water and riparian groundwater (Butturini *et al.*, 2002; Butturini *et al.*, 2003; Bernal *et al.*, 2003; Vázquez *et al.*, 2007), the temporal pattern of dissolved inorganic and organic nitrogen (Bernal *et al.*, 2002; Bernal *et al.*, 2005; Bernal *et al.*, 2006), stream hydrology (Bernal *et al.*, 2004), stream metabolism (Acuña *et al.*, 2004a, 2007) and organic matter dynamics and decomposition (Sabater *et al.*, 2001; Acuña *et al.*, 2004b, 2005, 2007; Artigàs *et al.*, 2004).

Previous studies initiated by researchers from the University of Barcelona have explored the influence of multiple factors, such as temperature, discharge, light and geology, on stream nutrient retention in nearby Mediterranean catchments (Martí *et al.*, 1994; Martí *et al.* 1995; Martí *et al.*, 1996; Butturini & Sabater, 1998; Sabater *et al.*, 2000). However, studies on stream nutrient retention in La Tordera catchment are scarce. Some studies have demonstrated how the retention efficiency of streams is altered by nutrient inputs from waste water treatment plants (Martí *et al.*, 2004; Merseburger *et al.*, 2005, 2006). More recently, in a study in Santa Fe del Montseny, a site also included in the present dissertation, Argerich *et al.*, (2008) have emphasized the influence of leaf litter and disturbance by flood on stream nitrogen and phosphorus retention.

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3. PUBLICATIONS

3.1 Influence of land use on stream ecosystem function in a Mediterranean catchment

3.1 Influencia del uso del suelo sobre la función del ecosistema fluvial en una cuenca mediterránea

Resumen

Debido a la organización jerárquica de las redes fluviales, los cambios en el uso del suelo que ocurren a escalas superiores (en la cuenca) pueden influir en las características físicas, químicas y biológicas a escalas inferiores, finalmente alterando la estructura y función del río. Los efectos antropogénicos sobre los ríos han sido principalmente estudiados mediante métricas estructurales como por ejemplo la química del agua, alteraciones hidromorfológicas y biomasa algal. Parámetros funcionales, incluyendo métricas de retención de nutrientes y metabolismo, también se utilizan actualmente como indicadores de la condición del río.

Dentro de dicho contexto jerárquico, en este estudio se utilizó una aproximación multivariante para examinar la relación entre atributos estructurales y funcionales (retención de nutrientes y metabolismo) de los ríos y variables de sus respectivas cuencas, incluyendo los usos del suelo. El estudio se llevó a cabo en 13 ríos situados en la misma cuenca mediterránea, pero que drenan sub-cuencas con diferentes usos del suelo.

A la escala de la cuenca, los resultados mostraron dos gradientes del uso del suelo: (i) de cuencas forestadas a cuencas urbanizadas y (ii) de cuencas poco agrícolas a cuencas moderadamente agrícolas. La variación en parámetros estructurales y funcionales se relacionó estrechamente con estos gradientes en los usos del suelo. Concretamente, la demanda de NH_4^+ (medida como la velocidad de captación, V_f) disminuyó a lo largo del gradiente desde cuencas forestadas a cuencas urbanizadas, principalmente en respuesta a un aumento en las concentraciones de nutrientes [NH_4^+ , nitrógeno orgánico disuelto (DON) y carbono orgánico disuelto (DOC)]. Tanto la producción primaria como la respiración aumentaron a lo largo del gradiente de agricultura en respuesta a aumentos en la biomasa algal (clorofila *a*). La demanda de fósforo reactivo soluble no se relacionó con ninguno de los gradientes de usos del suelo.

Nuestros resultados ponen de manifiesto las conexiones entre factores que operan a diferentes escalas espaciales (desde la cuenca hasta el río) y su distinta influencia en el funcionamiento del ecosistema fluvial. Los gestores deben tener en cuenta estas conexiones a la hora de diseñar planes de gestión y restauración fluvial. El uso de medidas de procesos funcionales es necesario debido a que una gestión ecológica con éxito debe restaurar tanto la función como la estructura del río. Los parámetros de retención de nutrientes y metabolismo son buenos candidatos para rellenar este hueco.

APPLIED ISSUES

Influence of land use on stream ecosystem function in a Mediterranean catchment

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SUMMARY

1. Due to the hierarchical organization of stream networks, land use changes occurring at larger spatial scales (i.e. the catchment) can affect physical, chemical and biological characteristics at lower spatial scales, ultimately altering stream structure and function. Anthropogenic effects on streams have primarily been documented using structural metrics such as water chemistry, channel alteration and algal biomass. Functional parameters, including metrics of nutrient retention and metabolism, are now being widely used as indicators of stream condition.
2. Within this hierarchical context, we used a multivariate approach to examine how structural and functional (i.e. nutrient retention and metabolism) attributes of streams are related to catchment variables, including land use. The study was done in 13 streams located within a single Mediterranean catchment, but draining sub-catchments with contrasting land use.
3. At the catchment scale, results showed two contrasting land use gradients: (i) from forested- to urban-dominated catchments and (ii) from low to moderate agricultural-dominated catchments. Variation in structural and functional parameters was strongly related to these land use gradients. Specifically, NH_4^+ demand (measured as the uptake velocity, V_f) decreased along the gradient from forested- to urban-dominated catchments primarily in response to increases in stream nutrient concentrations [NH_4^+ , dissolved organic nitrogen (DON) and carbon (DOC)]. Both primary production and respiration increased along the gradient of agricultural development in response to increases in algal biomass (chlorophyll *a*). Soluble reactive phosphorus demand was not related to any of the land use gradients.
4. Our results illustrate the connections among factors operating at different spatial scales (i.e. from catchments to streams) and their distinct influence on stream ecosystem function. Managers should take into consideration these connections when designing stream management and restoration plans. Because ecologically successful stream management and restoration is expected to restore function as well as structure to streams, the use of appropriate measures of functional processes is required. Nutrient retention and metabolism parameters are good candidates to fill this gap.

Keywords: land use, Mediterranean, metabolism, nutrient retention, streams

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Introduction

Stream networks are hierarchically organized systems such that land use changes occurring at larger spatial scales (i.e. the catchment) can affect physical, chemical

and biological characteristics at lower spatial scales, ultimately altering stream structure and function (Frissell *et al.*, 1986; Allan, 2004; Martí *et al.*, 2006). Because streams provide vital ecosystem services to humans (Palmer *et al.*, 2004), it is important to understand if and how different types of land use in the catchment compromise stream function.

Although human influences on physical, chemical and biological characteristics of streams are well documented, there have been far fewer studies on how humans affect ecosystem processes including biogeochemical cycling, production, respiration and decomposition (but see Bunn & Davies, 2000; Gessner & Chauvet, 2002; Meyer, Paul & Taulbee, 2005; Bott *et al.*, 2006). Ecosystem processes, however, can be ideal measures of stream condition, because they integrate environmental characteristics and may accurately reflect a broad range of catchment disturbances (Bunn, Davies & Mosisch, 1999). Stream management and restoration programs, such as the European Union's Water Framework Directive, are increasingly recognizing the importance of ecosystem processes and are designing programs directed towards maintaining these processes in addition to traditional goals of protecting biodiversity and improving water quality (Vighi, Finizio & Villa, 2006).

Stream nutrient retention, the set of processes by which nutrients are stored, transformed and removed from the water column, can mitigate problems associated with eutrophication, by reducing nutrient delivery to downstream and coastal ecosystems (Alexander, Smith & Schwarz, 2000). Because nutrient retention is now being used as an indicator of stream ecological condition, it is important to understand how human activities influence it (Meyer *et al.*, 2005; Newbold *et al.*, 2006; Roberts, Mulholland & Houser, 2007). Past research shows that some activities reduce retention efficiency (i.e. retention relative to nutrient flux), such as channel modification (Sweeney *et al.*, 2004; Bukaveckas, 2007), nutrient loading (Martí *et al.*, 2004; Bernot *et al.*, 2006; Newbold *et al.*, 2006) and other forms of water pollution that inhibit biological communities responsible for nutrient uptake (Newbold *et al.*, 2006; Lottig *et al.*, 2007). Conversely, retention efficiency may increase with other human activities, such as riparian vegetation removal, through increases in light for primary producers (Sabater *et al.*, 2000).

Stream metabolism can also be used to assess stream condition (e.g. Bunn *et al.*, 1999; Bott *et al.*, 2006; Fellows *et al.*, 2006). Primary production and respiration determine how carbon cycles through streams as well as the oxygen status of streams (Bott *et al.*, 2006). Human land use can influence stream metabolism by altering environmental variables, including light (Bunn *et al.*, 1999; Young & Huryn, 1999), organic matter inputs (Young & Huryn, 1999; Houser, Mulholland & Maloney, 2005) and nutrient availability (Bernot *et al.*, 2006; Gücker & Pusch, 2006).

The majority of studies of how land use changes affect nutrient retention and metabolism have been conducted in streams in temperate regions of North America. Here we study how land use influences stream ecosystems in a catchment situated in the Mediterranean region. Studying the influence of land use on stream function across cultures, landscapes and climates is critical for developing a global view of how land use affects streams. Specifically Mediterranean catchments, such as the ones in this study, differ from those in temperate North America because: (i) they have a longer history of human impact, (ii) they often have mixed land uses so that streams situated in urban and agricultural areas often have catchments dominated by second growth forests, and (iii) they may be more susceptible to human impacts due to the natural deficit of water resources (Alvarez-Cobelas, Rojo & Angeler, 2005).

The aim of this study was to explore how nutrient retention and metabolism are influenced by human land use in the catchment. With this purpose, we used a multivariate approach within a hierarchical context to examine the variability in parameters of nutrient retention and metabolism among streams located within the same catchment, but draining sub-catchments with contrasting land use composition.

Methods

Study sites

This study was conducted in the catchment of the river La Tordera (Catalonia, NE Spain), with an area of 868.5 km² and dominated by siliceous geology. Climate in this region is typically Mediterranean, with warm, dry summers and mild, humid winters. The long history of human settlement has created a highly heterogeneous mosaic of human land uses. Most of

the valley heads are protected areas dominated by deciduous forests of beech (*Fagus sylvatica* L.) at the higher altitudes and dry sclerophyllous forests of evergreen oaks (*Quercus ilex* L. and *Quercus suber* L.) and pine (*Pinus halepensis* Mill. and *Pinus pinea* L.) at the lower altitudes. In the valley plains the forests have been partially substituted by agricultural, urban and industrial areas.

Within this catchment, we selected experimental reaches with relatively unmodified channels from 13 headwater streams draining catchments subjected to a variety of land uses. The length of the reaches ranged from 20 to 200 m, and did not include any tributaries. The catchments above the experimental reaches were characterized for total area, mean altitude, mean slope and percent land use using geographic information system (GIS) data layers. Internet-accessible GIS databases from the Department of the Environment and Housing of Catalonia (http://www.gencat.net/cat/el_departament/cartografia) were used to obtain images, and data layers were subsequently combined using ArcGIS (Environmental Systems Research Institute, Redlands, CA, USA). Land uses were grouped into forested (including all types of forests), agricultural (including irrigated and dry-land crops) and urban (including towns, residential areas, industrial and commercial zones). Other types of land uses (e.g. water impoundments, areas without vegetation, grasslands) found only in discrete (or at single) catchments were not considered in this study.

Field sampling

Field experiments were performed in the early spring (20 March to 10 April) of 2006, a period characterized by moderate temperatures, base flow conditions and relatively high light availability at the stream bottom because leaf emergence had not yet occurred.

Solute additions. We measured nutrient retention metrics and hydraulic characteristics in each stream using short-term constant rate additions of ammonium (NH_4^+ , as NH_4Cl) and soluble reactive phosphorus [SRP, as $\text{Na}(\text{H}_2\text{PO}_4)\cdot 2\text{H}_2\text{O}$] in conjunction with chloride (Cl^- , as NaCl) as a conservative tracer (Webster & Valett, 2006). A Masterflex (Vernon Hills, IL, USA) L/S battery-powered peristaltic pump was used to deliver the addition solution to the stream until conductivity reached plateau (i.e. 1–3 h) at the bottom of the study

reach. Conductivity was automatically recorded at the bottom of the reach every 5 s using a WTW (Weilheim, Germany) 340i portable conductivity meter connected to a Campbell Scientific (Logan, UT, USA) data logger. We measured conductivity and collected water samples for NH_4^+ , SRP and nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$) at eight stations along the reach before the addition for background concentrations (two replicates per station), and once conductivity reached plateau for plateau concentrations (five replicates per station).

Metabolism measurements. Metabolism was estimated in each stream on cloudless days within 5 days of the nutrient addition using an open-system, single-station approach (Bott, 2006). Dissolved oxygen (DO) concentration and temperature were recorded at the bottom of the study reach at 10-min intervals during a 24-h period with a WTW 340i portable oxygen meter. Percent DO saturation was estimated using DO and temperature data together with a standard altitude-air pressure algorithm to correct for site altitude. To estimate mean daily temperature and percent DO saturation we averaged values recorded over the 24-h period. During the same period, photosynthetically active radiation (PAR) was measured every 10 s, and 10-min integrals were logged with a Skye (Powys, UK) SKP215 quantum sensor connected to a Campbell Scientific data logger. Unfortunately, PAR could only be determined in seven of the study streams due to a malfunctioning sensor.

Additional measurements. Water samples (three replicates) for total dissolved N (TDN) and dissolved organic carbon (DOC) were taken at the lowermost station of each reach before the addition. Wetted width (w) and percent reach coverage by different substratum types were determined on cross-sectional transects located at each sampling station along the reach. Six cobbles or sand core samples, in streams without cobbles, were randomly sampled from the streambed and transported to the laboratory for analysis of chlorophyll *a*. All water samples for nutrient chemistry were immediately filtered through pre-ashed Albet (Barcelona, Spain) FVF glass fibre filters (0.7 μm retention), stored on ice in the field and then refrigerated at 4 °C or frozen in the laboratory until analysis.

Laboratory analyses

Nutrient chemistry. Concentrations of $\text{NO}_3^- + \text{NO}_2^-$, NH_4^+ and SRP in stream water samples were analysed with a Bran + Luebbe (Norderstedt, Germany) TRAACS 2000 Autoanalyser following standard colorimetric methods (APHA, 1995). TDN and DOC concentrations were determined on a Shimadzu (Tokyo, Japan) TOC-VCSH analyser. Dissolved organic N (DON) concentration was calculated by subtracting the sum of the inorganic forms of dissolved N ($\text{NO}_3^- + \text{NO}_2^-$ and NH_4^+) from TDN.

Algal biomass. Chlorophyll *a* concentration on cobbles or sand cores was estimated following standard protocols (Steinman, Lamberti & Leavitt, 2006). Samples were extracted in 90% v/v acetone over 24 h at 4 °C, sonicated for 2 min and centrifuged for 10 min at 1108 g. Absorbance of the resultant supernatant was measured using a Shimadzu UV-spectrophotometer. The chlorophyll *a* content of each sample was corrected for phaeo-pigments by acidification and expressed per unit area of substratum.

Parameter calculations

Hydraulic parameters. Breakthrough curves of conductivity were analysed by visual inspection with a one-dimensional transport with inflow and storage model (OTIS; Runkel, 1998) to calculate stream hydraulic characteristics, including discharge (Q), cross-sectional area (A) and cross-sectional transient storage area (A_s). In this study, we used the ratio A_s/A to characterize water transient storage because it allows for comparison among streams of different size. Mean reach depth was calculated as A/w .

Nutrient retention parameters. Using data from the nutrient additions, we calculated three metrics of retention for each nutrient (i.e. NH_4^+ and SRP) and stream: uptake length (S_w), uptake velocity (V_f) and areal uptake (U). S_w , the average distance travelled by a nutrient molecule before being removed from the water column (Newbold *et al.*, 1981), was calculated as the negative inverse of the slope of the regression of the ln-transformed and background corrected nutrient : conductivity ratio versus distance downstream from the addition point. S_w is an indicator of the nutrient retention efficiency at the reach scale (Webster

& Valett, 2006). This metric was converted to V_f , calculated as the stream specific discharge (i.e. Q/w) divided by S_w . Because S_w is strongly dependent on discharge, V_f provides a more appropriate variable for comparison across streams of different size (Webster & Valett, 2006). V_f describes the velocity by which a nutrient molecule is removed from the water column, and it is an indicator of nutrient demand (Hall, Bernhardt & Likens, 2002). U , the mass of a nutrient taken up from the water column per unit streambed area and time, was calculated as V_f multiplied by the ambient nutrient concentration. Because U is an areal measurement it provides a more appropriate variable to examine relationships with areal metabolism measurements. Nitrification could not be estimated with our data for any of the study streams because no downstream increases in NO_3^- along the experimental reaches were observed during the additions.

High nutrient concentrations at plateau resulting from the addition experiments may overestimate S_w at ambient nutrient levels. This can be avoided by using multiple enrichments (Payn *et al.*, 2005) or stable isotope additions (Mulholland *et al.*, 2002). Because of the extensive character of our study, covering two nutrients across 13 streams, we used the traditional short-term addition method and tried to minimize the error associated with this method by maintaining a relatively low and similar nutrient enrichment factor (i.e. plateau/background) among streams. Enrichment factors for SRP (mean \pm 1SE = 23.4 ± 8.4) were higher than for NH_4^+ (4.5 ± 0.9), because relatively low ambient SRP concentrations had to be sufficiently increased to ensure reliable analytical detection of concentration changes downstream. Nevertheless, no relationship (Pearson correlation, $P \geq 0.386$) between enrichment factor and S_w was found for either of the two nutrients.

Metabolism parameters. We estimated gross primary production (GPP) and ecosystem respiration (ER), by integrating the DO measurements at a single station during a 24 h period following Bott (2006). Reaeration coefficients (range = $11.3\text{--}66.2 \text{ day}^{-1}$) and respiration at night were estimated based on DO change rates and DO deficits using the night-time regression method (Young & Huryn, 1996). Respiration at night was extrapolated to 24 h to estimate ER. GPP was computed by integrating the difference between the measured net DO change (corrected by the reaeration

flux) and the extrapolated day-time respiration. GPP and ER were multiplied by the mean reach depth to obtain areal estimates, which allow for comparison among streams of different size. Three other metabolic metrics were calculated: net ecosystem production (NEP = GPP – ER), the production/respiration ratio (GPP/ER) and total metabolism (TM = GPP + ER; *sensu* Meyer *et al.*, 2005).

Statistical analyses

All variables were divided into three groups: catchment variables (Table 1), stream structural variables (Table 2) and stream functional variables (Table 3). To examine which variables contributed to variation among streams at the two different scales (i.e. catchment and stream), we conducted separate principal component analyses (PCA) with the group of variables at each scale. Variables were standardized and a correlation matrix was used for the PCA. Results from the two PCA allowed us to test for correlations among variables within each scale. Results from each PCA are hereafter referred to as catchment-PCA and stream-PCA. The weight of a variable on a PCA component was considered significant when its loading was >0.7. Due to statistical constraints in the number of variables that can be included in the PCA based on the total number of cases, we only used percent fine substratum (i.e. sand + mud) to characterize streambed substratum composition, because this was the substratum type that showed the highest variability among streams. PAR measurements were

excluded from the PCA analysis because we only had data for seven streams.

We examined the relationship between catchment and stream environmental variables using simple linear regressions with the scores of the components of the catchment-PCA as independent variables and the scores of the components of the stream-PCA as dependent variables. The relationship between stream environmental variables and functional variables was explored using simple linear regression with the scores of the components of the stream-PCA as independent variables, and nutrient demand (V_f) and metabolism parameters (GPP, ER, NEP, GPP/ER and TM) as dependent variables. The relationship between nutrient retention and metabolism was examined using Pearson correlation analysis with the areal nutrient uptake (U) and metabolism (GPP, ER, NEP, GPP/ER and TM) as variables.

All variables were normalized prior to analysis by \log_{10} or arcsine $\sqrt{(x)}$ (for percent data) transformation. Results were considered significant if $P < 0.05$, and marginally significant if $0.05 < P < 0.10$. Statistical analyses were done with Statistica 6.0 (Statsoft, Tulsa, OK, USA).

Results

Catchment characteristics

Study catchments included a wide altitudinal range (122–1419 m a.s.l.; Table 1). Catchment size of all streams was relatively small, but values varied over

Table 1 Physiographic and land use characteristics of the catchments drained by the study streams. Geographical coordinates correspond to the lowermost station of the experimental reaches

Stream	Code	Longitude 2°E	Latitude 41°N	Mean altitude (m)	Mean slope (%)	Total area (km ²)	Forested area (%)	Urban area (%)	Agricultural area (%)
Aiguaviva Park	AGP	49°04''	44°54''	200	11.2	0.7	23.2	69.0	0.0
Castanyet	CAS	37°25''	53°28''	572	21.7	8.6	99.6	0.0	0.4
Sant Celoni	CEL	27°41''	42°44''	845	21.2	9.3	90.4	0.0	8.9
Santa Coloma Sur	COLs	39°32''	51°48''	554	19.9	45.0	93.7	3.6	2.6
Santa Coloma Norte	COLn	37°52''	52°18''	425	18.7	19.1	92.6	3.7	3.4
Font del Regàs	FR	27°00''	49°32''	929	24.5	12.7	99.7	0.0	0.2
Fuirosos	FUI	34°54''	42°14''	361	21.5	14.4	98.1	0.1	1.3
Gualba	GUA	30°17''	44°02''	940	22.6	13.5	96.0	0.6	2.1
Montbarbat	MB	46°54''	44°50''	182	10.8	0.4	8.7	91.0	0.0
Santa Fe	MON	27°42''	46°37''	1419	24.1	2.6	99.4	0.0	0.0
Residential Park	RES	42°08''	46°53''	122	6.9	0.8	27.7	57.2	15.0
Riells	RIE	32°50''	45°27''	716	19.9	15.3	96.1	0.6	3.1
Riudarenes	RIU	42°40''	50°15''	140	6.2	10.3	61.2	3.7	31.6

Table 2 Physical, chemical and biological characteristics of the study streams

Code	Discharge (L s ⁻¹)	A _s /A	Fine substratum (%)	Conductivity (µS cm ⁻¹)	PAR (mol m ⁻² day ⁻¹)	Temperature (°C)	NO ₃ ⁻ +NO ₂ ⁻ (µg N L ⁻¹)	NH ₄ ⁺ (µg N L ⁻¹)	DON (µg N L ⁻¹)	SRP (µg P L ⁻¹)	DOC (mg L ⁻¹)	DOsat (%)	Chlorophyll <i>a</i> (µg cm ⁻²)
AGP	0.5	0.44	95.0	772	2.2	12.6	296	48	294	19	3.0	88.7	0.6
CAS	27.0	0.06	57.0	170	14.4	10.0	193	16	190	0.4	1.1	95.5	1.5
CEL	21.5	0.08	5.5	98	N/A	12.6	557	11	223	26	1.0	93.9	1.0
COLs	130.4	0.08	34.8	251	N/A	12.4	673	13	180	2	1.1	93.0	5.8
COLn	35.2	0.04	60.8	253	N/A	12.6	633	15	177	1	1.2	101.4	7.9
FR	48.1	0.10	57.0	172	7.2	10.1	79	13	189	1	0.6	108.4	0.5
FUJ	12.8	0.05	57.9	170	N/A	11.5	79	9	290	0.4	1.5	100.8	1.0
GUA	65.4	0.07	21.1	85	14.1	12.9	115	10	195	0.5	0.9	98.0	1.2
MB	1.6	0.20	61.5	888	N/A	13.0	356	104	291	1	3.9	92.4	0.2
MON	48.6	0.13	8.2	34	15.4	7.1	115	14	207	2	0.6	97.2	1.1
RES	1.4	0.26	40.0	683	19.2	14.6	444	22	366	20	3.0	88.2	0.8
RIE	50.9	0.09	47.5	114	1.7	11.7	191	11	251	8	1.3	100.7	0.6
RIU	24.3	0.09	100.0	712	N/A	14.9	989	20	571	8	2.0	93.6	5.4

See Table 1 for site code.

A_s/A, relative transient storage; PAR, photosynthetically active radiation; NO₃⁻ + NO₂⁻, nitrate + nitrite; NH₄⁺, ammonium; DON, dissolved organic nitrogen; SRP, soluble reactive phosphorus; DOC, dissolved organic carbon; DOsat, dissolved oxygen saturation; N/A, data not available.

two orders of magnitude (0.7–45.0 km²; Table 1). Average catchment slope was relatively similar (*c.* 20%) among most of the streams, except for four streams with slopes ≤11%. In nine of the 13 sub-catchments over 90% of the land was forested (Table 1). In the most disturbed streams, urban land use accounted for >50% of the land area in three catchments, and agricultural land use accounted for 15% and 32% of the land area in two catchments (Table 1). Highly urbanized streams drained the smallest catchments, located at low altitudes with minor slope (Table 1).

The first component of the catchment-PCA explained 68.8% of the variance (Fig. 1a), with a positive loading of percent urban area (0.91), and a negative loading of percent forested area (-0.98), slope (-0.91), altitude (-0.91) and total area (-0.79). The second component accounted for 25.0% of the variance (Fig. 1a), with a negative loading of percent agricultural area (-0.94). Finally, the third component accounted for only 4.0% of the variance, and no variable had a significant loading on it. Thus, the first component of the catchment-PCA indicated a gradient from forested- to urban-dominated catchments associated with concomitant changes in physiographical characteristics. The second component of the catchment-PCA indicated a gradient of agricultural development among catchments.

Stream environmental characteristics

Discharge (0.5–130.4 L s⁻¹), A_s/A (0.04–0.44), percent fine substratum (5.5–100%), conductivity (34–888 µS cm⁻¹) and daily PAR (1.7–19.2 mol m⁻² day⁻¹) varied over an order of magnitude among the study streams (Table 2). Water temperature was less variable (7.1–14.9 °C) and >10 °C in all streams except MON, the stream at the highest altitude. Concentrations of inorganic solutes (NO₃⁻ + NO₂⁻ [79–989 µg N L⁻¹], NH₄⁺ [9–104 µg N L⁻¹] and SRP [0.4–26 µg P L⁻¹]) spanned wider ranges than those of organic solutes [DON (177–571 µg N L⁻¹) and DOC (0.6–3.9 mg L⁻¹); Table 2]. The range in mean daily percent DO saturation was relatively low (88.2–108%), and only four streams were oxygen supersaturated (*i.e.* DO saturation >100%; Table 2). Chlorophyll *a* per unit area of substratum varied >1 order of magnitude among streams (0.2–7.9 µg cm⁻²; Table 2).

Table 3 Parameters of nutrient retention and metabolism in the study streams

Code	NH ₄ ⁺			SRP			GPP (g O ₂ m ⁻² day ⁻¹)	ER (g O ₂ m ⁻² day ⁻¹)	NEP (g O ₂ m ⁻² day ⁻¹)	GPP/ER
	S _w (m)	V _f (mm min ⁻¹)	U (μg N m ⁻² min ⁻¹)	S _w (m)	V _f (mm min ⁻¹)	U (μg P m ⁻² min ⁻¹)				
AGP	335	0.2	8.3	145	0.4	7.3	0.11	0.28	-0.18	0.37
CAS	475	1.2	18.1	978	0.6	0.2	0.02	0.40	-0.39	0.04
CEL	476	1.0	11.7	1083	0.5	11.8	0.10	1.95	-1.85	0.05
COL _s	158	6.9	89.5	391	2.8	5.6	0.48	1.00	-0.52	0.48
COL _n	370	2.0	28.8	153	4.7	2.9	0.53	2.29	-1.76	0.23
FR	162	5.1	67.9	294	2.8	1.9	0.05	0.96	-0.91	0.05
FUI	173	1.4	12.1	441	0.5	0.2	0.85	0.71	0.15	1.21
GUA	249	3.2	30.6	502	1.6	0.7	0.57	1.22	-0.65	0.47
MB	238	0.5	47.5	64	1.7	1.5	0.01	0.41	-0.40	0.02
MON	162	5.0	68.0	655	1.2	2.0	0.25	1.34	-1.10	0.18
RES	104	1.0	23.4	62	1.8	35.7	1.19	2.42	-1.23	0.49
RIE	532	1.6	18.6	1171	0.7	6.2	0.15	0.34	-0.19	0.43
RIU	433	1.3	25.5	656	0.8	6.6	1.52	2.22	-0.70	0.68

See Table 1 for site code.

S_w, uptake length; V_f, uptake velocity; U, areal rate; GPP, gross primary production; ER, ecosystem respiration; NEP, net ecosystem production.

The first component of the stream-PCA explained 51.2% of the variance, with a positive loading of DOC (0.94), conductivity (0.89), NH₄⁺ (0.79), DON (0.73) and A_s/A (0.73), and a negative loading of discharge (-0.84) and percent DO saturation (-0.77; Fig. 1b). The second component accounted for 18.6% of the variance, with a negative loading of chlorophyll *a* (-0.89; Fig. 1b). Finally, the third component accounted for 11.4% of the variance, but no variable had a significant loading on it.

The first components of both the catchment-PCA and the stream-PCA were positively related ($r^2 = 0.94$, $P < 0.001$; Fig. 2a); the variation among streams in hydraulic and chemical characteristics was related to the gradient from forested- to urban-dominated catchments. Similarly, the second components of both the catchment-PCA and the stream-PCA were positively related ($r^2 = 0.71$, $P < 0.001$; Fig. 2b); algal biomass increased along the gradient of agricultural development. No other relationship ($P \geq 0.762$) between catchment-PCA and stream-PCA components was found.

Stream functional characteristics

Streams were generally more retentive for NH₄⁺ than for SRP (Table 3). Only in four streams, including the three most urbanized, S_w was shorter and V_f was higher for SRP than for NH₄⁺. Similarly, only in the two streams showing the highest SRP concentrations,

U was higher for SRP than for NH₄⁺. The V_f s for NH₄⁺ (0.2–6.9 mm min⁻¹) and SRP (0.4–4.7 mm min⁻¹) spanned similar ranges, and were positively correlated ($r = 0.57$, $P = 0.042$).

GPP and ER were also positively correlated ($r = 0.63$, $P = 0.020$). GPP (0.01–1.52 g O₂ m⁻² day⁻¹) was lower than ER (0.28–2.42 g O₂ m⁻² day⁻¹) in all streams except FUI, the only stream showing a positive NEP and a GPP/ER ratio >1 (Table 3). We found a marginally significant relationship ($r^2 = 0.26$, $P = 0.077$) between ER and U-SRP. No other relationship ($P \geq 0.141$) between metabolism parameters (i.e. GPP, ER, NEP, GPP/ER, TM) and retention parameters (U, V_f) was found for either nutrient.

V_f-NH₄⁺ and the first component of the stream-PCA were negatively related ($r^2 = 0.63$, $P = 0.002$; Fig. 3a); demand for NH₄⁺ decreased with stream hydraulic and chemical changes related to the degree of urbanization at the catchment scale. No relationship ($P \geq 0.457$) between the scores of the two stream-PCA components and V_f-SRP was found. GPP and the second component of the stream-PCA were negatively related ($r^2 = 0.34$, $P = 0.037$, Fig. 3b): GPP increased with increases in algal biomass related to the degree of agricultural development at the catchment scale. This relationship was only marginally significant for ER ($r^2 = 0.29$, $P = 0.060$, Fig. 3b). No relationship ($P \geq 0.148$) between the scores of the stream-PCA components and NEP, GPP/ER or TM was found.

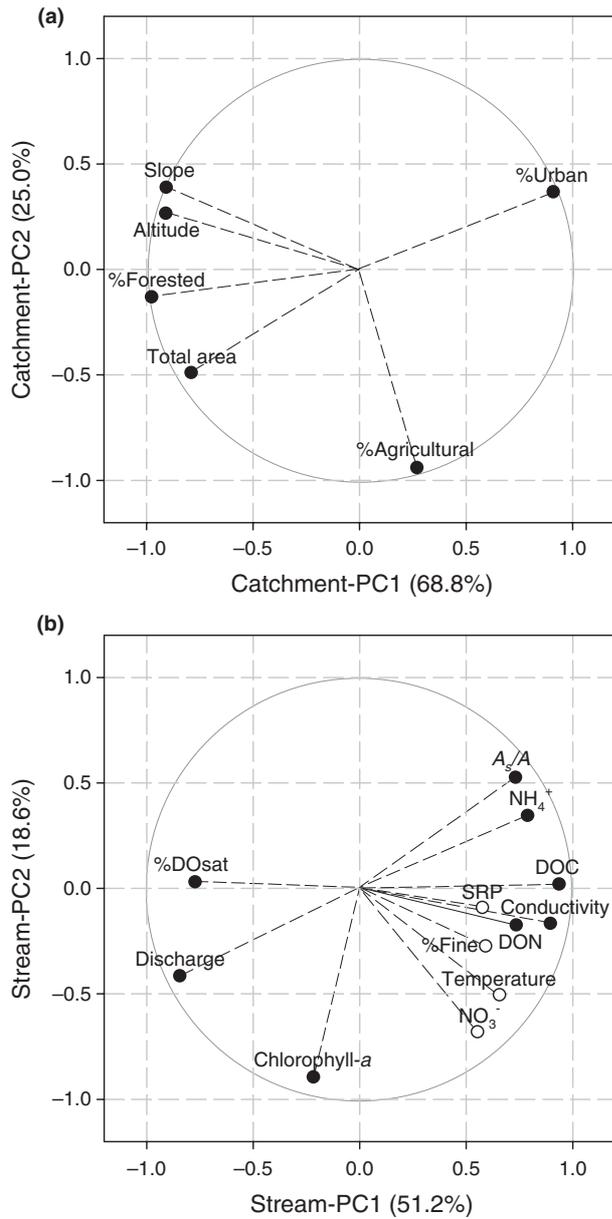


Fig. 1 Principal components analysis (PCA) of selected variables from two different hierarchical scales: (a) the catchment and (b) the stream. The percent values on each axis represent the amount of variance explained by each PCA component. Closed symbols denote significant variables (loading >0.7). See Tables 1 & 2 for a more detailed description of the variables included in each PCA.

Discussion

Stream functional and environmental parameters were strongly related to catchment land use composition. Catchment signatures detected in streams varied depending on the type of land use. Nutrient

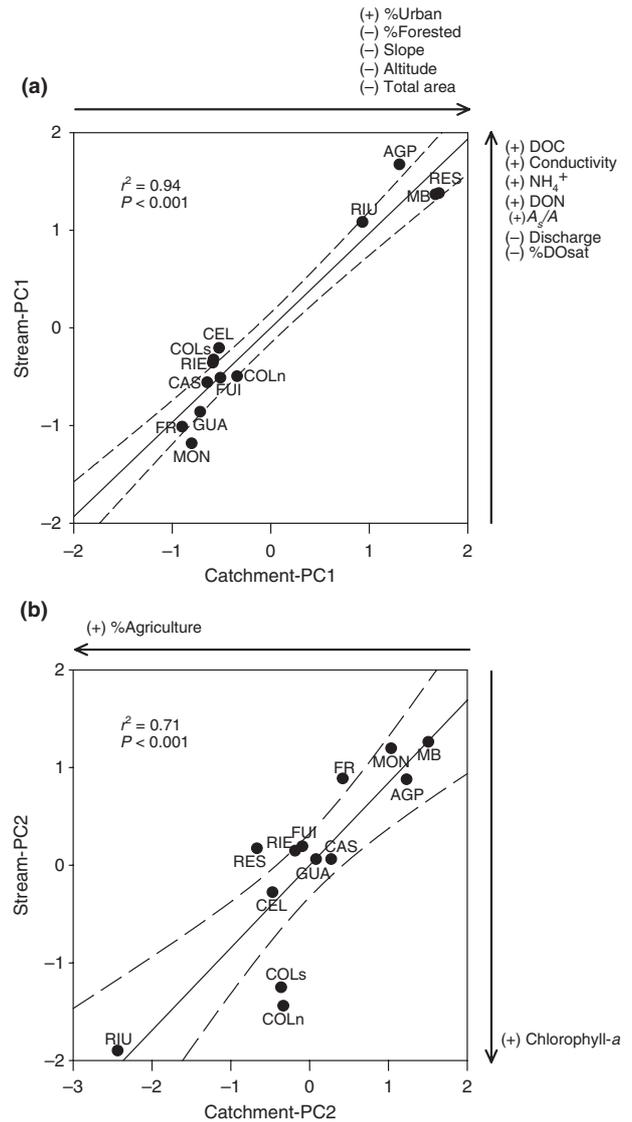


Fig. 2 (a) Linear regression between the scores of the first component of the catchment PCA (indicating a gradient from forested- to urban-dominated catchments) and the scores of first component of the stream PCA (indicating a gradient of stream chemical and hydraulic changes) and (b) Linear regression between the scores of the second component of the catchment PCA (indicating a gradient of catchment agricultural development) and the scores of the second component of the stream PCA (indicating a gradient of algal biomass). Significant variables (loading >0.7) associated with each PCA component are shown on the axes with their respective positive (+) or negative (-) weight. Dashed lines represent 95% confidence regression bands. Point labels correspond to the study streams (*n* = 13). See Table 1 for site code.

demand, in particular for NH₄⁺, was sensitive to catchment urbanization, whereas primary production and respiration were sensitive to agricultural

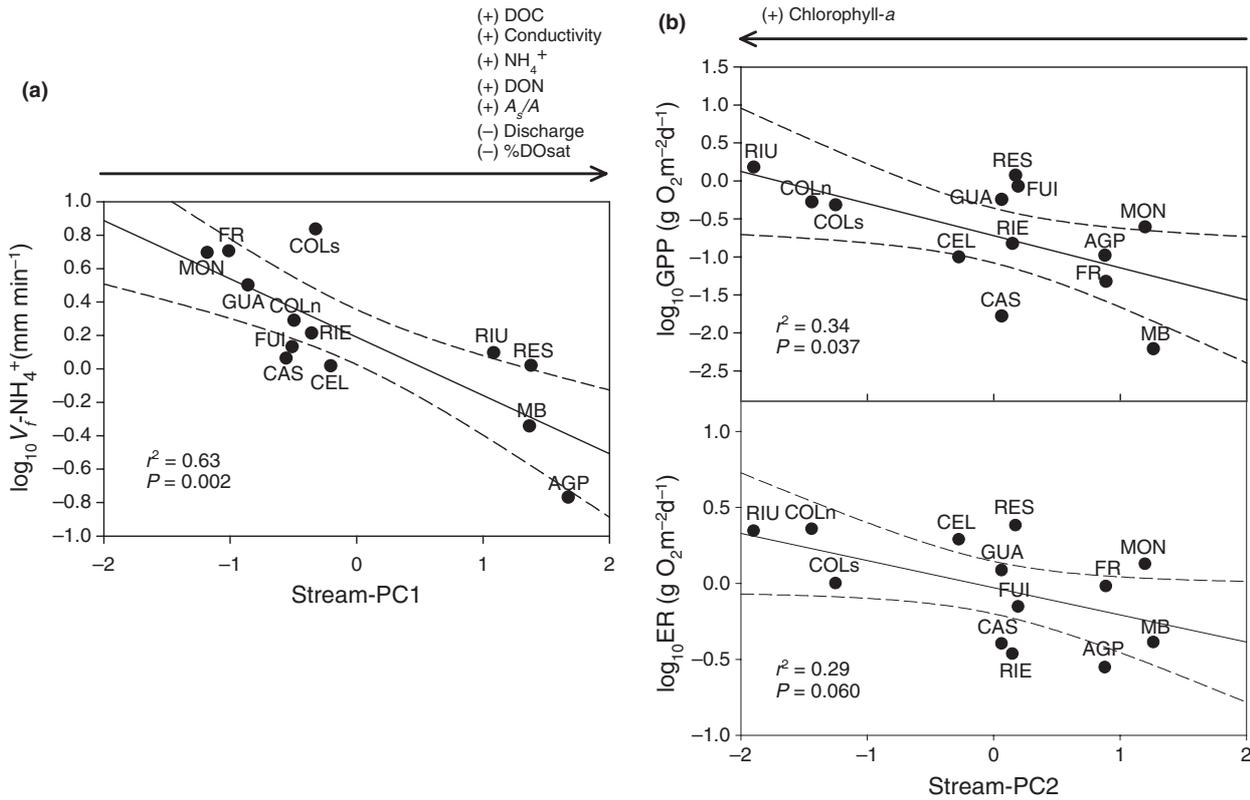


Fig. 3 (a) Linear regression between the scores of the first component of the stream PCA (indicating a gradient of stream chemical and hydraulic changes) and ammonium demand ($V_f\text{-NH}_4^+$) and (b) Linear regression between the scores of the second component of the stream PCA (indicating a gradient of algal biomass) and gross primary production (GPP) and ecosystem respiration (ER). Significant variables (loading >0.7) associated with each PCA component are shown on the axes with their respective positive (+) or negative (-) weight. Dashed lines represent 95% confidence regression bands. Point labels correspond to the study streams ($n = 13$). See Table 1 for site code.

development. A weak positive relationship between $U\text{-SRP}$ and ER indicated some coupling between SRP retention and metabolism in these streams, supporting results from previous studies (e.g. Mulholland *et al.*, 1997; Newbold *et al.*, 2006). In contrast to results from other previous studies (e.g. Hall & Tank, 2003; Gücker & Pusch, 2006; Newbold *et al.*, 2006), we did not find a coupling between NH_4^+ retention and metabolism. Stoichiometric imbalances (i.e. differences in the relative availability of nutrients) and variation in abiotic (e.g. sorption, volatilization) and dissimilatory (e.g. nitrification, denitrification) uptake processes among streams may have blurred this relationship in our study, but our data do not allow us to test this hypothesis.

