Hydrological and geomorphological controls on stream nutrient retention

Controls geomorfològics i hidrològics sobre la retenció de nutrients en els rius

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Vist-i-plau dels directors:

Dra. Eugènia Martí Roca
Centre d'Estudis Avançats de Blanes, CSIC

Dr. Francesc Sabater Comes
Universitat de Barcelona

La doctorant:

Alba Argerich Terradas

A en David,

It's a dangerous business, going out of your door. You step into the Road, and if you don't keep your feet, there is no telling where you might be swept off to.

J.R.R. TOLKIEN

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Contents

GENERAL INTRODUCTION

General Introduction

1. IMPORTANCE OF UNDERSTANDING THE CONTROL PROCESSES OF STREAM NUTRIENT RETENTION

Nitrogen (N) and phosphorus (P) are essential nutrients that may limit primary production in terrestrial and aquatic ecosystems (Elser et al., 2007). Because of the need to feed an increasing human population, agricultural practices during the history have evolved to counterbalance this natural limitation and have resulted in a significant alteration of N and P cycles in air, land, and water at local, regional, and global scales (Matson et al., 1997; Galloway, 2004). The "Green Revolution" occurring between 1950s and the late 1970s involved a series of research, development, and technology transfer initiatives involving the development of high-yielding varieties of cereal grains, expansion of irrigation infrastructure, and distribution of hybridized seeds, synthetic fertilizers, and pesticides to farmers (Evenson and Gollin, 2003). All those initiatives resulted in an increased industrialized agriculture production in many developing nations and, as a collateral effect, the mobilization of large amounts of N and P that were sequestrated in the atmosphere and earth, respectively. For instance, estimates of the annual load of P in the Earth's freshwater and terrestrial ecosystems has almost quadrupled since humans began mining and farming on a large scale from ~3.5 to ~13 Tg P yr^{-1} nowadays (Bennet, 2002). A part from food production, energy production is another anthropogenic activity that has greatly increased nutrient availability, especially for N. During combustion of fossil fuels N is emitted to the atmosphere as a waste product (NO, NO_x) from either the oxidation of atmospheric N_2 or organic N in the fuel (primarily coal). In global, energy and food production have lead to an increase in the reactive forms of N (i.e., all forms except N_2) from ~15 Tg N yr^{-1} in 1860 to ~187 Tg N yr^{-1} in 2005 (Galloway et al., 2008). As a result of those alterations in the nutrient cycles, nutrient loading of terrestrial and aquatic ecosystems has increased and it is still continuously increasing worldwide (Vitousek, 1997; Carpenter et al., 1998). As terrestrial environment saturates with nutrients the excess reach freshwaters through surface and subsurface runoff pathways (Aber et al., 1998), and ultimately discharges into coastal zones where it causes serious adverse effects on ecosystem functioning (Smith, 2003) such as eutrophication and the consequent oxygen depletion. For instance, seasonal hypoxia observed in the Gulf of Mexico has been related to high N and P loads delivered from the Mississippi River (Justic et al., 2002; Turner et al., 2006; Turner et al., 2008) and more than 70% of these loads have an agricultural origin (Alexander et al., 2008).

Over the last decades, there has been increasing evidence that streams and rivers, especially those draining catchments with low human pressure, play a central role in modulating the concentrations and forms of nutrients exported downstream (Lowe and Likens, 2005). Several studies have estimated that from 50 to 75%

of the N load of a watershed can be transformed and retained during passage through the stream network (Alexander et al., 2000; Seitzinger et al., 2002; Peterson et al., 2001) and 30% of the soluble reactive phosphorus (SRP) annually entering the stream can be retained by in-stream processes Mulholland (2004). For this reason and due to the global concern about ecological consequences derived from eutrophication of aquatic ecosystems, studies focusing on factors controlling stream nutrient transformation are of critical interest. In this sense, the present dissertation aims to contribute to a better understanding of how hydromorphology determines stream nutrient retention.

2. FROM STREAMS AS “PIPES” TO STREAMS AS “BIOREACTORS”

2.1. Perspectives on solute transport in streams

At the end of the 70’s there was an extensive knowledge of the mechanisms of solute transport in streams which basically relied on a hydraulic engineering perspective (Fischer et al., 1979). The basic approach to solute transport in streams was the one-dimensional advection-dispersion model, i.e., a model that describes the variation of solutes through time in a fixed location as the result of advection and dispersion processes occurring in the longitudinal direction. This model assumes a physically uniform channel, a constant discharge, no subsurface flow, and no variation of solute concentration with depth or width. Although these assumptions are hardly met in natural conditions, this model was widely used with fairly good results to describe solute transport in large rivers (Whitehead and Williams, 1982). Successful application of the model in these ecosystems can in part be explained due to the fact that solute dynamics in large rivers are less complex compared to small streams because large rivers generally have low slopes, are deeper than the roughest bed feature and have relatively uniform flows (Allan and Castillo, 2007). However, solute transport in small streams was not well described by the above mentioned model. To improve the predictive power of the model, Bencala and Walters (1983), who studied solute transport in a mountain stream with riffle-pool morphology, incorporated a term for transient storage in the initial model. The term transient storage was not new; it had been used before under a variety of nomenclatures (immobile domain, dead zone) to model solute transport in column and field experiments (Nordin and Troutman, 1980). Transient storage zones are assumed to be stagnant relative to the longitudinal flow of the stream and to obey a first-order mass transfer type of exchange relationship. That is, advection, dispersion, lateral inflow, and lateral outflow do not occur in these zones, and the exchange of solutes between the main stream channel and the storage zone is proportional to the difference in concentration between these two compartments (i.e., transport in or out the transient storage zone is only governed by diffusion). In their paper, Bencala and Walters (1983) recognized that those assumptions were not probably met in streams:

> “it is not easy to envision a linear physical driving mechanism that simultaneously transfers mass between the stream and the storage zone, distributes it uniformly throughout the storage zone and yet prevents the storage zone from moving longitudinally”

but they propose that the “transient storage zones” operating in streams (i.e., channel zones where water is not moving downstream such behind boulders, edges of pools, or backwaters) could be empirically simulated using identical equations than those used in the strict dead zone models. Several empirical studies have proved the utility of the Bencala and Walters model (Legrand-Marcq and Laudelout, 1985; Bencala et al., 1990; D’Angelo et al., 1993; Hart et al., 1999) and this transient storage compartment has been incorporated in several computer codes such as OTIS (Runkel, 1998) and STAMMT-L (Haggerty and Reeves, 2002) which have been widely used to model solute transport in small size streams.

2.2. Merging stream hydrology with ecology

The first studies I'm aware of, that examined the variation in chemical concentrations in streams, date from the first decade of 1900. They were technical studies performed mainly from a public health perspective of water supply to urban areas and implicitly recognized the existence of in-stream processes that modified the chemistry of the rivers by using the term "stream self-purification capacity". In this early stages self-purification was mainly attributed to be caused by hydrological dilution due to the water gaining condition of the rivers as they move downstream (Sedgwick, 1914) and it was not until later that the importance of in-stream biota in processing materials was recognized (Stehfest, 1975). Despite this early implicit recognition, before the 1970's, nutrient transport in streams was studied without paying much attention to the in-stream processes as potential mechanisms affecting nutrient concentrations (Mulholland and Webster, 2010).