Altered stream hydraulic and chemical characteristics were observed along the gradient of catchment urbanization, the predominant type of land use transformation in our study. Stream water conductivity and

concentrations of reduced forms of N (NH_4^+ and DON) and C (DOC) increased, while percent DO saturation decreased. These changes were likely a result of wastewater inputs from point and diffuse sources, which are characteristic of urban streams (Paul & Meyer, 2001; Kaplan *et al.*, 2006; Pellerin, Kaushal & McDowell, 2006). In addition, the more urbanized streams showed higher transient storage (A_s/A) and lower discharge, which enhanced the high solute concentrations in these streams by reducing their dilution capacity. Demand for NH_4^+ decreased with changes in these variables along the forested to urban gradient, but high levels of inter-correlation among them made it difficult to determine which were important in influencing this relationship. Some variables had the expected effect, while others had an effect opposite to that expected. For example, higher concentrations of both NH_4^+ and DON likely contributed to lower $V_f\text{-NH}_4^+$ through the saturation of the N

uptake capacity (Newbold *et al.*, 2006). Higher DOC (Strauss & Lamberti, 2000) and lower oxygen (Rysgaard *et al.*, 1994) concentrations may have reduced $V_f\text{-NH}_4^+$ through the inhibition of nitrification. In contrast, higher A_s/A was expected to positively affect $V_f\text{-NH}_4^+$ by increasing the contact time of dissolved nutrients to biogeochemically active surfaces (e.g. backwaters, eddies, sediments; Gücker & Boëchat, 2004). The observed result, however, was the opposite, indicating that other mechanisms, such as nutrient saturation, may have overridden the influence of transient storage on NH_4^+ retention. Results from other empirical studies are also conflicting, with some showing no relationship between nutrient retention and transient storage (e.g. Webster *et al.*, 2003; Niyogi, Simon & Townsend, 2004; Meyer *et al.*, 2005; Roberts *et al.*, 2007), or a relationship opposite to that expected (e.g. Hall *et al.*, 2002; Valett, Crenshaw & Wagner, 2002). Although V_{fs} for both NH_4^+ and SRP were positively correlated across streams, $V_f\text{-SRP}$ was not sensitive to the land use gradients. This result was likely due to the relatively low values and small range of SRP concentrations in comparison with dissolved N concentrations across the study streams. Only three streams showed concentrations $>9 \mu\text{g P L}^{-1}$ and previous studies have demonstrated SRP uptake saturation at concentrations $>5\text{--}13 \mu\text{g P L}^{-1}$ (Mulholland, Steinman & Elwood, 1990; Rosemond *et al.*, 2002; Newbold *et al.*, 2006). In addition, co-precipitation of SRP with calcium carbonate, which is an important abiotic removal process in calcareous streams, was probably negligible in the study streams due to the dominant siliceous geology (Reddy *et al.*, 1999).

Decreases in V_f due to increasing urbanization have been also reported from North American catchments. Meyer *et al.* (2005) found that demand for both NH_4^+ and SRP decreased as urbanization increased in streams located in Georgia (USA). The authors attributed this result to the decrease in fine benthic organic matter, an important resource for microbes, along the urbanization gradient. Similarly, in streams from the water-supply source areas of New York City (USA), demand for both NH_4^+ and SRP decreased from forested to more populated catchments mainly due to nutrient uptake saturation and possibly increases in toxic pollutants (Newbold *et al.*, 2006). Finally, in urbanized desert streams from the US Southwest, reduced areal NO_3^- uptake was attributed to reduced channel complexity and reduced primary production

due to the presence of algaecides in stream water (Grimm *et al.*, 2005). Results from these streams, located in different biomes of North America, together with our own results from streams in the Mediterranean region indicate that, regardless of the bioclimatic setting, urbanization has a negative effect on stream nutrient retention, a key ecosystem service of streams.

Although there was only a small amount of land allocated to agriculture in this study, our results indicate that the effect of agriculture was primarily through increasing algal biomass and metabolism, including both GPP and ER. Chlorophyll *a* was the only stream environmental variable that significantly responded to the gradient of agricultural development. Both nutrients (Borchard, 1996) and light (Hill, 1996) limit algal growth in many streams. The lack of significant changes in nutrient concentrations along the agricultural gradient indicates that either nutrients were not responsible for the observed increases in algal biomass or excess nutrients were efficiently transferred up the food chain. Although we did not detect a relationship between PAR and chlorophyll *a* among the streams where data were available, results from previous studies have demonstrated the higher importance of light over nutrient limitation on algal growth in some of these streams (von Schiller *et al.*, 2007). GPP and ER were positively correlated and increased with algal biomass along the agricultural development gradient. Despite high nutrient concentrations and light availability, stream metabolism was dominated by respiration (i.e. negative NEP and $\text{GPP/ER} < 1$) in all streams except FUI, supporting previous findings for headwater streams (reviewed by Battin *et al.*, 2008). Large patches of filamentous algae contributing to GPP peaks are typical in FUI during early spring (Acuña *et al.*, 2004).

High metabolism measurements associated with agriculture have been reported from previous studies. GPP increased with nutrient concentrations along an agricultural gradient located in the US Midwest (Bernot *et al.*, 2006). In the southern Appalachian Mountains (USA), GPP was higher in agricultural streams with little canopy cover but not in agricultural streams with well developed riparian forests, relative to streams draining forested catchments (McTammany, Benfield & Webster, 2007). Similarly, Young & Huryn (1999) in their study along a gradient of land use conversion to pasture located in New Zealand highlighted the effect of forest canopy on stream

metabolism through its control on light availability and organic matter supply. Results from these and other studies (e.g. Bunn & Davies, 2000; Bott *et al.*, 2006) together with our own results indicate that stream metabolism may be more susceptible to human influences on proximate factors operating at a near-stream spatial scale (e.g. riparian vegetation removal) than on distant factors operating at a greater spatial scale (e.g. catchment land use).

This study demonstrates that whole-stream N retention and metabolism are sensitive to human land use pressures. Demand for NH_4^+ was mainly influenced by changes in nutrient availability related to the degree of catchment urbanization, whereas both GPP and ER were influenced by increases in algal biomass related to the degree of agricultural development at the catchment. Both nutrient availability and algal biomass have been used as indicators of trophic state in streams (Dodds, 2007). Our study corroborates the ecological link between these key structural variables and functional attributes of streams in the context of catchment disturbance. Furthermore, by considering the hierarchical organization of stream networks, our results illustrate the connections among factors operating at different spatial scales (i.e. from catchments to streams), and their relative influence on stream ecosystem function. Land management practices can have wide repercussions on the ecological condition of streams (including their functional capacity) at varying scales through diverse pathways and involving complex interactions (Martí *et al.*, 2006). Managers should take into consideration these connections when designing stream management and restoration plans.

Ecologically successful stream management and restoration is expected to restore function as well as structure to streams (Bernhardt & Palmer, 2007), requiring indicators of functional processes. Results from this and other studies have demonstrated that nutrient retention and metabolism parameters are good candidates to fill this gap. This study reports the first measurements of nutrient retention and metabolism in most of these Mediterranean streams. It thus provides a baseline for assessing the impact of further deterioration or the benefits of management practices in these streams and their catchments in the future, which can now be based on measurements of stream ecosystem function in addition to the more traditional biotic and nutrient status indexes.

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3.2 Nitrate retention and removal in Mediterranean streams with contrasting land uses: a ^{15}N tracer study

3.2 Retención y eliminación de nitrato en ríos mediterráneos con distintos usos del suelo: un estudio con el ^{15}N como trazador

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Resumen

Se utilizaron adiciones de nitrato (NO_3^-) marcado con ^{15}N para investigar el reciclado de nitrógeno (N) a una escala de tramo completo en ríos mediterráneos sujetos a diferentes usos del suelo (forestado, urbano y agrícola). El objetivo fue examinar: (i) la magnitud e importancia relativa de la retención (captación asimilatoria) y la eliminación (desnitrificación) de NO_3^- , (ii) la contribución relativa de los diferentes compartimentos primarios de captación a la retención de NO_3^- , y (iii) las vías de regeneración, transformación y exportación del N retenido.

La concentración de NO_3^- aumentó y la de oxígeno disuelto (DO) disminuyó desde el río forestado al río agrícola, con valores intermedios en el río urbano. La biomasa de los compartimentos primarios de captación fue similar entre ríos y dominada por compartimentos detriticos (material orgánico particulado fino y grueso). En concordancia, el metabolismo fue netamente heterotrófico en los tres ríos, aunque el grado de heterotrofia aumentó desde el río forestado al agrícola. La distancia de captación de NO_3^- disminuyó a lo largo de este gradiente, mientras que la velocidad y la tasa de captación de NO_3^- mostraron los valores más altos en el río urbano. La desnitrificación no fue detectada en el río forestado, pero representó el 9% y el 68% de la captación total de NO_3^- en los ríos urbano y agrícola, respectivamente. La contribución relativa de los compartimentos detriticos a la captación asimilatoria de NO_3^- fue mayor en el río forestado y menor en el río agrícola. En los tres ríos, el N retenido fue rápidamente transferido a niveles tróficos superiores, y prontamente regenerado a la columna de agua. Debido a un estrecho acoplamiento entre los procesos de regeneración y nitrificación, la mayoría del N fue exportada de los tramos experimentales en forma de NO_3^- .

El presente estudio pone de manifiesto un rápido reciclado de N en ríos mediterráneos. Además, los resultados indican que la eliminación de NO_3^- vía desnitrificación puede verse incrementada frente a la retención de NO_3^- vía captación asimilatoria en ríos mediterráneos heterotróficos, alterados por la actividad humana, y caracterizados por concentraciones altas de NO_3^- y bajas de DO.

Nitrate retention and removal in Mediterranean streams with contrasting land uses: a ^{15}N tracer study

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Abstract. We used ^{15}N -labelled nitrate (NO_3^-) additions to investigate nitrogen (N) cycling at the whole-reach scale in three Mediterranean streams subjected to contrasting land uses (i.e. forested, urban and agricultural). Our aim was to examine: (i) the magnitude and relative importance of NO_3^- retention (i.e. assimilatory uptake), and removal, (i.e. denitrification), (ii) the relative contribution of the different primary uptake compartments to NO_3^- retention, and (iii) the regeneration, transformation and export pathways of the retained N.

The concentration of NO_3^- increased and that of dissolved oxygen (DO) decreased from the forested to the agricultural stream, with intermediate values in the urban stream. Standing stocks of primary uptake compartments were similar among streams and dominated by detritus compartments (i.e. fine and coarse benthic organic matter). In agreement, metabolism was net heterotrophic in all streams, although the degree of heterotrophy increased from the forested to the agricultural stream. The NO_3^- uptake length decreased along this gradient, whereas the NO_3^- mass-transfer velocity and the areal NO_3^- uptake rate were highest in the urban stream. Denitrification was not detectable in the forested stream, but accounted for 9% and 68% of total NO_3^- uptake in the urban and the agricultural stream, respectively. The relative contribution of detritus compartments to NO_3^- assimilatory uptake was highest in the forested and lowest in the agricultural stream. In all streams, the retained N was rapidly transferred to higher trophic levels and regenerated back to the water column. Due to a strong coupling between regeneration and nitrification, most retained N was exported from the experimental reaches in the form of NO_3^- .

This study evidences fast N cycling in Mediterranean streams. Moreover, results indicate that permanent NO_3^- removal via denitrification may be enhanced over temporary NO_3^- retention via assimilatory uptake in heterotrophic human-altered streams characterized by high NO_3^- and low DO concentrations.

1 Introduction

Biogeochemists have traditionally viewed fluvial ecosystems as relatively inactive conduits that transport nutrients from the land to the sea. However, streams and rivers have the ability to store, transform and remove nutrients during downstream transport, which results in variations in the form and amount of nutrients delivered to downstream ecosystems (Alexander et al., 2000; Peterson et al., 2001; Mulholland et al., 2008).

Nitrate (NO_3^-), the most abundant form of dissolved inorganic nitrogen (N) found in freshwaters, can be temporarily retained or permanently removed during downstream transport (Bernot and Dodds,

2005). Retention of NO_3^- occurs via assimilatory uptake by stream benthic organisms, often referred to as primary uptake compartments (i.e. those compartments that take up dissolved nutrients directly from the water column). The relative contribution of autotrophs (i.e. algae, macrophytes, and bryophytes) and heterotrophs (i.e. bacteria and fungi) to assimilatory uptake is influenced by the availability of nutrients, light and organic matter (Webster et al., 2003). The NO_3^- assimilated by the primary uptake compartments is transferred to consumers at higher trophic levels and regenerated back to the water column as ammonium (NH_4^+) via mineralization and excretion. As it is transported downstream, the released NH_4^+ is again taken up by the biota or

transformed to NO_3^- via nitrification, thus completing a whole cycle or spiral (Newbold, 1996). Removal of NO_3^- occurs via denitrification, a microbial dissimilatory process in which dissolved NO_3^- is reduced to gaseous forms of N that are permanently lost from the ecosystem (Seitzinger et al., 1988). Denitrification in streams takes place in the sediments, and is generally controlled by the availability of oxygen, organic carbon and NO_3^- (Holmes et al., 1996; Kemp and Dodds, 2002). This simple perspective of the processes involved in the fate of NO_3^- in streams can be further complicated by alternative dissimilatory uptake processes (Burgin and Hamilton, 2007), and abiotic N adsorption and burial (Bernot and Dodds, 2005).

Nitrogen pollution in aquatic ecosystems can cause acidification, eutrophication, and impairment of aquatic organisms, as well as problems to human health and economy (Camargo and Alonso, 2006). Understanding N retention and removal in streams is important because these processes can change the form and amount of N delivered to downstream and coastal ecosystems (Alexander et al., 2000; Alexander et al., 2007). Nitrate retention and removal differentially affect downstream N export; thus, it is critical to understand these pathways and to evaluate their relative contribution to total NO_3^- uptake. In addition, we know little about the role of streams as a source of N_2O , an intermediary product of denitrification that is considered an important greenhouse gas and a catalyzer of ozone destruction (Beaulieu et al., 2008).

Nitrogen uptake in streams has been mostly studied using nutrient enrichment experiments (Stream Solute Workshop, 1990; Webster and Valett, 2006), whereas particular processes such as nitrification and denitrification have been mainly investigated using incubation experiments (Holmes et al., 1996; Kemp and Dodds, 2002; Inwood et al., 2005). Both approaches, however, show evident methodological constraints. For instance, nutrient additions may underestimate actual uptake rates at ambient levels (Mulholland et al., 2002; Dodds et al., 2002), whereas measurements from incubation experiments are difficult to extrapolate to the whole-

reach scale (Dodds et al., 2000). More recently, N retention and removal pathways have been explored using ^{15}N tracer addition techniques, which allow quantifying processes simultaneously occurring in a stream at the whole-reach scale and at ambient conditions, while avoiding the limitations of enrichment experiments and incubations. Various studies have used ^{15}N tracer additions to investigate N retention and removal in reference headwater streams (e.g. Peterson et al., 1997; Hall et al., 1998; Peterson et al., 2001; Mulholland et al., 2004). More recent studies have expanded our knowledge on these processes using ^{15}N tracer additions in streams influenced by various human activities (e.g., Boehlke et al., 2004; Grimm et al., 2005; Bernot et al., 2006; Simon et al., 2007; O'Brien et al., 2007; Mulholland et al., 2008). Although results from these studies have emphasized the importance of N retention and removal in streams, both the relative contribution of each of these pathways to total N uptake and the regeneration, transformation and export of the retained N remain widely unknown. Moreover, most previous studies have been conducted in streams from temperate regions of North America, yet studies in different bioclimatic regions are needed for a global understanding of N dynamics in streams.

To our knowledge, this is the first study reporting results from ^{15}N tracer additions in Mediterranean streams. Catchments in the Mediterranean region are primarily characterized by a long history of human development and a natural deficit of water resources, which makes them highly susceptible to human impacts (Alvarez-Cobelas et al., 2005). Because these catchments are relatively small and located next to the sea, headwater streams are of greatest importance for the retention and removal of N that is quickly exported to downstream coastal ecosystems.

In this study, we used $^{15}\text{NO}_3^-$ tracer additions to investigate N cycling at the whole-reach scale in three headwater streams situated within the same Mediterranean catchment but subjected to contrasting land uses. Specifically, we aimed to examine i) the magnitude and relative importance of NO_3^- retention (i.e.

assimilatory uptake), and removal, (i.e. denitrification), ii) the relative contribution of the different primary uptake compartments to NO_3^- retention, and iii) the regeneration, transformation and export pathways of the retained N.

2 Methods

2.1 Study sites

This study was conducted in the catchment of the river La Tordera (Catalonia, NE Spain), with an area of 868.5 km² and dominated by siliceous geology. Climate in this region is typically Mediterranean, with warm, dry summers and mild, humid winters. Within this catchment, we selected an experimental reach with a relatively unmodified channel from each of three streams bordered by contrasting land use types (Table 1). From the stream Santa Fe (hereafter referred to as the forested stream) we selected a reach located within a beech (*Fagus sylvatica*) forest in the Montseny Natural Protected Area. From the stream Gualba (hereafter referred to as the urban stream) we selected a reach located in a park of a town with ~1000 inhabitants. Finally, from the stream Sant Celoni (hereafter referred to as the agricultural stream) we selected a reach bordered by a mixture of olive tree (*Olea europaea*) and pine (*Pinus halepensis*) plantations. Although this agricultural reach is intermittent, water flow did not cease until after the end of the experiments. In the forested stream riparian vegetation was dominated by beech, with some stems of common elder (*Sambucus nigra*) and a poorly developed herbaceous understory. In the other streams riparian vegetation consisted mainly of alder (*Alnus glutinosa*) and sycamore (*Platanus hispanica*), with a denser herbaceous understory, especially in the agricultural stream. The experiments were conducted from July to August 2004, a period characterized by base flow and full riparian cover.

2.2 Field methods

Tracer $^{15}\text{NO}_3^-$ addition experiments followed procedures adapted from Mulholland et al. (2004). A solution of $^{15}\text{NO}_3^-$ (as 99% enriched $\text{K}^{15}\text{NO}_3^-$) in conjunction with chloride (as NaCl) was injected at the top of the reach at a constant

rate (20 mL min⁻¹) using a Masterflex (Vernon Hills, Illinois, USA) L/S battery-powered peristaltic pump. Additions started at midnight (00:00) and lasted for ~12h. The amount of $\text{K}^{15}\text{NO}_3^-$ added to each stream was calculated to produce a target $\delta^{15}\text{N}$ enrichment of 10000 ‰ of the NO_3^- in the stream water.

The passage of the conservative tracer (i.e. chloride) was automatically recorded at the bottom of the reach every 10 s using a WTW (Weilheim, Germany) 340i portable conductivity meter connected to a Campbell Scientific (Logan, Utah, USA) data logger. We measured conductivity and collected water samples at six stations along the reach before the addition (i.e. background sampling). During the addition, we measured conductivity and collected water samples at the same stations at 06:00 (i.e. first plateau sampling) and 11:00 (i.e. second plateau sampling) to determine NO_3^- uptake parameters for night and day, respectively. One day after the addition was stopped (i.e. post-24 h sampling), we measured conductivity and collected water samples at the same stations and an additional upstream station. In the agricultural stream, we decided to bring forward the post-24 h sampling to 7 h after the end of the addition to avoid an imminent rain event. Nonetheless, conductivity along the reach was at background levels at the time of sampling, indicating that the conservative tracer had already left the experimental reach. Finally, we measured conductivity and collected water samples at the upstream and the most downstream station two days (i.e. post-48 h sampling) and three days (i.e. post-72 h sampling) after the addition was stopped.

All water samples were immediately filtered through ashed Albet (Barcelona, Spain) FVF glass fiber filters. Filtered samples for nutrient chemistry (40 mL, two replicates per station) and $^{15}\text{NO}_3^-$ for isotopic analysis (0.5 L, two replicates per station) were stored on ice in the field, and then refrigerated at 4 °C or frozen in the laboratory until further processing and analysis. Following procedures adapted from Holmes et al. (1998), filtered samples for $^{15}\text{NH}_4^+$ analysis (3 to 4 L, one sample per station) were amended with 3.0 g of MgO , 50 g of NaCl, and a Teflon filter

packet, and brought to the laboratory for further processing. The Teflon filter packet was constructed by sealing a 1-cm diameter ashed Whatman (Kent, UK) GF/D glass fiber filter, acidified with 25 μL of 2.5 M KHSO_4 , within a packet made of two pieces of Teflon plumbing tape.

Background and plateau samples of dissolved $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ (two replicates per station) were collected at 10 stations along the reach coinciding in time with water sampling following procedures by Hamilton and Ostrom (2007). Water samples (40 mL, two replicates per station) were collected in 60-mL plastic syringes fitted with stopcocks, taking care not to include bubbles in the samples. With the sample syringe submersed under water, 20 mL of high purity He were added to each syringe. Syringes were then shaken for ~ 10 min to allow equilibration of the N-gas into the He space. Afterwards, the He space was collected in evacuated 12-mL Labco (High Wycombe, UK) Type 3 exetainers. The exetainers were stored in water-filled centrifuge tubes and sent to the stable isotope laboratory.

Standing stocks of primary uptake compartments were estimated using patch-specific samplings at the seven (six downstream + one upstream) water sampling stations two days after the end of the $^{15}\text{NO}_3^-$ addition. Coarse benthic organic matter (CBOM), mostly consisting of leaves and small wood sticks, and water-submerged alder roots were sampled by collecting all material found within a 0.04 m^2 metal frame. Surface and sub-surface fine benthic organic matter (FBOM) was sampled using a syringe to collect an aliquot of the material suspended by manual agitation of the surface (~ 1 cm) and subsurface (~ 3 to 5 cm) sediment in a known volume within a 0.05 m^2 plastic corer. Collected FBOM samples were filtered onto ashed pre-weighted FVF glass fiber filters. We sampled suspended particulate organic matter (SPOM) by filtering a known volume of water onto ashed pre-weighted FVF glass fiber filters. Bryophytes and filamentous algae were sampled by scraping material found within a 0.002 m^2 plastic frame. Epilithon was sampled by scraping randomly collected cobbles and filtering onto ashed pre-

weighted FVF glass fiber filters. The cobble surface was estimated by covering with aluminum foil and applying a weight to area relationship. Composite samples for ^{15}N analysis (three to six replicates per station) were collected from all compartments at the seven water sampling stations during the post-24 h sampling using the same methods as for standing stock sampling. To follow the transfer of the retained N to higher trophic levels, we collected composite samples for the analysis of ^{15}N in the freshwater limpet *Ancylus fluviatilis* (~ 10 individuals per station), a scraper commonly found on rocks in these streams. Limpets were collected at the seven water sampling stations during the post-24 h sampling. Additional SPOM samples for ^{15}N analysis were collected from the upstream and the most downstream station during the post-48 h and the post-72 h sampling.

Whole-stream metabolism was estimated in each stream on the day of the $^{15}\text{NO}_3^-$ addition using the open-system, single station approach (Bott, 2006). Dissolved oxygen (DO) concentration and temperature were recorded at the bottom of the study reach at 10-min intervals during a 24 h period with a WTW (Weilheim, Germany) 340i portable oxygen meter. During the same period, photosynthetically active radiation (PAR) was measured every 10 min with a Skye (Powys, UK) SKP215 quantum sensor connected to a Campbell Scientific (Logan, Utah, USA) data logger.

On the day before the addition we determined the wetted width (w) and percent reach coverage of the different substrate types using cross-sectional transects located every 25 m along the reach. The percent reach coverage of stream habitats (riffle, run, and pool) and primary uptake compartments was visually estimated on the same date at 5-m intervals along the reach.

2.3 Laboratory methods

Stream water samples were analyzed for NO_3^- , NO_2^- , and soluble reactive phosphorus (SRP) concentrations on a Bran+Luebbe (Nordersted, Germany) TRAACS 2000 Autoanalyzer. The concentration of NH_4^+ was analyzed on a Skalar (Breda, The Netherlands) San⁺ Auto-

analyzer. All nutrient analyses were performed following standard colorimetric methods (APHA, 1995). The concentration of DOC was determined on a Shimadzu (Tokyo, Japan) TOC-VCSH analyzer.

The ^{15}N content of stream water NO_3^- was determined using an adaptation of the method by Sigman et al. (1997). Dissolved NH_4^+ was removed and stream water samples were concentrated (volume depending on the N content of each sample) by adding 3.0 g of MgO and 5.0 g of NaCl and boiling. Resulting samples (~0.1 L) were then transferred to acid-washed plastic bottles to which 0.5 g MgO and 0.5 g Devarda's alloy and a Teflon filter packet were added. The samples were incubated at 60 °C for 48 h to reduce NO_3^- to NH_4^+ , and then placed on a shaker for seven days to allow for diffusion of ammonia (NH_3) onto the acidified filter. Water samples for the determination of the ^{15}N content of stream water NH_4^+ , to which the MgO, NaCl, and Teflon filter packet had already been added in the field, were incubated in a shaker at 40 °C for three to four weeks to allow for diffusion of NH_3 onto the acidified filter. At the end of the processing, filters from $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ samples were removed from the bottles, placed in scintillation vials, dried in a desiccator for four days, encapsulated in tins, and sent to the stable isotope laboratory. Various sets of blanks and standards of known concentration for $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ samples were run for each addition.

Samples for standing stocks of all primary uptake compartments were oven-dried at 60 °C, weighted to the nearest 0.1 mg on a Sartorius (Goettingen, Germany) MC1 analytical balance, and combusted at 450 °C to estimate ash free dry mass (AFDM). To calculate whole-reach standing stocks (g AFDM m^{-2}) of the different primary uptake compartments, we corrected the patch-specific estimates by the percent reach coverage of each compartment. Bryophytes (only the tips), filamentous algae, root, and CBOM samples for ^{15}N analysis were oven-dried at 60 °C, ground to a fine powder, weighted to the nearest 0.001 mg on a Mettler-Toledo (Greifensee, Switzerland) MX5 microbalance, encapsulated in tins, and sent to the stable isotope laboratory. For FBOM,

SPOM and epilithon samples (on pre-weighted glass fiber filters), discs of a known surface area (diameter = 1 cm) were cut out from the previously oven-dried filters and processed as the rest of samples. Limpets were left in fresh stream water overnight to allow emptying their gut contents, and the fresh tissue was then separated from the shell and processed as the rest of samples.

Encapsulated samples of $^{15}\text{NO}_3^-$, $^{15}\text{NH}_4^+$, and primary uptake compartments were analyzed at the UC Davis Stable Isotope Facility (Davis, California, USA). The content (as a percent of dry mass) and the stable isotope ratios of C and N were measured by continuous flow isotope ratio mass spectrometry (20–20 mass spectrometer; PDZ Europa, Northwich, UK) after sample combustion in an on-line elemental analyzer (PDZ Europa ANCA-GSL). We estimated the C content of wood and root samples, for which the detection limit of the mass spectrometer was exceeded, as 45% of dry mass based on unpublished data from nearby streams. One set of N-gas samples was analyzed for $^{15}\text{N}_2\text{O}$ on a Finnigan (Sacramento, California, USA) MAT 251 Isotope Ratio Mass Spectrometer at the same stable isotope laboratory. The other set of N-gas samples was analyzed for $^{15}\text{N}_2$ on a multi-collector CV Instruments (Manchester, UK) Isoprime Mass Spectrometer at the MSU Stable Isotope Laboratory (Lansing, Michigan, USA).

2.4 Parameter calculations

Breakthrough curves of conductivity at the bottom of the reach were analyzed by visual inspection with a one-dimensional transport with inflow and storage model (OTIS; Runkel, 1998) to estimate stream hydraulic characteristics, including discharge, cross-sectional area (A) and cross-sectional transient storage zone area (A_s). Mean reach depth was calculated as A/w . Water transient storage was reported as the ratio A_s/A .

We calculated daily rates of gross primary production (GPP) and ecosystem respiration (ER) by integrating the DO measurements at the bottom of the reach during the 24 h period following Bott (2006). Percent DO saturation was

estimated using DO and temperature together with a standard altitude-air pressure algorithm to correct for site altitude. Reaeration coefficients were estimated based on the surface renewal model (Owens, 1974). Instantaneous respiration rates at night were extrapolated to 24 h to estimate ER. We computed GPP by integrating the difference between the measured net DO change (corrected by the reaeration flux) and the extrapolated daytime respiration. GPP and ER were multiplied by the mean reach depth to obtain areal estimates.

The fractional NO_3^- uptake rate per unit distance ($k_W \text{NO}_3^-$; m^{-1}) was estimated from the regression of the ln-transformed tracer $^{15}\text{NO}_3^-$ flux versus distance downstream with data from the plateau samplings (Mulholland et al., 2004). The tracer $^{15}\text{NO}_3^-$ flux at each station was calculated by multiplying the background-corrected $^{15}\text{NO}_3^-$ concentration by discharge at each station. Station-specific discharge was calculated from the dilution of the conservative tracer along the reach. The inverse of $k_W \text{NO}_3^-$ is the NO_3^- uptake length ($S_w \text{NO}_3^-$; m), which was converted to the NO_3^- mass transfer velocity ($V_f \text{NO}_3^-$; cm s^{-1}) as the stream specific discharge (Q/w) divided by $S_w \text{NO}_3^-$. The areal NO_3^- uptake rate ($U \text{NO}_3^-$; $\mu\text{g m}^2 \text{s}^{-1}$), the mass of NO_3^- taken up from the water column per unit streambed area and time, was calculated as $V_f \text{NO}_3^-$ multiplied by the mean ambient NO_3^- concentration. Data from the first and the second plateau sampling were used to calculate night and day $k_W \text{NO}_3^-$, respectively. Differences between day and night $k_W \text{NO}_3^-$ were tested with a *t*-test for the comparison of slopes (Fowler and Cohen, 1990). Mean $k_W \text{NO}_3^-$ for each stream was calculated as the mean of night and day values.

The fractional denitrification rates per unit distance via N_2 ($k_{DEN} \text{N}_2$; m^{-1}) and N_2O ($k_{DEN} \text{N}_2\text{O}$; m^{-1}) production were estimated by separately fitting the longitudinal tracer flux of $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ from the plateau samplings to the denitrification model proposed by Mulholland et al. (2004) using the Microsoft (Redmond, Washington, USA) Excel 2003 Solver tool. The tracer flux of $^{15}\text{N}_2$ or $^{15}\text{N}_2\text{O}$ at each station was calculated

by multiplying the background-corrected $^{15}\text{N}_2$ or $^{15}\text{N}_2\text{O}$ concentration by discharge at each station. Only data from stations with a significant tracer ^{15}N in N_2 or N_2O (defined as $\delta^{15}\text{N}$ values greater than the upper 97.5% confidence interval of background values) were used (Mulholland et al., 2008). The reaeration rates of N_2 and N_2O were estimated based on the surface renewal model (Owens, 1974) correcting for the respective Schmidt numbers. Total fractional denitrification rate per unit distance ($k_{DEN} \text{Total}$; m^{-1}) was calculated as the sum of $k_{DEN} \text{N}_2$ and $k_{DEN} \text{N}_2\text{O}$. The denitrification length (S_{DEN} ; m), the denitrification mass transfer velocity (V_{fDEN} ; cm s^{-1}) and the areal denitrification rate (U_{DEN} ; $\mu\text{g N m}^{-2} \text{s}^{-1}$) were calculated from k_{DEN} as previously indicated for $k_W \text{NO}_3^-$. Data from the first and the second plateau sampling were used to calculate night and day k_{DEN} , respectively. Mean k_{DEN} for each stream was calculated as the mean of night and day values.

The compartment-specific NO_3^- assimilatory uptake rate of each primary uptake compartment at each station was calculated as the reach-weighted mass of ^{15}N tracer per m^2 of each compartment divided by the time of the addition (~ 12 h) and the fraction of ^{15}N in the stream water NO_3^- flux. The areal NO_3^- assimilatory uptake rate by all primary uptake compartments together (U_{BIO} ; $\mu\text{g N m}^{-2} \text{s}^{-1}$) was calculated as the sum of the mean compartment-specific NO_3^- assimilatory uptake rates. We used the mean reach NO_3^- concentration and Q/w to convert U_{BIO} to the assimilatory uptake mass transfer velocity (V_{fBIO}), the assimilatory uptake length (S_{BIO}) and the fractional assimilatory uptake rate per unit distance (k_{BIO}). Finally, we estimated the proportion of the total NO_3^- assimilatory uptake (i.e. the difference between $k_W \text{NO}_3^-$ and k_{DEN}) accounted for by k_{BIO} .

We calculated the N turnover rate (s^{-1}) for each primary uptake compartment by dividing the compartment-specific NO_3^- assimilatory uptake rate ($\mu\text{g N m}^{-2} \text{s}^{-1}$) by the mass of N per m^2 of each compartment. This measure allows for comparisons of the role of the different primary uptake compartments in N regeneration. However, we should take this measurement as an

approximation to real N turnover for several reasons: this measurement does not take into account uptake of dissolved N forms other than NO_3^- , the additions were not long enough to reach a steady state, and much of the pool of N, especially in the CBOM compartments, is not involved in N cycling.

Because the post-24 h longitudinal tracer fluxes of $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ showed a similar tendency, the fractional N regeneration rates per unit time via NH_4^+ release (k_{AM} ; s^{-1}) and its subsequent transformation to NO_3^- via nitrification (K_{NIT} ; s^{-1}) were estimated by separately fitting both fluxes to an ammonification-nitrification model, based on the nitrification model proposed by Mulholland et al. (2000). We used the rate of decline in total biomass ^{15}N with distance as the N source. The tracer flux of $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ at each station was calculated by multiplying the background-corrected $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ concentration by discharge at each station. Because NH_4^+ is expected to be taken up preferentially over NO_3^- , we optimized a solution for k_{AM} with the Solver Tool using the condition of a fractional NH_4^+ uptake rate ($k_W \text{NH}_4^+$) $\leq k_W \text{NO}_3^-$. To estimate K_{NIT} , the mean $k_W \text{NO}_3^-$ value calculated from the $^{15}\text{NO}_3^-$ decline at plateau was used. We assumed that all labeled $^{15}\text{NO}_3^-$ at the post-24 h sampling must originate from nitrification of the labeled $^{15}\text{NH}_4^+$ released by the biota; thus the total fractional regeneration rate per unit distance (k_{REG} ; s^{-1}) was calculated as the sum of k_{AM} and k_{NIT} .

Export of the added ^{15}N from the experimental reach as NO_3^- , NH_4^+ , and SPOM was calculated using data from the post-24 h, post-48 h, and post-72 h samplings. The total export tracer ^{15}N flux for each sampling date was estimated as the sum of NO_3^- , NH_4^+ , and SPOM ^{15}N fluxes. A linear regression was fitted to the log-transformed export tracer ^{15}N flux versus time of sampling. The slope of this regression allows the comparison among streams of the velocity at which the reach was losing or exporting the retained N.

3 Results

3.1 Physical and chemical characteristics

Physical and chemical characteristics of the study streams during the $^{15}\text{NO}_3^-$ additions are summarized in Table 1. Selected reaches were riffle-run dominated and cobbles were the most abundant substrate type. Hydrological characteristics were typical of the summer base flow period in all streams, but the agricultural stream showed the lowest discharge, velocity, width and depth. Transient water storage was generally low, but higher in the forested stream than in the other streams. The lower temperature in the forested stream was due to its location at a higher altitude. Solar radiation (as PAR) was generally low and similar among streams reflecting the influence of the relatively well developed riparian canopies. Dissolved inorganic nitrogen (DIN) concentration, predominantly in the form of NO_3^- , was moderately low in the forested, intermediate in the urban and highest in the agricultural stream. Conversely, the daily mean DO concentration showed the inverse pattern among streams. The concentration of SRP was highest in the agricultural stream, whereas both conductivity and DOC concentration were highest in the urban stream. Physical and chemical characteristics were relatively constant during the experiments, although diel changes (i.e. lower discharge and higher temperature during the day) were observed, especially in the agricultural stream.

3.2 Stream metabolism and standing stocks

Results from stream metabolism measurements showed the importance of heterotrophic activity in these well shaded streams (Table 1). Both GPP and ER increased from the forested to the agricultural stream. All streams, however, were net heterotrophic (GPP:ER ratio < 1), and the degree of heterotrophy increased (i.e. the GPP:ER ratio decreased) from the forested to the agricultural stream.

The total standing stock of primary uptake compartments was similar among streams and dominated by detritus compartments (i.e. FBOM and CBOM), in agreement with the metabolism results (Table 2). The standing stock of FBOM, especially the subsurface fraction, was the largest compartment in all streams. Leaves were the largest CBOM fraction in the

forested and urban stream, whereas the wood fraction was larger in the agricultural stream. The standing stock of compartments dominated by primary producers, such as epilithon, bryophytes (only found in the forested stream), and filamentous algae (only found in the forested and urban streams), as well as the standing stock of submersed alder roots (only found in the urban and agricultural streams), were relatively small. In all streams, the %N content was highest for filamentous algae followed by roots, bryophytes, and leaves, whereas the remaining compartments showed an N content $\leq 2\%$ (Table 2). The C:N ratio was higher for detritus compartments and alder roots than for compartments dominated by primary producers (bryophytes, filamentous algae, and epilithon) (Table 2). For each compartment, the C:N ratio consistently decreased from the forested to the agricultural stream.

3.3 Nitrate removal and retention pathways

Results from the ^{15}N tracer additions showed considerable variation in total NO_3^- uptake among streams (Fig. 1, Table 3). Mean $k_w \text{NO}_3^-$ increased (i.e. mean $S_w \text{NO}_3^-$ decreased) from the forested to the agricultural stream. However, mean $V_f \text{NO}_3^-$ and mean $U \text{NO}_3^-$ were highest in the urban stream. Significant differences in NO_3^- uptake between day and night were only found in the agricultural stream, in which $k_w \text{NO}_3^-$ was three-fold higher during the day than during the night (Fig. 1).