It was not until 1975 that Webster introduced the concept of nutrient spiraling (Webster and Pattern, 1979), to describe the cycling of the nutrients in streams during their transport from headwaters to downstream. The term "spiraling" comes from the helical pattern that a nutrient molecule describes as it is moved downstream and at the same time is assimilated from the water column, it is temporally retained, and it is mineralized back into the water column. The nutrient spiraling concept was later developed by Newbold et al. (1981) who presented a mathematical framework to support the conceptual model. Newbold and colleagues (1981) introduced the first metric to estimate nutrient cycling in streams, i.e., the spiraling length (distance required for a nutrient atom to complete 1 cycle from its dissolved inorganic form in the water column). The spiraling length is the sum of the average distance traveled by the solute in a dissolved inorganic form (i.e., the uptake length) and the average distance traveled in a particulate organic form (the turnover length). Since these contributions, most of the studies on nutrient cycling in streams have focused on estimating the nutrient uptake length because it was initially found to be the longer/dominant component of the spiraling length (Newbold et al., 1983; Mulholland et al.,1985) and also because available methodology until late 90's had limitations to estimate nutrient turnover length.

During the Stream Solute Workshop (1990), there was a revision of the transport models for solutes affected by reactive processes, such as biological uptake and sorption. As a result, this paper presented a conceptual model for stream solute/nutrient transport that integrated physical, chemical, and biological processes. The paper reviewed various methodological and analytical approaches and identified advantages and limitations of each of them. It also introduced four basic uptake metrics: the uptake length (S_w, m), the uptake rate (total flux of nutrient transferred from the water column to the stream bottom; U, mg $m^{-2}h^{-1}$), the mass transfer or uptake velocity (vertical velocity at which a solute moves towards the sediment/water interface; V_f, m s^{-1}), and the uptake rate coefficient (k_c, s^{-1}); and the mathematical relation among these metrics.

2.3. Beyond the Stream Solute Workshop

After the Stream Solute Workshop (1990) studies addressing nutrient uptake in streams from a reach –scale perspective have multiplied (see reviews done by Ensign and Doyle (2006) and Mulholland and Webster (2010)) resulting in an increased knowledge of factors controlling stream nutrient retention. Most of this knowledge comes from the combined use of reactive (e.g., N and P) and conservative (e.g., Cl) tracers in field experiments in combination with the conceptual and mathematical framework of the nutrient spiraling concept.

The most commonly used method to study nutrient uptake at reach-scale is injecting a solution of the desired nutrient into the stream to raise in-stream nutrient concentration above ambient conditions and observe nutrient concentration decline in space along a selected reach to quantify uptake length. This method was first applied by McColl (1974) and is simple, fairly inexpensive, and widely used (Webster and Valett 2006). However, some studies have demonstrated that this method clearly overestimates uptake lengths of both P (Mulholland et al., 1990) and N (Mulholland et al., 2002) because the experimentally increased concentrations may fall above the linear range of the nutrient uptake-concentration relationship. One alternative to overcome this limitation is the use of isotope-labeled tracers, which allow to measure uptake lengths under environmental nutrient concentrations since isotope injections do not result in significant increases in ambient concentrations. Due to health and safety concerns there are few studies with radiotracer ^{32}P, but SRP uptake in several compartments have been calculated by Newbold et al. (1983), Mulholland et al. (1985) and Mulholland et al. (1990) using this technique. In contrast, the use of stable isotopes of C (13C) and N (15N) as tracers is less restrictive and has been applied in recent stream studies to gain insights of whole-reach nutrient processing and the mechanisms driving it. For instance, the N fate in streams and biogeochemical processes associated to it have been deeply studied using a stable isotope tracer, ^{15}N as both ammonium (NH_4-N) and nitrate (NO_3-N), in the large-scale collaborative project "Lotic Intersite Nitrogen eXperiment" (LINX I and LINX II, NSF) which began on 1996. The project involved several streams spanning a broad range of ecosystem types across North America, and has produced numerous papers (www.biol.vt.edu/faculty/webster/linx/). Key results from this project demonstrated the important role of headwater streams in controlling N exports to rivers (Peterson et al., 2001); indicated factors influencing ammonium uptake variability among the studied streams (Webster at al. 2003); and allowed the quantification of denitrification rates at whole-reach scale and how they varied among streams from several biogeoclimatic regions draining catchments with different lad use composition; and thus, subjected to different nitrate loading (Mulholland et al., 2008 and 2009). Unfortunately, the high cost of isotope-labeled tracers and the analysis of samples by mass spectrometry limit the extensive use of this technique (but see, von Schiller et al., 2009).

Overall, results from studies done over the past 3 decades converge to indicate that stream nutrient uptake is a function of the combination among chemical and biological processes, and hydrologic retention (Valett et al., 1996; McClain et al., 2003). Chemical uptake of nutrients is mainly attributed to sorption-desorption processes, precipitation, and flocculation, and it is mainly determined by the physical and chemical characteristics of both the sediments and water (Meyer, 1979; Reddy, 1999). Biological uptake of nutrients is determined by the communities inhabiting the streams. Among them, microbial communities have a paramount role. For instance, Elwood et al. (1980), and Newbold et al. (1983) showed that microbial uptake could contribute to 80 - 91% of total P retention, being the remaining percentage attributed to abiotic processes. Hydrologic retention determines the residence time of nutrients in a given stream reach as well as their interaction with stream substrata on which microbial communities develop. Hydrologic retention is determined by a combination of stream hydrology and channel geomorphology, and is affected by the extent, direction and strength of the hydrological linkages between the stream channel, the hyporheic zone and the riparian zone. This dissertation focuses on the influence of hydrologic retention on stream nutrient uptake responses, specifically on how zones of hydrologic retention, i.e., transient storage zones, affect nutrient transformation processes.

3. TRANSIENT STORAGE ZONES IN STREAMS

Transient storage zones can be defined as locations of stagnant water that is not subjected to downstream transport (Bencala and Walters 1983); however, they can also we viewed as stream zones where water flow is much slower than that in the free flowing water channel. Size of the transient storage zone is usually reported

Figure 1. Transient storage zones generated by different types of in-stream structures: (A) pools, and (B) leaf litter accumulation.

as a cross-sectional area (A_s) or as its relative proportion in relation to the main-channel cross-sectional area (A_s/A). The proportion of water allocated in transient storage zones can be as large or larger than that allocated in surface free flowing channel in some streams (D'Angelo et al., 1993; Morrice et al., 1997). One of the major contributors to transient storage is surface water exchange with the hyporheic zone (Triska et al., 1989a; Morrice et al., 1997; Butturini and Sabater, 1999). The hyporheic zone is the saturated subsurface compartment located beneath and adjacent to the stream surface channel which has a hydrological interaction with stream surface water (White, 1993). This definition separates the hyporheic zone from the groundwater zone in the classical sense that groundwater represents water beneath the water table that has not yet been influenced by channel processes. In addition to the hyporheic zone, water transient storage can be also generated in the surface stream channel by pools (Hall et al., 2002), debris dams (Lautz et al., 2006; Roberts et al., 2007; Jin et al., 2009), eddies, and backwaters (Bukaveckas, 2007) among other structures (Fig. 1).

3.1. Factors that influence the size of transient storage

Among the factors that control transient storage there are the streambed morphology (Harvey and Bencala, 1993; Kasahara and Wondzell, 2003; Cardenas et al., 2004; Gooseff et al., 2005) and hydraulic conductivity of the stream substrate (Valett et al., 1996, Morrice et al., 1997) since they control water exchange fluxes between surface and hyporheic zones. Additionally, transient storage zone size is influenced by geomorphic complexity (Gücker and Boëchat, 2004; Gooseff et al., 2007) and the presence of obstacles for surface flow such as vegetation (Salehin et al., 2003, Harvey et al., 2003) or leaf litter and woody debris accumulation (Haggard and Storm, 2003; Lautz et al., 2006; Roberts et al., 2007). However, the effect of all those factors may be modulated by discharge (Tonina et al., 2007). For instance, D'Angelo et al. (1993) when comparing streams of different size found that the relative storage zone size (i.e. the ratio of the cross-sectional area of the storage zone to that of the main channel) decreased with increasing discharge. However, because these streams not only had different discharge but also different morphologies, the relationships found in this work may not be directly attributable to differences in streamflow. Nevertheless, several field studies have found the same negative relationship between discharge and transient storage (D'Angelo et al., 1993; Martí et al 1997; Morrice et al., 1997; Butturini and Sabater, 1999; Hall et al., 2002; Zarnetske et al., 2007) while others (Legrand-Marcq and Laudelout, 1985; Hart et al., 1999) found a somewhat contrary relationship.