The $^{15}\text{N}_2$ and $^{15}\text{N}_2\text{O}$ tracer fluxes observed in the urban and the agricultural stream were relatively well fitted by the denitrification model (Fig. 2), whereas no evidence of denitrification was observed in the forested stream. Mean k_{DEN} increased from the forested stream, in which it was below detection, to the urban and agricultural streams, in which denitrification accounted for 9% and 68% of NO_3^- uptake, respectively (Table 3). Similarly, both mean V_{DEN} and mean U_{DEN} increased along this gradient. Denitrification tended to be higher at day than at night in the two streams where denitrification was measurable (Fig. 2). Overall, N_2O production accounted for $< 3\%$ of total denitrification (Table 3).

However, the ^{15}N tracer was only detected in the form of N_2O in the urban stream at night.

The ^{15}N tracer was detected in all primary uptake compartments collected during the experiments. However, in the urban stream k_{BIO} accounted for only 15% of the total assimilatory uptake calculated from the difference between $k_w \text{NO}_3^-$ and k_{DEN} , whereas it accounted for 92% and 100% in the forested and agricultural stream, respectively (Table 3). U_{BIO} was similar among streams, but the relative importance of the different primary uptake compartments varied considerably among streams (Fig. 3). The contribution of detritus compartments to NO_3^- uptake decreased from the forested to the agricultural stream. Conversely, the contribution of epilithon and alder roots showed the opposite pattern, together accounting for up to 60% of U_{BIO} in the agricultural stream. The CBOM was relatively unimportant with respect to FBOM except in the urban stream, in which leaves accounted for 26% of U_{BIO} . Both bryophytes (only found in the forested stream) and filamentous algae (only found in the forested and urban streams) represented $< 0.1\%$ of U_{BIO} .

Epilithon showed the highest N turnover rate in all streams, exceeded only by alder roots in the urban stream (Fig. 3). Where present, bryophytes and filamentous algae presented the lowest N turnover rates. Detritus compartments tended to show intermediate N turnover rates in all streams.

The ^{15}N tracer was detected in primary consumers (i.e. *Ancylus fluviatilis* limpets) in all streams (Fig. 4). The $\delta^{15}\text{N}$ signal of the limpets tended to follow the $\delta^{15}\text{N}$ signal of epilithon along the experimental reach, especially in the agricultural stream. The mean proportion of $\delta^{15}\text{N}$ in epilithon over $\delta^{15}\text{N}$ in consumers was similar among streams ranging from 10 in the forested to 18 in the agricultural stream.

3.4 Nitrogen regeneration pathways

The ^{15}N tracer was detected in the form of NH_4^+ and NO_3^- in all streams during the post-24 h sampling (Fig. 5). The longitudinal data of $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ were well fitted by the ammonification-

nitrification model, especially in the forested and urban streams. The Solver tool gave the best fit for k_{AM} when $k_W \text{NH}_4^+$ and $k_W \text{NO}_3^-$ were identical. In all streams, k_{NIT} was over one order of magnitude greater than k_{AM} . The highest k_{AM} was found in the urban and the lowest in the agricultural stream, whereas k_{NIT} was highest in the agricultural and lowest in the urban stream. As a result, k_{REG} was highest in the agricultural and lowest in the urban stream, with k_{NIT} accounting for 95% to 100% of k_{REG} in all streams (Table 4).

3.5 Nitrogen export pathways

Results from the post-addition samplings at the most downstream station showed that the regenerated N was being exported out of the experimental reaches (Fig. 6). The tracer ^{15}N flux as NO_3^- accounted for > 90% of exported ^{15}N in all reaches except in the urban stream, in which NH_4^+ became the most important ^{15}N export pathway over time, accounting for up to 96% of total export 72 h after the addition was stopped. Conversely, in the forested and agricultural stream the tracer ^{15}N flux as NH_4^+ accounted for < 9% of total ^{15}N export throughout the experiment. SPOM was a relatively unimportant export pathway, accounting for < 0.4% of total ^{15}N export in all study reaches. The slope of the regression of total export versus time since the end of the addition increased from the forested to the agricultural stream.

4 Discussion

4.1 Total NO_3^- uptake

The study streams were moderately efficient in taken up NO_3^- , with $S_W \text{NO}_3^-$ ranging from a few hundred to a few thousand meters. Values of $V_f \text{NO}_3^-$, a measure of nutrient demand that is commonly used to compare uptake among streams because it corrects for differences in stream size (Webster and Valett, 2006), were in the lower range of values reported from 72 streams subjected to various land uses across USA and Puerto Rico (Mulholland et al., 2008). Our $V_f \text{NO}_3^-$ values, however, were generally above those reported from streams affected by wastewater treatment plant inputs (e.g., Ruggiero et al., 2006; Gücker and Pusch, 2006) and below those reported from

reference headwater streams (e.g., Davis and Minshall, 1999; Simon et al., 2005; Hoellein et al., 2007).

Significant diel changes in NO_3^- uptake were only found in the agricultural stream, which showed higher $k_W \text{NO}_3^-$ at day than at night. Previous studies in reference streams showed similar findings and attributed higher NO_3^- uptake during daylight to higher photoautotrophic activity (Fellows et al., 2006; Mulholland et al., 2006). This was likely the case in the agricultural stream, which showed the highest GPP among the study streams. In addition, the agricultural stream showed higher temperature and lower discharge during the day, a common pattern in Mediterranean streams in summer due to high evapotranspiration by riparian trees (Butturini et al., 2002). These diel changes in temperature and discharge likely contributed to higher $k_W \text{NO}_3^-$ at day by respectively enhancing metabolic activity (Kaplan and Bott, 1989) and providing a greater ratio of streambed area to water volume (Butturini and Sabater, 1998).

Although the number of streams in our study was limited, we expected $S_W \text{NO}_3^-$ and $U \text{NO}_3^-$ to increase and $V_f \text{NO}_3^-$ to decrease along the gradient of increasing NO_3^- concentration due to saturation processes (O'Brien et al., 2007). However, the observed pattern was nearly opposite: $S_W \text{NO}_3^-$ decreased along the NO_3^- concentration gradient, whereas both $V_f \text{NO}_3^-$ and $U \text{NO}_3^-$ were higher in the urban than in the other streams. The considerably lower discharge in the agricultural stream in comparison to the other streams may have contributed to the observed pattern in $S_W \text{NO}_3^-$ by increasing the contact surface between the sediments and the water column. However, results from $V_f \text{NO}_3^-$ and $U \text{NO}_3^-$, which correct $S_W \text{NO}_3^-$ for differences in stream size and NO_3^- concentration, respectively (Webster and Valett, 2006), indicate that other factors were influencing NO_3^- uptake in these streams. The notably lower water temperature in the forested stream may have contributed to the low GPP and ER, which may in turn have reduced biological N demand in this stream (Fellows et al., 2006). Based on temperature and metabolism rates, however, we would have

expected a higher N demand in the agricultural stream than in the urban stream, which was not observed. A relatively greater standing stock of primary uptake compartments and higher solar radiation in the urban stream may partially explain its higher NO_3^- demand relative to the agricultural stream.

Results from a previous study using NH_4^+ enrichments during spring, at conditions of higher discharge and light availability than in the present study, showed that, in contrast to NO_3^- , the efficiency to take up NH_4^+ decreased from the forested to the agricultural stream (von Schiller et al., 2008). In addition, values of V_f for NH_4^+ were greater than those found for NO_3^- in this study, indicating that demand for NH_4^+ is higher than demand for NO_3^- in these streams. However, a comparison of both studies indicates that the magnitude of the preference for NH_4^+ over NO_3^- may decrease in more N polluted streams with higher NO_3^- and lower DO concentrations. In these conditions, relative demand for NO_3^- may increase because it may be used as an electron acceptor in dissimilatory uptake processes such as denitrification. This is further supported by results from nutrient diffusing substrata experiments conducted in these streams during the same period as the present study (von Schiller et al., 2007).

4.2 Comparison of NO_3^- removal and retention pathways

The importance of NO_3^- retention over removal decreased from the forested to the agricultural stream along the gradient of increasing NO_3^- and decreasing DO concentrations. Assimilatory uptake accounted for all or most of total NO_3^- uptake in the forested and the urban streams, respectively, whereas denitrification dominated total NO_3^- uptake in the agricultural stream. However, similarly to $V_f \text{NO}_3^-$, values of V_{fDEN} were in the lower range of values reported from streams subjected to various land uses across USA and Puerto Rico (Mulholland et al., 2008). In that study, denitrification accounted for a median of 16% of total NO_3^- uptake and exceeded 45% of total uptake in a quarter of them (Mulholland et al., 2008). Despite a narrower NO_3^-

concentration range, denitrification was highly variable among streams in our study, accounting for 0% to 68% of total NO_3^- uptake.

The decrease in S_{DEN} and the increase in V_{fDEN} and U_{DEN} from the forested to the agricultural stream were likely due to differences in physicochemical characteristics among streams. Low temperature, DIN, and DOC, and high oxygen availability did not favor denitrification in the forested stream (Piña-Ochoa and Álvarez-Cobelas, 2006). Variation among streams in these parameters enhanced denitrification as a NO_3^- retention pathway in the urban and agricultural stream. An increase in ER and its relative importance over GPP from the forested to the agricultural stream indicates that heterotrophic activity may promote dissimilatory uptake pathways in streams (Christensen et al., 1990). In contrast to our results, Mulholland et al. (2008) showed that V_{fDEN} decreased with increasing NO_3^- concentration; however, their study covered a much wider range of NO_3^- concentrations and other stream physicochemical parameters.

Denitrification rates tended to be higher at day than at night in both streams in which denitrification was detected, this difference being more important in the urban stream. O'Brien et al. (2007) found the opposite pattern (i.e. higher denitrification at night than at day) in prairie streams located in Kansas (USA), likely due to the inhibitory effect of high algal photoautotrophic oxygen production during daylight in open-canopy streams. In contrast, our streams were well shaded and photoautotrophic activity was relatively unimportant, as indicated by the low GPP:ER ratios. Nevertheless, Mulholland et al. (2008) found no difference in denitrification between day and night, and a recent review of denitrification in aquatic ecosystems found no significant differences between light and dark conditions (Piña-Ochoa and Álvarez-Cobelas, 2006).

The main end product of denitrification was N_2 , except in the urban stream at night, when denitrification was detected only in the form of N_2O . On average, N_2O production accounted for < 3% of total stream denitrification,

supporting results from previous studies indicating that stream denitrification consumes nearly the entire N_2O intermediary (Mulholland et al., 2004; Mulholland et al., 2008; Beaulieu et al., 2008).

The proportion of total NO_3^- assimilatory uptake (i.e. the difference between $k_W \text{NO}_3^-$ and k_{DEN}) accounted for by k_{BIO} was high in the forested and the urban stream, whereas only a small proportion of total NO_3^- assimilatory uptake was accounted for by k_{BIO} in the urban stream. This underestimation was likely due to either errors in the sampling of standing stocks, alternative dissimilatory NO_3^- uptake pathways (e.g., DNRA), rapid transfer of the retained N to higher trophic levels, or high regeneration rates of the retained N (Tank et al., 2000; Burgin and Hamilton, 2007). For this reason, results from NO_3^- assimilatory uptake should be viewed with great caution, especially in the urban stream.

The relative contribution of the different primary uptake compartments to U_{BIO} varied considerably among streams. Detritus compartments, especially FBOM, dominated standing stocks and NO_3^- assimilatory uptake in the forested and urban stream as has been previously reported in studies from well-shaded streams with low autotrophic activity (e.g., Mulholland et al., 2000; Tank et al., 2000). Despite its small standing stocks, epilithon was the most important NO_3^- uptake compartment in the agricultural stream and also contributed notably to NO_3^- uptake in the other streams. Previous studies have emphasized the high N uptake capacity of epilithon even in well-shaded streams (e.g., Tank et al., 2000; Merriam et al., 2002). Although leaves were an important standing stock compartment in both the forested and the urban stream, they contributed considerably more to NO_3^- uptake in the urban stream. An important part of leaf litter found in the urban stream was fresh due to the summer leaf fall, which is characteristic of Mediterranean streams affected by hydric stress (Sabater et al., 2001). Conversely, leaves found in the forested stream were old (i.e. from the previous autumn leaf fall) and highly decomposed at the time of study (von

Schiller, personal observation). This observation was confirmed by the C:N ratios of leaves, which were higher in the forested than in the urban stream. Small wood standing stocks characteristic of Mediterranean streams with young riparian vegetation (Sabater et al., 2001) were reflected in low uptake by this detritus compartment. Similarly, autotrophic organisms such as bryophytes and filamentous algae showed small standing stocks and low uptake rates. In these streams, filamentous algae were in a decaying state during the experiments and are usually more abundant in spring, when light availability is higher, whereas bryophytes are relatively scarce during the whole year (von Schiller, personal observation).

Despite their small standing stock, submersed alder roots were an important uptake compartment, especially in the agricultural stream. Because we did not sample non-submersed parts of the alders, we could not determine if NO_3^- was taken up by the trees or by biofilms growing on the root surface. Even if trees were taking up NO_3^- , we would not know whether it was directly for nutrient use or indirectly through water transport. However, the role of riparian vegetation in stream N retention has been previously demonstrated using long-term ^{15}N tracer additions in both arid (Schade et al., 2005) and temperate streams (Ashkenas et al., 2004). Results indicate that riparian vegetation may not only act as an effective filter for nutrients moving from the surrounding catchment to the stream (Naiman et al., 1997), but it may also contribute to nutrient movement in the opposite direction by taking up and retaining dissolved nutrients that are being transported in the stream water, thereby increasing whole-stream N retention. This stream-riparian linkage may be enhanced in arid and semi-arid regions, where riparian vegetation is strongly limited by water availability.

4.3 Fate of retained NO_3^-

The tracer ^{15}N was found in the limpets growing on the epilithon only a few hours after the addition was stopped, indicating that the NO_3^- assimilated by primary uptake compartments was being

rapidly transferred to higher trophic levels, as has been reported in other streams (e.g., Mulholland et al., 2000; Dodds et al., 2000). The similar longitudinal pattern in the ^{15}N signal along the experimental reaches confirms that epilithon was the most likely food source for the limpets in these streams (Tank et al., 2000; Ashkenas et al., 2004). Moreover, a similar proportion of $\delta^{15}\text{N}$ between epilithon and limpets indicates that the assimilated N was transferred with a similar efficiency in all streams.

Labeled NH_4^+ released from stream benthic compartments was found shortly after the end of the addition, indicating that regeneration of the assimilated NO_3^- was rapid in these streams. This NH_4^+ was likely released via excretion by aquatic organisms and mineralization of organic matter and labile organic molecules like aminoacids (Newbold, 1996). Total regeneration was greatest in the agricultural stream, intermediate in the forested stream, and lowest in the urban stream. Results from the N turnover rates indicate that epilithon was the primary uptake compartment that most contributed to N regeneration in these streams. The low C:N ratio of epilithon in comparison with detritus compartments, which indicates high metabolic activity and highly labile N, may explain the observed result (Dodds et al. 2000). We also expected a high N turnover in other compartments dominated by primary producers showing a low C:N ratio (i.e. filamentous algae and bryophytes). However, these compartments generally showed the lowest turnover in the study streams, likely due to their decaying state. In contrast, alder roots showed a high N turnover rate, but this probably reflected N allocation from the roots to other parts of the plant rather than N regeneration back to the water column.

Most released NH_4^+ was rapidly transformed to NO_3^- via nitrification within the experimental reach, indicating that NH_4^+ release and nitrification were strongly coupled in these streams. However, the proportion of NH_4^+ transformed differed among streams, being lowest in the urban stream. The produced NO_3^- was then transiently retained again via assimilatory uptake by primary uptake compartments,

permanently removed via denitrification, or exported downstream. As a result of rapid nitrification, most retained N was exported from the study reaches in the form of NO_3^- . Export as NH_4^+ was more important in the urban than in the other streams due to the lower nitrification rate in the urban stream. Export as SPOM was relatively unimportant in the study streams, as has been observed in previous studies (Tank et al., 2000; Ashkenas et al. 2004). However, we must highlight that SPOM is expected to be retained for a longer time within the reach because it is subjected to more physical retention mechanisms than DIN. In fact, our results indicate that the proportion of retained N exported as SPOM tended to increase with time after the addition was stopped. We did not measure export as dissolved organic nitrogen (DON), although previous studies using ^{15}N tracer additions have demonstrated that export of retained N as DON may exceed that of NH_4^+ or SPOM, but not that of NO_3^- (Merriam et al. 2002; Ashkenas et al. 2004). The slopes of the regressions of total export versus time since the end of the addition increased from the forested to the agricultural stream, indicating that the velocity by which the reach was exporting the transiently retained N increased along this gradient.

5 Conclusions

Despite relatively small differences in stream physicochemical characteristics and standing stocks of primary uptake compartments, we found large differences in N uptake, regeneration, transformation and export pathways among Mediterranean streams subjected to contrasting land uses. This study provides evidence of fast N cycling in Mediterranean streams through a strong coupling among uptake, regeneration and transformation processes. Moreover, our results indicate that permanent NO_3^- removal via denitrification may be enhanced over temporary NO_3^- retention via assimilatory uptake in heterotrophic human-altered streams characterized by high NO_3^- and low DO concentrations. Changes in the relative importance of retention and removal may substantially influence the amount and form of N delivered to downstream and coastal ecosystems. Further studies addressing N

retention, transformation and removal processes in streams located in different biomes and subjected to various human alterations are required to gain a complete understanding of N cycling and its controlling factors in fluvial ecosystems.

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Table 1. Geographical, physical, chemical, and metabolism characteristics of the stream reaches during the $^{15}\text{NO}_3^-$ additions.

	Santa Fe (forested)	Gualba (urban)	Sant Celoni (agricultural)
Geographical			
Latitude (41°N)	46' 37"	44' 02"	42' 44"
Longitude (2°E)	27' 42"	30' 17"	27' 41"
Altitude (m)	1120	168	246
Drainage area (km ²)	2.6	13.5	9.3
Reach length (m)	262	400	400
Physical			
Pool/Riffle (m ² m ⁻²)	0.19	0.40	0.28
Dominant substrate type	cobbles	cobbles	cobbles
Discharge (L s ⁻¹)	8.8	11.6	1.2
Velocity (cm s ⁻¹)	5.1	6.7	2.4
Width (m)	3.7	3.0	2.0
Depth (cm)	4.6	5.8	2.5
A_w/A	0.29	0.06	0.08
Temperature (°C)	12.2	19.7	19.8
PAR (moles m ⁻² d ⁻¹)	1.8	2.6	2.3
Chemical			
Conductivity (μS cm ⁻¹)	62	156	101
Dissolved oxygen (mg L ⁻¹)	9.0	8.2	5.7
NO ₃ ⁻ (μg N L ⁻¹)	172	394	601
NO ₂ ⁻ (μg N L ⁻¹)	2	4	3
NH ₄ ⁺ (μg N L ⁻¹)	7	9	8
SRP (μg P L ⁻¹)	16	8	22
DOC (mg L ⁻¹)	1.2	2.4	2.0
Metabolism			
GPP (g O ₂ m ⁻² d ⁻¹)	0.7	2.0	4.6
ER (g O ₂ m ⁻² d ⁻¹)	1.3	5.1	32.9
GPP:ER	0.54	0.39	0.14

Table 2. Reach-weighted standing-stock (g AFDM m⁻²), percent nitrogen (%N of dry mass), and carbon to nitrogen ratio (C:N by mass) of primary uptake compartments in the study reaches. Data reported are means with SE in parenthesis.

	Santa Fe (forested)			Gualba (urban)			Sant Celoni (agricultural)		
	AFDM	%N	C:N	AFDM	%N	C:N	AFDM	%N	C:N
Leaves	10.8 (7.8)	1.5 (0.1)	29.6 (1.7)	11.4 (2.6)	9 (0.2)	24.5 (2.6)	1.8 (0.9)	2.0 (0.02)	19.5 (0.2)
Wood	0.4 (0.2)	0.8 (0.1)	4.58 (0.8)	5.8 (0.03)	0 (0.1)	0.49 (0.1)	6.9 (4.2)	1.3 (0.05)	33.6 (0.2)
Surface FBOM	26.1 (11.6)	1.3 (0.1)	17.7 (0.8)	19.7 (5.3)	0.4 (0.02)	12.1 (0.1)	10.2 (1.6)	0.6 (0.01)	11.1 (0.05)
Subsurface FBOM	39.7 (16.4)	1.0 (0.2)	17.0 (0.4)	46.9 (16.9)	0.4 (0.1)	12.3 (0.4)	59.4 (17.7)	0.5 (0.02)	10.3 (0.1)
Epilithon	1.2 (0.2)	1.4 (0.1)	7.5 (0.1)	2.0 (0.4)	2 (0.1)	6.5 (0.1)	0.6 (0.5)	0.7 (0.02)	5.8 (0.03)
Bryophytes	0.2 (0.2)	2.1 (0.2)	18.4 (0.9)	-	-	-	-	-	-
Filamentous	0.04 (0.01)	4.5 (0.1)	8.8 (0.1)	0.1 (0.04)	3 (0.1)	7.5 (0.1)	-	-	-
Roots				0.6 (0.1)	2 (0.1)	9 (0.1)	0.4 (0.3)	2.4 (0.04)	18.4 (0.1)
Total AFDM	78.5 (36.3)			86.0 (25.3)			78.9 (25.1)		

* %C assumed to be 45% of dry mass

Table 3. Summary of total NO_3^- uptake, denitrification and NO_3^- assimilatory uptake in the study reaches. Parameters of total NO_3^- uptake and denitrification were calculated from the mean of night and day values shown in Figs. 1 and 2. Parameters of NO_3^- assimilatory uptake were calculated from the NO_3^- uptake rates of primary uptake compartments shown in Fig. 3.

	Santa Fe (forested)	Gualba (urban)	Sant Celoni (agricultura l)
Total NO_3^- uptake			
$k_W \text{NO}_3^-$ (m^{-1})	3.82×10^{-4}	7.05×10^{-4}	1.25×10^{-3}
$S_W \text{NO}_3^-$ (m)	2620	1419	802
$V_f \text{NO}_3^-$ (cm s^{-1})	9.07×10^{-5}	2.71×10^{-4}	7.47×10^{-5}
$U \text{NO}_3^-$ ($\mu\text{g N m}^{-2} \text{s}^{-1}$)	1.56×10^{-1}	1.07	4.49×10^{-1}
Denitrification			
$k_{DEN} \text{N}_2$ (m^{-1})	0	5.98×10^{-5}	8.24×10^{-4}
$k_{DEN} \text{N}_2\text{O}$ (m^{-1})	0	1.48×10^{-7}	1.85×10^{-5}
$k_{DEN} \text{Total}$ (m^{-1})	0	5.99×10^{-5}	8.42×10^{-4}
$S_{DEN} \text{Total}$ (m)	-	16681	1187
$V_{fDEN} \text{Total}$ (cm s^{-1})	0	2.30×10^{-5}	4.50×10^{-5}
$U_{DEN} \text{Total}$ ($\mu\text{g N m}^{-2} \text{s}^{-1}$)	0	8.68×10^{-2}	2.70×10^{-1}
% of total NO_3^- uptake	0	8.5	67.6
Assimilatory uptake			
k_{BIO} (m^{-1})	3.50×10^{-4}	9.92×10^{-5}	4.27×10^{-4}
S_{BIO} (m)	2860	10077	2342
V_{fBIO} (cm s^{-1})	8.30×10^{-5}	3.82×10^{-5}	2.50×10^{-5}
U_{BIO} ($\mu\text{g N m}^{-2} \text{s}^{-1}$)	1.43×10^{-1}	1.50×10^{-1}	1.50×10^{-1}
% of total NO_3^- uptake	91.6	14.1	34.3

Table 4. Summary of N regeneration rates in the study reaches estimated with results from the ammonification-nitrification model shown in Fig. 5.

	Santa Fe (forested)	Gualba (urban)	Sant Celoni (agricultura l)
Ammonium release			
$k_{AM} (s^{-1})$	5.48×10^{-5}	7.59×10^{-5}	1.88×10^{-5}
Nitrification			
$k_{NIT} (s^{-1})$	4.89×10^{-3}	1.52×10^{-3}	8.21×10^{-2}
Total N regeneration			
$k_{REG} (s^{-1})$	4.94×10^{-3}	1.59×10^{-3}	8.22×10^{-2}
% as NH_4^+	1.1	4.8	2.3×10^{-2}
% as NO_3^-	98.9	95.2	100

Figure Captions

Fig. 1. Plot of the \ln tracer $^{15}\text{NO}_3^-$ flux versus distance downstream from the addition point. The slopes of the linear regression lines are the fractional NO_3^- uptake rates per unit distance ($k_W \text{NO}_3^-; \text{m}^{-1}$). Closed symbols represent samples from the night plateau; open symbols represent samples from the day plateau. Solid lines (for night plateau) and dashed lines (for day plateau) show the best fit of linear regressions. Significant differences between night and day were only found in the agricultural stream as shown by the t -tests for the comparison of slopes. Missing points are either lost samples or outliers.

Fig. 2. Plot of the tracer $^{15}\text{N}_2$ (left) and tracer $^{15}\text{N}_2\text{O}$ (right) flux versus distance downstream from the addition point in the two streams where denitrification was detected. Closed symbols represent samples from the night plateau; open symbols represent samples from the day plateau. Solid lines (for night plateau) and dashed lines (for day plateau) show the best fit of the denitrification model to the tracer ^{15}N -gas flux data using the estimated reaeration coefficients ($k_2; \text{m}^{-1}$) and the fractional NO_3^- uptake rate per unit distance ($k_W \text{NO}_3^-; \text{m}^{-1}$) shown in Fig. 1. Notice the different units of the tracer $^{15}\text{N}_2$ and tracer $^{15}\text{N}_2\text{O}$ fluxes.

Fig. 3. Nitrate assimilatory uptake rates (mean + SE) and N turnover rates (mean + SE) of the different primary uptake compartments within each stream calculated with ^{15}N content data from the post-24 h sampling. Asterisks (*) represent uptake rates $< 0.001 \mu\text{g m}^{-2} \text{s}^{-1}$. Uptake compartments not found in a stream are marked as not available (n/a). Notice the same scale on the y-axes to facilitate comparisons among streams.

Fig. 4. Plot of the $\delta^{15}\text{N}$ of epilithon and consumer (*Ancylus fluviatilis* limpet) versus reach distance with data from the post-24 h sampling. Dashed lines separate upstream from downstream of the addition point. Closed symbols represent epilithon samples (scale on the left axis); open symbols represent limpet samples (scale on the right axis). Limpets were not found in two sampling stations of the forested stream.

Fig. 5. Plot of the tracer $^{15}\text{NH}_4^+$ (left) and tracer $^{15}\text{NO}_3^-$ (right) flux versus distance downstream from the addition point with data from the post-24 h sampling. Solid lines show the best fit of the ammonification-nitrification model to the tracer ^{15}N flux data. The estimated parameters were: the fractional NH_4^+ release rate per unit time ($k_{AM}; \text{s}^{-1}$), the fractional NH_4^+ uptake rate per unit distance ($k_W \text{NH}_4^+; \text{m}^{-1}$), and the fractional nitrification rate per unit time ($k_{NT}; \text{s}^{-1}$).

Fig. 6. Plot of the tracer ^{15}N export as NO_3^- , NH_4^+ and suspended particulate organic matter (SPOM) versus time since the end of the addition with data from the post-24 h, post-48 h, and post-72 h samplings. Notice that the post-24 h sampling was performed 7 h after the end of the addition in the agricultural stream. Dashed lines show the best fit of a linear regression of the log-transformed total export versus time for the forested stream ($\log \text{Total export} = -0.0232 \times \text{Time} - 0.1690$; $r^2 = 0.99$; $p = 0.035$), the urban stream ($\log \text{Total export} = -0.0245 \times \text{Time} - 0.0245$; $r^2 = 0.95$; $p = 0.146$), and the agricultural stream ($\log \text{Total export} = -0.0285 \times \text{Time} + 0.9792$; $r^2 = 0.95$; $p = 0.113$).

Fig. 1.

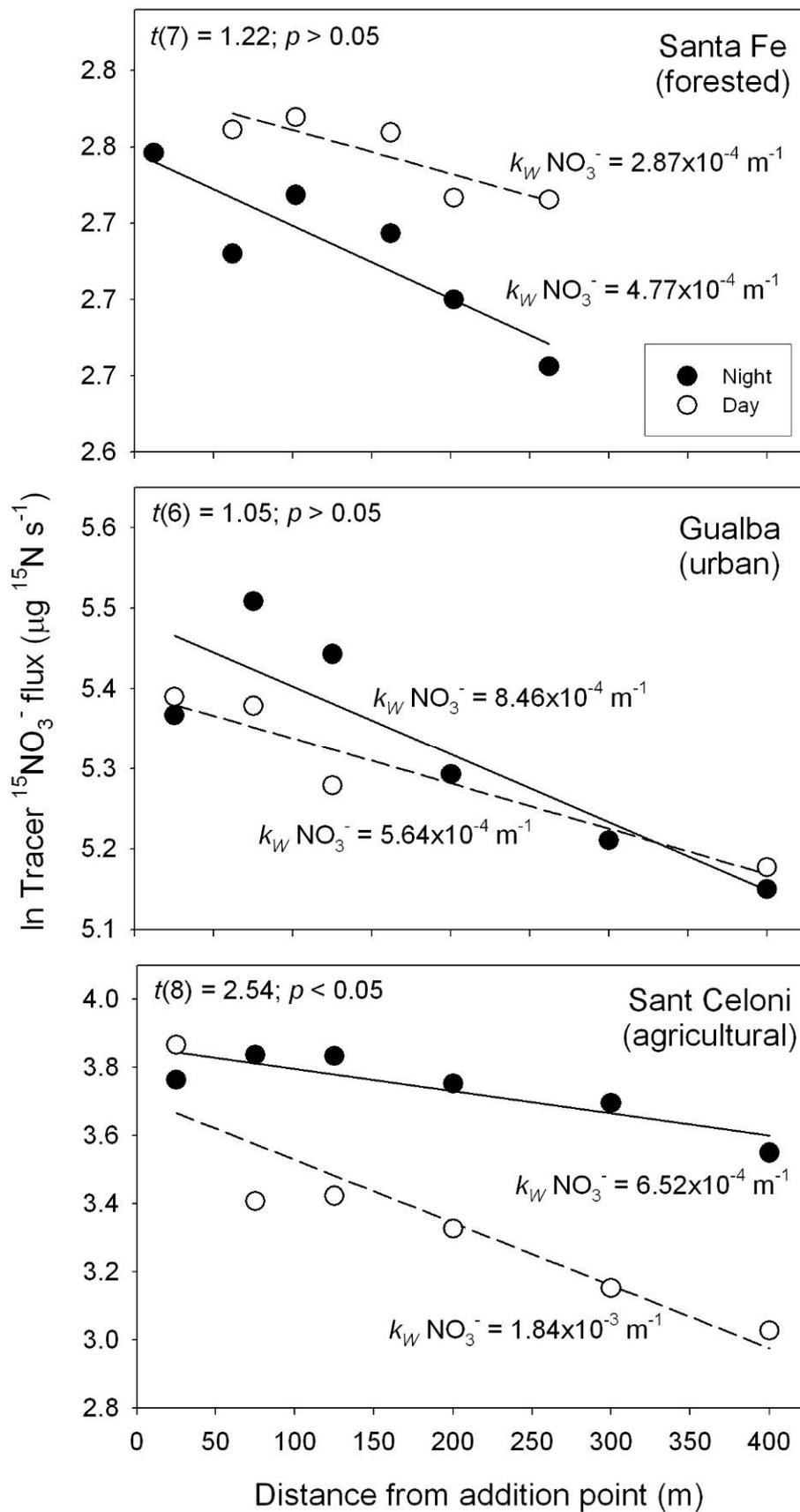


Fig. 2.

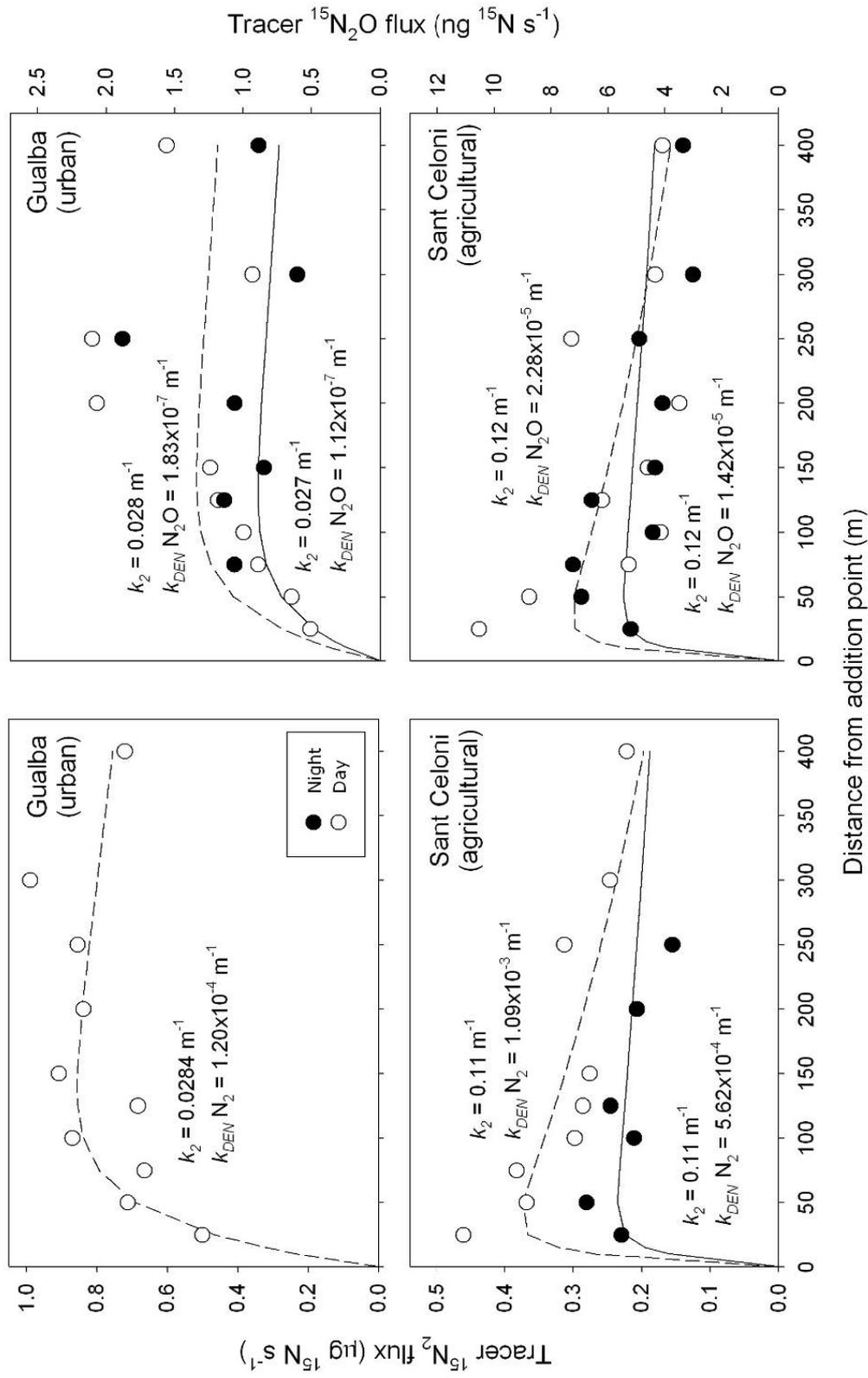


Fig. 3.

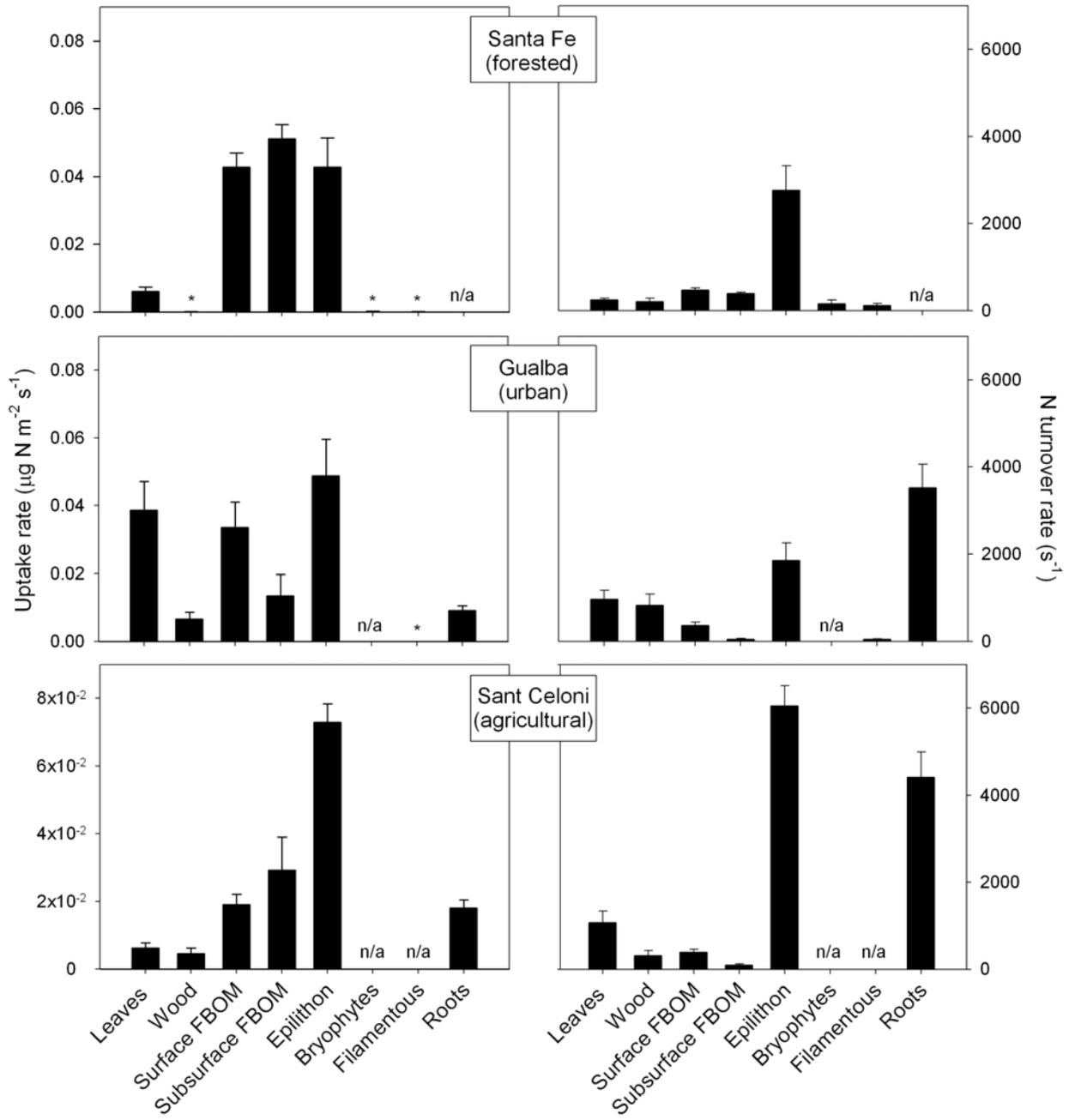


Fig. 4.

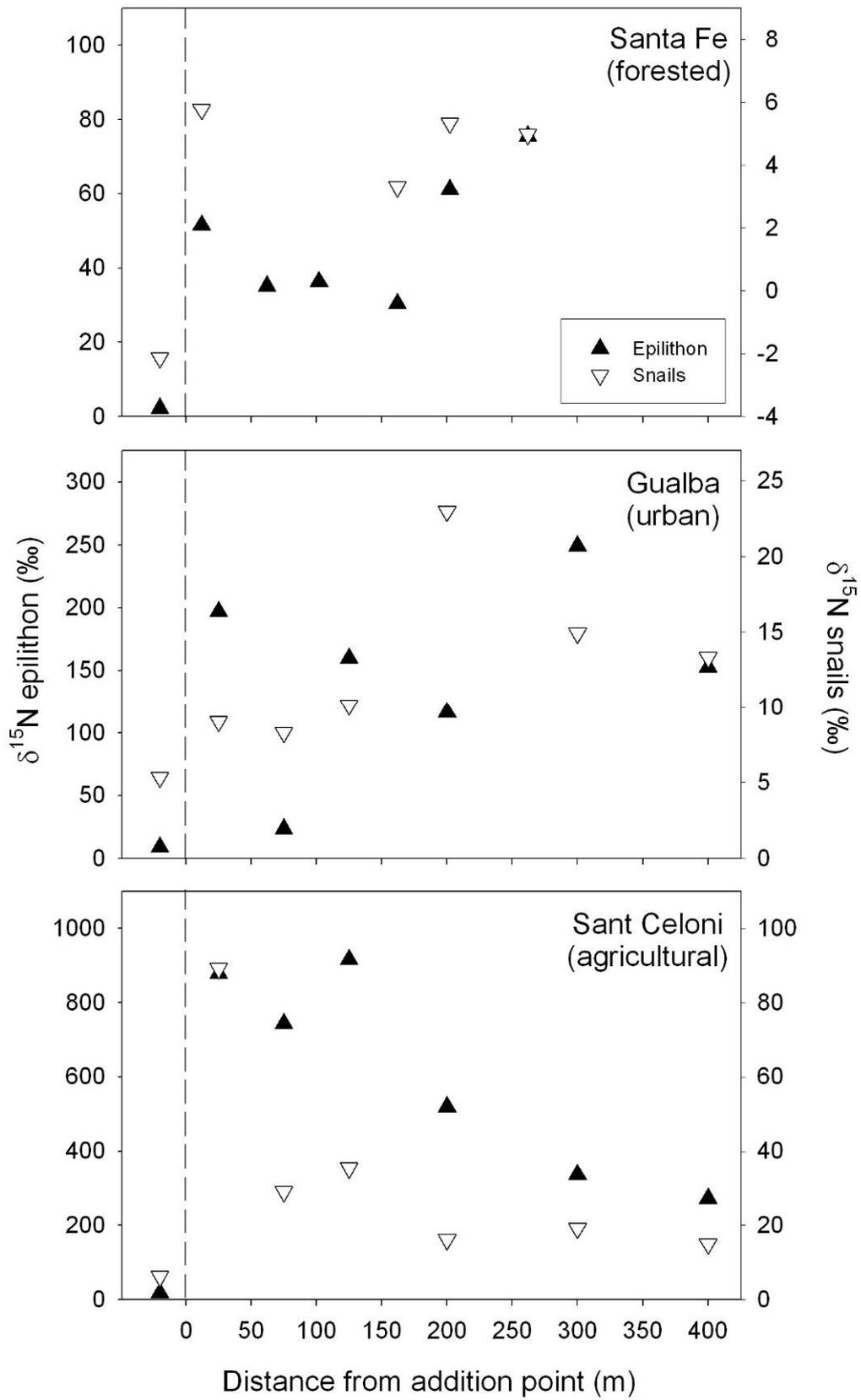


Fig. 5.

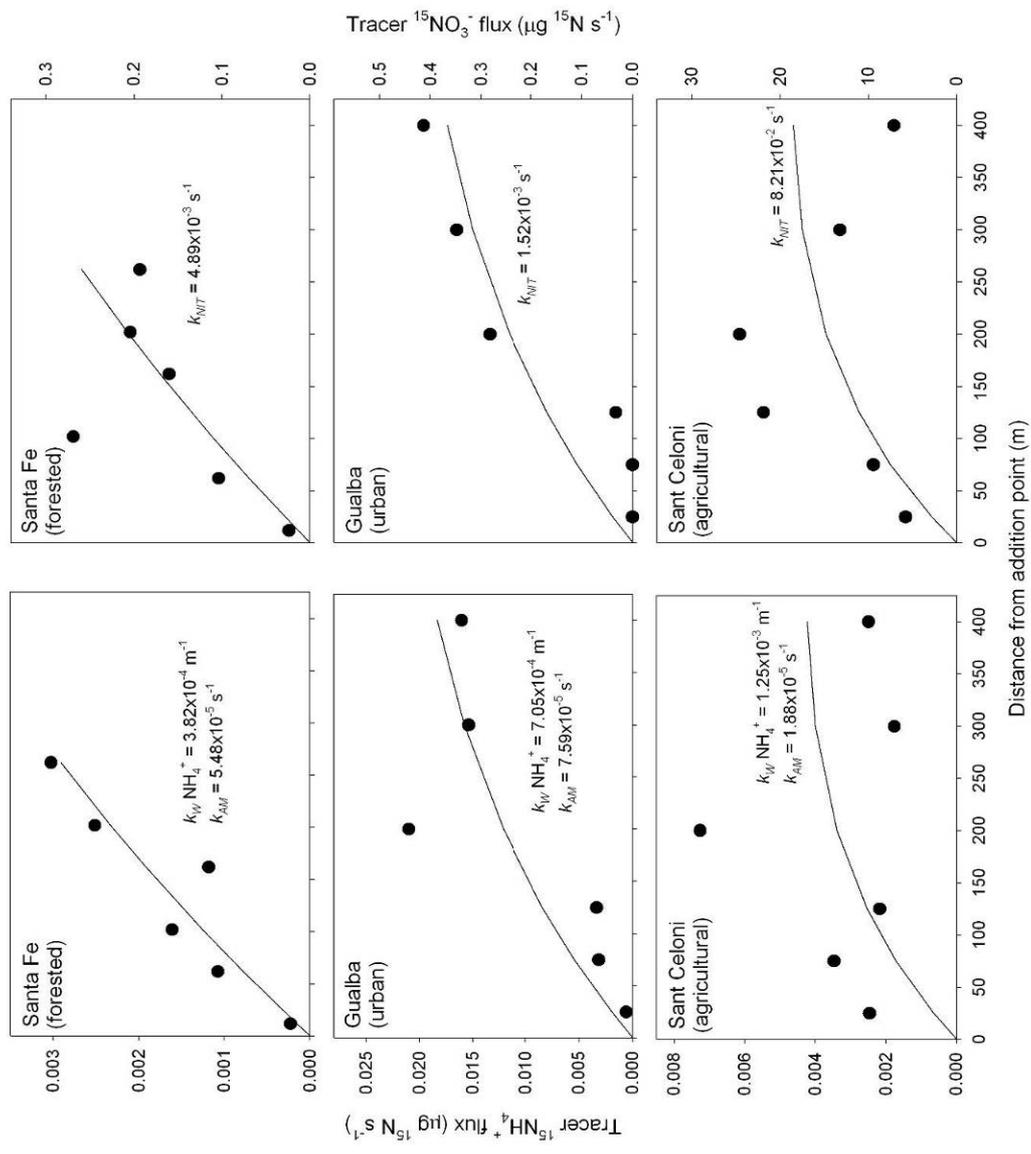
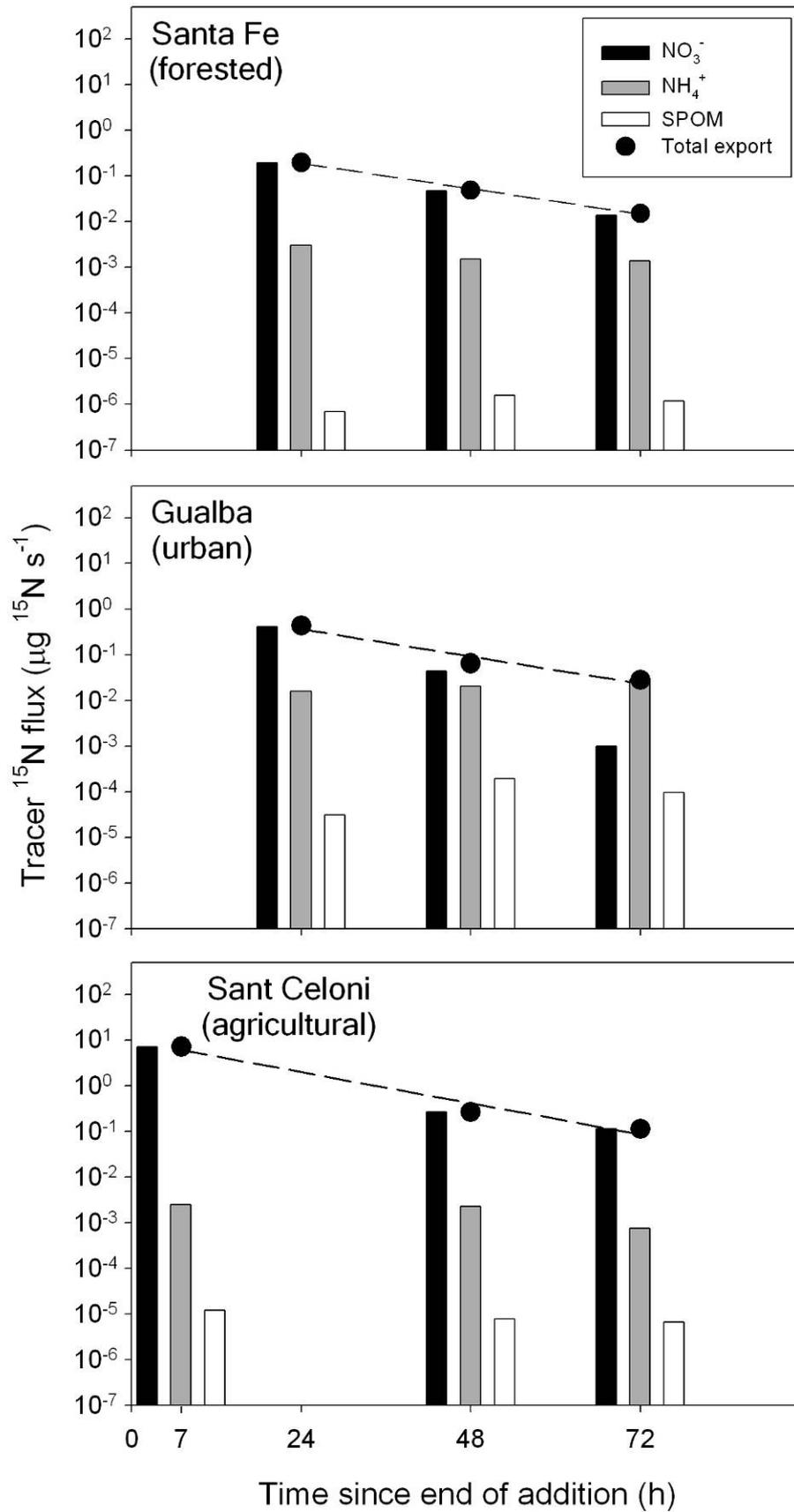


Fig. 6.



3.3 Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses

3.3 Efectos de los nutrientes y la luz sobre la biomasa y captación de nitrógeno del perifiton en ríos mediterráneos con distintos usos del suelo

Resumen

Se utilizaron sustratos difusores de nutrientes (NDS) para determinar la importancia relativa de los nutrientes y la luz como factores limitantes potenciales de la biomasa y capacidad de captación de nitrógeno (N) del perifiton en ríos mediterráneos sometidos a diferentes impactos humanos. Los nutrientes examinados fueron el fósforo (P) y el N, y además diferenciamos entre la respuesta de las comunidades de perifiton a dos fuentes de N ($\text{NO}_3\text{-N}$ y $\text{NH}_4\text{-N}$). Para examinar el efecto de la luz y los nutrientes sobre la biomasa del perifiton, comparamos las tasas de incremento de la clorofila *a* en NDS situados en zonas de luz y de sombra. El efecto de la disponibilidad de nutrientes sobre la captación del perifiton se midió por los cambios en el contenido de ^{15}N en los NDS tras adiciones de $\text{NO}_3\text{-}^{15}\text{N}$ de corta duración.

Los resultados muestran que la luz fue el factor que más afectó a la biomasa algal en los ríos estudiados. La biomasa algal fue generalmente más alta en zonas de luz que en zonas de sombra. La mayor disponibilidad de nutrientes simulada mediante los experimentos de NDS no incrementó el crecimiento de la biomasa algal en ninguna de las dos condiciones de luz.

En los tratamientos control (concentraciones ambientales), las tasas de captación de $\text{NO}_3\text{-N}$ incrementaron y el ratio C:N molar disminuyó consistentemente con el aumento de la disponibilidad de N entre ríos. Las tasas de captación de $\text{NO}_3\text{-N}$ fueron alteradas en los NDS con concentraciones de N incrementadas artificialmente. Las comunidades de perifiton que crecieron sobre los sustratos enriquecidos en N tendieron a captar preferentemente el N proveniente del sustrato que el N proveniente de la columna de agua. Esta respuesta varió entre ríos y dependió de la disponibilidad ambiental de N.

La biomasa de perifiton no difirió significativamente entre los sustratos expuestos a las dos formas de N disponibles. Sin embargo, encontramos diferencias en los efectos de ambas formas de N sobre la captación de N desde la columna de agua. El $\text{NH}_4\text{-N}$ pareció la fuente de N preferida por el perifiton desarrollado sobre los NDS.

Los resultados sugieren que el efecto de las zonas de ribera sobre la disponibilidad de luz, pese a ser poco considerado por los gestores del agua, puede ser más importante que los nutrientes a la hora de controlar los efectos de la eutrofización derivados de las actividades humanas. Finalmente, nuestros resultados confirman que no sólo los incrementos en la concentración, sino también los desequilibrios estequiométricos deben ser tenidos en cuenta a la hora de examinar la retención de N en ríos alterados por la acción humana.

Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses

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SUMMARY

1. Nutrient diffusing substrata (NDS) were used to determine the relative importance of nutrients and light as potential limiting factors of periphyton biomass and nitrogen (N) uptake in Mediterranean streams subjected to different human impacts. The nutrients examined were phosphorus (P) and N, and we also further differentiated between the response of periphyton communities to N species (i.e. $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$). To examine the effect of light and nutrients on periphyton biomass, chlorophyll *a* accrual rates on NDS located at open and closed canopy sites were compared. The effect of nutrient availability on periphyton uptake was measured by ^{15}N changes on the NDS after $\text{NO}_3\text{-}^{15}\text{N}$ short-term nutrient additions.

2. Results show that light was the main factor affecting algal biomass in the study streams. Algal biomass was in general higher at open than at closed canopy sites. Nutrient availability, as simulated with the NDS experiments, did not enhance algal biomass accrual in either of the 2 light conditions.

3. In the control treatments (i.e. ambient concentrations), periphyton $\text{NO}_3\text{-N}$ uptake rates increased and C : N molar ratios decreased consistently with increases in N availability across streams. $\text{NO}_3\text{-N}$ uptake rates were altered when ambient N concentrations were increased artificially in the N amended NDS. Periphyton assemblages growing on N enriched substrata seemed to preferentially take up N diffusing from the substratum rather than N from the water column. This response differed among streams, and depended on ambient N availability.

4. Periphyton biomass was not significantly different between substrata exposed to the two forms of available N sources. Nonetheless, we found differences in the effects of both N sources on the uptake of N from the water column. $\text{NH}_4\text{-N}$ seemed to be the preferred source of N for periphyton growing on NDS.

5. Results suggest that the effect of riparian zones on light availability, although seldom considered by water managers, may be more important than nutrients in controlling eutrophication effects derived from human activities. Finally, our results confirm that not only increases in concentration, but also stoichiometric imbalances should be considered when examining N retention in human altered streams.

Keywords: biofilm, light, N uptake, nutrients, periphyton, streams

Introduction

Nutrient availability (Borchardt, 1996; Francoeur *et al.*, 1999) and light (Hill & Knight, 1988; Hill, 1996) are both essential resources that can limit growth of periphyton (i.e. the algal component of benthic microbial communities) in lotic ecosystems. Although normally weak due to the effect of other factors, positive relationships between stream benthic chlorophyll and nutrient concentrations in the water column have been documented (Dodds, Smith & Zander, 1997; Dodds, Smith & Lohman, 2002). High nutrient concentrations can cause a dramatic increase in biomass and changes in species composition of periphyton, which leads to eutrophication of running waters (Smith, Tilman & Nekola, 1999). Dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) have often been identified as the key nutrients limiting periphyton in streams (Borchardt, 1996). In addition, periphyton communities, or the biofilm (i.e. the benthic microbial assemblage of algae, bacteria and fungi embedded in a muco-polysaccharide matrix) to a greater extent, play a key role in nutrient cycling in stream ecosystems through nutrient uptake, transfer of nutrients to higher levels of the trophic food web, and remineralisation. The greater the external nutrient supply, the less important nutrient cycling is within the biofilm to meet its biological demands (Mulholland, 1996). Therefore, a feedback is established between the biofilm and stream nutrient availability.

Light availability is prominent among the abiotic factors that limit primary production in lotic ecosystems (Hill, Ryon & Schilling, 1995). As outlined by Hill (1996), environmental variation in the quantity and quality of light potentially accounts for much of the variation in the physiology, population growth and community structure of benthic algae. In shallow streams with low amounts of suspended organic matter, riparian vegetation is the main factor affecting light availability at the stream bottom. It also contributes to habitat heterogeneity for algae and determines their seasonal variation. Weak or lack of relationship between periphyton growth and nutrient concentrations or light availability in streams may be attributed to other limiting factors, such as physical disturbances (Biggs & Close, 1989; Biggs & Thomsen, 1995), grazing (Rosemond, 1994; Steinman, 1996),

substratum stability (Power & Stewart, 1987; Grimm & Fisher, 1989) and temperature (Bothwell, 1988).

Interactive effects of nutrients and light on benthic algae can be more important than their independent effects (Rosemond, Mulholland & Elwood, 1993). Researchers have reported greater algal biomass accrual in response to nutrient addition in streams or stream reaches with open canopies than in those that were densely shaded (for example see, Hill & Knight, 1988; Mosisch, Bunn & Davies, 2001). The consistency of these results has led these researchers to conclude that light is the primary resource limiting stream algal biomass, with nutrients becoming limiting only at relatively unshaded sites. However, other studies have indicated that algal growth can be primarily nutrient limited (or co-limited by light and nutrients) in oligotrophic streams, even in heavily shaded sections (Taulbee, Cooper & Melack, 2005). Much less is known about the effect of these factors on periphyton nutrient uptake.

Human activities can dramatically alter the availability of nutrients and light in lotic ecosystems. Streams draining catchments subjected to land use changes derived from human activities show higher nutrient concentrations and changes in their stoichiometric ratios (i.e. DIN : SRP or $\text{NO}_3 : \text{NH}_4$ ratios) compared with their pristine counterparts (Bennet, Carpenter & Caraco, 2001; Boyer *et al.*, 2002). Likewise, light regimes, to which stream communities are exposed, are substantially altered through removal of riparian vegetation for urban or agricultural development. These changes may affect structural and functional properties of stream biofilms, which can be reflected at the whole stream ecosystem level. Despite a natural deficit of water resources, climate and historical reasons have made Mediterranean regions particularly suitable for human settlement and intensive agricultural production. Consequently, streams from the Mediterranean region are particularly susceptible to human impacts (Alvarez-Cobelas, Rojo & Angeler, 2005). The relatively sparse riparian vegetation and high solar radiation levels in Mediterranean regions suggest that photoautotrophic processes may play a relatively more important role in whole stream metabolism here than in streams located in temperate regions. Reductions in riparian vegetation or increases in nutrient loading can further enhance photoautotrophic processes in Mediterranean-type

streams (Gasith & Resh, 1999), which may have important consequences on nutrient dynamics.

Nutrient limitation, and to a lesser extent light limitation, of lotic periphyton community biomass is a well-studied subject (Borchardt, 1996; Hill, 1996). However, relatively few studies have examined the combined effects of nutrients and light (but see for example, Rosemond, 1993; Mosisch *et al.*, 2001), and to our knowledge none of those studies were carried out in human altered streams. Even fewer studies have examined the effect of different forms of DIN (i.e. the quality of the nutrient supply) on algal accrual in streams (but see Bushong & Bachmann, 1989), or experimentally examined *in situ* the effect of nutrient availability on periphyton nutrient uptake rates. In addition, benthic algae limitation studies are scarce in streams of the Mediterranean region (Alvarez-Cobelas *et al.*, 2005). This paper aims to provide insights into some of these gaps.

The general goal of this study was to determine the relative importance of nutrients and light as potential limiting factors of periphyton biomass and N uptake in Mediterranean streams subjected to different human impacts. To examine this, we selected three streams draining forested, agricultural and urban-dominated catchments and assessed light and nutrient effects on periphyton by means of *in situ* experiments with nutrient diffusing substrata (NDS) bioassays located at open and closed canopy sites. The effect of nutrient availability on periphyton uptake was measured by ^{15}N changes on the NDS after $\text{NO}_3\text{-}^{15}\text{N}$ short-term nutrient additions. The nutrients examined were phosphorus (P) and nitrogen (N), and we also further differentiated between the response of periphyton communities to N species (i.e. $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$). Knowledge of how nutrient and light availability influence periphyton growth is critical to understand how these factors may control stream ecosystem function in humanised landscapes. The implications of our results for stream management will be discussed.

Methods

Study sites

This study was conducted in La Tordera catchment (Catalonia, NE Spain; Fig. 1), with an area of 868.5 km² dominated by siliceous geology and sub-

jected to a large variety of land use activities. Climate in this region is typically Mediterranean, with hot, dry summers, and cool, wet winters. The hydrologic regime of the streams draining catchments in this region follows climatological patterns, showing low or intermittent flows during the late spring–early autumn period and higher and permanent flows from late autumn to early spring. However, the interannual variability is high.

Within this catchment, we selected three streams draining sub-catchments differing in land use. One was predominantly forested (Santa Fe); the second had the influence of an urban development (Gualba); and agricultural activities were important in the third one (Sant Celoni). The reaches where the experiments took place were situated at an altitude of 1180 (Santa Fe), 176 (Gualba) and 240 (Sant Celoni) m.a.s.l. Experiments were performed during July and August 2004, and repeated during the same months of 2005. During the second year, the agricultural site had to be changed to a different stream (Santa Coloma), situated at 298 m.a.s.l., because Sant Celoni had dried out due to an extended period (>360 days) of high temperatures and scarce precipitation.

All four study streams were comparable in terms of substratum type and canopy cover from riparian vegetation. The reaches where the experiments took place were riffle-pool dominated and stream substratum was basically composed of cobbles and pebbles with patches of sand. Riparian vegetation was well developed along the study reaches and dominated by riparian deciduous trees that completely shade the stream channel during spring and summer (i.e. the study period). Dominant riparian tree species varied according to altitude. At the highest elevation (i.e. the forested stream), riparian vegetation was dominated by *Fagus sylvatica* L., with some stems of *Sambucus nigra* L. and poorly developed herbaceous understory. At the lower elevation streams (i.e. the urban and agricultural streams), riparian vegetation was dominated by *Alnus glutinosa* (L.) Gaertn., *Platanus hispanica* Mill. ex Muench and *Robinia pseudoacacia* L., with a well developed herbaceous understory dominated by *Pteridium aquilinum* (L.) Kuhn, and *Urtica* sp.

In situ bioassays

To assess the effect of nutrient availability on stream periphyton biomass, NDS experiments were per-

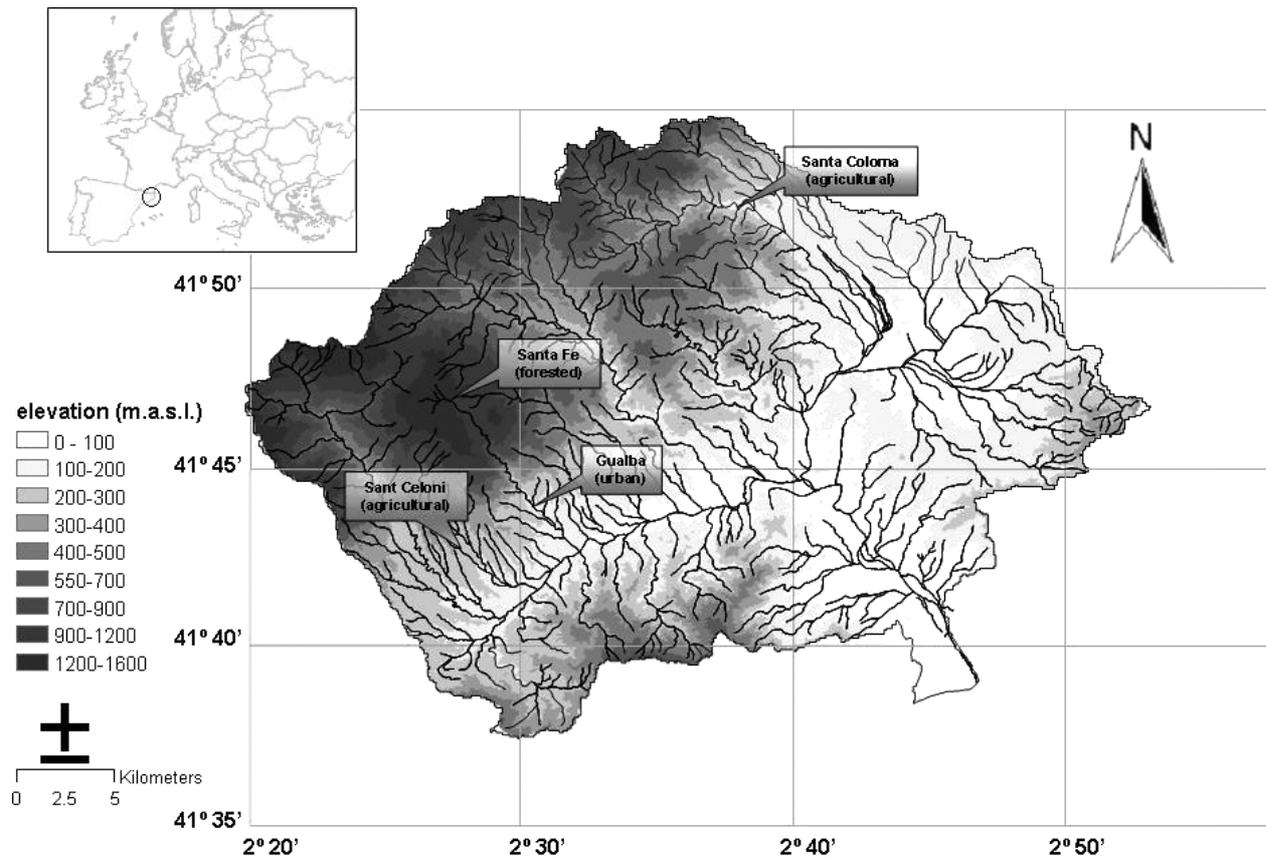


Fig. 1 Map of the location of la Tordera catchment and the location of the study streams within the catchment.

formed in the summer of 2004 and 2005 following similar procedures as outlined by Tank & Dodds (2003). NDS were constructed using 60 mL plastic containers filled with a 2% (by weight) agar solution amended with different combinations of nutrients, resulting in six different treatments: C (control, agar only), NO (nitrate-N, 0.5 M NaNO_3), NH (ammonium-N, 0.5 M NH_4Cl), P (phosphate-P, 0.5 M NaH_2PO_4), NOP (nitrate-N + phosphate-P, amendments as above) and NHP (ammonium-N + phosphate-P, amendments as above). These acronyms are used hereafter to refer to the different nutrient treatments. Whatman (Kent, U.K.) GF/F glass fibre filters (0.7 μm retention) were placed on the top of the containers to cover the agar completely and serve as the inorganic surface for periphyton colonisation. Experimental NDS sets (Fig. 2) consisted of plastic baskets, each containing one replicate of each treatment ($n = 6$), which were displayed in hexangles such that an upstream substratum would not leach nutrients towards a substratum immediately downstream that did not already have a

supply of those particular nutrients. Baskets were deployed on the streambed at enough depth to level the top of the NDS with the surrounding stream substratum in riffle-run areas of similar velocity (i.e. 0.10–0.25 m s^{-1}) and depth (i.e. 0.08–0.15 m). After the placement of each basket, any settled organic matter and other debris were cleared from the colonising surfaces. Further entangled material was removed from the NDS at each inspection during the experiments (i.e. three to four times in each stream).

In the 2004 experiment, six to seven replicates of each experimental set were deployed at closed canopy sites, which were the characteristic conditions of the streams; and removed 37, 49 and 64 days later from the forested (Santa Fe), agricultural (Sant Celoni) and urban (Gualba) sites, respectively. Incubation time at each stream was set based on visual observations of algal growth on the NDS, to ensure that the community was well developed on each site when $\text{NO}_3^-^{15}\text{N}$ additions (see description below) were conducted. Nutrient release rates were not measured in our

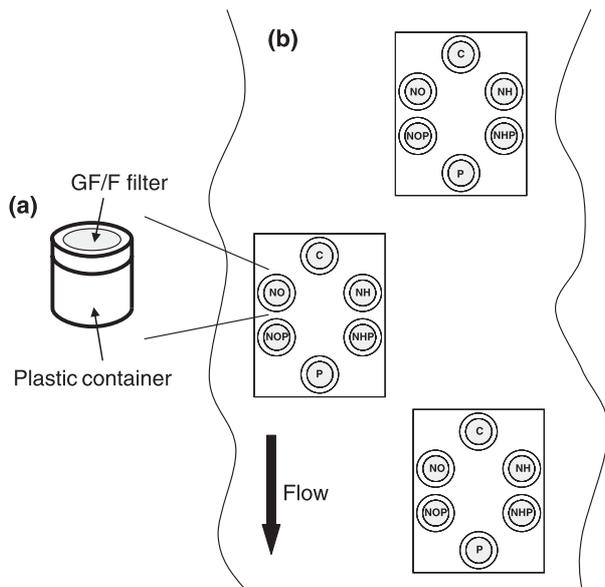


Fig. 2 Scheme of the nutrient diffusing substrata (NDS) experimental setting in the study streams: (a) Detail of a NDS unit, (b) Orientation of experimental NDS sets with respect to flow direction in the stream. Nutrient treatments were: C (control), NO (nitrate-N), NH (ammonium-N), P (phosphate-P), NOP (nitrate-N + phosphate P) and NHP (ammonium-N + phosphate-P).

experiments; however, previous research using similar NDS has demonstrated that although release rates decrease with time after deployment, lower rates can persist for several weeks (Tank & Dodds, 2003; Bernhardt & Likens, 2004), and thus contribute to a nutrient enriched environment at the water-filter interface relative to the water column. Constrained by the need to perform the $\text{NO}_3\text{-}^{15}\text{N}$ additions under optimal conditions, we decided to amend the NDS with high nutrient concentrations and assumed that treatments would be releasing nutrients in at least low amounts during the whole incubation period.

Results from the first year experiment suggested that factors other than nutrients, especially light availability, may have influenced algal growth in the study streams. Consequently, in summer 2005 we repeated the NDS experiment in order to explore the influence of both nutrient and light availability on periphyton biomass. With this aim, six treatment sets were placed at closed canopy sites (i.e. as in the previous year), while six additional sets were placed at open canopy sites where riparian vegetation was absent and light availability at the stream surface was much higher than at the shaded sites. Open and closed canopy locations in

each stream were <100 m apart and no tributaries joined the stream reach along this stream length. Freed from the constraints imposed by $\text{NO}_3\text{-}^{15}\text{N}$ additions, the effects of diminishing release rates with time were minimised by varying incubation times from 18 days in the agricultural (Santa Coloma) and urban (Gualba) streams, to 33 days in the forested (Santa Fe) stream. In the latter, and following a recommendation by J.L. Tank (University of Notre Dame, personal communication), agar containers for all treatments were replaced with new ones after 18 days of incubation while retaining the original filters. No spates occurred during any of the experiments.

Stream water samples for nutrient chemistry were collected on four evenly spaced dates during the NDS incubation period. All water samples were immediately filtered through Whatman (Kent, U.K.) GF/F glass fibre filters (0.7 μm retention), stored on ice in the field, and then refrigerated at 4 $^\circ\text{C}$ in the laboratory until analysis. In addition, instantaneous measures of conductivity and temperature were obtained in the field with a WTW (Weilheim, Germany) 340i portable conductivity meter.

$\text{NO}_3\text{-}^{15}\text{N}$ constant rate additions

To evaluate the effects of nutrient availability on periphyton N uptake, 99% enriched K^{15}NO_3 was released at a constant rate into each stream at a tracer level (i.e. there was no significant fertilisation) for a period of 12 h. These additions were conducted only in summer 2004, just before the end of the NDS incubation period, following similar addition procedures as outlined by Mulholland *et al.* (2004). The ^{15}N addition point was placed 50 m upstream of the site where the NDS were located, and NDS were collected for ^{15}N analysis 24 h after the end of the release. Addition solution and flow rate was set to achieve a $\text{NO}_3\text{-}^{15}\text{N}$ target enrichment of 10 000‰ in each stream. Samples of stream water were collected at the site where NDS were located for the analysis of $\text{NO}_3\text{-}^{15}\text{N}$ before, during and after the additions.

Laboratory analyses

At the end of the incubation period, filters were carefully removed from the NDS and cut into half. One half of the filter was frozen for later analysis of chlorophyll *a*, and the other half dried in the oven at

60 °C until constant weight for later analysis of dry mass, C and N content, and ^{15}N signature. In 2005, only chlorophyll *a* was measured and thus, the whole filter was used for this analysis.

Chlorophyll *a* was analysed following the method described in Steinman & Lamberti (1996). Each frozen filter was placed in a centrifuge tube containing a known volume of 90% v/v acetone and left at 4 °C overnight in the dark. This was followed by sonication for 2 min and centrifugation for 10 min at $1108 \times g$. Absorbance of the resultant supernatant was measured using a Shimadzu (Tokyo, Japan) UV- spectrophotometer. The chlorophyll *a* content of each sample was corrected for phaeo-pigments by acidification and expressed in $\mu\text{g cm}^{-2}$.

Stream water samples for $\text{NO}_3\text{-}^{15}\text{N}$ analysis from the 2004 additions were processed as described in Mulholland *et al.* (2004). Samples ranged in volume from 0.1 to 0.5 L depending on $\text{NO}_3\text{-N}$ concentration at each study stream. For the analysis of ^{15}N in periphyton (only NDS filters from 2004), discs of a known surface area (diameter = 1 cm) were cut out from the previously dried filters and weighted to the nearest 0.001 mg on a Mettler-Toledo (Greifensee, Switzerland) MX5 microbalance. Samples for both $\text{NO}_3\text{-}^{15}\text{N}$ in the water and ^{15}N in periphyton were encapsulated in tins, and sent to the Stable Isotope Facility at the University of California-Davis (Davis, CA, U.S.A.). The content (as a percentage of dry mass) and the stable isotope ratios of C and N were measured by continuous flow isotope ratio mass spectrometry (20–20 mass spectrometer; PDZEuropa, Northwich, U.K.) after sample combustion to CO_2 and N_2 at 1000 °C in an on-line elemental analyzer (PDZ-Europa ANCA-GSL).

Stream water samples collected during the NDS incubation period were analysed for $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, and SRP concentrations on a Bran + Luebbe (Norderstedt, Germany) TRAACS 2000 Autoanalyzer II. $\text{NH}_4\text{-N}$ concentration was analysed on a Skalar (Breda, the Netherlands) San⁺ Autoanalyzer. All nutrient analyses were performed following standard colorimetric methods as described in APHA (1995).

Data calculation and statistical analysis

For the comparison of results among streams and treatments, chlorophyll *a* absolute values ($\mu\text{g cm}^{-2}$) were converted to chlorophyll *a* accrual rates ($\mu\text{g cm}^{-2} \text{ day}^{-1}$), by dividing the absolute values by

the NDS incubation time at each stream site (Stevenson, 1996). Lost and broken NDS replicates, representing <1%, were discarded for the statistical analyses.

For the first year, chlorophyll *a* accrual rates and C : N ratios were compared using two-way ANOVA with stream ($n = 3$) and nutrient treatment ($n = 6$) as factors. To determine ambient differences in chlorophyll *a* accrual and C : N ratios among the three streams, control treatments were compared using a one-way ANOVA (stream as a factor). For the second year experiment, a three-way ANOVA was used to test for the effects of stream ($n = 3$), nutrient treatment ($n = 6$) and canopy cover ($n = 2$) on chlorophyll *a* accrual rates. As with the first year experiment, control treatments were compared among streams using a two-way ANOVA with stream and canopy cover as factors. Chlorophyll *a* concentrations and accrual rates varied considerably for all treatments both among streams and among set locations within each stream. As this may have masked any nutrient effects, periphyton responses to nutrient amendments were expressed as ratios relative to the control treatment of each particular set. The response ratios (i.e. value of chlorophyll *a* on nutrient treatment versus control) estimated with data from the second year experiment were then subjected to a three-way ANOVA to determine the effect of stream ($n = 3$), nutrient treatment ($n = 5$) and canopy cover ($n = 2$).

Because ^{15}N was determined from the NDS filter, measured N uptake does not discriminate between algal uptake and uptake by other organisms (e.g. bacteria and fungi) that may have also colonised the filter. However, the use of glass fibre filters as inorganic colonising surfaces should have promoted autotrophic over heterotrophic growth in our experiments. For this reason, periphyton N uptake rather than biofilm N uptake is used throughout the text. To compare periphyton N uptake among streams and treatments, we calculated the tracer ^{15}N in periphyton after the ^{15}N addition for each bioassay using the following equation:

$$^{15}\text{N}_{\text{periphyton}} (\text{mg N m}^{-2}) = B_{\text{periphyton}} \times (\%N/100) \times (MF_i - MF_b) \quad (1)$$

where $^{15}\text{N}_{\text{periphyton}}$ is the ^{15}N tracer in periphyton biomass, $B_{\text{periphyton}}$ is the biomass of periphyton per unit area (mg m^{-2}), %N is the percentage of N in periphyton biomass, MF_i is the molar fraction of ^{15}N

in periphyton growing on the assay and MF_b is the background molar fraction of ^{15}N obtained from natural periphyton samples upstream from the ^{15}N addition point. Periphyton $\text{NO}_3\text{-N}$ uptake rate ($U_{\text{periphyton}}$) for each bioassay was estimated as

$$U_{\text{periphyton}}(\text{mg N m}^{-2}\text{h}^{-1}) = \frac{{}^{15}\text{N}_{\text{periphyton}}}{T_{\text{addition}} \times ({}^{15}\text{N}_{\text{flux}}/N_{\text{flux}})} \quad (2)$$

where ${}^{15}\text{N}_{\text{periphyton}}$ is the ^{15}N tracer in periphyton biomass from eqn (1) (mg N m^{-2}), T_{addition} is the duration of the ^{15}N addition (i.e. 12 h), ${}^{15}\text{N}_{\text{flux}}$ is the tracer $\text{NO}_3\text{-}^{15}\text{N}$ flux at the steady state (at plateau during ^{15}N release) in stream water at the NDS experiments distance downstream from the addition point ($\mu\text{g}^{15}\text{N s}^{-1}$), and N_{flux} is the total $\text{NO}_3\text{-N}$ flux at the same distance based on stream water concentration and discharge ($\mu\text{g s}^{-1}$). $\text{NO}_3\text{-N}$ uptake rates were compared using a two-way ANOVA with stream ($n = 3$) and nutrient treatment ($n = 6$) as factors. Control treatments were compared among streams using a one-way ANOVA (stream as a factor) to determine ambient differences in $\text{NO}_3\text{-N}$ uptake rates among the three streams.