3.2. Methods for estimating transient storage parameters in streams

One of the common methods to quantify transient storage in streams is by the use of conservative tracers (Harvey and Wagner, 2000). Similarly as for measuring nutrient uptake, the method consists on the injection of a conservative tracer (e.g., Cl) into the stream and the examination of its break-through curve at one or several downstream sites. This examination is usually done by fitting the observed data to a solute transport model that considers solute mass partitioning between main channel and transient storage. Among the simplest models are those that consider the exchange of solutes between the main channel and the transient storage zones as a linear first-order mass transfer process with lumped exchange coefficients, such as the model by Bencala and Walters (1983) which has been used in this dissertation (more complicated models are reviewed by Cardenas, 2004). The numerical solution to those models can be obtained through the use of several codes such as the One-Dimensional Transport with Inflow and Storage (OTIS, Runkel, 1998) or STAMMT-L (Haggerty and Reeves, 2002).

One of the limitations of this method lies on the fact that because the descriptions of mass transfer is phenomenological, the cross-sectional area of the transient storage zone estimated by the models is not directly related to any specific physical feature in real stream channels, i.e., is a conceptualization of the process (see Fig. 2), so that it is difficult to use for the quantification of the biogeochemical effects of hyporheic exchange flow in stream ecosystems (Kasahara, 2000). In addition, commonly used tracers are not capable to distinguish between stream compartments that contribute to transient storage zones. The parameters estimated by the models, including the cross-sectional area of the storage zone (A_s), and water exchange coefficients (k_1 or α, k_2 or α_2 –the notation varies among studies) are therefore interpreted as "lumped" parameters that represent a spectrum of storage processes that occur simultaneously in multiple types of storage zones. For instance, in-stream transient storage can yield similar arrival times than those from hyporheic transient storage (Gooseff et al., 2005). Transient storage models generally aggregate all transient storage zones (e.g., in-channel and hyporheic transient storage) in one single storage zone although several attempts have been made to find a method able to separate those two compartments (Gooseff et al., 2004 and 2005; Briggs et al., 2009). Since biogeochemical processes are expected to differ between those compartments, this limitation is particularly critical when trying to understand the relation between transient storage parameters and whole-reach metabolic and biogeochemical processes. In this dissertation we will present a new tracer aimed to overcome this methodological limitation by providing information of the transient storage zones from a functional perspective, in addition to that from a hydraulic perspective.

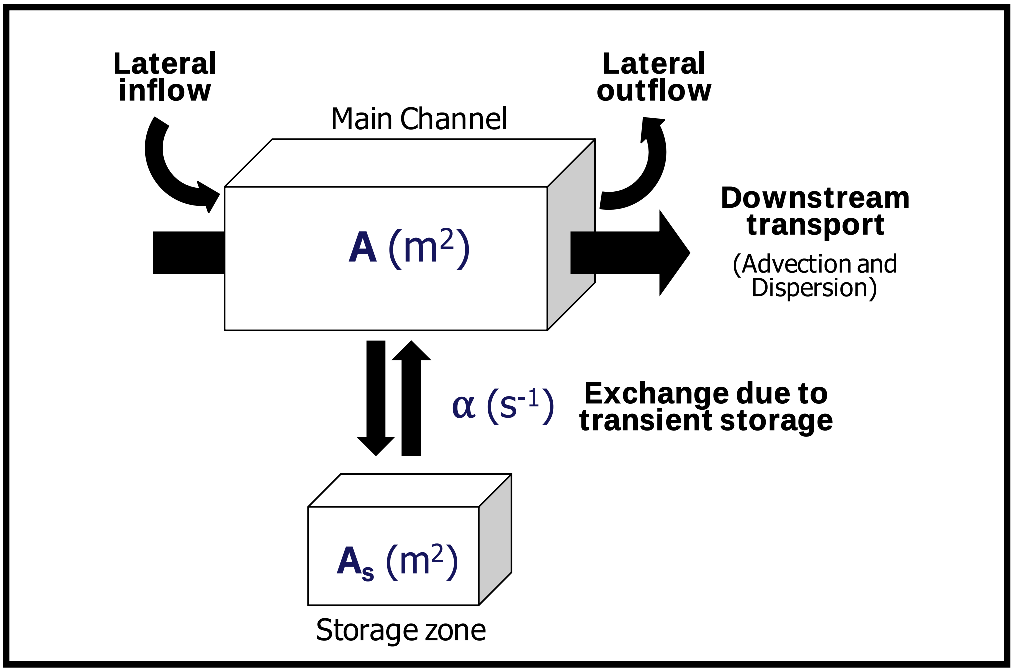

Figure 2. Conceptual model for transient storage (from Runkel, 1998)

4. TRANSIENT STORAGE AND NUTRIENT UPTAKE

As said previously, transient storage zones increase the contact time of main-channel water with biogeochemically reactive sediments, and thus, increased transient storage is often presumed to increase stream nutrient retention. For instance, Triska et al. (1989b) found that transient storage of NO_3-N accounted for 10% of the NO_3-N added over 10 days to Little Lost Man Creek, California. Valett et al. (1996) found that S_w-NO_3 was inversely related to A_s/A in three southwestern streams. Martí et al. (1997) hypothesized that short S_w-NO_3 were associated with the transient storage provided by high algal biomass in Sycamore Creek, Arizona, although no direct relation was observed between transient storage and uptake metrics. Mulholland et al. (1997) found transient storage to be responsible for 43% of SRP retention in Hugh White Creek (North Carolina), but had little effect on SRP uptake in Walker Branch (Tennessee). Lautz and Siegel (2007) created a regression model relating transient storage and measures of stream flow to nitrate uptake length from results from 18 NO_3-N-addition tracer tests. The model explained 44% of the variability in nitrate uptake length.

However, most of those studies have used uptake length as a metric to measure nutrient retention. Since uptake length is strongly dependant on discharge it does not provide effective comparison of nutrient affinity between streams or in a stream over time as velocity changes, results obtained from these studies should be taken with caution (Hall et al., 2002). As an alternative, the use of V_f, which normalize S_w for stream depth and velocity, or U, which normalizes V_f by background nutrient concentrations, may be better parameters for comparison among streams and for determining the underlying biotic factors that influence nutrient retention. Hall et al. (2002), in a study involving 13 streams and 37 injections, found that transient storage explained 14% of the annual variation in NH_4-N uptake velocities while SRP uptake was not related to transient storage. Gücker and Boëchat (2004) reported that between 52% and 85% of ecosystem NH_4-N retention occurred in transient storage zones in a study encompassing 12 streams with different morphology. Buckaveckas (2007) found increased nutrient uptake velocities after the restoration of a stream channel that increased the size of the transient storage

Other studies have not found a direct relation between nutrient uptake and transient storage or even a negative relation. Valett et al. (2002) found lower V_f-SRP as A_s/A increased in a study involving 6 Appalachian mountain streams. Ensign and Doyle (2006) in analysis of the results obtained in 404 individual experiments from 52 published found a negative correlation between A_s/A and S_w-SRP and S_w-NH_4 but results regarding uptake velocities were contradictory: they found a positive relation between A_s/A and V_f-SRP and no relation between A_s/A and V_f-NH_4, and Webster et al. (2003) did not a find a relation between NH_4-N uptake and hyporheic exchange in a study encompassing data from 11 streams from different biomes. This lack of relationship was attributed to a domination of the variability in the environmental factors over the hydraulic variability suggesting that a relationship between transient storage and nutrient uptake may be found in more regional comparisons.

Despite the growing interest on the processes of stream nutrient retention, little is still known about the control that the location of transient storage exerts on nutrient retention. Hyporheic transient storage exposes stream water to interstitial sediment pore water zones with lower redox potential, higher concentrations of organic carbon, and heterotrophic biofilms that may transform N and P between inorganic and organic forms (Ensign and Doyle, 2005). In contrast, in-channel storage only extends the contact time of water with superficial sediments and their associated biofilms, and thus, it is generally believed that the importance of hyporheic processes outweighs the nutrient removal functions of in-channel storage (Hall et al., 2002). However, Ensign and Doyle (2005) examined the effect of in-channel flow obstructions such as vegetation and coarse woody debris (CWD) on transient storage and nutrient uptake and found that in-channel transient storage, rather than hyporheic storage, can be a substantial portion of overall transient storage and nutrient retention in streams. Butturini and Sabater (1999) concluded that the hyporheic zone did not influence nutrient cycling in Riera Major. Gücker and Boëchat (2004) reported surface transient storage to be the major contributor of transient storage in swamp and step-pool reaches and a good relation between surface transient storage and ammonium uptake. Investigations of hyporheic and in-channel nutrient uptake in Brougham Creek, a N-rich agricultural stream in Ontario (Canada), suggested that in-channel storage zones might have been the predominant location of nutrient uptake in this stream (Hill and Lymburner, 1998). Some of the results presented in this dissertation will address those questions.

OBJECTIVES

Objectives

The main goal of this dissertation is to study the influence of hydromorphological factors on stream nutrient uptake. As introduced in the previous section, nutrient uptake in streams results from the combination between nutrient transport rates (determined by the morphology of the channel and the hydrology) and biological processing rates (determined by the biological communities residing in the stream). Within this context, hydromorphology regulates the “opportunity” of process to take place, whereas biotic activity regulates the processing “capacity”. The balance between these two components will ultimately determine the degree to which streams regulate nutrient exports (Battin et al., 2008). From this perspective, the central hypothesis of this dissertation is that water transient storage zones should play an important role in stream nutrient uptake since they increase the time of interaction between nutrients and biota; and thus, may increase the opportunities for the biota to process nutrients, which may result in an increase of nutrient uptake at whole-reach scale.

This dissertation is articulated in two parts and contains five chapters. The first part examines the influence of several drivers of transient storage characteristics and their implications on nutrient uptake using conventional tracer methodologies. This part is composed by three chapters which examine this influence from different perspectives. The first and second chapters use a whole-reach approach and focus on both surface processes (first chapter) and hyporheic processes (second chapter). The third chapter uses a field mesocosm experimental approach and focuses on the contribution of different substrata types on both transient storage and nutrient uptake.

The second part of the dissertation presents the development and application of a new tracer technique to characterize transient storage zones from a metabolic perspective. This part includes two chapters. The first chapter focuses on the laboratory studies performed to develop the tracer and the second chapter focuses on the application of the tracer in a field experiment and the development of the metrics to estimate the metabolically active transient storage. The detailed objectives of each publication are:

The first chapter, “**Combined effects of leaf litter inputs and a flood on nutrient retention in a Mediterranean mountain stream during fall**”, aims to study the temporal variability of nutrient retention in relation to hydraulic parameters under natural conditions. The study was done during the leaf fall period in a Mediterranean headwater stream. Leaf litter accumulation during this period has a dramatic effect on the morphology of this stream, increasing the number of pools and the channel wet width. On the other hand, leaf fall period in Mediterranean climate is one of the periods with more variability in precipitation; and thus, when more variability in discharge is observed. With this study we want to test if stream nutrient processing capacity would be affected by changes in hydromorphological characteristics derived from leaf litter inputs and discharge variation. Several authors have examined this relationship although none with such an intensive temporal frequency. The specific objectives of this study are:

a) to examine how autumnal accumulation of leaf litter modifies hydraulic properties of the channel, including transient water storage,
b) to evaluate nutrient retention response over this period,
c) to examine the relationship between nutrient retention and leaf accumulation and hydraulic properties.

The second chapter, “**Temporal variation of hydrological exchange and hyporheic biogeochemistry in a headwater stream during fall**”, aims to study the effects of hydromorphological changes caused by leaf litter accumulation and varying discharge on the biogeochemistry of the hyporheic zone, which has been often presented in the literature as the main transient storage compartment in streams. The study explores the variability in the connectivity between the surface channel and the hyporheic zone and how this affects nutrient and oxygen concentrations as well as nutrient retention in the hyporheic zone. The specific objectives of this study are:

a) to examine how the accumulation of leaf litter and variations in discharge modifies surface-hyporheic water exchange,
b) to evaluate possible consequences for the biogeochemistry of the hyporheic zone.

The third chapter “**Influence of transient storage on stream nutrient uptake based on substrata manipulation**”, studies the relation between nutrient retention and different types of substrata creating transient storage using an experimental approach. The experiment was done in a man-made channel with regulated flow where we artificially increased transient storage using deflectors made of different substrata types, which were naturally colonized by stream microbial communities. The specific objective of this study is:

a) to examine the influence of physical and biological characteristics of stream substrata that generate water transient storage on nutrient uptake.

The fourth chapter, “**Development of a “smart” tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: The resazurin-resorufin system”**, presents a new tracer aimed to provide a metabolic characterization of transient storage zones and to differentiate types of transient storage zones based on this characteristic. The specific objective of this study is:

a) to identify and characterize a smart tracer to measure microbiological activity associated with stream water-sediment interaction.

The fifth chapter, “**Quantification of metabolically active transient storage (MATS) in two reaches with contrasting transient storage and ecosystem respiration**”, aims to quantify the portion of transient storage that is significantly contributing to whole-reach metabolic processes by using the “smart” tracer developed presented in the fourth publication. The specific objective of this study is:

a) to quantify the metabolically active transient storage zone in two reaches that are very different in terms of transient storage.

PUBLICATIONS

Combined effects of leaf litter inputs and a flood on nutrient retention in a Mediterranean mountain stream during fall

Argerich et al. (2008) Limnology and Oceanography

Efectes combinats de l'acumulació de fullaraca i una riuada en la retenció de nutrients en un riu mediterrani durant la tardor

Alba Argerich[1], Eugènia Martí[2], Francesc Sabater[1], Miquel Ribot[2], Daniel von Schiller[2], i Joan L. Riera[1]

[1]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028; [2]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona.

RESUM

Aquest estudi examina l'efecte de l'acumulació de fullaraca en la retenció hidràulica i de nutrients en un riu de capçalera mediterrani. Una riuada al final del període de caiguda de fulles creà la oportunitat per estudiar l'efecte del "rentat" sobtat de la major part de la biomassa de fulles que s'havia acumulat a la llera durant la tardor. L'estudi es va portar a terme a la riera de Santa Fe, dins del Parc Natural del Montseny entre l'octubre i el desembre del 2004. En total, es van realitzar vint-i-una addicions de clorurs per mesurar els paràmetres hidràulics i en 13 d'aquestes, també es va afegir amoni i fosfat per calcular la distància (S_w) i la velocitat (V_f) de captació de nutrients. Els paràmetres hidràulics del tram d'estudi es van estimar mitjançant l'OTIS, un codi computacional que permet resoldre les equacions de transport de soluts considerant processos d'advecció, dispersió, dilució i zones de retenció temporal d'aigua (ZRT).