Chlorophyll *a* accrual rates and response ratios, C : N ratios, and $\text{NO}_3\text{-N}$ uptake rates were log transformed prior to analysis in order to meet assumptions of homogeneity of variance and normality (Zar, 1996). *Post hoc* pair-wise comparisons were made in all significant ANOVA tests using Tukey HSD test to further explore the effects of particular factors

or the interactions among them. All statistical tests were done using STATISTICA 5.1. (Statsoft, Tulsa, OK, U.S.A.).

Results

Physical and chemical characteristics of the study streams

Table 1 summarises some physical and chemical characteristics of each stream during the NDS incubations. Discharge was higher and water temperature lower in the first year experiment than in the second year experiment in all streams, reflecting a warmer and dryer summer in 2005. Water temperature was considerably lower in the forested stream relative to the other streams in both years, because of its location at a higher altitude. Significant differences among streams were associated with their nutrient concentrations and nutrient ratios. The human altered streams (i.e. urban and agricultural) showed higher DIN concentrations, mainly in the form of $\text{NO}_3\text{-N}$, than the forested stream. Concentrations of SRP and $\text{NH}_4\text{-N}$ were lower relative to $\text{NO}_3\text{-N}$ and similar among all four streams. As a consequence, in the human altered streams DIN : SRP and $\text{NO}_3 : \text{NH}_4$ ratios were higher than in the forested stream. Stream water DIN : SRP ratios suggestive of P limitation (i.e. ratios >16) were observed at all but the forested site, where we measured ratios close to 16 : 1, suggesting the possibility of N limitation in this stream (Redfield, 1958). Differences in conductivity, nutrient concentra-

Table 1 Physical and chemical characteristics of the study streams. Data reported are mean values \pm 1 SEM of samples collected on four different dates ($n = 4$) during the NDS incubation periods (i.e. summers of 2004 and 2005).

	Forested stream		Urban stream		Agricultural streams	
	Sta. Fe		Gualba		St. Celoni	Sta. Coloma
	2004	2005	2004	2005	2004	2005
Discharge (L s^{-1})	18.4 \pm 2.2	7.9 \pm 1.2	50.3 \pm 19.6	5.6 \pm 0.8	42.3 \pm 10.9	23.2 \pm 1.8
Water temperature ($^{\circ}\text{C}$)	12.1 \pm 0.8	13.5 \pm 0.3	18.8 \pm 1.6	19.2 \pm 0.3	18.2 \pm 1.2	19.8 \pm 0.3
Conductivity ($\mu\text{S cm}^{-1}$)	59.8 \pm 1.1	61.6 \pm 0.2	148.2 \pm 5.2	156.3 \pm 5.4	103.4 \pm 1.0	331.0 \pm 2.5
NH_4^+ ($\mu\text{g N L}^{-1}$)	4.1 \pm 0.6	8.4 \pm 0.7	9.3 \pm 2.5	16.7 \pm 3.7	9.6 \pm 2.4	20.5 \pm 2.8
NO_3^- ($\mu\text{g N L}^{-1}$)	133.9 \pm 21.0	228.6 \pm 3.6	397.6 \pm 76.8	369.9 \pm 19.3	533.3 \pm 41.6	791.3 \pm 38.2
NO_2^- ($\mu\text{g N L}^{-1}$)	2.3 \pm 0.1	1.8 \pm 0.3	11.4 \pm 4.0	3.4 \pm 0.3	4.9 \pm 0.8	5.2 \pm 0.7
SRP ($\mu\text{g P L}^{-1}$)	16.6 \pm 4.3	22.9 \pm 1.2	14.0 \pm 3.0	12.7 \pm 0.8	38.0 \pm 8.1	15.4 \pm 1.1
DIN:SRP (molar)	20.8 \pm 4.7	23.6 \pm 0.9	72.7 \pm 18.5	72.4 \pm 6.3	36.0 \pm 7.4	123.9 \pm 10.3
$\text{NO}_3^-:\text{NH}_4^+$ (molar)	31.3 \pm 2.5	29.8 \pm 2.6	42.2 \pm 12.1	52.7 \pm 14.1	56.1 \pm 13.3	49.3 \pm 7.8

SRP, soluble reactive phosphorus; DIN, dissolved inorganic nitrogen.

tions and nutrient ratios were negligible in the forested and urban sites between years. On the contrary, differences in these parameters were more evident between the two agricultural sites, although differences with the other two streams were kept similar.

Effects of nutrients and light on periphyton biomass

Results from the two-way ANOVA on data from year 2004 showed that both stream and nutrient treatment had a statistically significant effect on chlorophyll *a* accrual rate (Fig. 3, Table 2). The agricultural and forested streams showed higher rates than the urban stream (Tukey HSD test, $P < 0.001$). Pair-wise *post hoc* comparisons of chlorophyll *a* accrual rates among nutrient treatments within each stream, however, showed no significant differences (Tukey HSD test, $P > 0.05$), indicating no nutrient limitation in any of the three streams. Similar results were obtained when comparing chlorophyll *a* accrual rates in control treatments (i.e. ambient concentrations) among

Table 2 Results from a two-way ANOVA, using stream (forested, agricultural, urban) and nutrient treatment (C, NH, NO, P, NHP, NOP) as factors, on chlorophyll *a* accrual rates. Data are from nutrient diffusing substrata experiments conducted in summer 2004

Source	d.f.	MS	F	P-level
Stream	2	0.0016	22.57	<0.001
Nutrients	5	0.0002	2.39	0.042
Stream × nutrients	10	0.0001	1.77	0.076

streams (one-way ANOVA, $F_{(2,16)} = 8.70$, $P = 0.003$). Control treatments in the agricultural and forested streams showed higher rates compared to the urban stream (Tukey HSD test, $P < 0.05$).

Results from the three-way ANOVA on data from year 2005 showed that stream and canopy cover were the major factors explaining differences in the accrual rates of algal biomass on the experimental substrata, whereas nutrient treatment had no significant effect (Fig. 3, Table 3). After the incubation period, chlorophyll *a* accrual rates were significantly higher in the agricultural stream than in the other two streams

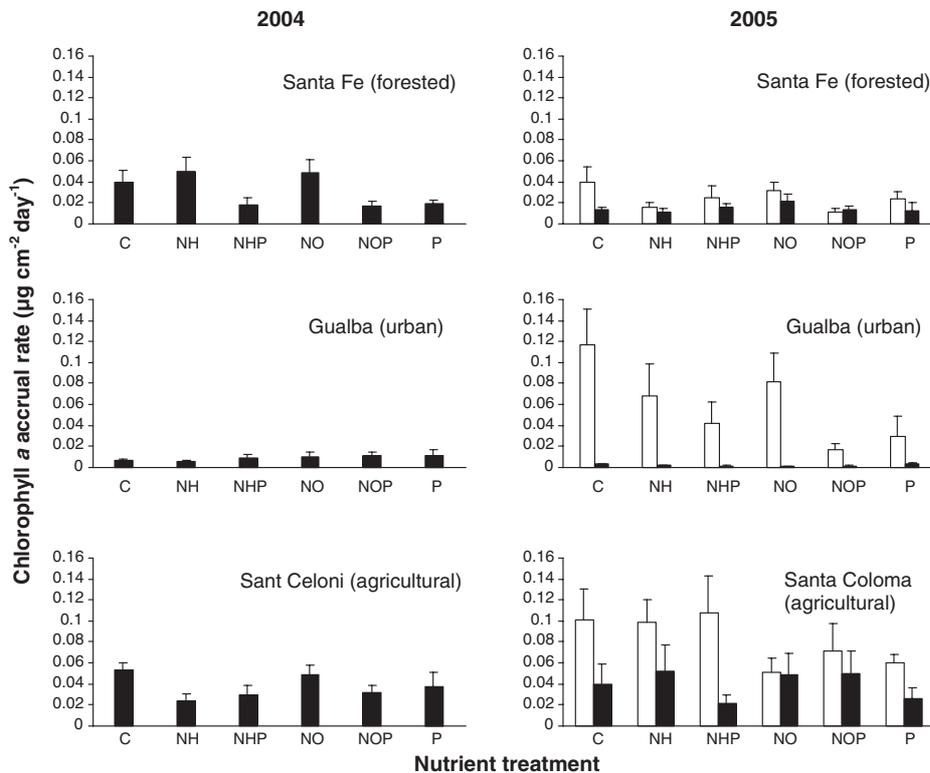


Fig. 3 Chlorophyll *a* accrual rates of periphyton in the different streams (forested, urban, agricultural) and nutrient treatments (C, NH, NO, P, NHP, NOP) from nutrient diffusing substrata experiments conducted in the summer of 2004 (left panel) and 2005 (right panel). Open and closed bars represent open and closed canopy sites, respectively. Data reported are mean \pm 1 SEM.

(Tukey HSD test, $P < 0.001$), and at open sites than at sites with closed canopy cover (Tukey HSD test, $P < 0.001$). A significant effect of the interaction between stream and canopy cover was also found (Table 3). *Post hoc* comparisons of chlorophyll *a* accrual rates between canopy cover types within each stream showed that rates were higher at open sites compared with closed canopy sites in the agricultural and urban streams (Tukey HSD test, $P < 0.001$); in contrast, no significant differences were found between canopy cover types in the forested stream (Tukey HSD test, $P = 0.899$). Canopy cover (two-way ANOVA, $F_{(1,30)} = 16.77$, $P < 0.001$), but not stream type (two-way ANOVA, $F_{(1,30)} = 2.44$, $P = 0.104$), had a significant effect on chlorophyll *a* accrual rates in the control treatments (i.e. ambient concentrations). Rates were significantly higher at open sites than at sites with closed canopy cover (Tukey HSD test, $P < 0.001$).

Nutrients did not have a significant limitation effect on algal accrual rates either across streams or across treatments in each stream. We further examined whether there were differences in the response of nutrient treatments relative to the controls. Results from a three-way ANOVA based on values for the relative responses were consistent with those based on accrual rates (Tables 3 & 4). Both stream type and canopy cover had a significant effect on the relative response of nutrient treatments compared with controls (Table 4). Mean response of chlorophyll *a* was significantly higher in the agricultural stream than in the other two streams (Tukey HSD test, $P < 0.05$). Additionally, chlorophyll *a* response at closed canopy sites was 2.1 times that of open sites (Tukey HSD test, $P = 0.029$). No significant differences in chlorophyll *a* response were found among nutrient treatments

Table 3 Results from a three-way ANOVA, using stream (forested, agricultural, urban), nutrient treatment (C, NH, NO, P, NHP, NOP), and riparian canopy cover (open, closed) as factors, on chlorophyll *a* accrual rates. Data are from nutrient diffusing substrata experiments conducted in summer 2005

Source	d.f.	MS	F	P-level
Stream	2	0.0053	20.59	<0.001
Nutrients	5	0.0006	2.16	0.061
Canopy	1	0.0118	45.21	<0.001
Stream × nutrients	10	0.0002	0.91	0.521
Stream × canopy	2	0.0017	6.70	0.002
Nutrients × canopy	5	0.0005	2.05	0.073
Stream × nutrients × canopy	10	0.0003	1.14	0.333

Table 4 Results from a three-way ANOVA, using stream (forested, agricultural, urban), nutrient treatment (C, NH, NO, P, NHP, NOP), and riparian canopy cover (open, closed) as factors, on chlorophyll *a* response to nutrient enrichment relative to control treatments. Data are from nutrient diffusing substrata experiments conducted in summer 2005

Source	d.f.	MS	F	P-level
Stream	2	0.4930	7.71	<0.001
Nutrients	4	0.0227	0.35	0.841
Canopy	1	0.3070	4.80	0.030
Stream × nutrients	8	0.0202	0.32	0.959
Stream × canopy	2	0.1120	1.76	0.176
Nutrients × canopy	4	0.0207	0.32	0.862
Stream × nutrients × canopy	8	0.0390	0.61	0.769

(Table 4). Therefore, no significant differences were found in the response of periphyton when comparing results from the treatments that were exposed to $\text{NO}_3\text{-N}$ and those that were exposed to $\text{NH}_4\text{-N}$ as a source of N in any of the streams.

Results from the two-way ANOVA with C : N data from the 2004 experiment showed that stream (two-way ANOVA, $F_{(2,101)} = 21.01$, $P < 0.001$) and nutrient treatment (two-way ANOVA, $F_{(5,101)} = 7.96$, $P < 0.001$) had a significant effect on C : N ratios. The forested site showed the highest ratios, the urban site intermediate ratios, and the agricultural site the lowest ratios (Tukey HSD test, $P < 0.01$). Mean C : N ratio was significantly lower in the NOP treatment than in the rest of the treatments (Tukey HSD test, $P < 0.01$). A significant effect of the interaction between stream and nutrient treatment was also found (two-way ANOVA, $F_{(5,101)} = 9.61$, $P < 0.001$). *Post hoc* comparisons of C : N ratios within each stream revealed that this interaction was only due to an unexpectedly low mean C : N ratio (i.e. 4.9) in the NOP treatment of the forested stream compared with the other nutrient treatments (Tukey HSD test, $P < 0.001$). Hence, results indicate no general effect of nutrient amendment on C : N periphyton stoichiometry in the study streams. C : N ratios in the control treatments (i.e. ambient concentrations) were significantly different among streams (one-way ANOVA, $F_{(2,17)} = 27.59$, $P < 0.001$), decreasing from the forested to the agricultural site (Tukey HSD test, $P < 0.05$; Fig. 4).

Effects of nutrient availability on $\text{NO}_3\text{-N}$ uptake

Final isotopic enrichment of the $\text{NO}_3\text{-}^{15}\text{N}$ pool of stream water (i.e. $\delta \text{NO}_3\text{-}^{15}\text{N}$ in ‰) at the distance

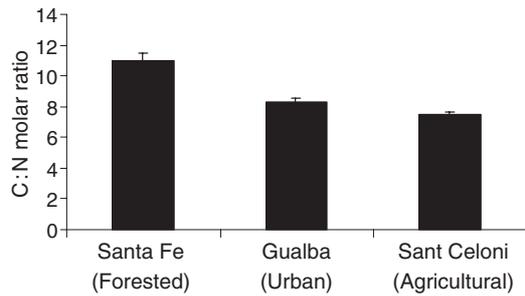


Fig. 4 C : N molar ratios of periphyton growing on control treatments (i.e. ambient concentrations) in the study streams (forested, urban, agricultural). Data reported are mean \pm 1 SEM from nutrient diffusing substrata experiments conducted in summer 2004.

where the NDS were incubated was 2960‰, 13850‰ and 28870‰ in the forested, urban and agricultural streams, respectively. Differences in isotopic enrichment among streams were due to contrast between estimated and actual conditions during the addition, as well as to among-stream differences in whole reach N retention. However, none of these $\text{NO}_3\text{-}^{15}\text{N}$ additions resulted in significant increases in the ambient $\text{NO}_3\text{-N}$ concentration of the stream water. $\text{NO}_3\text{-N}$ uptake rates in control treatments (i.e. ambient concentrations) were significantly different among streams (one-way ANOVA, $F_{(2,15)} = 39.17$, $P < 0.001$). The agricultural stream (Sant Celoni) showed the highest rates, the urban site (Gualba) intermediate rates and the forested site (Santa Fe) the lowest rates (Tukey HSD test, $P < 0.01$; Fig. 5). Taking data from all nutrient treatments together, results from a two-way ANOVA on $\text{NO}_3\text{-N}$ uptake rates showed a significant effect of nutrient treatment (two-way ANOVA, $F_{(5,98)} = 19.24$, $P < 0.001$), but no significant effect of stream type (two-way ANOVA, $F_{(2,98)} = 2.19$, $P = 0.118$). Mean $\text{NO}_3\text{-N}$ uptake rate in the $\text{NH}_4\text{-N}$ amended substrata (i.e. NH or NHP treatments) was significantly lower compared to the other nutrient treatments (Tukey HSD test, $P < 0.01$). The interaction between stream and nutrient treatment was significant (two-way ANOVA, $F_{(10,98)} = 5.28$, $P < 0.001$), thus indicating that the availability of nutrients affected the uptake rate of NO_3 differently depending on the stream considered. Results from Tukey HSD *post hoc* tests comparing the uptake rates in the control treatment with those in the other treatments showed no significant differences for any treatment in the forested site (Tukey HSD test, $P > 0.05$). In the urban

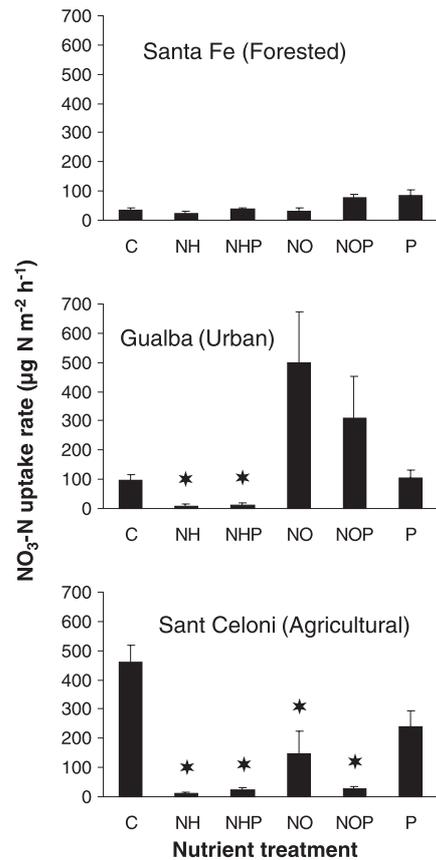


Fig. 5 $\text{NO}_3\text{-N}$ uptake rates of periphyton in the different nutrient treatments (C, NH, NO, P, NHP, NOP) for the forested (top), the urban (middle), and the agricultural (bottom) streams calculated from $^{15}\text{NO}_3$ additions conducted in the summer of 2004. Data reported are mean \pm 1 SEM. Asterisks (*) above bars indicate a significant difference ($P < 0.05$) relative to the control treatment based on *post-hoc* Tukey HSD test on log transformed data.

site, *post hoc* comparisons indicated that only rates of the NH (Tukey HSD test, $P < 0.001$) and NHP (Tukey HSD test, $P < 0.001$) treatments were significantly lower than those of the control treatment. In the agricultural stream, uptake rates were significantly higher in the control treatment than in the rest of the treatments (Tukey HSD test: NH, $P < 0.001$; NO, $P < 0.001$; NHP, $P = 0.008$; NOP, $P < 0.001$), with the exception of the P treatment (Tukey HSD test, $P = 0.272$). Tukey HSD *post hoc* tests were also performed to do pair-wise comparisons between $\text{NH}_4\text{-N}$ (i.e. NH or NHP treatments) and $\text{NO}_3\text{-N}$ (i.e. NO or NOP treatments) amended substrata, to examine the effect of different N sources on $\text{NO}_3\text{-N}$ uptake rates. In the forested stream, no significant differences

in uptake rates were found between NH and NO treatments or between NHP and NOP treatments (Tukey HSD test, $P > 0.05$). In the urban stream, we found significant differences between both NH and NO treatments (Tukey HSD test, $P < 0.001$), and NHP and NOP treatments (Tukey HSD test, $P < 0.001$). Significant differences were also found between NH and NO treatments (Tukey HSD test, $P = 0.002$) in the agricultural stream, but not between NHP and NOP (Tukey HSD test, $P = 0.775$). In the cases where differences were significant, the $\text{NH}_4\text{-N}$ amended substrata always showed lower $\text{NO}_3\text{-N}$ uptake rates than the $\text{NO}_3\text{-N}$ amended substrata.

Discussion

Effects of nutrients and light on periphyton biomass

Results from the experiments demonstrate that light was the main factor affecting algal biomass in the study streams. Differences in chlorophyll *a* accrual rates among streams differing in nutrient concentrations and other physical and chemical characteristics were less important than differences between closed and open canopy locations within the same stream. High nutrient availability, as simulated with the NDS experiments, did not enhance algal biomass accrual at either open or closed canopy sites, confirming the key role of light as a major factor limiting algal growth in these streams, at least during the study period (i.e. summer).

DIN : SRP ratio was not a reliable indicator of nutrient limitation in our streams, as suggested in previous studies (Dodds, 2003). Our results indicate that DIN and SRP ambient concentrations were above the limitation threshold and the stoichiometric imbalance did not affect algal accrual. This supports the observation by Snyder *et al.* (2002) that in-stream DIN : SRP ratios are not good predictors of nutrient limitation at high ambient nutrient concentrations because, at these saturated levels, the absolute rather than the relative availability is of overriding importance. Thus in our study, at least in the human-altered streams, ambient nutrient concentrations were sufficient to support an increase in algal biomass in response to higher light availability at open canopy sites.

Nutrients are expected to play a more important role in streams where the riparian canopy has been

reduced or removed (Rosemond, 1994). In those situations, N and P have been shown to be secondarily limiting (Borchardt, 1996). Contrary to our expectations, response to nutrient enrichment in the absence of riparian shading was lower than at closed canopy sites. This disagrees with previous findings from other studies (Mosisch *et al.*, 2001; Tank & Dodds, 2003). If nutrients were secondarily limiting, we would have expected a significant positive response of nutrient amended treatments compared with controls at open canopy sites, where light was not a limiting factor. However, mean response to nutrient additions was 2.1 times greater at closed than at open canopy locations where, despite relatively low NH_4 and SRP concentrations, even NH, P and NOP treatments showed lower mean chlorophyll *a* values than the control treatment. Other authors have shown the inhibitory response of N and P in experiments using NDS (Tank & Dodds, 2003; Bernhardt & Likens, 2004). Stimulation of heterotrophic growth, and thus enhanced competition with algae, in nutrient (i.e. $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) enriched substrata was put forward by Bernhardt & Likens (2004) to explain their results. These same authors suggested other possible explanations for inhibitory responses of periphyton grown on NDS, such as preference of grazing invertebrates for nutrient-rich periphyton grown on enriched substrata, toxic levels of nutrient enrichment and changes in algal community structure to taxa with lower chlorophyll content. Our experiments do not allow us to determine whether these or other mechanism could explain the obtained results.

The ability of algae to adjust their intracellular chlorophyll *a* concentration is a limitation of the use of chlorophyll *a* as an index of algal biomass (Stevenson, 1996), and could explain the lack of a significant effect of nutrients on algal biomass accrual rates. However, despite its limitations, chlorophyll *a* has been shown to be an effective measure of algal biomass at the community level (Hill & Boston, 1991), and a very practical measure to compare results across studies. Some authors have also suggested that it is preferable to use chlorophyll *a* over other measures such as ash free dry mass, because the latter usually incorporates non-algal organic detritus captured in the periphyton matrix (Snyder *et al.*, 2002). In fact, NDS incubated in our streams, mainly in the urban site, showed accumulations of fine organic matter on their colonising surface. This may have caused the unexpected low

algal accrual rates in the urban stream. Another possible explanation for the lack of effect of nutrients might be the effect of grazers (Steinman, 1996). In this study, our intention was to assess nutrient and light limitation under natural conditions, and therefore we did not attempt to exclude grazers from the colonising substrata. Nevertheless, field observations confirmed that invertebrates on NDS surfaces were generally unusual. Therefore, it is not likely that grazers were responsible for differences in algal accrual among nutrient and light treatments.

As riparian trees were deciduous, the influence of canopy cover on periphyton development may be seasonal. Thus, light might be limiting algal growth only during the vegetative period. We conducted the experiments in summer when differences in light availability between open and closed canopy sites were at its maximum. Moreover, nutrient concentrations in the study streams reach their highest annual values during this period (D. von Schiller, unpublished data). We cannot rule out that limitation of periphyton biomass accrual by nutrients in these streams may occur during other seasons, when ambient nutrient concentrations and the shading effect of canopy cover are lower. Temporal variation in nutrient limitation has been documented for other streams (Elwood *et al.*, 1981; Francoeur *et al.*, 1999; Wold & Hershey, 1999; Francoeur, 2001). Nevertheless, in this paper we aimed to assess how nutrient and light may control algal growth across a gradient of human land uses, rather than to characterise any individual stream in time.

The use of glass fibre filters as our NDS growth surfaces makes it difficult to correctly assess the community structure of periphyton. Therefore, in this study we do not know if *in situ* periphyton communities were structurally similar to those that colonised the experimental artificial substrata, nor if there were differences in community structure among the different nutrient treatments. Within the periphyton community, each taxon/species may differ in their nutrient demands or preferences (Borchardt, 1996). Some authors have reported changes in community structure after nutrient enrichment (Lowe, Golladay & Webster, 1986; Pringle, 1990), whereas others have shown no response (Snyder *et al.*, 2002; Greenwood & Rosemond, 2005). Nevertheless, other environmental factors such as light seem to have a more important effect than nutrients on community structure (Wellnitz, Rader & Ward, 1996).

Effects of nutrient availability on NO₃-N uptake

In contrast to the results from biomass accrual rates, periphyton NO₃-N uptake rates were affected by nutrient availability at both ambient and substratum-enriched nutrient levels. We must highlight that measured uptake rates do not take into account simultaneous remineralisation rates of nutrients (Mulholland, 1996), and do not separate algal uptake from uptake by other organisms (e.g. bacteria and fungi), which may develop within the periphyton matrix (Findlay & Howe, 1993); and thus, are more representative of the whole biofilm community. However, as previously pointed out, the use of glass fibre filters as inorganic colonising surfaces should have promoted autotrophic over heterotrophic growth in our experiments.

As expected, NO₃-N uptake rates at ambient concentrations (i.e. in control treatments) increased from the forested to the agricultural stream following the N availability gradient. There is evidence that the greater the external supply of nutrients, the less important internal nutrient cycling is within the periphyton community in meeting its nutrient demands (Mulholland, 1996). Mulholland & Rosemond (1992) showed that declines in stream water PO₄ concentrations with distance downstream from springs were accompanied by increased phosphatase activity and reduced P content of periphyton communities, suggesting both increased P limitation and cycling. In our case, an increase in N availability across the study streams following a land use gradient was consistent with an increase in N uptake rates as well as a decrease in C : N ratios of the periphyton communities, although no explicit nutrient limitation was found. Ambient N concentrations did not saturate periphyton uptake rates in the study streams, as indicated by higher N uptake rates at higher N concentrations.

Nevertheless, periphyton N uptake rates were altered when ambient N concentrations were increased artificially in the N amended NDS. This response differed among streams, and depended on ambient nutrient availability. At the stream with the highest DIN availability (i.e. the agricultural stream), NO₃-N uptake was significantly lower in all N amended substrata with respect to the control treatment. In the urban site, the stream with intermediate DIN concentration, N uptake was significantly lower

only in the $\text{NH}_4\text{-N}$ amended substrata. Finally, nutrient amendment did not have any significant effect on N uptake in the most oligotrophic stream (i.e. the forested stream). The decreased nutrient demand from the water column may be explained by saturation of N uptake of periphyton communities growing on N enriched NDS. In the forested stream, the additional N source from the N enriched substrata compensated the periphyton community for lower N availability in the water column. However, with higher N availability in the water column along the land use gradient, additional N from enriched substrata was sufficient to complete the N requirements of the periphyton community and thus, decreased N uptake from the water column.

Results suggest that periphyton assemblages growing on N enriched substrata seem to preferentially take up N diffusing from the substratum rather than N from the water column. As suggested by Burkholder (1996), benthic algae may prefer colonised substrata rather than the water column as a nutrient source, although the former has been traditionally assumed to be a more limited and energy-costly source. The physical structure of the biofilm as well as its capacity to adsorb organic and inorganic substances creates a microhabitat that is chemically distinct and nutrient enriched relative to the overlying water (Lock *et al.*, 1984). There is empirical evidence that benthic algae can take up nutrients from natural substrata. For instance, Klotz (1985) found that stream periphyton was less P-limited when growing on sediments with high P content than on sediments with lower quantities of adsorbed P, regardless of stream water P concentration. Efficient use of P from the sediment by algae grown in laboratory aquaria was also demonstrated by Hansson (1992).

Differences among N sources

We hypothesised that algal accrual rates would be greater in $\text{NH}_4\text{-N}$ than in $\text{NO}_3\text{-N}$ amended substrata, basically because of the higher bioavailability of the first (Ward & Wetzel, 1980; McCarthy, Wynne & Berman, 1982). Our results show that in all streams and under different light conditions periphyton accrual was not significantly different between substrata exposed to the two forms of available N sources. Nonetheless, we found differences in the effects of both N sources on the uptake of $\text{NO}_3\text{-N}$ from

the water column. $\text{NH}_4\text{-N}$ seems to be the preferred source of N for periphyton growing on NDS, as indicated by the generally lower $\text{NO}_3\text{-N}$ uptake rates of periphyton communities growing on $\text{NH}_4\text{-N}$ amended substrata (i.e. NH or NHP) in comparison to those growing on $\text{NO}_3\text{-N}$ amended substrata (i.e. NO or NOP).

Little research has been undertaken to elucidate the differential effect of N sources on biomass of benthic algae, and even less on N uptake. The few published works on this issue show no clear patterns (for example see, Bushong & Bachmann, 1989; Maberly *et al.*, 2002), although it is commonly assumed that NH_4 is an energetically less costly source. Theoretically, less energy is required to assimilate $\text{NH}_4\text{-N}$ than $\text{NO}_3\text{-N}$ because the former is available for direct utilisation after uptake. $\text{NO}_3\text{-N}$, in contrast, must be first reduced to $\text{NH}_4\text{-N}$ before utilisation, a process that requires substantial energy. Observed differences in N uptake between $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ treatments could indicate energy limitation of periphyton as suggested by Bushong & Bachmann (1989). Additionally, $\text{NH}_4\text{-N}$ is much more easily retained through abiotic mechanisms such as adsorption onto the periphyton matrix than is $\text{NO}_3\text{-N}$ (Grobbelaär, 1983), and thus may become much more biologically available. It is also possible that $\text{NH}_4\text{-N}$ levels in the $\text{NH}_4\text{-N}$ amended treatments were sufficient to inhibit $\text{NO}_3\text{-N}$ assimilation by suppressing the enzyme nitrate reductase as suggested by Eppley, Coatsworth & Solorzano (1969).

Implications for stream management

Riparian zones not only act as buffers preventing nutrients derived from human activities from entering streams and rivers (Naiman & Décamps, 1997; Sabater *et al.*, 2003), but can also have an effect on in-stream function by regulating light availability to stream communities (Sabater *et al.*, 2000; Sweeney *et al.*, 2004). Unfortunately, riparian zones are highly vulnerable to human activities that directly remove vegetation from these zones to gain land for development (Hohensinner *et al.*, 2004). They are especially vulnerable in Mediterranean regions, where hydrologic and stream channel alterations due to human activities can cause rapid shifts in riparian plant community composition, senescence of woody communities, loss of riparian vegetation

and bank erosion (Gasith & Resh, 1999; Alvarez-Cobelas *et al.*, 2005).

Excess algal biomass accrual presents aesthetic and management problems in lotic ecosystems. In providing both shade and retention of terrestrial nutrient inputs, riparian zones can help restrict periphyton growth in streams draining human-altered catchments. Our results suggest that a potential impact of a nutrient point source could be buffered by a well maintained riparian zone, which may limit the development of algal biomass through regulation of light availability, and thus, reduce the potential for eutrophic conditions with their undesirable effects on the integrity of stream ecosystems (Smith *et al.*, 1999). Furthermore, eutrophic conditions observed in many agricultural streams having high nutrient loads from diffuse sources could be regulated to some extent if riparian vegetation was well preserved, because of both a reduction of diffuse nutrient inputs from the surrounding landscape and a control on algal growth through light limitation. Our results indicate that shade may be more important than nutrients in controlling eutrophication effects derived from human activities. Nevertheless, the role of riparian zones in controlling light availability has seldom been considered by water managers. Finally, periphyton as part of the biofilm, plays an important role in stream nutrient cycling, controlling and being controlled by nutrient availability. Our results confirm that not only concentration increases, but also stoichiometric imbalances should be considered when examining N retention in human altered streams.

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3.4 Inter-annual, annual and seasonal variation of P and N retention in a perennial and an intermittent stream

3.4 Variación interanual, anual y estacional de la retención de P y N en un río permanente y un río estacional

Resumen

Los ríos de cabecera son sitios clave en la retención de nutrientes pero se conoce poco sobre la variación temporal de este proceso. Se utilizaron medidas mensuales tomadas a lo largo de dos años para examinar la variación en la retención de fósforo reactivo soluble (SRP) y amonio (NH_4^+) en dos ríos mediterráneos de cabecera con distinto régimen hidrológico (permanente vs. estacional).

Las diferencias en la retención entre ríos fueron más evidentes para el NH_4^+ , probablemente debido a las grandes diferencias en el potencial de limitación por nitrógeno observadas. En ambos ríos, la eficiencia en la retención de nutrientes fue negativamente afectada por los cambios bruscos en el caudal, mientras que los cambios graduales en la demanda de SRP fueron parcialmente controlados por la dinámica de la vegetación de ribera a través de cambios en la disponibilidad de materia orgánica y luz. Las concentraciones de nutrientes se mantuvieron por debajo del nivel de saturación en ambos ríos. Sin embargo, en el río estacional la demanda de SRP aumentó en relación a la demanda de NH_4^+ cuando el potencial de limitación por fósforo se incrementó (ratio de nitrógeno inorgánico disuelto:SRP más elevado).

Inesperadamente, la variabilidad en la retención de nutrientes no fue más elevada en el río estacional, lo cual sugiere una alta resiliencia de las comunidades responsables de la captación de nutrientes. No obstante, la variabilidad de todas las métricas de retención en cada río aumentó con el aumento de la escala temporal. Una revisión de estudios sobre variación temporal en la retención de nutrientes a diferentes escalas temporales avala este hallazgo e indica un aumento de la variabilidad en la retención de nutrientes con el incremento de la variabilidad en los factores ambientales desde la escala diaria a la escala interanual.

En general, este estudio pone de manifiesto la importancia de las condiciones climáticas locales en la regulación de la retención de nutrientes, e indica efectos potenciales de los cambios en el uso del suelo y el régimen climático sobre el funcionamiento de los ecosistemas fluviales.

Inter-annual, Annual, and Seasonal Variation of P and N Retention in a Perennial and an Intermittent Stream

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ABSTRACT

Headwater streams represent the key sites of nutrient retention, but little is known about temporal variation in this important process. We used monthly measurements over 2 years to examine variation in retention of soluble reactive phosphorus (SRP) and ammonium (NH_4^+) in two Mediterranean headwater streams with contrasting hydrological regimes (that is, perennial versus intermittent). Differences in retention between streams were more evident for NH_4^+ , likely due to strong differences in the potential for nitrogen limitation. In both streams, nutrient-retention efficiency was negatively influenced by abrupt discharge changes, whereas gradual seasonal changes in SRP demand were partially controlled by riparian vegetation dynamics through changes in organic matter and light availability. Nutrient concentrations were below saturation in the two streams; however, SRP demand increased relative to NH_4^+ demand in the intermittent stream as the potential for phosphorus limitation increased (that

is, higher dissolved inorganic nitrogen:SRP ratio). Unexpectedly, variability in nutrient retention was not greater in the intermittent stream, suggesting high resilience of biological communities responsible for nutrient uptake. Within-stream variability of all retention metrics, however, increased with increasing time scale. A review of studies addressing temporal variation of nutrient retention at different time scales supports this finding, indicating increasing variability of nutrient retention with concomitant increases in the variability of environmental factors from the diurnal to the inter-annual scale. Overall, this study emphasizes the significance of local climate conditions in regulating nutrient retention and points to potential effects of changes in land use and climate regimes on the functioning of stream ecosystems.

Key words: nutrient retention; nutrient spiraling; uptake length; temporal variation; nitrogen; phosphorus; intermittent stream.

INTRODUCTION

Along the land-to-ocean aquatic continuum, headwater streams represent the key sites of

nutrient storage, transformation, and removal (Alexander and others 2000; Peterson and others 2001), processes generally compiled under the term nutrient retention. Over the past three decades, the nutrient spiraling concept (Webster and Patten 1979; Newbold and others 1981; Stream Solute Workshop 1990), which combines the processes of

nutrient uptake and transport, has provided an excellent framework to advance research on nutrient retention in streams. Numerous studies have used this concept to evaluate the variation of nutrient retention among different streams and to examine controlling factors (for a review see Ensign and Doyle 2006). Even though streams are highly dynamic ecosystems (Ward 1989; Palmer and Poff 1997), few studies have addressed the temporal variation of nutrient retention within streams.

Information on temporal variation in stream nutrient retention is important for several reasons. Firstly, it allows researchers to evaluate how many measurements of nutrient retention are needed to characterize adequately a stream. Secondly, it may help determine key factors driving nutrient-retention processes. Thirdly, it is critical for improving models of nutrient dynamics at larger spatial and temporal scales (Doyle 2005; Ensign and Doyle 2006; Wollheim and others 2006). Finally, it is needed to evaluate the utility of reference conditions as a managing tool (Nijboer and others 2004) and allows better predictions of how the ability of streams to retain nutrients will vary in response to changes in land use and climatic regimes.

Results from the few studies that have examined temporal variation of nutrient retention within streams indicate substantial variation at different time scales, from diurnal (for example, Martí and Sabater 1994; Mulholland and others 2006) to annual (for example, Simon and others 2005; Hoellein and others 2007). This finding is not surprising, considering that many of the environmental factors shown to influence nutrient retention, such as discharge (Wollheim and others 2001; Peterson and others 2001), temperature (Butturini and Sabater 1998; Simon and others 2005), and nutrient concentrations (Mulholland and others 2002; O'Brien and others 2007), may change dramatically over time. The temporal pattern of these environmental factors varies among streams, ultimately due to the influence of local catchment and climate conditions (Hynes 1975; Allan 1995). Thus, each stream is characterized by its own "heartbeat" of environmental factors, which results from a combination of factors following gradual seasonal patterns (for example, temperature), factors displaying an abrupt temporal regime (for example, discharge), and factors showing gradual seasonal patterns with abrupt changes associated with periods of flood or drought (for example, nutrient concentrations). The autotrophic (that is, algae, macrophytes, and bryophytes) and heterotrophic (that is, bacteria and fungi) compartments responsible for biotic nutrient

uptake vary with time in response to changes in some of these factors (Minshall 1978; Bott and Kaplan 1985; Francoeur and others 1999). Nutrient retention is expected to follow these changes and, thus, to exhibit a characteristic temporal pattern within each stream.

Most studies on the temporal variation of stream nutrient retention have been constrained to time scales of 1 year or less (but see Martí and Sabater 1996). Nutrient retention is, however, expected to vary not only within and among seasons but also among years. This is of particular interest in Mediterranean streams, where a pronounced seasonality and high inter-annual variability are typical (Gasith and Resh 1999). Additionally, although studies of nutrient retention performed over 1 year have been used to draw conclusions about seasonality (for example, Mulholland and others 1985; Simon and others 2005; Hoellein and others 2007), the consistency of seasonal patterns can be verified only by examining its repeatability among years. Additionally, the variability of nutrient retention is expected to increase with the time scale covered by the study because of a higher probability of longer-term studies in capturing a greater variability in environmental factors.

Because processing rates in streams are strongly influenced by transport, the study of nutrient retention under variable flow conditions or hydrologic regimes can contribute to a better understanding of temporal variation in stream nutrient retention (Fisher and others 2004). Hydrological intermittency, which is also characteristic of Mediterranean and other arid and semi-arid streams, can exert a strong influence on nutrient dynamics (Dahm and others 2003; Bernal and others 2005; Lillebo and others 2007), including the effects of prior drought conditions on nutrient retention, which are largely unknown. Temporal variation in nutrient retention is, therefore, expected to be larger in intermittent than in perennial streams because of the additional effect of droughts on the biological communities responsible for nutrient uptake (Lake 2003).