L'entrada de fulles durant la tardor causà modificacions en els paràmetres hidràulics del riu i en la disponibilitat d'hàbitat. L'acumulació de fulles provocà un increment gradual de la mida de les ZRT (A_s) i una disminució dels coeficients d'intercanvi d'aigua entre el canal principal i les ZRT. Durant aquest període, les S_w no van mostrar cap tendència temporal clara per cap dels nutrients i, en general, es van mostrar relativament curtes (S_W-PO_4=219 ± 57 m i S_W-NH_4=49 ± 11 m). Les V_f tendiren a augmentar gradualment a mesura que les fulles s'acumularen en el canal. La riuada provocà la disminució d'A_s, l'augment de les S_w i la disminució de les V_f. Anàlisis de regressió van mostrar una relació positiva entre la V_f-PO_4 i la biomassa de fullaraca i entre la V_f-PO_4 i la temperatura mentre que la V_f-NH_4 es va relacionar negativament amb la concentració de DIN (nitrogen inorgànic dissolt) i la relació molar DIN:P. Ambdues V_f es van mostrar positivament relacionades amb A_s.

Combined Effects of Leaf Litter Inputs and a Flood on Nutrient Retention in a Mediterranean Mountain Stream During Fall

Alba Argerich[1], Eugènia Martí[2], Francesc Sabater[1], Miquel Ribot[2], Daniel von Schiller[2], and Joan L. Riera[1]

[1]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028, Spain; [2]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona, Spain.

ABSTRACT

This study examined the effect of increasing in-channel leaf standing stocks on hydrologic transient storage and nutrient retention in a Mediterranean mountain stream. A flood at the end of the leaf fall period provided the opportunity to examine the effect of abrupt removal of much of the leaf material. Twenty-one chloride additions were performed from October to December 2004. In 13 of these, we also added ammonium and phosphate to estimate nutrient uptake lengths and uptake velocities to assess nutrient retention. The One-dimensional Transport with Inflow and Storage (OTIS) model was used to estimate transient water storage parameters. Although discharge remained constant during leaf fall, water residence time increased because of in-channel litter accumulation, as did nutrient uptake velocity. Flooding reduced leaf benthic standing stocks by 65% and dramatically altered hydraulic and nutrient retention properties of the channel. Following recession, the stream rapidly recovered in terms of nutrient retention, especially for phosphate. Abrupt changes in discharge under flood conditions largely determined the variability in stream nutrient retention. However, leaf litter inputs played an important role in nutrient dynamics during constant flow. Because both the flood regime and the timing of leaf fall are being regionally altered by climate change, our results have implications for stream nutrient dynamics under climate change scenarios.

1. INTRODUCTION

Intense leaf fall from deciduous riparian vegetation is of major importance for both the community structure (Wallace et al. 1997) and metabolism (Crenshaw et al. 2002) of streams. Leaf litter inputs provide large quantities of energy to headwater streams that typically exhibit low levels of primary productivity (Fisher and Likens 1973). The ecological relevance of these inputs is well recognized, especially in temperate regions (Wallace et al. 1997). Streams in Mediterranean regions, in contrast to those from more humid climates, are characterized by high interannual variability in the intensity and

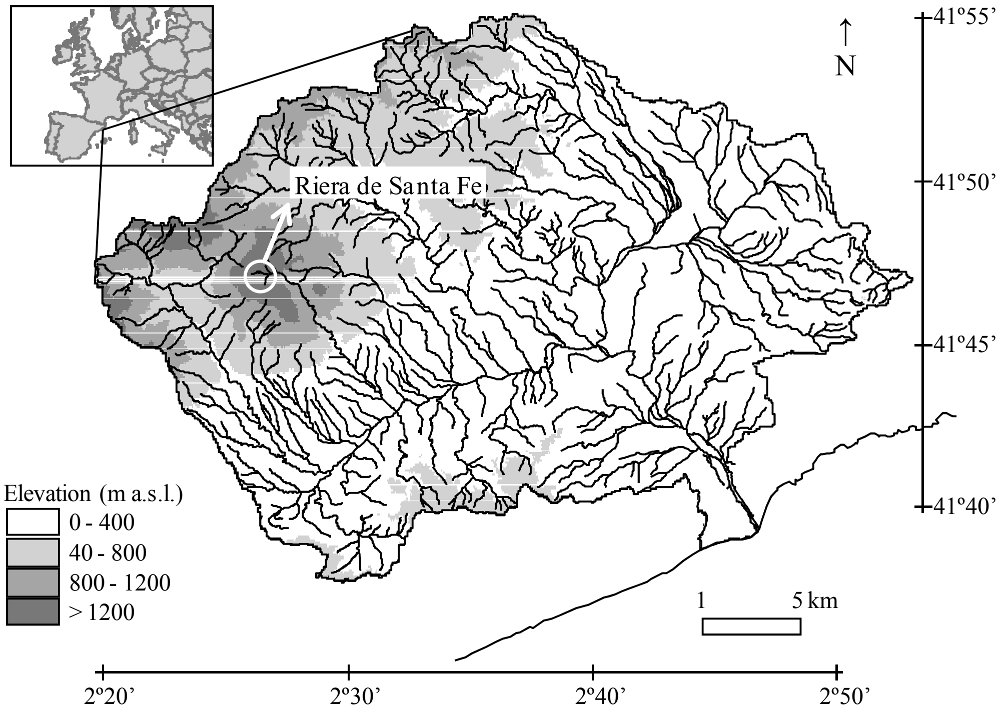

Figure 1. Map showing general location of Riera de Santa Fe in Europe and in the La Tordera catchment.

frequency of floods as well as by periods of intermittent flow. In addition, in Northern Hemisphere Mediterranean regions higher leaf litter input coincides with periods of high flood frequency, low temperature, and low autotrophic activity (Gasith and Resh, 1999). Therefore, the residence time of the allochthonous matter in Mediterranean streams may be much shorter than in temperate streams because it enters shortly before or in conjunction with flooding. In consequence, the ecological relevance of this allochthonous energy source may depend on the timing of flood events relative to leaf inputs.

Mediterranean regions are among the most vulnerable to climate change (Schröter et al. 2005). In these regions, both a change in the frequency and intensity of rains and an increase in temperatures are expected (Christensen and Christensen 2004). Under this scenario, it is likely that alterations in the regimes of autumnal leaf inputs and flooding will have implications for stream metabolism and biogeochemistry. The effects of autumnal leaf litter inputs on stream biota (Maamri et al. 1997) and metabolism (Acuña et al. 2004) have been studied previously in Mediterranean streams, but little is known about their effect on nutrient retention. In addition, methodological constraints on measuring nutrient retention under flood conditions, together with the unpredictability and high rate of change of these episodic events, have restricted the number of studies assessing flood effects on nutrient retention (but see Martí et al. 1997).

Nutrient retention in stream ecosystems is a combination of hydrologic, biologic and chemical retention (Valett et al. 1996). Hydrologic retention is influenced by discharge and the hydraulic and morphologic properties of the stream channel, which determine the extent of the transient water storage (i.e., water moving at slower velocity than the average velocity in the channel, relative to the free flowing water). The magnitude of the transient water storage can be relevant for nutrient retention, because the interaction between stream biota and available nutrients is greater in transient storage zones than in the free flowing water (Triska et al. 1989). Therefore, it has been hypothesized that the influence of in-stream processes on stream nutrient concentrations increases with transient water storage. However, literature results conflict (Mulholland et al. 1997, Hall et al. 2002, Ensign and Doyle 2005), possibly because transient water storage can originate in a variety of channel structures that delay water transport (e.g., eddies, back waters, side channels, streambed irregularities, pools, mats of filamentous green algae, hyporheic zones, and organic debris dams), which may contribute differently to nutrient retention across streams or within streams over time.

The presence of in-channel vegetation (Harvey et al. 2003), debris dams (Lautz et al. 2006), flow obstacles (Ensign and Doyle 2005), and complex stream morphology (Gücker and Boëchat 2004) have been demonstrated to enlarge transient storage zones relative to main channel cross-sectional area. In temperate streams with well-developed riparian forests, autumnal litter inputs may naturally modify channel hydraulic properties, increasing transient water storage (Haggard and Storm, 2003). Ecologically, leaf litter constitutes an external input of energy and resources to stream communities, and provides new colonization substrata for microbial communities. Therefore, an increase in transient water storage coupled to enhanced microbial colonization during leaf fall can result in a hot nutrient retention moment (sensu McClain et al. 2003). Mulholland et al. (1985) attributed temporal variation in phosphate retention efficiency to the availability of benthic organic matter in streams, showing that it was greatest during leaf fall. Nevertheless, several studies have also shown that discharge is a key factor in controlling stream nutrient retention efficiency. In general, rising discharge lowers nutrient retention, which may override or counterbalance other effects such as the accumulation of benthic organic matter. This pattern is consistent both for variation across streams (Peterson et al. 2001, Hall et al. 2002, Martí et al. 2004) and for temporal variation within a stream (Butturini and Sabater 1998). Floods not only suddenly increase discharge, but also dramatically affect stream biota and decrease stream nutrient retention (Martí et al. 1997). Little is currently known about the subsequent recovery of streams from high discharge events.

In this study, we present insights into the combined effects of leaf litter inputs and flooding on in-stream nutrient retention. The aims of this study were a) to examine how autumnal accumulation of leaf litter modifies hydraulic properties of the channel, including transient water storage, b) to evaluate nutrient retention response over this period, and c) to examine its relationship with leaf accumulation and hydraulic properties. The occurrence of a flood just after all trees had completely lost their leaves allowed us to examine flood effects and subsequent responses of both hydraulic properties and in-stream nutrient retention to the abrupt removal of much of the accumulated leaf litter.

2. METHODS

2.1. Study site

The study was conducted in Riera de Santa Fe, a 2nd order tributary of La Tordera stream in the natural protected area of Parc Natural del Montseny in the northeastern Iberian Peninsula (50 km north of Barcelona, Spain; Fig. 1). At the study site (1180 m a.s.l.) the stream drains a 2.15 km^2 catchment dominated by siliceous geology and forested primarily with *Fagus sylvatica* at lower elevations and *Abies alba* at higher elevations. Mean annual precipitation is 1200 mm and occurs mostly during autumn and spring. Precipitation occasionally falls as snow during the coldest months (December and January). Stream flow is permanent, with a mean discharge of 16.2 L s^{-1} (biweekly 2004-2005). During floods, which occur mostly during spring and autumn, stream discharge can increase by more than two orders of magnitude.

We selected a morphologically homogeneous 140 m reach, dominated by pools and riffles and 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 (5%) and sand (1%). The riparian vegetation was well developed and dominated by trees (*Fagus sylvatica*) with some shrubs (*Sambucus nigra*).

2.2. Sampling strategy

Between October-December 2004 we assessed the temporal variation in hydraulic and nutrient retention parameters relative to variation in leaf benthic standing stocks and stream discharge. The leaf fall period began in the first week of October and lasted through November. We sampled benthic litter weekly in October, then at least twice weekly through November until all the trees had lost their leaves. In the first week of December, a major flood washed out 65% of the leaf biomass. To assess the temporal variation in hydraulic and nutrient retention parameters as the flood receded, we then sampled three times a week through December. In total, 21 samplings were completed during the study period (Oct-Dec 2004).

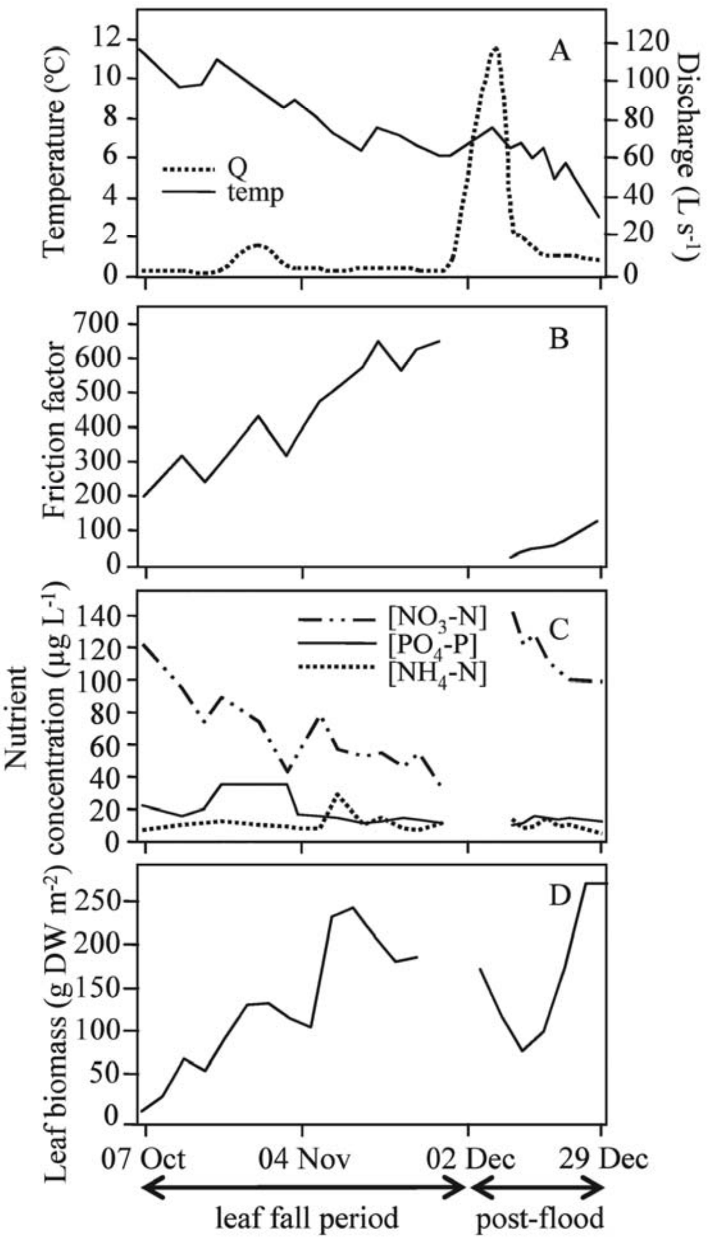

Figure 2. Temporal variation of (A) stream discharge and water temperature, (B) Darcy–Weisbach friction factor, (C) ambient nutrient concentrations, and (D) leaf standing stocks in the stream channel during the study period. The break in the lines corresponds to the onset of flooding.