Phosphorus (P) and nitrogen (N) are the nutrients that most commonly limit primary production in streams (Elwood and others 1981; Borhardt 1996). Thus, studies of nutrient retention have focused on one or both of these elements, normally using soluble reactive phosphorus (SRP) and ammonium (NH_4^+) because these are the P and N forms taken up preferentially by most organisms. Yet, simultaneous experiments with both nutrients can provide interesting information from a stoichiometric perspective because the retention of one

nutrient is expected to be dependent on its relative availability with respect to other nutrients. Both SRP and NH_4^+ are subjected to abiotic and assimilatory biotic retention processes, but NH_4^+ can additionally undergo dissimilatory processes (for example, nitrification). Consequently, NH_4^+ retention is expected to be less influenced by changes in environmental variables and, therefore, to be less variable than SRP retention.

Our goal was to explore temporal variation in nutrient retention by addressing the three aspects of temporal extent, hydrologic regime, and nutrient stoichiometry. Thus, we analyzed temporal variation of both SRP and NH_4^+ retention in two Mediterranean streams with contrasting hydrological regimes: a perennial and an intermittent stream. The study was done over two complete hydrologic years to cover seasonal, annual, and inter-annual variation. To examine within-stream temporal variation, we considered the magnitude, temporal pattern, and range of retention metrics in relation to changes in associated environmental drivers (that is, discharge, temperature, and nutrient concentrations). Finally, we compiled published data on nutrient retention worldwide to explore further the relationship between variability of nutrient retention and environmental drivers over different time scales.

METHODS

Study Sites

This study was conducted in La Tordera catchment (Catalonia, NE Spain). With an area of 868.5 km² dominated by siliceous geology, the catchment covers an altitudinal gradient of approximately 1,700 m in less than 30 km horizontal distance from the highest peaks (Montseny mountain range) to the river mouth at the Mediterranean Sea. The climate is Mediterranean, with warm, dry summers and mild, humid winters, but the pronounced altitudinal gradient results in a mosaic of microclimates with contrasting local temperature, precipitation, and evapotranspiration regimes. Within this catchment, we selected a perennial and an intermittent stream located at the extremes of the altitudinal gradient. The perennial stream (Santa Fe: 2° 27' 40"E, 41° 46' 34"N) is located in the Montseny Natural Protected Area. Monthly mean temperatures range from 3 in January to 20°C in August. Mean annual precipitation is approximately 900 mm, occurring mostly as rain in autumn and spring but with occasional snow in winter. At the study site (1,136 m asl), the stream drains a 2.6-km² granitic catchment forested primarily with silver fir (*Abies alba*) at higher eleva-

tions and beech (*Fagus sylvatica*) at lower elevations. Human use is mainly recreational, with some dispersed shepherding. Based on the influence of temperature, precipitation, and vegetation dynamics on stream hydrology, we divided each hydrologic year into two periods: (1) from December to April (the cool-wet and dormant period, or *wet period*), and (2) from May to November (the warm-dry and vegetative period, or *dry period*).

The intermittent stream (Fuirosos, 2° 34' 55"E, 41° 42' 12"N) is located in the Montnegre-Corredor Natural Protected Area. The climate is warmer and drier than at the perennial stream, with monthly mean temperatures ranging from 5 in January to 24°C in August. Annual precipitation varies greatly among years, with a mean of approximately 750 mm. At the study site (115 m asl), the stream drains a 14.4-km² granitic catchment covered mostly by perennial cork oak (*Quercus suber*) and Aleppo pine (*Pinus halepensis*) at the lower elevations and a deciduous forest of chestnut (*Castanea sativa*), hazel (*Corylus avellana*), and oak (*Quercus pubescens*) at the higher elevations. Human land use is restricted to the periodic harvesting of bark from cork trees and agricultural fields that occupy less than 2% of the catchment area. Stream discharge is intermittent in summer with no flow periods of variable duration among years. Only during the wettest years (annual precipitation >800 mm), does the stream not dry in summer. Due to the influence of drought, we divided each hydrologic year into three periods: (1) from December to mid-March (the cool-wet and dormant period, or *wet period*), (2) from mid-March to August (the warm-dry and vegetative period or *dry period*), and (3) from September to November (the transition from dry to wet conditions, or *transition period*).

We selected a representative reach of each stream for experiments. In the perennial stream, the reach was 140-m long and riffle-pool dominated with a slope of 0.094 m m⁻¹. The streambed was composed of cobbles (47%), boulders (25%), and pebbles (21%), with patches of gravel and sand. Riparian vegetation was well developed and dominated by beech, with some stems of common elder (*Sambucus nigra*) and poorly developed herbaceous understory. In the intermittent stream, the study reach was 80-m long and riffle-pool dominated with a slope of 0.074 m m⁻¹. Dominant substrate was finer than in the perennial stream and was composed of sand (56%) and boulders (30%), with patches of cobbles, pebbles, gravel, and bedrock. Riparian vegetation was dense, consisting mainly of alder (*Alnus glutinosa*) and sycamore (*Platanus hispanica*), with substantial herbaceous understory.

Field Sampling and Laboratory Analyses

This study covered two hydrologic years, from September 2004 through August 2006. During this period, the two streams were sampled approximately bi-weekly for water temperature, discharge, and concentrations of nitrate (NO_3^-) + nitrite (NO_2^-), ammonium (NH_4^+), and SRP. These data allowed a detailed characterization of the temporal variation of environmental variables known to affect nutrient retention. To examine temporal variation in nutrient retention, we conducted short-term, constant-rate additions of SRP (as $\text{Na}(\text{H}_2\text{PO}_4) \cdot 2\text{H}_2\text{O}$) and NH_4^+ (as NH_4Cl) into each stream once a month (coinciding with some of the regular sampling dates). Chloride (Cl^- , as NaCl) was added as a conservative tracer. A total of 25 additions in the perennial stream and 20 additions in the intermittent stream were completed. No flow or high-discharge conditions were avoided due to methodological constraints.

The methodology for nutrient additions followed Webster and Valett (2006). A Masterflex (Vernon Hills, Illinois, USA) L/S battery-powered peristaltic pump was used to deliver nutrient/tracer solution to the stream. Additions began at approximately 11 a.m. and lasted until conductivity reached a plateau at the bottom of the study reach (that is, 1–4 h, depending on stream discharge on each date). Conductivity was automatically recorded at the bottom of the reach every 5 s using a WTW (Weilheim, Germany) 340i portable conductivity meter connected to a Campbell Scientific (Logan, Utah, USA) data logger. We measured conductivity and collected water samples at eight stations along the reach prior to the addition to determine background concentrations (two to three replicates per station), and we collected samples at the same stations when conductivity reached a plateau (hereafter, “plateau” concentrations; five replicates per station). Water samples for nutrient chemistry were immediately filtered through Whatman (Kent, UK) GF/F glass-fiber filters (0.7 μm), stored on ice in the field, and refrigerated at 4°C or frozen in the laboratory until analysis.

On each addition date, stream discharge was estimated based on a mass-balance approach using the time-curve conductivity data recorded at the bottom of the reach (Gordon and others 2004). Discharge values from additions were compared with discharge measurements done at a single transect on the regular sampling dates, and the calculated correction factor was applied to the latter. Wetted width was determined on cross-sectional transects located at each sampling station

along the reach. Water temperature was measured with the conductivity meter at each station during background and plateau samplings, and the values were averaged.

Concentrations of $\text{NO}_3^- + \text{NO}_2^-$ and SRP in stream water samples were analyzed in the laboratory using a Bran + Luebbe (Norderstedt, Germany) TRAACS 2000 Autoanalyzer II. NH_4^+ concentration was analyzed on a Skalar (Breda, The Netherlands) San⁺ Autoanalyzer. All nutrient analyses were performed following standard colorimetric methods as described in APHA (1995). Analysis of the concentration of NO_2^- in more than 50% of the samples showed that it was negligible, accounting for less than 4% of dissolved inorganic nitrogen (DIN) in both study streams; thus, NO_3^- is used hereafter to refer to the concentration of $\text{NO}_3^- + \text{NO}_2^-$. DIN concentration was calculated as the sum of NO_3^- and NH_4^+ concentrations.

Calculation of Nutrient Retention Metrics

Using data from the nutrient additions, we calculated three metrics of retention for each nutrient (that is, NH_4^+ and SRP) and each addition date and stream: uptake length (S_w , m), uptake velocity (V_f , mm min^{-1}), and uptake rate (U , $\mu\text{g m}^{-2} \text{s}^{-1}$). S_w , the average distance traveled by a nutrient molecule before being removed from the water column (Newbold and others 1981), was calculated as the negative inverse of the longitudinal uptake rate (k_w , m^{-1}). This rate is the slope of the regression of the ln-transformed and background-corrected nutrient:conductivity ratio versus distance downstream from the addition point. S_w is an indicator of the nutrient-retention efficiency at the reach scale (Webster and Valett 2006). V_f , the velocity at which a nutrient is removed from the water column, was calculated as the stream-specific discharge (that is, discharge/width) divided by S_w . V_f is an indicator of nutrient demand relative to concentration in the water column (Hall and others 2002). U , the mass of a nutrient taken up from the water column per unit streambed area and time, was calculated as V_f multiplied by ambient nutrient concentration. U is used as an indicator of stream nutrient-retention capacity (Webster and Valett 2006). Finally, we calculated the nutrient-demand ratio ($V_f\text{-NH}_4^+ : V_f\text{-SRP}$) by dividing the demand of NH_4^+ over that of SRP.

Nutrient concentrations in plateau samples are slightly enriched relative to background and, thus, may overestimate ambient S_w (Mulholland and others 2002). To minimize this effect, nutrient concentration of the added solution and pump flow

rate was adjusted for each addition to reach a relatively low and similar nutrient-enrichment level among addition dates and streams. In the perennial stream, increases in nutrient concentration relative to background nutrient concentration at the top sampling station (mean \pm SE) were $65 \pm 8 \mu\text{g P l}^{-1}$ for SRP and $54 \pm 9 \mu\text{g N l}^{-1}$ for NH_4^+ . In the intermittent stream, these increases were $79 \pm 16 \mu\text{g P l}^{-1}$ for SRP and $65 \pm 14 \mu\text{g N l}^{-1}$ for NH_4^+ . We found no relationship between the increase in enrichment concentration and S_w , except for NH_4^+ in the intermittent stream ($r^2 = 0.23$, $P = 0.032$, $n = 20$).

Data Analysis

All k_w used to estimate S_w values reported in this study are from statistically significant ($P < 0.05$) regressions of nutrient-addition data. Standard errors for each nutrient retention metric (that is, S_w , V_f , and U) were calculated based on error estimates for k_w . This error estimate is a practical approach to real measurement uncertainty associated with each nutrient addition (Hanafi and others 2007). We used Pearson's product-moment correlation to explore relationships between environmental parameters and nutrient-retention metrics. The independent t -test was used to compare environmental parameters and nutrient-retention metrics between streams, and the dependent t -test was used to compare retention metrics between nutrients in each stream. We applied linear regression analysis to examine relationships between environmental parameters and specific nutrient-retention metrics as follows. Firstly, the influence of discharge was explored using S_w . Secondly, the influence of water temperature was analyzed using V_f because this metric corrects S_w for discharge. Finally, we examined the influence of nutrient availability using either V_f or U . Additionally, potential U saturation was investigated with a Michaelis-Menten model (with Levenberg-Marquardt estimation algorithm and non-log-transformed data). To explore whether or not the influence of measured environmental drivers varied depending on time of year, regressions were also estimated separately with data for each hydrological period (as described in the "Study site" section). Variability of environmental parameters and retention metrics was compared within and among streams employing the range quotient, R_q (that is, maximum value/minimum value). Patterns of variability across time scales were further examined using linear regressions between R_q of nutrient-retention metrics and environmental parameters, based on

data compiled from studies addressing temporal variation of stream nutrient retention at different time scales. In this analysis, we considered only studies for which data on retention metrics and environmental factors were available for three or more dates. Data were log-transformed as needed to meet assumptions of parametric statistics. Statistical analyses were done using Statistica 6.0 (Statsoft, Tulsa, Oklahoma, USA).

RESULTS

Environmental Parameters

Water temperature and discharge followed seasonal patterns (Figure 1) and were negatively correlated in both the perennial ($r = -0.62$, $P < 0.001$, $n = 49$) and the intermittent stream ($r = -0.45$, $P = 0.004$, $n = 39$). Temperature was higher and spanned a broader range in the intermittent stream (Table 1). Conversely, discharge was relatively similar in both streams, but the range was larger in the intermittent stream (Table 1). Discharge was generally low from late-spring to early autumn (that is, dry period), and high, especially in the second study year, from late-autumn to early spring (that is, transition and wet periods) (Figure 1). The intermittent stream dried out in the summer, with a shorter no flow period in the first year (20 days) than in the second year (65 days).

Ambient concentrations of SRP and NO_3^- and their temporal patterns differed between streams. The concentration of SRP was higher, and that of NO_3^- was lower, in the perennial stream (Table 1). The concentration range of both nutrients was broader in the intermittent stream, especially for NO_3^- (Table 1). In the perennial stream, SRP and NO_3^- concentrations were positively correlated ($r = 0.55$, $P < 0.001$, $n = 49$) and followed a marked seasonal pattern that was consistent between years, with higher values during the warm, dry period than during the cold, wet period (Figure 1). Accordingly, both nutrients correlated positively with water temperature (SRP: $r = 0.72$, $P < 0.001$, $n = 49$; NO_3^- : $r = 0.73$, $P < 0.001$, $n = 49$) and negatively with discharge (SRP: $r = -0.71$, $P < 0.001$, $n = 49$; NO_3^- : $r = -0.27$, $P = 0.065$, $n = 49$). In contrast, SRP concentration showed a similar pattern as in the perennial stream in the intermittent stream, whereas NO_3^- concentration showed a nearly opposite pattern (Figure 1). The concentration of NO_3^- correlated negatively with water temperature ($r = -0.55$, $P < 0.001$, $n = 39$) and correlated positively with stream discharge ($r = 0.59$, $P < 0.001$, $n = 39$), whereas SRP concentration only correlated

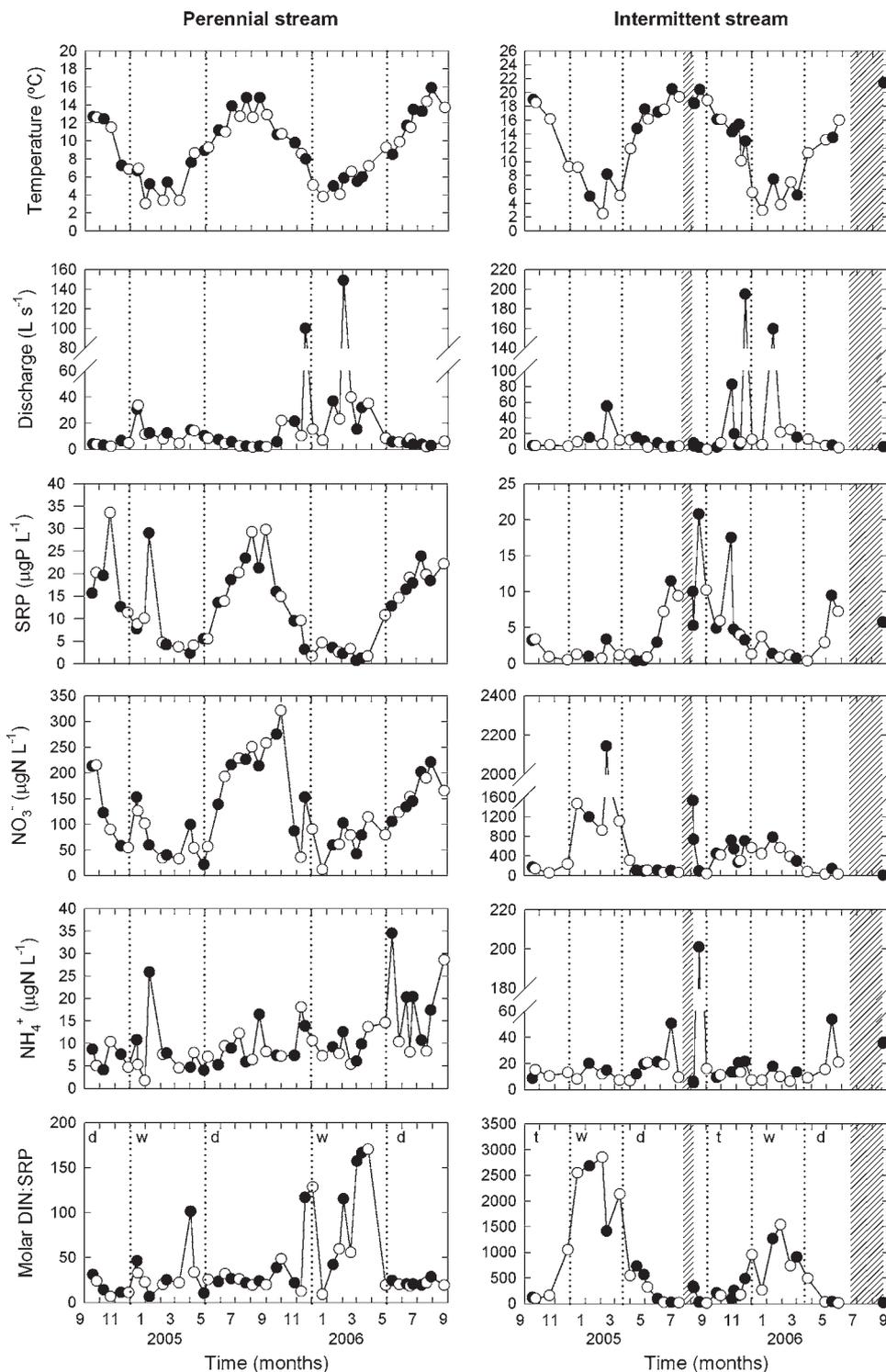


Figure 1. Temporal variation of stream water temperature, discharge, soluble reactive phosphorus (SRP), nitrate (NO_3^-), ammonium (NH_4^+), and molar DIN:SRP in the perennial stream ($n = 49$) and the intermittent stream ($n = 39$). Data are from regular samplings carried out during the 2-year study period. *Open symbols* denote days when nutrient additions were conducted. *Dotted lines* separate the hydrological periods, which are labeled with *small letters* in the lower panels (d, dry period; w, wet period; t, transition period). *Dashed bars* indicate the no flow periods in the intermittent stream. Notice different scales in y-axis for clarity of patterns.

positively with water temperature ($r = 0.50$, $P < 0.001$, $n = 39$). Concentrations of both nutrients increased dramatically after the first year's drought in the intermittent stream (Figure 1).

The concentration of NH_4^+ averaged less than 13% of DIN in both streams, but it was higher and

showed a broader range in the intermittent stream (Table 1; Figure 1). The NH_4^+ concentration was positively related to water temperature ($r = 0.34$, $P = 0.035$, $n = 39$) and SRP ($r = 0.39$, $P = 0.015$, $n = 39$) and negatively related to NO_3^- concentration ($r = -0.42$, $P = 0.008$, $n = 39$) in this stream

Table 1. Mean, Standard Error (SE), Minimum (min), Maximum (max), and Range Quotient (R_q , max/min) of Physicochemical Parameters in the Perennial Stream ($n = 49$) and the Intermittent Stream ($n = 39$) over the 2-year Study Period

Parameter	Perennial stream				Intermittent stream				P
	Mean	SE	Min–Max	R_q	Mean	SE	Min–Max	R_q	
Temperature (°C)	9.3 <i>(8.8)</i>	0.5 <i>(0.5)</i>	3.1–15.9 <i>(3.1)–(14.4)</i>	5 <i>(5)</i>	13.1 <i>(11.5)</i>	0.9 <i>(0.9)</i>	2.5–21.4 <i>(2.5)–(19.4)</i>	9 <i>(8)</i>	<0.001 <i>(0.054)</i>
Discharge (L s ⁻¹)	15.9 <i>(12.6)</i>	3.6 <i>(1.6)</i>	1.6–148.8 <i>(4.5)–(52.0)</i>	93 <i>(12)</i>	20.0 <i>(9.0)</i>	6.4 <i>(1.0)</i>	0.1–194.9 <i>(0.7)–(24.9)</i>	1,820 <i>(36)</i>	0.558 <i>(0.107)</i>
SRP (µg P L ⁻¹)	13 <i>(13)</i>	1 <i>(1)</i>	1–34 <i>(2)–(34)</i>	49 <i>(20)</i>	5 <i>(3)</i>	1 <i>(1)</i>	0–21 <i>(0)–(10)</i>	56 <i>(26)</i>	<0.001 <0.001
NO ₃ ⁻ (µg N L ⁻¹)	129 <i>(125)</i>	11 <i>(12)</i>	12–321 <i>(12)–(321)</i>	26 <i>(26)</i>	452 <i>(369)</i>	78 <i>(64)</i>	14–2,143 <i>(35)–(1,468)</i>	156 <i>(42)</i>	<0.001 (0.019)
NH ₄ ⁺ (µg N L ⁻¹)	10 <i>(10)</i>	1 <i>(1)</i>	2–35 <i>(2)–(29)</i>	20 <i>(19)</i>	20 <i>(12)</i>	5 <i>(1)</i>	5–201 <i>(6)–(21)</i>	39 <i>(3)</i>	0.034 (0.047)
Molar DIN:SRP	40.8 <i>(35.4)</i>	6.0 <i>(5.3)</i>	6.5–170.7 <i>(6.6)–(170.7)</i>	26 <i>(26)</i>	614.1 <i>(695.9)</i>	125.6 <i>(127.4)</i>	11.5–2,853.1 <i>(11.5)–(2,550.4)</i>	247 <i>(222)</i>	<0.001 <0.001

Data in italics within parenthesis represent values of parameters on nutrient addition dates (perennial stream, $n = 25$; intermittent stream, $n = 20$). SRP, soluble reactive phosphorus; NO₃⁻, nitrate; NH₄⁺, ammonium; DIN, dissolved inorganic nitrogen. The P values are the results of independent t-tests comparing physicochemical parameters between streams. Significant ($P < 0.05$) t-test results are highlighted in bold.

only. Pre- and post-drought increases of NH₄⁺ were also observed in the intermittent stream (Figure 1).

The mean and range of the DIN:SRP molar ratio was larger in the intermittent stream (Table 1), although it showed a similar seasonal pattern in both streams, with its highest values during the wet period (Figure 1). Water temperature and DIN:SRP correlated negatively in both the perennial ($r = -0.34$, $P = 0.015$, $n = 49$) and the intermittent stream ($r = -0.74$, $P < 0.001$, $n = 49$), whereas discharge was positively related to DIN:SRP only in the perennial stream ($r = 0.54$, $P < 0.001$, $n = 49$).

Dates when nutrient additions were performed captured a considerable part of the variation in environmental parameters observed in the richer data set from the regular bi-weekly samplings (Table 1).

Nutrient Retention

Nutrient-retention Efficiency. In the perennial stream, mean S_w -NH₄⁺ was 4.2-fold shorter than mean S_w -SRP (Table 2), and S_w -NH₄⁺ never exceeded S_w -SRP. S_w for the two nutrients was

Table 2. Mean, Standard Error (SE), Minimum (min), Maximum (max), and Range Quotient (R_q , max/min) of Retention Metrics for Both Added Nutrients on Nutrient Addition Dates in the Perennial Stream ($n = 25$) and the Intermittent Stream ($n = 20$) over the 2-year Study Period

Retention metric	Nutrient	Perennial stream				Intermittent stream				P
		Mean	SE	Min–Max	R_q	Mean	SE	Min–Max	R_q	
Uptake length, S_w (m)	SRP	406.4	81.3	97.3–1,556.8	16	384.6	86.0	66.1–919.2	14	0.800
	NH ₄ ⁺	96.4	19.3	18.6–618.4	33	223.4	50.0	23.8–568.1	24	0.002
P		<0.001				0.012				
Uptake velocity, V_f (mm min ⁻¹)	SRP	0.7	0.1	0.2–1.4	8	0.6	0.1	0.1–2.2	32	0.166
	NH ₄ ⁺	3.4	0.7	0.7–11.5	17	1.1	0.2	0.2–3.1	14	<0.001
P		<0.001				0.012				
Uptake rate, U (µg m ⁻² min ⁻¹)	SRP	8.5	1.7	0.3–33.2	112	1.5	0.3	0.1–5.9	77	<0.001
	NH ₄ ⁺	31.1	6.2	3.7–83.1	23	13.8	3.1	2.7–59.1	22	0.001
P		<0.001				<0.001				

NH₄⁺, ammonium; SRP, soluble reactive phosphorus. The P values are the results of independent t-tests comparing retention metrics between streams and dependent t-tests comparing retention metrics between nutrients. Significant ($P < 0.05$) t-test results are highlighted in bold.

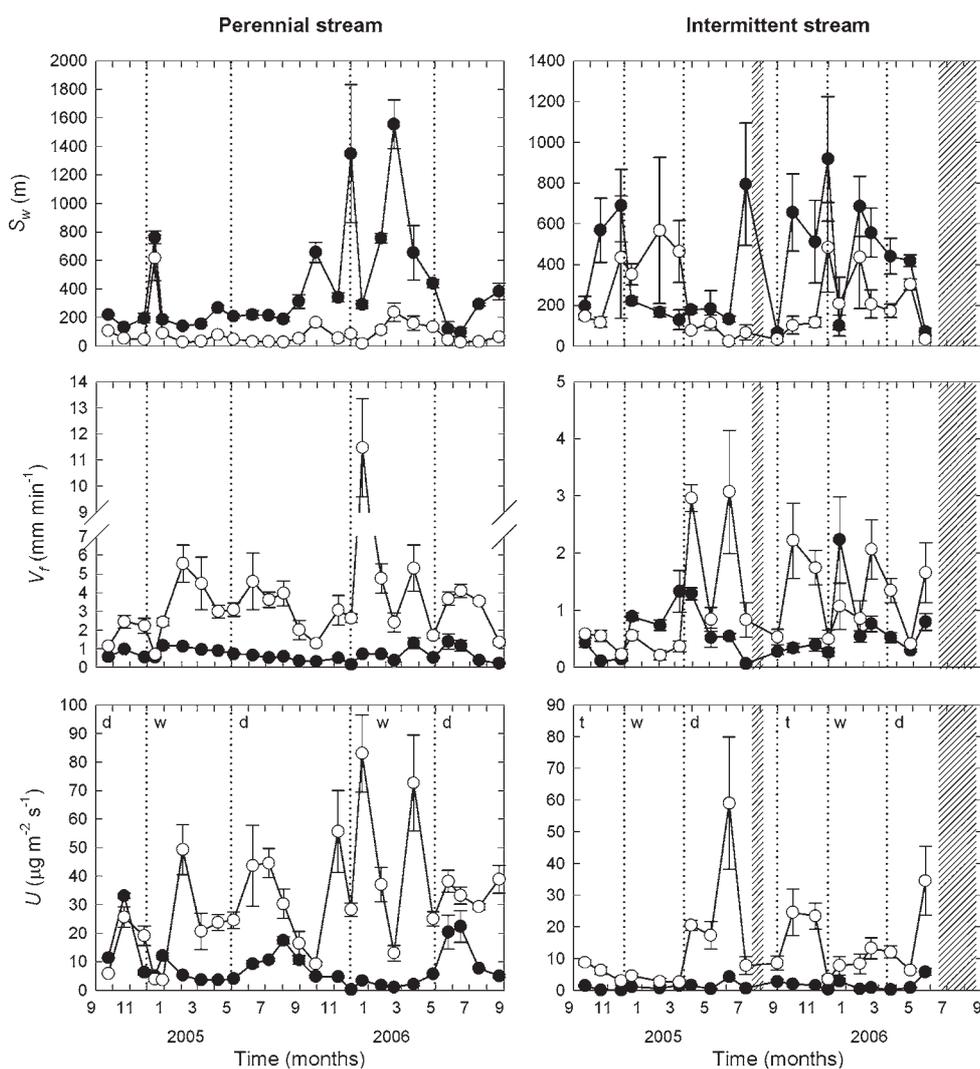


Figure 2. Temporal variation of the uptake length (S_w), uptake velocity (V_f), and uptake rate (U) of soluble reactive phosphorus (SRP) (closed symbols) and ammonium (NH_4^+ ; open symbols) in the perennial stream ($n = 25$) and the intermittent stream ($n = 20$). Data are from monthly nutrient additions done over the 2-year study period. Error bars are \pm SE (see text for detailed explanation). Dotted lines separate the hydrological periods, which are labeled with small letters in the lower panels (d, dry period; w, wet period; t, transition period). Dashed bars indicate the no flow periods in the intermittent stream. Notice different scales in y-axis for clarity of patterns.

positively correlated ($r = 0.72$, $P < 0.001$, $n = 25$) and tended to be longest during the wet period, especially in the second study year (Figure 2). In the intermittent stream, mean $S_w\text{-NH}_4^+$ was 1.7-fold shorter than mean $S_w\text{-SRP}$ (Table 2), but they were not correlated. $S_w\text{-NH}_4^+$ tended to be longest during the wet period when it exceeded $S_w\text{-SRP}$, but no clear seasonal pattern was observed for $S_w\text{-SRP}$ (Figure 2). A comparison between streams showed that mean $S_w\text{-NH}_4^+$ was 2.3-fold shorter in the perennial than in the intermittent stream, whereas $S_w\text{-SRP}$ was similar between streams (Table 2).

Nutrient Demand. In the perennial stream, $V_f\text{-NH}_4^+$ was positively correlated with $V_f\text{-SRP}$ ($r = 0.46$, $P = 0.022$, $n = 25$), although it was consistently higher (4.9-fold, on average; Table 2) and reached maximum value during the wet period (Figure 2). In the intermittent stream, mean $V_f\text{-NH}_4^+$ was 1.8-fold higher but uncorrelated with

mean $V_f\text{-SRP}$ (Table 2). $V_f\text{-SRP}$ was highest during the wet period when it exceeded $V_f\text{-NH}_4^+$, which showed no clear seasonal pattern (Figure 2). In this stream, $V_f\text{-SRP}$ was higher than $V_f\text{-NH}_4^+$ only during the wet period, especially in the first study year (Figure 2). A comparison between streams showed that mean $V_f\text{-NH}_4^+$ was 3.1-fold higher in the perennial stream, whereas a similar mean $V_f\text{-SRP}$ was found in both streams (Table 2).

Nutrient Uptake Capacity. In the perennial stream, mean $U\text{-NH}_4^+$ was 3.7 times higher than mean $U\text{-SRP}$ (Table 2), and they were not correlated. Only $U\text{-SRP}$ showed a clear seasonal pattern, with its highest values during the dry period (Figure 2). In the intermittent stream, mean $U\text{-NH}_4^+$ was 9.2 times higher than mean $U\text{-SRP}$ (Table 2). U for both nutrients correlated positively ($r = 0.53$, $P = 0.015$, $n = 20$), showing its highest values during the dry period (Figure 2). An increase in $U\text{-NH}_4^+$ during the transition period was

also apparent, especially in the second study year (Figure 2). A comparison between streams showed that mean U -SRP and U - NH_4^+ were 5.7 and 2.3-fold higher, respectively, in the perennial than in the intermittent stream (Table 2).

Variability of Nutrient-retention Metrics. Retention of both nutrients showed high variability in our study streams. Metrics spanned ranges of one ($R_q > 10$) or even two ($R_q > 100$) orders of magnitude, except for V_f -SRP in the perennial stream (Table 2). Furthermore, R_q increased as the time scale was expanded from seasonal to inter-annual for all three retention metrics and in both streams (Figure 3). R_q was lowest when computed per period (that is, seasonal scale) and highest when computed for the two study years (that is, inter-annual scale), with intermediate R_q at the annual

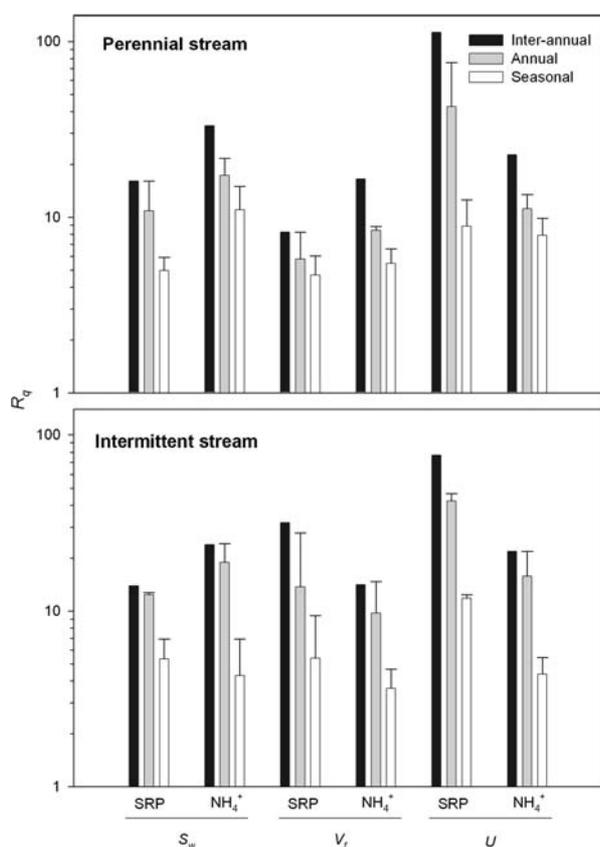


Figure 3. Average range quotient (R_q) of uptake length (S_w), uptake velocity (V_f), and uptake rate (U) of soluble reactive phosphorus (SRP) and ammonium (NH_4^+) in the perennial stream (upper panel) and the intermittent stream (lower panel) computed for the 2 years together (that is, inter-annual scale), each year separately (that is, annual scale; $n = 2$ for both streams), and each period of each year separately (that is, seasonal scale; $n = 6$ for the perennial stream, and $n = 6$ for the intermittent stream). Error bars are $+ SE$.

scale (Figure 3). A comparison between streams showed that R_q was similar or higher in the perennial stream, except in the case of V_f -SRP (Table 2; Figure 3).

Environmental Drivers of Nutrient Retention

Discharge. In the perennial stream, both S_w -SRP and S_w - NH_4^+ increased with discharge following power functions (Table 3). These relationships held for both nutrients when the analysis was restricted to the wet period (Table 3). When data from the dry period were analyzed, only S_w - NH_4^+ showed a significant relationship with discharge, and it was best fitted by a linear function (Table 3). In the intermittent stream, the relationships of discharge with both S_w -SRP and S_w - NH_4^+ also followed power functions, but these accounted for a lower proportion of the variation of S_w than in the perennial stream (Table 3). When each period was analyzed separately, only S_w -SRP showed a linear relationship with discharge during the wet period (Table 3).

Temperature. With data from all addition dates used together, V_f -SRP was not related to temperature in the perennial stream. When the extremely low V_f -SRP value measured in December 2005 (Figure 2) was excluded, however, a negative exponential relationship was found (Figure 4). Considering data from each period separately, we found only a negative exponential relationship of V_f -SRP with temperature during the dry period (Figure 4). In the intermittent stream, using data from all addition dates together, V_f -SRP showed a negative exponential relationship with temperature (Figure 4). This relationship lost its significance when data from each hydrological period were analyzed separately. No relationship between V_f - NH_4^+ and temperature was found in any of the streams, but the nutrient-demand ratio (that is, the ratio between V_f - NH_4^+ and V_f -SRP) exponentially increased with temperature in the intermittent stream (Figure 5).

Nutrient Availability. Considering data from all addition dates used together, neither V_f -SRP nor V_f - NH_4^+ was related to their respective nutrient concentrations (that is, SRP and NH_4^+) in any of the streams. When we analyzed each hydrological period separately, we found only a positive logarithmic relationship for V_f -SRP in the intermittent stream during the transition period (V_f -SRP = $0.285 \cdot \log \text{SRP} + 0.202$, $r^2 = 0.76$, $P = 0.023$, $n = 6$). The nutrient-demand ratio did, however, decline exponentially with the DIN:SRP molar ratio in the intermittent stream with data from all addition dates

Table 3. Relationships of the Uptake Length, S_w (m) of Soluble Reactive Phosphorus (SRP) and Ammonium (NH_4^+) with Discharge, Q (L s^{-1}) in the Perennial and the Intermittent Stream using All Data Together (that is, ALL) or Separated into Periods (that is, Dry, Wet, Transition)

Nutrient	Period	Perennial stream	Intermittent stream
SRP	ALL	$S_w\text{-SRP} = 49.73 \cdot Q^{0.79}$ $r^2 = 0.49, p < 0.001$	$S_w\text{-SRP} = 122.01 \cdot Q^{0.45}$ $r^2 = 0.20, p = 0.045$
	Dry	n.s.	n.s.
	Wet	$S_w\text{-SRP} = 37.13 \cdot Q^{0.89}$ $r^2 = 0.54, P = 0.004$	$S_w\text{-SRP} = 30.44 \cdot Q - 130.50$ $r^2 = 0.82, P = 0.013$
	Transition	–	n.s.
NH_4^+	ALL	$S_w\text{-NH}_4^+ = 9.27 \cdot Q^{0.85}$ $r^2 = 0.47, P < 0.001$	$S_w\text{-NH}_4^+ = 41.89 \cdot Q^{0.68}$ $r^2 = 0.38, P = 0.004$
	Dry	$S_w\text{-NH}_4^+ = 12.25 \cdot Q - 24.87$ $r^2 = 0.47, P = 0.014$	n.s.
	Wet	$S_w\text{-NH}_4^+ = 6.45 \cdot Q^{0.96}$ $r^2 = 0.46, P = 0.010$	n.s.
	Transition	–	n.s.

n.s. not significant ($P > 0.05$).

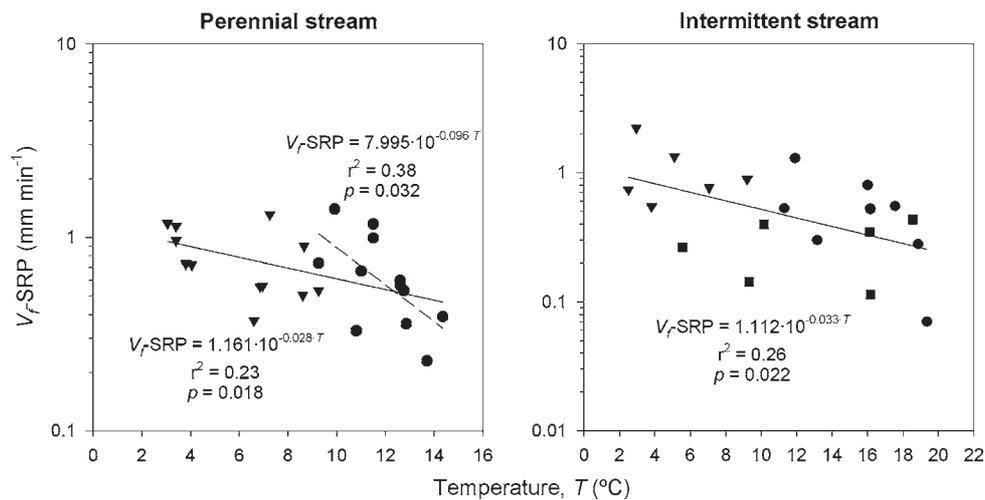


Figure 4. Relationships of the uptake velocity of soluble reactive phosphorus ($V_f\text{-SRP}$) with stream water temperature (T), in the perennial stream ($n = 24$, outlier from December 2005 excluded) and the intermittent stream ($n = 20$). Symbols indicate the different hydrological periods as follows: dry period (●), wet period (▼), and transition period (■). Straight lines represent significant ($P < 0.05$) linear regressions for data from all seasons in each stream. Dashed line represents a significant ($P < 0.05$) linear regression for the dry period in the perennial stream ($n = 12$).

(Figure 5). No relationship between the nutrient-demand ratio and DIN:SRP was found in the perennial stream or in any of the streams when data from each period were considered separately.