On each sampling date, we conducted a short-term conservative tracer (Cl^- as NaCl) addition at constant rate to estimate transient water storage parameters. In 13 additions we also added NH_4-N (as NH_4Cl) and PO_4-P (as $Na(H_2PO_4){\cdot}2H_2O$) to estimate nutrient retention metrics. Additions began at approximately 11:00 h and lasted until conductivity reached plateau indicating complete mixing at the bottom of the reach (i.e., 2-3 h). Conductivity was automatically recorded every 5 seconds using a conductivity meter (WTW®, model LF 340) connected to a data logger (Campbell Scientific®) placed at the bottom of the reach. On the dates of nutrient addition, we measured conductivity and collected water samples at eight locations along the reach before the addition and once conductivity reached plateau. Water samples for chemical analyses were collected using 50 mL syringes. At each location, two replicates were collected for ambient concentrations and five for plateau concentrations. Water samples were filtered in the field using Whatman® GF/F fiberglass filters (0.7 µm pore diameter) and refrigerated until analysis. Ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N) and soluble reactive phosphorus (SRP) concentrations were analyzed following standard colorimetric methods (APHA 1998) using Bran+Luebbe® autoanalyzers (TRAACS for NO_3-N, NO_2-N, and SRP, and Technicon for NH_4-N). On average, the nutrient additions increased NH_4-N and SRP concentrations by 14.4±7.6 and 6.8±2.7 fold above ambient concentrations, respectively. Despite this variability in enrichment levels, we did not find a

Table 1. Temporal variation in nutrient uptake length (S_w), uptake length ratio (S_w NH_4-N:S_w PO_4-P), nutrient uptake velocity (V_f), uptake velocity ratio (V_f NH_4-N:V_f PO_4-P), and DIN:P molar ratio during the study period.

	S_w (m)			V_f (mm min^{-1})			DIN:P
Date	PO_4-P	NH_4-N	S_w ratio	PO_4-P	NH_4-N	V_f ratio	molar ratio
Leaf fall period							
07 Oct 04	287.3	60.5	0.21	0.40	1.89	4.75	14.8
21 Oct 04	135.8	51.3	0.38	0.71	1.87	2.65	6.6
04 Nov 04	223.1	44.4	0.20	0.71	3.56	5.03	9.3
11 Nov 04	251.0	55.7	0.22	0.49	2.22	4.51	14.9
26 Nov 04	196.6	51.9	0.26	0.66	2.49	3.79	11.5
30 Nov 04	-	30.0	-	-	5.70	-	10.3
Post-flood period							
13 Dec 04	1312.2	416.4	0.32	0.55	1.73	3.15	45.4
15 Dec 04	825.2	551.5	0.67	0.68	1.01	1.50	32.9
17 Dec 04	665.6	299.9	0.45	0.68	1.50	2.22	24.0
19 Dec 04	585.2	240.8	0.41	0.55	1.34	2.43	24.0
21 Dec 04	429.3	227.6	0.53	0.61	1.15	1.89	22.8
23 Dec 04	287.2	148.6	0.52	0.88	1.70	1.93	21.0
29 Dec 04	202.5	92.5	0.46	1.16	2.54	2.19	22.9

significant relationship between the degree of enrichment and uptake lengths or uptake velocities.

Water temperature at each sampling station was determined several times over the course of each addition and values were averaged. Discharge (Q, L s^{-1}) and average water velocity (u, m s^{-1}) were measured using the time-curve conductivity data recorded at the bottom of the reach. Calculation of u was done by dividing the reach length by the time needed to increase the conductivity to one half of the plateau (i.e., nominal travel time). Calculation of Q was based on a tracer mass balance approach.

Along the reach, we defined 29 channel transects at 5 m intervals. On each sampling date at each transect we measured the channel wetted width (w, m), a cross-sectional profile (every 20 cm) of water depth (h, m), and estimated the percentage coverage by different kinds of substrata. A measure of channel roughness was calculated by solving the Darcy–Weisbach equation:

$$f = (8ghs)/u^2 \qquad (1)$$

where f is the friction factor coefficient, g is the gravitational acceleration (9.8 m s^{-2}), and s (m m^{-1}) is the streambed slope. This coefficient was used as an integrative measure of the temporal changes in both discharge and leaf standing stocks.

Finally, on each sampling date we visually estimated the percentage of stream surface that was covered by leaves and the percentage occupied by pools and riffles. These estimates were made for each stream segment between every consecutive transect. Pools and riffles were categorized according to flow types following River Habitat Survey guidelines (EA, 2003). Estimated percentages for each stream segment were then used to calculate the surface area for each habitat type (i.e., pools or riffles). Leaf standing stock was determined by sampling twenty-four 20 by 20 cm quadrats on each sampling date. Half of the samples were collected in pools and half in riffles to account for differences in leaf accumulation patterns associated with each habitat type. Leaf samples were dried and weighed to determinate dry weight (g DW m^{-2}).

2.3. Estimation of transient water storage parameters

The magnitude of transient water storage zones was quantified using OTIS (One-dimensional Transport model with Inflow and Storage, Runkel 1998) to obtain two parameters: a) the transient water storage zone (A_s, m^2) and b) the water exchange coefficient (k_1, s^{-1}) between the free flowing water and the transient storage zone.

The cross-sectional area of the stream channel (A) was calculated by dividing Q by *u* and was used to obtain the ratio between the cross section of the transient water storage zone and that of the surface stream channel (A_s:A). This was used to estimate k_2 (the water exchange coefficient between the transient storage zone and the free flowing water) as:

$$\frac{A_s}{A} = \frac{k_1}{k_2} \qquad (2)$$

Before applying the OTIS model to the conductivity time-series data, we calculated the Damköhler number (DaI) to evaluate whether the selected reach length (L) was appropriate (Wagner and Harvey 1997). DaI was estimated as:

$$\mathrm{DaI} = \left(\left(k_1 + k_2\right)L\right)/u \qquad (3)$$

Although estimated hydraulic uncertainties are lowest at DaI values ~1.0, values of 0.5 to 5 have been considered acceptable (Hall et al. 2002, Ensign and Doyle 2005). If the reach length is too short (DaI<0.5), not enough water enters the transient storage zone to estimate transient water storage parameters properly. Conversely, DaI values >5 indicate that dispersion dominates the shape of the solute concentration curve.

Relationships between hydraulic parameters (i.e., *w*, *h*, *u*, A_s, A_s:A, k_1, and k_2) and discharge or leaf benthic standing stocks were examined using univariate regression analyses. The level of significance considered was $p<0.05$.