Michaelis-Menten models were poor predictors of nutrient uptake rates in both streams, regardless of whether data from all additions were used

together or separated into hydrological periods. The model fits were not significant, or estimated half-saturation constants were outside the range of nutrient concentrations measured in this study when significant. The relationships between ambient nutrient concentrations and uptake rates were better explained by linear regression models.

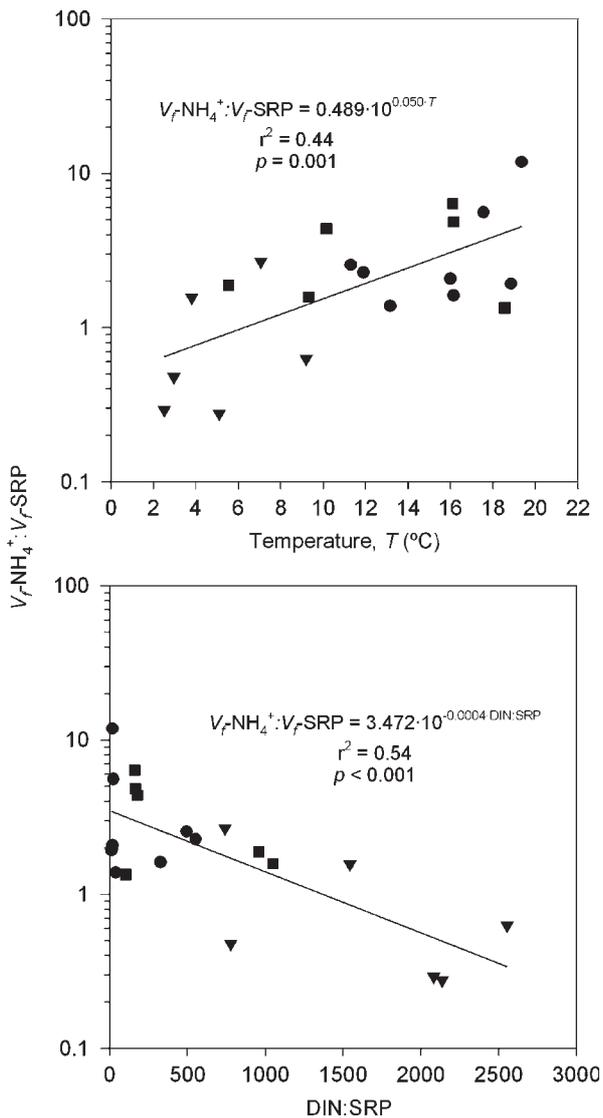


Figure 5. Relationships of the nutrient-demand ratio ($V_f\text{-NH}_4^+ : V_f\text{-SRP}$) with stream water temperature (T) and the DIN to SRP molar ratio (DIN:SRP) in the intermittent stream ($n = 20$). Symbols indicate the different hydrological periods as follows: dry period (●), wet period (▼), and transition period (■). Straight lines represent significant ($P < 0.05$) linear regressions.

In the perennial stream, both $U\text{-SRP}$ and $U\text{-NH}_4^+$ were positively related to their respective ambient nutrient concentrations following a power function (Table 4). This relationship held when each period was analyzed separately. In the intermittent stream, $U\text{-SRP}$ was related to the ambient SRP concentration following a power function with both all data together or separated into periods (Table 4). Conversely, this relationship was linear in the case of $U\text{-NH}_4^+$, and no relationships were found when periods were analyzed separately.

Patterns of Variability Across Time Scales

The compilation of data from studies on the temporal variation of stream nutrient retention showed that R_q for all three retention metrics increased as the time scale considered was expanded from diurnal to inter-annual (Figure 6). Furthermore, variation in R_q of retention metrics among studies was related to the estimated R_q of specific environmental drivers. In particular, $S_w\text{-}R_q$ was positively related to discharge- R_q , whereas $V_f\text{-}R_q$ and $U\text{-}R_q$ were positively related to concentration- R_q (Figure 6). No relationship between R_q of retention metrics and temperature- R_q was found.

DISCUSSION

Comparison of the Magnitude of Nutrient Retention Between Streams and Nutrients

Nutrients in our study streams traveled, on average, only a few hundred meters before being removed from the water column, indicating high efficiency in nutrient retention, as has been observed in other headwater streams (see for a review Ensign and Doyle 2006). One major difference between the two streams was found for NH_4^+ retention, which, based upon all three retention metrics, was greater in the perennial than in the intermittent stream. This difference could be attributed to the relatively high DIN and low SRP availability in the intermittent stream, indicative of a lower potential for N limitation. Although the average retention efficiency (that is, uptake length) and demand (that is, uptake velocity) for SRP were similar between streams, the SRP uptake capacity (that is, uptake rate) was higher in the perennial stream due to its higher SRP availability because uptake capacity is a function of the ambient nutrient concentration (Webster and Valett 2006).

Both streams showed a higher ability to retain NH_4^+ than SRP. NH_4^+ is subject to a greater diversity of biotic assimilatory and dissimilatory retention processes than SRP, especially in reaches with fine substrata (Butturini and Sabater 1999). In addition, co-precipitation of SRP with calcium carbonate, which is an important abiotic removal process in calcareous streams, was probably negligible in our study streams due to the siliceous geology (Reddy and others 1999). In fact, Martí and Sabater (1996) reported similar findings in nearby catchments for a sand-cobble reach of the siliceous Riera Major, but higher SRP retention for a similar

Table 4. Relationships of the Uptake Rate, U ($\mu\text{g m}^{-2} \text{min}^{-1}$) of Soluble Reactive Phosphorus (SRP) and Ammonium (NH_4^+) with their Respective Nutrient Concentration in the Perennial and the Intermittent Stream using All Data Together (that is, ALL) or Separated into Periods (that is, Dry, Wet, Transition)

Nutrient	Period	Perennial stream	Intermittent stream
SRP	ALL	$U\text{-SRP} = 0.70 \cdot \text{SRP}^{0.95}$ $r^2 = 0.71, P < 0.001$	$U\text{-SRP} = 0.53 \cdot \text{SRP}^{0.81}$ $r^2 = 0.49, P = 0.001$
	Dry	$U\text{-SRP} = 1.04 \cdot \text{SRP}^{0.80}$ $r^2 = 0.34, P = 0.046$	$U\text{-SRP} = 0.59 \cdot \text{SRP}^{0.69}$ $r^2 = 0.54, P = 0.037$
	Wet	$U\text{-SRP} = 0.57 \cdot \text{SRP}^{1.09}$ $r^2 = 0.65, P = 0.001$	$U\text{-SRP} = 1.29 \cdot \text{SRP}^{3.12}$ $r^2 = 0.70, P = 0.037$
	Transition	–	$U\text{-SRP} = 0.18 \cdot \text{SRP}^{1.51}$ $r^2 = 0.81, P < 0.001$
NH_4^+	ALL	$U\text{-NH}_4^+ = 2.69 \cdot \text{NH}_4^{+1.03}$ $r^2 = 0.47, P < 0.001$	$U\text{-NH}_4^+ = 1.56 \cdot \text{NH}_4^{-4.95}$ $r^2 = 0.28, P = 0.017$
	Dry	$U\text{-NH}_4^+ = 2.99 \cdot \text{NH}_4^{+0.94}$ $r^2 = 0.39, P = 0.031$	n.s.
	Wet	$U\text{-NH}_4^+ = 2.47 \cdot \text{NH}_4^{+1.12}$ $r^2 = 0.52, P = 0.005$	n.s.
	Transition	–	n.s.

n.s., not significant ($P > 0.05$).

reach of the calcareous La Solana stream, a pattern that did not hold when bedrock-dominated reaches were included. Published studies conducted at an annual scale have found either higher retention of NH_4^+ (for example, Simon and others 2005), higher retention of SRP (for example, Hanafi and others 2006), or no differences between nutrients (for example, Hoellein and others 2007). These contrasting findings could be explained by differences in geology, substrate types, or nutrient limitation status among streams.

Drivers of the Temporal Patterns of Nutrient Retention in the Perennial and Intermittent Streams

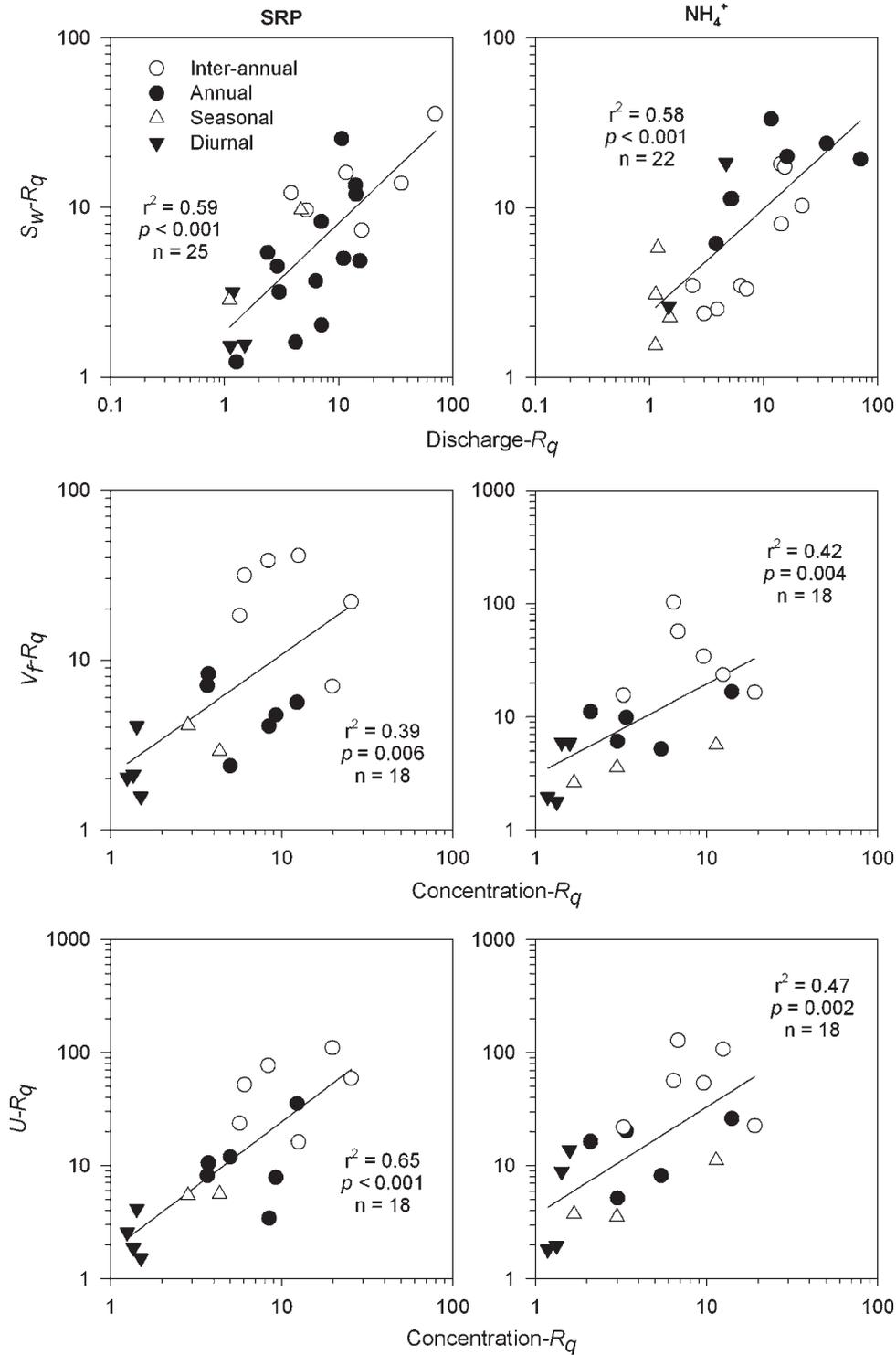
The two study streams exhibited differences in the temporal patterns of nutrient retention. In the perennial stream, temporal variation in both NH_4^+ and SRP retention efficiency and demand were coupled, as shown in other studies (for example, Butturini and Sabater 1998; Martí and Sabater 1996; Simon and others 2005). In contrast, retention efficiency and demand for the two nutrients exhibited contrasting temporal patterns in the intermittent stream, as found in other studies (Sabater and others 2000; Simon and others 2005; Hanafi and others 2006). Coupled temporal variation of NH_4^+ and SRP retention suggests that the same factors or mechanisms influence retention of both nutrients in the perennial stream, whereas, in the intermittent stream, each of the nutrients may be subjected to different driving factors. Uptake

rates of NH_4^+ and SRP, however, showed the opposite pattern: they were coupled in the intermittent stream but not in the perennial stream. This reflects the strong influence that temporal patterns of concentration exert on this metric, which were similar for the two nutrients in the intermittent stream and nearly opposite in the perennial stream.

Figure 6. Relationships between the range quotients (R_q) of: (upper panels) uptake length (S_w) and discharge, (middle panels) uptake velocity (V_f) and ambient nutrient concentration, and (lower panels) uptake rate (U) and ambient nutrient concentration, for soluble reactive phosphorus (SRP) (left panel) and ammonium (NH_4^+ ; right panel). We used data reported in the literature from stream reaches in which $n \geq 3$ additions were performed, and data for both retention metrics and environmental factors were available. Studies were grouped into four different time scales, indicated in the graphs with different symbols, based on the study period covered: (○) Inter-annual (two full years), including Martí and Sabater (1996) and this study; (●) Annual (at least three seasons of 1 year), including Mullholland and others (1985), Butturini and Sabater (1998), Hall and others (2002), Simon and others (2005), Hoellein and others (2007) (Δ) Seasonal (1–2 seasons of 1 year), including Sabater and others (2000), Haggard and Storm (2003), Hall and others (2003), Argerich and others (2008), and (▼) Diurnal (one full day), including Martí and Sabater (1994), Martí (1995). Straight lines represent significant ($P < 0.05$) linear regressions. The discrepancy in the number of points (n) among regressions is due to differences in the availability of data.

Seasonal patterns observed for most environmental factors considered in this study, which were consistent between the 2 years, did not translate into equally clear patterns of variation in nutrient retention. The patterns of temporal variation of nutrient retention were instead a result of the

combined influence of antecedent weather conditions and of different environmental drivers, as indicated by regression results. More importantly, as our regression analysis indicates, the influence of each factor differed between periods; therefore, no model of environmental controls on nutrient



retention can be formulated for a full hydrologic year that does not fully take into account seasonal differences.

The use of three standard retention metrics allowed us to explore the relative importance of each environmental driver on the variation of nutrient-retention response over time. Nutrient-retention efficiency was negatively influenced by discharge in the two study streams. This is consistent with previous studies and has been attributed to a decrease in contact time between the water column and the stream bottom in faster and deeper streams (Wollheim and others 2001; Peterson and others 2001). Our results also indicate that the effect of discharge was more relevant during the wet period in both streams, when base flow was higher as a result of lower evapotranspiration and frequent floods. The latter can additionally reduce nutrient retention by scouring benthic organic matter (Argerich and others 2008) and periphyton growing on substrates (Martí and others 1997), thus disturbing heterotrophic and autotrophic biological communities responsible for nutrient uptake. Previous studies in the intermittent stream suggest, however, that removal of benthic organic matter by floods reduces ecosystem respiration but enhances gross primary production by allowing light to reach primary producers until then covered by stored organic matter (Acuña and others 2004). Thus, floods may enhance the relative role of autotrophic over heterotrophic nutrient retention processes in these streams, but our data do not allow us to test this hypothesis. During the dry period, consistently low discharge became less important as a driver of the variation in nutrient retention, and other factors assumed a greater influence.

To examine the influence of these other factors, we focused on uptake velocity (that is, nutrient demand), a metric that corrects uptake length for the influence of stream size (that is, discharge) (Webster and Valett 2006). In contrast to discharge, which showed an episodic temporal regime, changes in water temperature were gradual and followed a consistent seasonal pattern. Thus, relationships with temperature would indicate seasonality. In this sense, we found no clear seasonal patterns in NH_4^+ demand in any of the two streams, likely due to the high complexity of mechanisms governing NH_4^+ dynamics in streams. In contrast, SRP demand was negatively related to water temperature in the two streams, suggesting similar mechanisms at work in the two streams. SRP demand was highest during the wet period and lowest during the dry and transition periods, in

contrast to what would have been expected from a direct effect of temperature on the physiological activity of stream biofilms (DeNicola 1996). We should note, however, that, in these well-forested streams, the wet period includes “hot moments” of energy availability, which may favor nutrient demand by both heterotrophic and autotrophic organisms. In both study streams, leaf fall occurs mainly in autumn (that is, October to November), but, in the intermittent stream, leaf fall may extend from late summer to autumn (that is, August to November) during dry years, due to hydrologic stress (Acuña and others 2007). As a consequence, large inputs of organic matter accumulate on the streambed and may fuel heterotrophic activity during the transition and wet periods. Mulholland and others (1985) found that retention efficiency of SRP was highest in a perennial stream of a temperate region during the leaf-fall period. Although ecosystem respiration peaks are characteristic in the intermittent stream during autumn (Acuña and others 2004), neither our data nor other studies in Mediterranean catchments (Martí and Sabater 1996; Argerich and others 2008) showed evidence of a clear peak in nutrient demand associated with this period. Heterotrophic organisms may fulfill most of their nutrient needs through uptake of nutrients contained in the fresh organic matter during decomposition, thus showing low demand for nutrients in the water column. Additionally, the leaf-fall period coincides with high flood frequency in streams from the Mediterranean region (Gasith and Resh 1999), which may reduce the accumulation of large amounts of benthic organic matter (Acuña and others 2004; Argerich and others 2008). Inputs of leaves that accumulate in adjacent riparian soils during winter, when floods are less frequent than during fall, may further mask the relevance of a discrete period of organic matter inputs for heterotrophic organisms in these streams, compared to streams in temperate regions.

The wet period also is characterized by higher light availability than the dry period, especially before leaf out, due to the absence of riparian shading. Although forested headwater streams are typically heterotrophic (Battin and others 2008), increases in light availability can favor the development of photoautotrophic organisms, which are important players in nutrient retention. In fact, an increase in algal biomass with a concomitant shift from net heterotrophy to net autotrophy is characteristic in the intermittent stream during spring just before leaf out (Acuña and others 2004). In addition, light was shown to be an important limiting factor of periphyton growth in our perennial

stream and other streams with well-developed riparian vegetation located in the same catchment during summer (von Schiller and others 2007). Although heterotrophic processes related to inputs of organic matter have been posited to control nutrient retention in forested streams (Webster and Meyer 1997), many other studies demonstrate that autotrophic processes can be important during periods of high light availability (for example, Mulholland and others 1992; Mulholland and others 2006; Hoellein and others 2007). Moreover, Sabater and others (2000) showed that removal of riparian vegetation-enhanced growth of photosynthetic organisms and increased SRP retention in a nearby stream. A stronger relationship of water temperature with SRP demand during the dry period, found only in the perennial stream, indirectly suggests a contrasting influence of riparian vegetation on SRP retention between the two streams through the regulation of the light regime. In fact, although riparian vegetation was well developed in the two streams, the structure and dynamics differed between the streams due to local climate conditions. In the perennial stream, riparian vegetation was very dense and dominated by a single-tree species, the phenology of which created two clearly contrasting seasons (that is, dormant and vegetative). In contrast, the riparian vegetation in the intermittent stream was sparser and dominated by a variety of species with differing phenologies and was also subject to hydrologic stress in the summer that caused leaf fall during this season and, thus, relatively higher light availability (Acuña and others 2007).

The lack of relationships between demand of SRP and NH_4^+ and their respective nutrient concentrations, as well as a better fit of uptake rates to linear rather than Michaelis-Menten models, suggests that nutrient concentrations were below saturation in the two study streams. These results support previous findings in headwater streams (for example, Simon and others 2005; Hanafi and others 2006; Hoellein and others 2007). Results from regressions between uptake rate and nutrient concentration done separately for each period, however, suggest saturation conditions for SRP in both streams during the dry period. The relationship between SRP uptake rate and concentration was best explained by a power function for each of the periods in the two streams, but exponents less than 1, which indicate potential saturation conditions (O'Brien 2007), were obtained only with data from the dry period in the two streams, coinciding with the highest SRP concentrations. Uptake of NH_4^+ did not show any signs of saturation, proba-

bly due to the relatively low concentrations and weak seasonal changes of NH_4^+ in both streams.

Although variation in nutrient demand was not influenced by nutrient availability, variation in the relative demand of the two nutrients (that is, $V_f\text{-NH}_4^+ : V_f\text{-SRP}$ ratio) in the intermittent stream responded to the relative availability of both N and P (that is, DIN:SRP ratio). Demand of SRP tended to increase relative to that of NH_4^+ as the potential for P limitation increased (that is, higher DIN:SRP ratios). In fact, the few dates when demand of SRP surpassed that of NH_4^+ coincided with times of extremely high DIN:SRP ratios. This pattern was driven by dramatic increases in NO_3^- concentration during the wet period, which may be attributed to a combination of a decline in NO_3^- uptake by terrestrial vegetation and soil microbial activity (Bernal and others 2005) and NO_3^- release in the stream edge zone due to the elevation of the groundwater table (Butturini and others 2003). This pattern did not hold in the perennial stream, likely due to the less clear seasonality and relatively low range of DIN:SRP ratios in this stream. Other studies have demonstrated temporal changes in the relative demand of N over P (Martí and others 1996; Simon and others 2005), which may be attributed to structural and functional adaptations of biofilm communities to the temporal variation of relative nutrient availability.

Variability of Nutrient Retention Between and Within Streams

Results from this study demonstrated differences not only in the magnitude and pattern of nutrient retention between the streams but also in the range of variation of this functional response. We expected larger variability in the intermittent stream in response to the higher variability observed in most measured environmental factors and to the anticipated negative effect of droughts on biological communities responsible for nutrient uptake (Lake 2003). Results were contrary to our expectations, however, with the perennial stream showing a similar or even broader range of variation in most retention metrics. This unexpected result suggests high resilience of biotic communities in the intermittent stream after disturbance (that is, drought), as supported by previous studies in the same stream (Acuña and others 2005, 2007). Our initial expectation held, however, when we analyzed the patterns of temporal variability measured across a variety of published studies. Our review analysis indicated that within-stream temporal variability in nutrient retention metrics tends to increase as the

variability of specific environmental drivers becomes larger.

More evident patterns of variability emerged when we considered different time scales within each stream. We found an increasing range of variation of all retention metrics with increasing time scale in both streams, with the highest value at the inter-annual scale. This indicates that sampling any one period, or even a whole year, would not have been enough to capture the full range of nutrient retention observed over the two study years. In addition, we must take into account that we likely did not capture all the potential variability in nutrient retention of our study streams due to the constraints of the methodology employed (Webster and Valett 2006).

The high variability observed in our streams was comparable to that found by Martí and Sabater (1996) in two other Mediterranean streams located in nearby catchments. To the best of our knowledge, this is the only other existing study covering the variation of nutrient retention over a similarly extensive period. Our analysis of the variability across time scales with data from compiled studies further supports our findings. The variability of nutrient retention tended to increase as the time scale considered increased; it was lowest in studies at the diurnal scale, intermediate at the annual or seasonal scales, and highest in studies performed over an inter-annual scale. This interesting result may be explained by the higher probability of longer-term studies capturing greater variability in environmental drivers. Nevertheless, one unexplored subject of great interest is if the within-stream pattern across time scales observed for Mediterranean streams holds for streams found in other climate regimes.

Overall, results from this study emphasize the significance of local climate conditions in regulating the magnitude, temporal pattern, and variability of nutrient retention by dictating the disturbance regime and temporal windows of energy and resource availability in streams. As these factors may vary in response to changes in land use and climate regimes, our study contributes to a better understanding of the potential effect of these changes on stream ecosystem functioning and highlights the importance of long-term studies for the correct characterization of stream nutrient retention and its controlling factors. Models of nutrient dynamics at larger spatial and temporal scales that incorporate the temporal variation of stream nutrient retention would become valuable tools for the prediction and management of nutri-

ent-related environmental problems in present and future scenarios.

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4. GENERAL CONCLUSIONS

The present dissertation aimed to explore the implications of global change for stream nutrient retention in Mediterranean streams. With this purpose, a space for time approach was used to address the implications of two important aspects of global change: (i) land transformation and (ii) climate change.

The first three articles focused on the effects of land transformation on nutrient retention processes by measuring nutrient retention metrics and additional parameters in selected streams subjected to contrasting land uses. The fourth article focused on the effects of climate change on nutrient retention processes by measuring temporal variation of nutrient retention metrics and additional parameters in a perennial and an intermittent stream. The conclusions from each of the four articles are the following:

Influence of land use on stream ecosystem function in a Mediterranean catchment (Chapter 3.1)

1. Urbanization had a negative effect on stream nutrient retention. Ammonium (NH_4^+) demand (measured as the uptake velocity, V_f) decreased along the gradient from forested to human dominated catchments due to uptake saturation by increased stream nutrient concentrations [NH_4^+ , dissolved organic nitrogen (DON) and carbon (DOC)] and inhibition of nitrification by reduced dissolved oxygen (DO) availability along the gradient.
2. Stream metabolism was more susceptible to human influences on proximate factors operating at a near-stream spatial scale (e.g., riparian vegetation removal) than on distant factors operating at greater spatial scale (e.g. catchment land use). Gross primary production (GPP) and ecosystem respiration (ER) increased along the gradient of agricultural development in response to increases in algal biomass (chlorophyll *a*) due to higher light availability.
3. The retention of nitrogen (N) and phosphorus (P) were differently influenced by human activities. Soluble reactive phosphorus (SRP) demand was not related to catchment land use due to the relatively low values and small range of SRP concentrations among the study streams. In addition, co-precipitation of SRP with calcium carbonate was negligible due to the dominant siliceous geology in the study catchment.
4. Functional parameters such as nutrient retention and metabolism were sensitive measures of the effect of human activities on stream ecosystem function, and thus could be used in stream management and restoration plans in addition to more traditional structural parameters.

Nitrate retention and removal in Mediterranean streams with contrasting land uses: a ^{15}N tracer study (Chapter 3.2)

5. Cycling of N was fast in all streams due to a strong coupling among uptake, regeneration and transformation processes. Despite relatively small differences in stream physicochemical characteristics and standing stocks of primary uptake compartments, large differences in N cycling pathways were found among streams.
6. The importance of permanent removal via denitrification increased over that of temporary nitrate (NO_3^-) retention via assimilatory uptake from the forested to the agricultural stream. This result was due to increases in the degree of heterotrophy and NO_3^- concentration, and decreases in DO concentration along the gradient. In these conditions, NO_3^- may be preferentially used as an electron acceptor in dissimilatory uptake processes such as denitrification.
7. The contribution of detritus compartments to NO_3^- assimilatory uptake decreased and that of compartments dominated by primary producers increased from the forested to the agricultural stream. Riparian vegetation greatly contributed to N retention, especially in the agricultural stream. This stream-riparian linkage may be enhanced in arid and semiarid regions, where riparian vegetation is strongly limited by water availability.
8. Changes in the relative importance of retention and removal due to changes in land use may substantially affect the amount and form of N delivered to downstream ecosystems, which will have important consequences on ecosystem structure and function.

Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses (Chapter 3.3)

9. Light was the main factor affecting algal biomass in the study streams, whereas nutrients did not have a significant effect. The influence of riparian zones on light availability, although seldom considered by water managers, may be more important than nutrients in controlling eutrophication effects derived from human activities. This may be enhanced in Mediterranean stream ecosystems due to the relatively sparse riparian vegetation and high solar radiation levels.
10. Ambient N concentrations did not saturate periphyton N uptake in these streams. Rates of NO_3^- uptake increased and C:N ratios decreased consistently with increases in ambient N availability across streams. However, periphyton

assemblages preferentially took up N from the substratum rather than N from the water column.

11. Ammonium was the preferred form of N, which indicates that not only increases in concentration, but also stoichiometric imbalances should be considered when examining N retention in human-altered streams

Inter-annual, annual and Seasonal variation of P and N retention in a perennial and an intermittent stream (Chapter 3.4)

12. Local climate conditions regulated the magnitude, temporal pattern and variability of stream nutrient retention by dictating the disturbance regime and temporal windows of energy and resource availability.
13. Nutrient retention efficiency was negatively influenced by abrupt increases in discharge, whereas nutrient demand was regulated by riparian vegetation dynamics through gradual changes in organic matter and light availability. Between-stream differences in the temporal variation of nutrient demand were partly due to differences in the phenology of riparian trees in two contrasting local climate conditions.
14. Local climate influenced nutrient processing at the catchment and near-stream zones, which had consequences on nutrient availability in stream water and concomitantly caused changes in the magnitude and temporal variation of stream nutrient retention.
15. The variability in stream nutrient retention increased with increasing time scale due to concomitant increases in the variability of environmental factors. This finding was supported by results from a review of studies addressing temporal variation of stream nutrient retention at different time scales.
16. Variability in nutrient retention was not greater in the intermittent stream, indicating high resilience of biological communities responsible for nutrient uptake.

In conclusion, results from the present dissertation evidence that changes in land use and climate regimes may affect nutrient retention in fluvial ecosystems. This important ecosystem service of streams and rivers may be reduced through changes in several important environmental drivers derived from human activities.

Conversion of forested land for urban and agricultural development causes increases in the concentrations of dissolved N and P, which may saturate stream nutrient uptake, thus decreasing the capacity and efficiency of these ecosystems to retain excess nutrients. In addition, other changes in stream environmental characteristics associated with human activities (e.g, low DO, high algal biomass, channelization) may inhibit important retention processes. Changes in environmental characteristics of streams due to the effects of human land transformation may also substantially modify nutrient cycling pathways in streams. For instance, results from this dissertation show that NO_3^- removal may be enhanced over NO_3^- retention pathways in human-altered streams. These changes may have profound consequences on the amount and form of nutrients delivered to downstream and coastal ecosystems.

Climate change in the Mediterranean region is expected to cause an increase in temperature and a decrease in precipitation. Physical, chemical and biological parameters of fluvial ecosystems may be altered through direct effects at the stream level or indirect effects at the level of the catchment and near-stream zones. Due to the effects of climate change, intermittency is expected to increase in many streams and rivers, especially in arid and semiarid regions such as the Mediterranean. Results from this dissertation demonstrate that nutrient dynamics strongly differed between a perennial and an intermittent stream situated within the same catchment, and point out to some of the potential effects of climate change on stream ecosystem function.

To better understand the effects of land transformation on fluvial ecosystems and to build efficient management and restoration plans, future studies should address the influence of the spatial distribution of the different land uses within the catchment on stream structural and functional characteristics. Moreover, to make better predictions of the impacts of climate change on fluvial ecosystems, future research should focus on the implications of intermittency for stream ecosystem function. Finally, further studies on the interactions between land transformation and climate change are needed to gain a better understanding of the overall effects of global change on the Earth's ecosystems.

**5. INFORME DE LOS DIRECTORES DE LA TESIS
DOCTORAL**

Informe dels directors de la Tesi Doctoral referent al factor d'impacte i a la contribució del doctorand en cadascun dels articles publicats

La Dra. Eugènia Martí Roca del Centre d'Estudis Avançats de Blanes (CSIC) i el Dr. Joan Lluís Riera Rey del Departament d'Ecologia (UB), directors de la Tesi Doctoral elaborada pel Sr. Daniel von Schiller Calle, amb el títol "Implications of global change for stream nitrogen and phosphorus retention in a Mediterranean catchment (Implicaciones del cambio global sobre la retención de nitrógeno y fósforo en ríos de una cuenca Mediterránea)",

INFORMEN

Que els treballs de recerca portats a terme pel Sr. Daniel von Schiller Calle com a part de la seva formació predoctoral i inclosos a la seva Tesi Doctoral han donat lloc a tres publicacions i a un manuscrit **que es troba en fase de revisió**. A continuació es detalla la llista d'articles així com els índexs d'impacte (segons el SCI de la ISI Web of Knowledge) de les revistes **on han estat o està previst** que es publiquin els treballs.

1. von Schiller, D., E. Martí, and J.L. Riera, and F. Sabater. 2007. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Freshwater Biology*. 52:891-906.

L'índex d'impacte de la revista *Freshwater Biology* a l'any de publicació de l'article va ser de 2,650. Aquesta revista està inclosa a la categoria "Marine and Freshwater Biology". Aquesta categoria té una mediana d'índex d'impacte de 1.155 i inclou un total de 86 revistes. Tenint en compte l'índex d'impacte de *Freshwater Biology*, aquesta ocupa el 6^è lloc de la seva categoria.

2. von Schiller, D., E. Martí, J.L. Riera, M. Ribot, A. Argerich, P. Fonollà and F. Sabater. 2008. Inter-annual, annual and seasonal variation of P and N retention in a perennial and an intermittent stream. *Ecosystems*. Printed online, DOI: 10.1007/s10021-008-9150-3.

L'índex d'impacte de la revista *Ecosystems* al 2007 va ser de 2,684. Aquesta revista està inclosa a la categoria "Ecology". Aquesta categoria té una mediana d'índex d'impacte de 1.532 i inclou un total de 116 revistes. Tenint en compte l'índex d'impacte d'*Ecosystems*, aquesta ocupa el 33^è lloc de la seva categoria.

3. von Schiller, D., E. Martí, J.L. Riera, M. Ribot, J.C. Marks and F. Sabater. 2008. Influence of land use on stream ecosystem function in a mediterranean catchment. *Freshwater Biology*. Printed online, DOI: 10.1111/j.1365-2427.2008.02059.x

L'índex d'impacte de la revista *Freshwater Biology* al 2007 va ser de 2,650. Aquesta revista està inclosa a la categoria "Marine and Freshwater Biology". Aquesta categoria té una mediana d'índex d'impacte de 1.155 i inclou un total de 86 revistes. Tenint en compte l'índex d'impacte de *Freshwater Biology*, aquesta ocupa el 6^é lloc de la seva categoria.

4. von Schiller, D., E. Martí, and J.L. Riera. 2008. Nitrate retention and removal in Mediterranean streams with contrasting land uses: a ¹⁵N tracer study. A punt per ser enviat a *Biogeosciences*.

L'índex d'impacte de la revista *Biogeosciences* al 2007 va ser de 2,813. Aquesta revista està inclosa a la categoria "Ecology". Aquesta categoria té una mediana d'índex d'impacte de 1.532 i inclou un total de 116 revistes. Tenint en compte l'índex d'impacte de *Biogeosciences*, aquesta ocupa el 31^é lloc de la seva categoria.

Alhora CERTIFIQUEN

Que el Sr. Daniel von Schiller Calle ha participat activament en el desenvolupament del treball de recerca associat a cadascun d'aquests articles així com en la seva elaboració. En concret, la seva participació en cadascun dels articles ha estat la següent:

- Participació en el plantejament inicial dels objectius de cadascun dels treballs els quals estaven emmarcats en dos projectes del Plan Nacional del MEC (RENITRAC i NICON) i en un projecte de la Comissió Europea (EUROLIMPACS).
- Desenvolupament de la part experimental de camp i posada a punt de les metodologies de camp i de laboratori associades a cadascun dels experiments. Una part de aquesta tasca va comportar una estada a Puerto Rico amb el grup del Dr. Bill McDowell per a l'aprenentatge de metodologies de camp per a l'estima de taxes de processos associats al cicle del nitrogen en rius utilitzant isòtops estables.
- Realització dels diversos experiments.
- Processat i anàlisi de totes les mostres obtingudes.
- Càlcul de resultats i anàlisi de dades.
- Redacció dels articles i seguiment del procés de revisió dels mateixos.

Finalment, certifiquem que cap dels coautors dels articles abans esmentats i que formen part de la Tesis Doctoral del Sr. Daniel von Schiller Calle ha utilitzat o bé té previst utilitzar implícita o explícitament aquests treballs per a l'elaboració d'una altra Tesis Doctoral.

Atentament,

Blanes, 1 de setembre de 2008

Eugènia Martí Roca

Joan Lluís Riera Rey

6. RESUMEN

1. Introducción general

1.1 Los ecosistemas fluviales y el cambio global

El cambio global, el conjunto de cambios en el medio ambiente derivados de la acción humana, está transformando sustancialmente el paisaje y el clima de la Tierra. Aproximadamente un 50% de la superficie de la Tierra ha sido transformada por la acción humana (Vitousek *et al.*, 1997), y los efectos del cambio climático ya están siendo observados (IPCC, 2007). El conjunto de estos cambios está teniendo efectos importantes sobre los ecosistemas de la Tierra.

Aunque únicamente el 0,01% del agua dulce de la Tierra se encuentra en los ríos (Giller & Malmqvist, 1998), los ecosistemas fluviales son de gran importancia para la biosfera y además aportan un buen número de valores y servicios a los humanos. Debido al fuerte ligamen que les une a sus cuencas, los ríos son altamente susceptibles a la transformación del paisaje (Hynes, 1975, Likens & Borman, 1995). Asimismo, los ecosistemas fluviales se caracterizan por su flujo unidireccional, de tal manera que cualquier contaminante que llega a estos ecosistemas puede tener un efecto mucha distancia aguas abajo (Giller & Malmqvist, 1998). Consecuentemente, el cambio global ha alterado los ecosistemas de agua dulce del planeta aún más que los ecosistemas terrestres (Vitousek *et al.*, 1997). El desarrollo urbano, agrícola e industrial, la minería y las alteraciones de los cursos fluviales influyen directamente en los factores naturales que regulan los ecosistemas fluviales, incluyendo la hidrología, la química del agua, la estructura del hábitat y las interacciones bióticas. Múltiples perturbaciones contribuyen a la degradación ecológica local, lo cual puede llevar a cambios irreversibles y a una pérdida significativa de los valores y servicios que los ecosistemas fluviales aportan a la humanidad (Malmqvist & Rundle, 2002).

Junto a la transformación del paisaje, el cambio climático es la alteración de los ecosistemas de la tierra que resulta más evidente (Vitousek *et al.*, 1997). Descubrimientos científicos recientes confirman que el cambio climático, causado por las emisiones de gases invernadero provenientes de las actividades humanas, puede ejercer una presión adicional sobre los ecosistemas (EEA, 2007; IPCC, 2007). La temperatura media es hoy 0,8 °C más alta que antes de la revolución industrial (CRU, 2006; GISS/NASA, 2006), y está previsto que aumente entre 1,8 y 4,0 °C durante este siglo, aunque algunos estudios apuntan a un rango más amplio de entre 1,1 y 6,4 °C (IPCC, 2007). Cambios en el régimen de precipitación también han sido registrados y se espera que se vean acelerados en el futuro (EEA, 2007).

En general, se espera que el cambio climático influya en los ecosistemas fluviales a través de alteraciones de los regímenes hidrológico y térmico (Carpenter *et al.*, 1992). Se prevé que el incremento de la temperatura del aire provoque aumentos en la temperatura del agua y la evapotranspiración de la cuenca. Estos cambios, junto con modificaciones en la magnitud y la distribución temporal de la precipitación pueden causar cambios en la cantidad y variabilidad del caudal (Carpenter *et al.*, 1992). Las alteraciones de la temperatura y la hidrología pueden tener fuertes implicaciones para muchos organismos que habitan el río o la zona de ribera que poseen estrechos rangos de tolerancia térmica e hidrológica (Malmqvist & Rundle, 2002). Se espera que todo esto cause profundos cambios en la estructura y función de los ecosistemas fluviales. Sin embargo, los efectos del cambio global sobre los ecosistemas fluviales son difíciles de predecir, ya que pueden interactuar con los efectos de otras actividades antropogénicas como la transformación del paisaje (Dale, 1997). Además, existen importantes diferencias regionales en la gravedad y tendencia del cambio climático (IPCC, 2007).

1.2 Vulnerabilidad de los ecosistemas fluviales mediterráneos frente al cambio global

El clima de la región mediterránea se caracteriza por veranos cálidos y secos, e inviernos fríos y húmedos, además de una alta variabilidad interanual (Strahler & Strahler, 1989). La evapotranspiración potencial es alta y puede ser responsable de más de un 80% de la precipitación anual (Piñol *et al.*, 1991). Este marco climático resulta en una hidrología compleja, caracterizada por una frecuente sequera estival, una marcada estacionalidad y una gran variabilidad interanual (Gasith & Resh, 1999). La falta de agua durante largos periodos hace que los ecosistemas fluviales mediterráneos sean particularmente vulnerables a los efectos del cambio global (EEA, 2007). Debido al déficit hídrico, muchos de los valores y servicios que proporcionan los ecosistemas fluviales son especialmente importantes en la región mediterránea. Los ríos suministran la mayor parte del agua dulce en muchas zonas (EEA, 2007, Mas-Pla, 2005). Además, los ecosistemas fluviales representan *islas húmedas* en un *paisaje seco*, de forma que mantienen una alta diversidad de vida acuática endémica y constituyen corredores de fauna para muchas especies terrestres (Naiman *et al.*, 1993). Cabe destacar que la región mediterránea es considerada una de las zonas calientes de biodiversidad más amenazadas del planeta (Sala *et al.*, 2000). Asimismo, los ecosistemas fluviales bien conservados son cruciales para el control de inundaciones periódicas y la dilución de contaminantes (Mas-Pla, 2005). Finalmente,

debido a su escasez, los ecosistemas acuáticos de la región mediterránea son muy apreciados por su valor estético, artístico y espiritual (Meyer *et al.*, 1997).

La región mediterránea ha estado sujeta al desarrollo humano durante milenios. Sin embargo, el impacto de la transformación del paisaje ha aumentado considerablemente durante las últimas décadas. El suelo forestado y dedicado tradicionalmente a la agricultura extensiva ha sido sustituido en muchas zonas por suelo urbano o agricultura intensiva de regadío. Asimismo, el abandono de muchas zonas rurales está llevando a una reforestación natural del suelo antes dedicado a la agricultura, lo cual ha hecho aumentar el riesgo de incendios en muchas zonas (EEA, 2006a). Múltiples factores tales como el crecimiento de la población local, el turismo, el aumento de la movilidad, y la inversión comercial han hecho aumentar espectacularmente el desarrollo urbano. La expansión urbana durante la última década ha sido especialmente intensa en países con un alto crecimiento económico. Por ejemplo en España hasta un 50% de la costa mediterránea está ya cubierta por superficies impermeables (EEA, 2006b). La expansión agrícola va frecuentemente acompañada de alteraciones en los cursos fluviales y trasvases (EEA, 2006a). Todas estas transformaciones del paisaje ejercen un fuerte efecto sobre los frágiles ecosistemas fluviales mediterráneos.

Además de la transformación del paisaje, el cambio climático puede afectar a la integridad de los ecosistemas de agua dulce mediterráneos. Europa se ha calentado más rápidamente que la media global, mostrando una temperatura unos 1,4 °C más alta que antes de la revolución industrial (EEA, 2007). Todo el continente probablemente se calentará entre 2,1 y 4,4 °C hasta el 2080, o posiblemente entre 2,0 y 6,3 °C. Se prevé que este calentamiento sea especialmente intenso en la región mediterránea (Schröter *et al.*, 2005). De hecho, estudios previos en la zona de estudio de la presente Tesis Doctoral han mostrado evidencias de alteración de los ciclos de vida de algunas plantas y animales debido al calentamiento global (Peñuelas *et al.*, 2002, 2003). La precipitación anual ha disminuido hasta un 20% en el sur de Europa durante el s. XX, y está previsto que continúe disminuyendo en el futuro (Schröter *et al.*, 2005). Además, en muchas zonas se prevé una mayor frecuencia de eventos de precipitación extrema (de Castro *et al.*, 2005).

En general, está previsto que una mayor temperatura media y una menor precipitación media provoquen un caudal menor (Carpenter *et al.*, 1992). De hecho, una disminución del caudal ya ha sido observada en varios ríos mediterráneos durante las últimas décadas (EEA, 2007). Está previsto que el cambio climático convierta muchos ríos permanentes en estacionales, y algunos pueden incluso desaparecer

(Alvarez-Cobelas *et al.*, 2005). El riesgo de inundaciones puede aumentar debido a los eventos de precipitación extrema (Mas-Pla, 2005). Finalmente, la biodiversidad de mucho de estos ecosistemas puede disminuir, y las tasas de los procesos metabólicos y biogeoquímicos pueden verse alterados (Carpenter *et al.*, 1992, Alvarez-Cobelas *et al.*, 2005).

1.3 Procesos de retención de nutrientes en ecosistemas fluviales

Los ecosistemas fluviales han sido tradicionalmente vistos como simples conductos que transportan nutrientes y otros materiales desde la tierra hasta el mar. Sin embargo, los ríos tienen la capacidad de almacenar, transformar y eliminar nutrientes durante su transporte aguas abajo. Estos procesos se agrupan normalmente bajo el término retención de nutrientes. Nutrientes como el nitrógeno (N) y el fósforo (P) que frecuentemente limitan la producción primaria en los ecosistemas fluviales, son especialmente afectados por la capacidad de los ríos para retenerlos (e.g., Triska *et al.*, 1989; Martí *et al.*, 1996; Simon *et al.*, 2005).

El concepto de la espiral de nutrientes (“nutrient spiralling”; Webster & Patten, 1979; Newbold *et al.*, 1981) revolucionó la biogeoquímica fluvial al establecer un marco teórico y matemático para el estudio de la retención de nutrientes en ríos. Los primeros estudios de la espiral de nutrientes utilizaron el ^{32}P como trazador para investigar los procesos de retención de nutrientes en ríos (Newbold *et al.*, 1981; Newbold *et al.*, 1983; Mulholland *et al.*, 1985). Sin embargo, el uso de este trazador radiactivo fue rápidamente abandonado debido a los riesgos para la salud humana y de los ecosistemas que conlleva. Desde entonces, la retención de N y P en ríos ha sido ampliamente estudiada mediante experimentos de enriquecimiento (Stream Solute Workshop, 1990; Webster & Valett, 2006). Sin embargo, esta técnica no permite distinguir entre diferentes vías de retención y se ha demostrado que subestima las tasas de captación en condiciones ambientales (Mulholland *et al.*, 2002; Dodds *et al.*, 2002). Más recientemente, la retención de N en ríos ha sido explorada mediante adiciones de ^{15}N , las cuales permiten cuantificar en condiciones ambientales los diferentes procesos de retención de N que ocurren simultáneamente en el río y evitando las limitaciones de los experimentos de enriquecimiento (Peterson *et al.*, 1997; Peterson *et al.*, 2001; Mulholland *et al.*, 2008).

Muchos estudios han destacado la importancia de los ríos prístinos de cabecera en la retención de N y P disueltos (Alexander *et al.*, 2000; Peterson *et al.*, 2001; Lowe & Likens, 2005). El cambio global ya ha alterado algunos de los factores ambientales que afectan a la capacidad de retención de estos ecosistemas. Sin embargo, son

escasos los estudios sobre los efectos de la transformación del paisaje y sólo se han hecho proyecciones indirectas de los efectos del cambio climático. Los resultados de estudios recientes muestran que las actividades humanas reducen la eficiencia de la retención de nutrientes (la retención relativa al flujo de nutrientes) mediante el aumento de las cargas de nutrientes (e.g., Martí *et al.*, 2004, Newbold *et al.*, 2006; Ruggiero *et al.*, 2006; Mulholland *et al.*, 2008), la disminución del material orgánico bentónico (Meyer *et al.*, 2005), modificaciones del canal (Sweeney *et al.*, 2004; Grimm *et al.*, 2005; Bukaveckas, 2007) y otras formas de contaminación acuática que inhiben a organismos responsables de la captación de nutrientes (Grimm *et al.*, 2005; Newbold *et al.*, 2006; Lottig *et al.*, 2007). Por el contrario, la eficiencia en la retención puede aumentar con otras acciones humanas tales como la eliminación de la vegetación de ribera, lo cual aumenta la disponibilidad de luz para los productores primarios (Sabater *et al.*, 2000). El cambio climático puede alterar directamente la retención de nutrientes a través de cambios en los regímenes hidrológico y térmico, e indirectamente mediante interacciones con otros componentes del cambio global. Sin embargo, la dirección de estos cambios es difícil de predecir (Carpenter *et al.*, 1992; Meyer *et al.*, 1999).

Comprender como afecta el cambio global a los procesos biogeoquímicos es importante debido a que estos procesos son imprescindibles para el mantenimiento de muchos de los servicios que los ríos aportan a los humanos (Palmer *et al.*, 2004). La retención de nutrientes en los ríos representa un servicio del ecosistema por sí mismo, ya que puede ayudar a mitigar los problemas asociados a la contaminación por nutrientes mediante la reducción de la exportación de nutrientes hacia ecosistemas situados aguas abajo (Peterson *et al.*, 2001). Además, los procesos de los ecosistemas pueden ser medidas integradoras ideales del estado ecológico de los ríos, aunque su uso aún sea limitado (Bunn *et al.*, 1999). La retención de nutrientes puede ser un buen candidato para tapar este hueco, ya que tiene una buena base teórica, es fácilmente medible y comparable entre diferentes tipos de ecosistemas y es sensible a los impactos humanos. Finalmente, la mayoría de los estudios sobre el efecto de las acciones humanas sobre la retención de nutrientes en ríos se han concentrado en ríos situados en regiones templadas de Norteamérica. Sin embargo, son necesarios estudios en diferentes zonas bioclimáticas para conseguir un entendimiento completo sobre la influencia del cambio global en la biogeoquímica fluvial.

1.4 Objetivos de la presente Tesis Doctoral

El objetivo general de la Tesis Doctoral era explorar las implicaciones del cambio global para la retención de nutrientes en ríos de una cuenca mediterránea. Se usó una aproximación espacio por tiempo para examinar la influencia de dos aspectos importantes del cambio global: (i) la transformación del paisaje y (ii) el cambio climático.

Esta Tesis Doctoral está dividida en cuatro publicaciones independientes. Las primeras tres publicaciones tratan de los efectos de la transformación del paisaje sobre los procesos de retención de nutrientes, mientras que la cuarta publicación examina los efectos del cambio climático. Los objetivos específicos de cada uno de las cuatro publicaciones fueron:

1. *Influencia del uso del suelo sobre la función del ecosistema fluvial en una cuenca mediterránea (Capítulo 3.1)*

Se utilizó una aproximación multivariante para examinar cómo los atributos fisicoquímicos y funcionales (retención de nutrientes y metabolismo) se relacionan con las variables de la cuenca, incluyendo el uso del suelo. Es estudio se llevó a cabo en 13 ríos situados en la misma cuenca, pero que drenan sub-cuencas con distintos usos del suelo.

2. *Retención y eliminación de nitrato en ríos mediterráneos con distintos usos del suelo: un estudio con el ^{15}N como trazador (Capítulo 3.2).*

Se realizaron adiciones de nitrato (NO_3^-) marcado con ^{15}N para examinar las vías de retención (captación asimilatoria) y eliminación (desnitrificación) de NO_3^- en tres ríos situados en la misma cuenca, pero que drenan sub-cuencas con distintos usos del suelo.

3. *Efecto de los nutrientes y la luz sobre la biomasa y captación de nitrógeno del perifiton en ríos mediterráneos con distintos usos del suelo (Capítulo 3.3).*

Se usaron experimentos de sustratos difusores de nutrientes junto con las adiciones de NO_3^- marcado con ^{15}N para determinar la importancia relativa de los nutrientes y la luz como factores limitantes de la biomasa y la captación de nutrientes en tres ríos situados en la misma cuenca, pero que drenan sub-cuencas con distintos usos del suelo.

4. *Variación interanual, anual y estacional de la retención de P y N en un río permanente y un río estacional (Capítulo 3.4).*

Se usaron medidas mensuales de retención de nutrientes obtenidas mediante adiciones por enriquecimiento durante un periodo de dos años para examinar

la variación de la retención de fósforo reactivo soluble (SRP) y amonio (NH_4^+) en dos ríos forestados con distinto régimen hidrológico (permanente vs. estacional).

2. Área de estudio

2.1 Características geográficas, climáticas y paisajísticas de la cuenca de estudio

La investigación se llevó a cabo en una serie de ríos de pequeño tamaño situados en la cuenca del río Tordera (Cataluña, NE de España; Fig. 2.1). Esta cuenca ocupa un área de 868,5 km² dominada por geología de tipo silíceo y cubre un gradiente de altitud de unos 1.600 m en menos de 30 km de distancia horizontal desde los picos más altos (Macizo del Montseny) hasta la desembocadura en el Mar Mediterráneo.

El clima es típicamente mediterráneo, con temperaturas mínimas durante el invierno y máximas durante el verano. La precipitación se concentra en la primavera y el otoño, aunque existe una gran variabilidad interanual (Piñol *et al.*, 1991). El gradiente pronunciado de altitud crea un mosaico de microclimas con distintos regímenes de temperatura y precipitación. De hecho, durante el periodo de estudio se encontraron marcadas diferencias en el régimen de temperatura y precipitación en dos sitios situados en los extremos del rango de altitud. Este marco climático provoca que haya una gran variabilidad en el régimen hidrológico entre ríos. La mayoría de los ríos situados en zonas de baja altitud son estacionales y muestran un patrón anual característico formado por tres periodos hidrológicos distintos (Bernal *et al.*, 2005): (i) de finales de otoño a principios de primavera (el periodo frío, húmedo y no vegetativo, o *periodo húmedo*), (ii) de finales de primavera a finales de verano (el periodo cálido, seco y vegetativo, o *periodo seco*), y (iii) a principios de otoño (la transición de condiciones secas a húmedas, o *periodo de transición*). La duración de cada periodo hidrológico puede cambiar sustancialmente entre años dependiendo de la cantidad y distribución de la precipitación.

La cuenca del río Tordera está formada por una mezcla heterogénea de paisajes debido a la influencia conjunta de cambios de altitud y transformación del paisaje por la acción humana. Del área total de la cuenca, un 78% está forestado (incluyendo todos los tipos de bosques), un 13% se dedica a actividades agrícolas (incluyendo cultivos de secano y de regadío), y un 7% está urbanizado (incluyendo núcleos

urbanos, urbanizaciones, infraestructuras viarias y zonas industriales y comerciales). Otros tipos de usos del suelo (incluyendo embalses, praderas o áreas sin vegetación) ocupan menos de un 2% del área total de la cuenca.

La mayoría de las áreas forestadas están dentro de áreas naturales protegidas localizadas en las cabeceras de los valles. Los bosques de abeto (*Abies alba* Mill.) y haya (*Fagus sylvatica* L.) dominan en las altitudes más altas, mientras que bosques esclerófilos de encinas (*Quercus ilex* L.), alcornoques (*Quercus suber* L.) y pinos (*Pinus halepensis* Mill. y *Pinus pinea* L.) dominan en altitudes más bajas. En las cabeceras de los valles, los bosques de ribera están generalmente bien conservados y dominados por el haya en altitudes altas y el aliso (*Alnus glutinosa* L.) en altitudes más bajas. En las llanuras, muchas de las zonas forestadas han sido transformadas por el desarrollo urbano y agrícola, y la vegetación de ribera autóctona ha sido mayormente eliminada o sustituida por plantaciones de chopo (*Populus sp.*) o plátano (*Platanus hispanica* Mill ex Muench). Quedan muy pocos bosques de ribera bien conservados, y especies invasoras como la acacia blanca (*Robinia pseudoacacia* L.) o la caña común (*Arundo donax* L.) son muy abundantes (Observatori de la Tordera, 2006).

La cuenca del río Tordera tiene una población permanente de aproximadamente 299.000 personas, pero este número se duplica durante los meses de verano debido a las segundas residencias y el turismo, especialmente cerca de la costa (ACA, 2006). La demanda de agua dulce es principalmente para uso doméstico (41%) e industrial (32%), mientras que el uso agrícola (27%) es menos importante (ACA, 2006). El agua proviene principalmente de acuíferos subterráneos (71%), mientras que las aguas superficiales sólo aportan un 2% (Observatori de la Tordera, 2006). El resto de la demanda de agua queda cubierta por desalinización (14%) y trasvases de agua desde otras cuencas (13%). La implementación de numerosas plantas depuradoras durante la década pasada ha disminuido el impacto de muchas fuentes puntuales de origen urbano sobre los ecosistemas fluviales. Sin embargo, el impacto de los fertilizantes agrícolas a través de fuentes difusas sigue siendo un problema a resolver (ACA, 2006).

2.2 Características de los ríos estudiados

Las investigaciones se llevaron a cabo en 13 ríos de pequeño tamaño situados en la cuenca del río Tordera que fueron muestreados bisemanalmente durante el periodo de estudio (2004-2006). Los ríos fueron seleccionados con el objetivo de cubrir un amplio rango de condiciones micro-climáticas y de uso del suelo. La altitud media de las cuencas (122–1419 m) y el tamaño (0,7-45 km²) mostraron rangos amplios. La

pendiente media de la cuenca fue relativamente similar (c. 20%) entre ríos, exceptuando cuatro ríos con pendientes menores al 11%. En nueve de las 13 cuencas más del 90% del suelo estaba forestado. El suelo urbano ocupó más del 50% del área de la cuenca en tres casos, mientras que el suelo agrícola ocupó el 15% y el 32% del área de la cuenca en dos casos.

Las características de la cuenca se reflejaron en las características fisicoquímicas de los ríos estudiados. La temperatura del agua (9,3-13,9 °C) fue similar y mayor de 11 °C en todos los ríos menos MON, el río situado a la altitud más alta. El caudal medio (0,9-180,8 L s⁻¹) varió más de dos órdenes de magnitud entre ríos, y fue más bajo en los ríos más urbanizados. Siete de los 13 ríos resultaron ser estacionales, con un periodo seco de duración variable durante el verano. La conductividad media (53-1.117 µS cm⁻¹) y la concentración media de NO₃⁻+NO₂⁻ (129-1.093 µg N L⁻¹), NH₄⁺ (10-604 µg N L⁻¹) y SRP (3-99 µg N L⁻¹) aumentaron desde los ríos más forestados situados a mayor altitud a los ríos más urbanizados situados a menor altitud. También se observó la tendencia a aumentar de la concentración de NO₃⁻+NO₂⁻ en los ríos más agrícolas.

La morfología del canal de los tramos experimentales estaba relativamente poco modificada. El sustrato arenoso dominó en la mayoría de los ríos, aunque en cuatro de ellos (MON, RIE, GUA y CEL) el sustrato dominante fueron los cantos y las rocas de tamaño grande. Excepto en algunos de los ríos más alterados por la acción humana (RES, MB, RIU, COLn), la vegetación de ribera estaba bien desarrollada.

2.3 Estudios previos sobre biogeoquímica fluvial en la cuenca del río Tordera

Los estudios sobre biogeoquímica fluvial en la cuenca del río Tordera fueron iniciados por investigadores de la Universidad de Barcelona a finales de los años 80. Estos estudios siguieron la aproximación de Likens *et al.* (1977) para examinar los balances de agua y solutos en la cuenca experimental del río La Castanya, situado en el Macizo del Montseny (Àvila & Rodá, 1988; Àvila *et al.*, 1992; Piñol & Àvila, 1992; Piñol *et al.*, 1992). Estudios más recientes sobre biogeoquímica fluvial se han llevado a cabo por investigadores de la Universidad de Barcelona y la Universidad de Girona en la cuenca del río Furiosos, situado en la cordillera litoral del Montnegre-Corredor y también incluido en la presente Tesis Doctoral. Estos estudios se han concentrado en la interacción entre el canal del río y la zona de ribera (Butturini *et al.*, 2002; Butturini *et al.*, 2003; Bernal *et al.*, 2003; Vázquez *et al.*, 2007), el patrón temporal del N inorgánico y orgánico (Bernal *et al.*, 2002; Bernal *et al.*, 2005; Bernal *et al.*, 2006), la hidrología (Bernal *et al.*, 2004), el metabolismo fluvial (Acuña *et al.*, 2004a, 2007) y la dinámica y

descomposición de la materia orgánica (Sabater *et al.*, 2001; Acuña *et al.*, 2004b, 2005, 2007; Artigás *et al.*, 2004).

Estudios previos iniciados por investigadores de la Universidad de Barcelona han explorado la influencia de múltiples factores, tales como la temperatura, el caudal, la disponibilidad de luz y la geología, sobre la retención de nutrientes en cuencas mediterráneas cercanas (Martí *et al.*, 1994; Martí *et al.* 1995; Martí *et al.*, 1996; Butturini & Sabater, 1998; Sabater *et al.*, 2000). Sin embargo, son escasos los estudios sobre retención de nutrientes en la cuenca del río Tordera. Algunos estudios han demostrado cómo la eficiencia en la retención de los ríos se ve alterada por los aportes de nutrientes de las plantas depuradoras (Martí *et al.*, 2004; Merseburger *et al.*, 2005, 2006). Más recientemente Argerich *et al.*, (2008) han mostrado la importancia de la hojarasca y las crecidas para la retención de N y P en Santa Fe del Montseny, un río también incluido en la presente Tesis Doctoral.

3. Publicaciones

3.1 *Influencia del uso del suelo sobre la función del ecosistema fluvial en una cuenca mediterránea.*

Debido a la organización jerárquica de las redes fluviales, los cambios en el uso del suelo que ocurren a escalas superiores (en la cuenca) pueden influir en las características físicas, químicas y biológicas a escalas inferiores, finalmente alterando la estructura y función del río. Los efectos antropogénicos sobre los ríos han sido principalmente estudiados mediante métricas estructurales como por ejemplo la química del agua, alteraciones hidromorfológicas y biomasa algal. Parámetros funcionales, incluyendo métricas de retención de nutrientes y metabolismo, también se utilizan actualmente como indicadores de la condición del río.

Dentro de dicho contexto jerárquico, en este estudio se utilizó una aproximación multivariante para examinar la relación entre atributos estructurales y funcionales (retención de nutrientes y metabolismo) de los ríos y variables de sus respectivas cuencas, incluyendo los usos del suelo. El estudio se llevó a cabo en 13 ríos situados en la misma cuenca mediterránea, pero que drenan sub-cuencas con diferentes usos del suelo.

A la escala de la cuenca, los resultados mostraron dos gradientes del uso del suelo: (i) de cuencas forestadas a cuencas urbanizadas y (ii) de cuencas poco agrícolas a cuencas moderadamente agrícolas. La variación en parámetros

estructurales y funcionales se relacionó estrechamente con estos gradientes en los usos del suelo. Concretamente, la demanda de NH_4^+ (medida como la velocidad de captación, V_f) disminuyó a lo largo del gradiente desde cuencas forestadas a cuencas urbanizadas, principalmente en respuesta a un aumento en las concentraciones de nutrientes [NH_4^+ , N orgánico disuelto (DON) y carbono orgánico disuelto (DOC)]. Tanto la producción primaria como la respiración aumentaron a lo largo del gradiente de agricultura en respuesta a aumentos en la biomasa algal (clorofila *a*). La demanda de SRP no se relacionó con ninguno de los gradientes de usos del suelo.

Nuestros resultados ponen de manifiesto las conexiones entre factores que operan a diferentes escalas espaciales (desde la cuenca hasta el río) y su distinta influencia en el funcionamiento del ecosistema fluvial. Los gestores deben tener en cuenta estas conexiones a la hora de diseñar planes de gestión y restauración fluvial. El uso de medidas de procesos funcionales es necesario debido a que una gestión ecológica con éxito debe restaurar tanto la función como la estructura del río. Los parámetros de retención de nutrientes y metabolismo son buenos candidatos para tapar este hueco.

3.2 Retención y eliminación de nitrato en ríos mediterráneos con distintos usos del suelo: un estudio con el ^{15}N como trazador.

Se utilizaron adiciones de NO_3^- marcado con ^{15}N para investigar el reciclado de N a una escala de tramo completo en ríos mediterráneos sujetos a diferentes usos del suelo (forestado, urbano y agrícola). El objetivo fue examinar: (i) la magnitud e importancia relativa de la retención (captación asimilatoria) y la eliminación (desnitrificación) de NO_3^- , (ii) la contribución relativa de los diferentes compartimentos primarios de captación a la retención de NO_3^- , y (iii) las vías de regeneración, transformación y exportación del N retenido.

La concentración de NO_3^- aumentó y la de oxígeno disuelto (DO) disminuyó desde el río forestado al río agrícola, con valores intermedios en el río urbano. La biomasa de los compartimentos primarios de captación fue similar entre ríos y dominada por compartimentos detríticos (material orgánico particulado fino y grueso). En concordancia, el metabolismo fue netamente heterotrófico en los tres ríos, aunque el grado de heterotrofia aumentó desde el río forestado al agrícola. La distancia de captación de NO_3^- disminuyó a lo largo de este gradiente, mientras que la velocidad y la tasa de captación de NO_3^- mostraron los valores más altos en el río urbano. La desnitrificación no fue detectada en el río forestado, pero representó el 9% y el 68% de la captación total de NO_3^- en los ríos urbano y agrícola, respectivamente. La

contribución relativa de los compartimentos detríticos a la captación asimilatoria de NO_3^- fue mayor en el río forestado y menor en el río agrícola. En los tres ríos, el N retenido fue rápidamente transferido a niveles tróficos superiores, y prontamente regenerado a la columna de agua. Debido a un estrecho acoplamiento entre los procesos de regeneración y nitrificación, la mayoría del N fue exportada de los tramos experimentales en forma de NO_3^- .

El presente estudio pone de manifiesto un rápido reciclado de N en ríos mediterráneos. Además, los resultados indican que la eliminación de NO_3^- vía desnitrificación puede verse incrementada frente a la retención de NO_3^- vía captación asimilatoria en ríos mediterráneos heterotróficos, alterados por la actividad humana, y caracterizados por concentraciones altas de NO_3^- y bajas de DO.

3.3 Efectos de los nutrientes y la luz sobre la biomasa y captación de nitrógeno del perifiton en ríos mediterráneos con distintos usos del suelo.

Se utilizaron sustratos difusores de nutrientes (NDS) para determinar la importancia relativa de los nutrientes y la luz como factores limitantes potenciales de la biomasa y capacidad de captación de N del perifiton en ríos mediterráneos sometidos a diferentes impactos humanos. Los nutrientes examinados fueron el P y el N, y además diferenciamos entre la respuesta de las comunidades de perifiton a dos fuentes de N ($\text{NO}_3\text{-N}$ y $\text{NH}_4\text{-N}$). Para examinar el efecto de la luz y los nutrientes sobre la biomasa del perifiton, comparamos las tasas de incremento de la clorofila *a* en NDS situados en zonas de luz y de sombra. El efecto de la disponibilidad de nutrientes sobre la captación del perifiton se midió por los cambios en el contenido de ^{15}N en los NDS tras adiciones de $\text{NO}_3\text{-}^{15}\text{N}$ de corta duración.

Los resultados muestran que la luz fue el factor que más afectó a la biomasa algal en los ríos estudiados. La biomasa algal fue generalmente más alta en zonas de luz que en zonas de sombra. La mayor disponibilidad de nutrientes simulada mediante los experimentos de NDS no incrementó el crecimiento de la biomasa algal en ninguna de las dos condiciones de luz.

En los tratamientos control (concentraciones ambientales), las tasas de captación de $\text{NO}_3\text{-N}$ incrementaron y el ratio C:N molar disminuyó consistentemente con el aumento de la disponibilidad de N entre ríos. Las tasas de captación de $\text{NO}_3\text{-N}$ fueron alteradas en los NDS con concentraciones de N incrementadas artificialmente. Las comunidades de perifiton que crecieron sobre los sustratos enriquecidos en N tendieron a captar preferentemente el N proveniente del sustrato que el N proveniente

de la columna de agua. Esta respuesta varió entre ríos y dependió de la disponibilidad ambiental de N.

La biomasa de perifiton no difirió significativamente entre los sustratos expuestos a las dos formas de N disponibles. Sin embargo, encontramos diferencias en los efectos de ambas formas de N sobre la captación de N desde la columna de agua. El $\text{NH}_4\text{-N}$ pareció la fuente de N preferida por el perifiton desarrollado sobre los NDS.

Los resultados sugieren que el efecto de las zonas de ribera sobre la disponibilidad de luz, pese a ser poco considerado por los gestores del agua, puede ser más importante que los nutrientes a la hora de controlar los efectos de la eutrofización derivados de las actividades humanas. Finalmente, nuestros resultados confirman que no sólo los incrementos en la concentración, sino también los desequilibrios estequiométricos deben ser considerados a la hora de examinar la retención de N en ríos alterados por la actividad humana.

3.4 Variación interanual, anual y estacional de la retención de P y N en un río permanente y un río estacional.

Los ríos de cabecera son sitios clave en la retención de nutrientes pero se conoce poco sobre la variación temporal de este proceso. Se utilizaron medidas mensuales tomadas a lo largo de dos años para examinar la variación en la retención de SRP y NH_4^+ en dos ríos mediterráneos de cabecera con distinto régimen hidrológico (permanente vs. estacional).

Las diferencias en la retención entre ríos fueron más evidentes para el NH_4^+ , probablemente debido a las grandes diferencias en el potencial de limitación por N observadas. En ambos ríos, la eficiencia en la retención de nutrientes fue negativamente afectada por los cambios bruscos en el caudal, mientras que los cambios graduales en la demanda de SRP fueron parcialmente controlados por la dinámica de la vegetación de ribera a través de cambios en la disponibilidad de materia orgánica y luz. Las concentraciones de nutrientes se mantuvieron por debajo del nivel de saturación en ambos ríos. Sin embargo, en el río estacional la demanda de SRP aumentó en relación a la demanda de NH_4^+ cuando el potencial de limitación por P se incrementó (ratio de N inorgánico disuelto:SRP más elevado).

Inesperadamente, la variabilidad en la retención de nutrientes no fue más elevada en el río estacional, lo cual sugiere una alta resiliencia de las comunidades responsables de la captación de nutrientes. No obstante, la variabilidad de todas las métricas de retención en cada río aumentó con el aumento de la escala temporal. Una

revisión de estudios sobre variación temporal en la retención de nutrientes a diferentes escalas temporales avala este hallazgo e indica un aumento de la variabilidad en la retención de nutrientes con el incremento de la variabilidad en los factores ambientales desde la escala diaria a la escala interanual.

En general, este estudio pone de manifiesto la importancia de las condiciones climáticas locales en la regulación de la retención de nutrientes, e indica efectos potenciales de los cambios en el uso del suelo y el régimen climático sobre el funcionamiento de los ecosistemas fluviales.

4. Conclusiones generales

El objetivo de la presente Tesis Doctoral era explorar las implicaciones del cambio global sobre la retención de nutrientes en ríos mediterráneos. Se utilizó una aproximación de espacio por tiempo para examinar las implicaciones de dos importantes aspectos del cambio global: (i) la transformación del paisaje y (ii) el cambio climático.

Las primeras tres publicaciones se centraron en los efectos de la transformación del paisaje sobre los procesos de retención mediante la medición de parámetros de retención y variables adicionales en una serie de ríos con distintos usos del suelo. La cuarta publicación se centró en los efectos del cambio climático sobre los procesos de retención de nutrientes mediante la medición de la variación temporal de las métricas de retención y variables adicionales en un río permanente y un río estacional. Las conclusiones de cada una de las cuatro publicaciones fueron las siguientes:

Influencia del uso del suelo sobre la función del ecosistema fluvial en una cuenca mediterránea (capítulo 3.1).

1. El desarrollo urbano tuvo un efecto negativo sobre la retención de nutrientes en los ríos estudiados. La demanda de NH_4^+ (medida como la velocidad de captación, V_f) disminuyó a lo largo del gradiente desde cuencas forestadas a cuencas urbanizadas debido a la saturación de la captación por el aumento de las concentraciones de nutrientes (NH_4^+ , DOC y DON) e inhibición de la nitrificación por una reducción de la disponibilidad de DO a lo largo del gradiente.
2. El metabolismo fluvial fue más susceptible a la influencia de la acción humana sobre los factores que operan a una escala espacial cercana al río (por ejemplo la vegetación de ribera) que sobre los factores que operan a una escala

espacial mayor (por ejemplo los usos del suelo en la cuenca). Tanto la producción primaria bruta como la respiración del ecosistema aumentaron a lo largo del gradiente de agricultura en respuesta a aumentos en la biomasa algal (clorofila *a*), probablemente causados por una mayor disponibilidad de luz.

3. La demanda de SRP no se relacionó con ninguno de los gradientes de usos del suelo debido a los valores relativamente bajos y al estrecho rango de las concentraciones de SRP. Asimismo, la co-precipitación de SRP con el carbonato cálcico fue insignificante debido a la geología silíceo dominante en la cuenca de estudio.
4. Los parámetros funcionales de retención de nutrientes y metabolismo fueron medidas sensibles al efecto de la acción humana sobre la función del ecosistema. Por tanto, estos parámetros podrían ser utilizados en planes de gestión y restauración fluvial junto con parámetros estructurales más tradicionales.

Retención y eliminación de nitrato en ríos mediterráneos con distintos usos del suelo: un estudio con el ^{15}N como trazador (Capítulo 3.2).

5. El reciclado de N fue muy rápido en todos los ríos estudiados debido a un estrecho acoplamiento entre los procesos de captación, regeneración y transformación. Sin embargo, encontramos grandes diferencias entre los ríos en las vías de reciclado de N, pese a las diferencias relativamente pequeñas en las características fisicoquímicas y la biomasa de los compartimentos responsables de la captación.
6. La eliminación permanente de NO_3^- vía desnitrificación aumentó respecto de la retención temporal vía captación asimilatoria a lo largo del gradiente desde el río forestado al río agrícola. Este resultado se debió a un aumento en el grado de heterotrofia y en la concentración de NO_3^- , y a una disminución en la concentración de DO a lo largo del gradiente. En estas condiciones, el NO_3^- puede ser preferentemente utilizado como aceptor de electrones en procesos disimilatorios de captación, como por ejemplo la desnitrificación.
7. La contribución de los compartimentos detríticos a la asimilación de NO_3^- disminuyó y la de los compartimentos dominados por productores primarios aumentó a lo largo del gradiente desde el río forestado al río agrícola. La vegetación de ribera contribuyó notablemente a la retención de N, especialmente en el río agrícola. Esta conexión entre el río y la ribera puede

ser de gran importancia en zonas áridas y semiáridas en las cuales la vegetación de ribera está fuertemente limitada por la disponibilidad de agua.

8. Los cambios en la importancia relativa de los procesos de retención frente a los de eliminación causados por cambios en el uso del suelo pueden afectar sustancialmente a la cantidad y forma del N exportado a ecosistemas situados aguas abajo, lo cual tendrá consecuencias en la estructura y función de estos ecosistemas.

Efectos de los nutrientes y la luz sobre la biomasa y captación de nitrógeno del perifiton en ríos mediterráneos con distintos usos del suelo (Capítulo 3.3).

9. La luz fue el factor que más afectó a la biomasa algal en los ríos estudiados. La influencia de los bosques de ribera sobre la disponibilidad de luz, aunque es raramente considerada por los gestores del agua, puede ser más importante que los nutrientes para el control de los efectos de la eutrofización derivados de la acción humana. Este efecto puede ser más acentuado en ecosistemas fluviales mediterráneos debido a la relativamente poco densa vegetación de ribera y los altos niveles de radiación solar.
10. Las concentraciones ambientales de nutrientes no saturaron la captación de N por parte del perifiton en los ríos estudiados. Las tasas de captación de NO_3^- aumentaron y los ratios C:N disminuyeron consistentemente con el aumento de la disponibilidad de N entre ríos. Las comunidades de perifiton prefirieron captar N proveniente del sustrato que de la columna de agua.
11. El NH_4^+ fue la forma de N preferida por el perifiton, lo cual sugiere que no sólo se debe considerar el aumento en la concentración, sino también los desequilibrios estequiométricos a la hora de estudiar la retención de N en ríos alterados por la acción humana.

Variación interanual, anual y estacional de la retención de P y N en un río permanente y un río estacional (Capítulo 3.4).

12. Las condiciones climáticas locales regularon la magnitud, el patrón temporal y la variabilidad de la retención de nutrientes imponiendo el régimen de perturbaciones y las ventanas temporales de disponibilidad de energía y recursos.
13. La eficiencia en la retención de nutrientes fue negativamente afectada por aumentos abruptos del caudal, mientras que la demanda de nutrientes fue

regulada por cambios graduales en la disponibilidad de materia orgánica y luz. Las diferencias entre ríos en la variación temporal de la demanda se debieron en parte a diferencias en la fenología de la vegetación en dos condiciones climáticas locales contrastadas.

14. Las condiciones climáticas locales influyeron en el procesamiento de nutrientes en la cuenca y las zonas cercanas al río, lo cual tuvo consecuencias en la disponibilidad de nutrientes en el agua y a su vez causó cambios en la magnitud y variación temporal de la retención de nutrientes.
15. La variabilidad de la retención de nutrientes aumentó con la escala temporal debido al aumento de la variabilidad de los factores ambientales. Esta observación fue constatada mediante una revisión de estudios sobre variación temporal de la retención temporal de nutrientes a diferentes escalas temporales.
16. Inesperadamente, la variabilidad de la retención de nutrientes no fue mayor en el río estacional que en el río permanente, lo cual sugiere una gran resiliencia de las comunidades biológicas responsables de la captación de nutrientes.

En conclusión, los resultados de la presente Tesis Doctoral evidencian que la transformación del paisaje y el cambio climático tienen un efecto sobre la retención de nutrientes en los ecosistemas fluviales, Este importante servicio que los ríos proporcionan a la humanidad puede verse reducido por los cambios en varios factores ambientales derivados de la acción humana.

La transformación del suelo forestado a suelo urbano y agrícola provoca un aumento de las concentraciones de N y P disueltos que pueden saturar la capacidad de captación de nutrientes de los ríos, disminuyendo así la capacidad y eficiencia de estos ecosistemas para retener nutrientes en exceso. Además, otros cambios en las características ambientales de los ecosistemas fluviales asociados a la alteración antrópica (baja concentración de DO, gran biomasa algal, canalización) pueden inhibir procesos importantes de retención. Cambios en las características físicas y químicas de los ecosistemas fluviales pueden modificar sustancialmente las vías de reciclado de N. Por ejemplo, en los ríos estudiados los procesos de eliminación de NO_3^- fueron favorecidos frente a los de retención. Estos cambios pueden tener importantes consecuencias sobre la cantidad y forma de los nutrientes exportados a ecosistemas situados aguas abajo.

Se prevé que el cambio climático en la región mediterránea provoque un aumento de la temperatura y una disminución de la precipitación. Los parámetros

físicos, químicos y biológicos de los ecosistemas fluviales se verán alterados mediante efectos directos a nivel del río, o efectos indirectos a nivel de la cuenca o zonas cercanas al río. Los resultados de la presente Tesis Doctoral demuestran que la dinámica de nutrientes difirió notablemente entre el río permanente y el estacional. Las predicciones indican que el grado de estacionalidad o intermitencia de los ríos de regiones áridas o semiáridas como la región mediterránea aumentará durante las próximas décadas. Los resultados de la presente Tesis sugieren algunos de los efectos que estos cambios provocarán en el funcionamiento de los ecosistemas fluviales.

Para entender mejor los efectos de la transformación del paisaje sobre los ecosistemas fluviales, futuros estudios deben examinar la influencia de la distribución espacial de los diferentes usos del suelo dentro de la cuenca en la estructura y función de los ríos que las drenan. Asimismo, para poder predecir mejor los impactos del cambio climático, las nuevas investigaciones deben concentrarse en las implicaciones de la estacionalidad y/o intermitencia en el funcionamiento de los ecosistemas fluviales. Finalmente, son necesarios más estudios sobre las interacciones entre la transformación del paisaje y el cambio climático para poder entender mejor los efectos generales del cambio global sobre los ecosistemas de la Tierra.