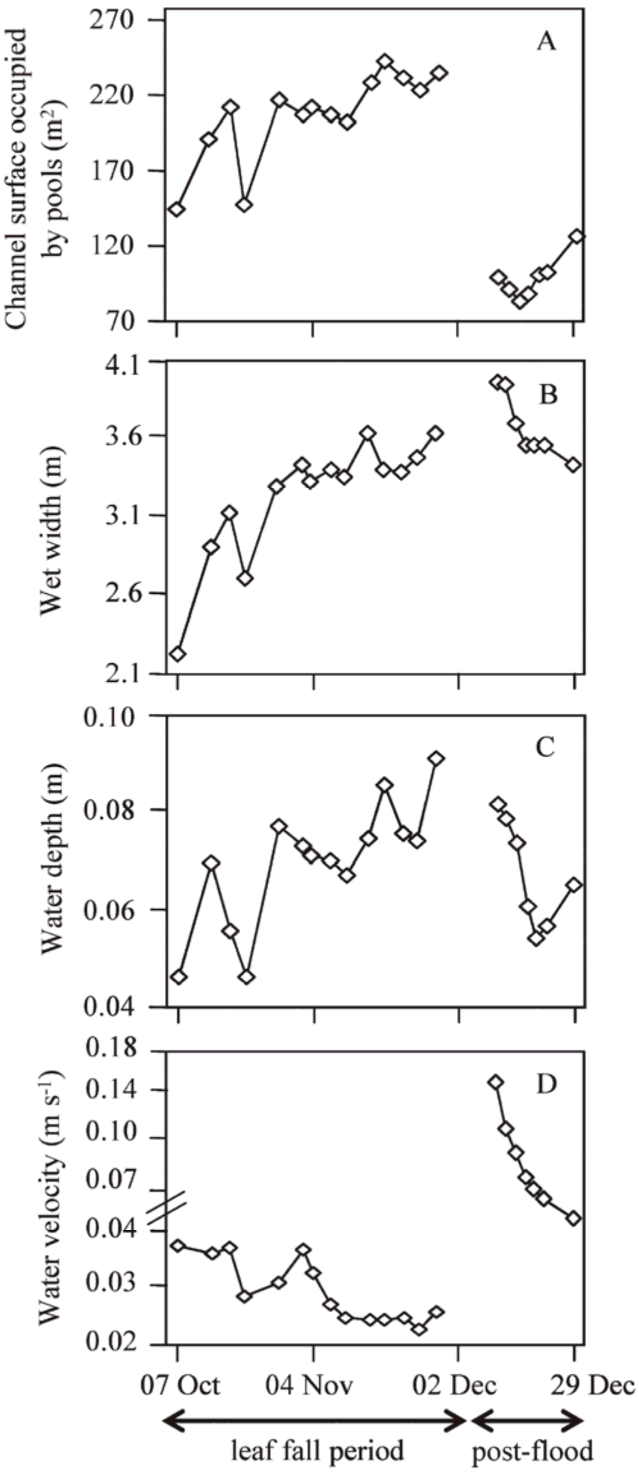

Figure 3. (A) Surface area of the stream channel dominated by pools, (B) average channel wetted width, (C) average water depth, and (D) average water velocity during the study period. The break in the lines corresponds to the onset of flooding.

Table 2. Temporal variation of hydraulic parameters during the study period. Discharge (Q), dispersion (D), cross-sectional area of the transient storage (A_s), transient storage exchange rates (k_1, from channel to transient storage and k_2, from transient storage to main channel), ratio of the cross-sectional area of the transient storage zone to the cross-sectional area of the stream (A_s:A), and Damköhler number (DaI).

Date	Q (L s^{-1})	D ($m^2 s^{-1}$)	A_s (m^2)	k_1x10^{-3} (s^{-1})	k_2x10^{-3} (s^{-1})	A_s:A	DaI
Leaf fall period							
14 Oct 04	6.53	0.07	0.036	0.25	0.88	0.29	3.96
18 Oct 04	8.90	0.08	0.045	0.14	0.61	0.23	2.58
21 Oct 04	6.19	0.09	0.039	0.14	0.54	0.26	2.79
28 Oct 04	20.76	0.14	0.042	0.08	0.54	0.07	2.28
02 Nov 04	9.70	0.08	0.036	0.14	0.82	0.17	3.33
04 Nov 04	8.18	0.10	0.041	0.12	0.56	0.22	2.55
16 Nov 04	8.34	0.08	0.050	0.10	0.43	0.23	2.44
19 Nov 04	8.55	0.07	0.063	0.12	0.42	0.29	2.44
23 Nov 04	7.76	0.08	0.054	0.10	0.39	0.26	2.20
30 Nov 04	6.69	0.07	0.053	0.12	0.40	0.30	2.31
Post-flood period							
13 Dec 04	28.72	0.22	0.022	0.50	4.05	0.12	4.27
15 Dec 04	23.65	0.23	0.029	0.22	1.48	0.15	2.00
17 Dec 04	18.94	0.20	0.032	0.20	1.08	0.18	1.74
19 Dec 04	15.34	0.18	0.033	0.21	1.02	0.21	1.95
21 Dec 04	14.25	0.17	0.030	0.21	1.11	0.19	2.30
23 Dec 04	15.47	0.17	0.041	0.20	0.93	0.22	2.13
29 Dec 04	11.61	0.15	0.043	0.19	0.75	0.25	2.18

2.4. Calculation of nutrient retention metrics

Two nutrient retention metrics were estimated from the short-term nutrient additions at constant rate: nutrient uptake length (S_w, m) and nutrient uptake velocity (V_f, m s^{-1}), also referred to as mass-transfer coefficient (Stream Solute Workshop 1990). S_w, the average distance traveled by a nutrient molecule before being removed from the water column, was calculated based on the variation in tracer nutrient concentrations along the reach at plateau conditions (corrected for ambient levels) and by the variation in conductivity using the following equation:

$$N_x = N_0 e^{-bx} \qquad (4)$$

where N is the nutrient concentration at the first sampling station (N_0) and at the sampling station located x m downstream (N_x), and b is the nutrient retention coefficient (m^{-1}). S_w is the inverse of this coefficient (i.e., S_w= $-b^{-1}$) and is inversely proportional to stream nutrient retention efficiency. Simple linear regression analysis was used to determine the degree of significance of the S_w values (accepted significance level was $p<0.05$). The ratio between S_w for N and P (i.e., S_w-NH_4:S_w-PO_4, Martí and Sabater 1996) was calculated to compare the relative retention efficiency of the 2 nutrients among sampling dates.

We calculated V_f by dividing *u* times *h* by S_w. V_f indicates the rate at which a molecule of a nutrient is removed from the water column, and is a good indicator of the stream nutrient demand across variable flows (Hall et al. 2002).

Relationships between nutrient retention metrics (i.e., S_w and V_f) and hydrological and hydraulic parameters or leaf benthic standing stocks were examined using univariate regression analyses. The level of significance considered was $p<0.05$. All statistical analyses were done using SPSS for Windows (version 12.0, SPSS Inc., Chicago).

3. RESULTS

3.1. Environmental characterization during the study period

Over the leaf fall period (07 October to 02 December), Q remained almost constant at around 8 L s^{-1}. However, just after defoliation was complete on 10 December, a large storm increased Q from 7 to 124 L s^{-1} (Fig. 2A). After the flood, Q receded rapidly, but at the end of the study the stream was still twice its pre-storm discharge. During the study water temperature steadily declined from 12 to 3 ºC (Fig. 2A) and was unaffected by changes in discharge. The values of the friction factor *f* increased 3-fold during the leaf fall period from 205 to 657 (Fig. 2B), dramatically decreased just after the flood, and gradually increased afterwards. By the end of the study, *f* was still 5-fold less than the pre-flood values. Concentrations of SRP and NH_4-N remained low throughout the study (mean ± 1 SE were 14.4±8.2 µg P L^{-1} and 8.3±5.0 µg N L^{-1}, respectively; Fig. 2C). In contrast, NO_3-N concentration decreased from 122 to 30 µg N L^{-1} during leaf fall, increased sharply after the flood (to 252 µg N L^{-1}), and then decreased again. Due to the variation in NO_3-N concentration, the DIN:P molar ratio varied from a mean of 11.3±4.0 before the flood to 45.4 just after the flood, after which it decreased gradually to 22.9 by the end of the study (Table 1). Leaves accumulated in the stream channel over the leaf fall period from 9.3 to 217.5 g DW m^{-2} (Fig. 2D). After the flood, 65% of leaf benthic standing stocks were washed from the reach, decreasing stocks to 77.1 g DW m^{-2}. Two weeks after the disturbance, in-channel leaf standing stocks had recovered to pre-flood levels (Fig. 2D).

3.2. Temporal variation of hydraulic parameters

During leaf fall, the percentage of reach surface area dominated by pools increased from 51 to 67% (141 m^2 to 339 m^2; Fig. 3A). The decrease of leaf standing stocks after the flood coincided with a sharp decrease in the proportion of reach surface area dominated by pools. Once the flood receded, pool area increased again, but by the end of the study was still less than before (Fig. 3A).

Because Q was relatively constant over the leaf fall period, the observed changes in the relative proportion of habitat types resulted in a gradual increase of the average channel *w* and *h* (Figs. 3B and 3C) and a concomitant decrease in average *u* (Fig. 3D). During this period, the variation in these parameters was significantly related to the total biomass (g DW m^{-2}) of leaves accumulated in the stream channel ($w=1.7\text{biomass}^{0.14}$, $R^2=0.87$, $p<0.001$, df=12; $h=0.03\text{biomass}^{0.17}$, $R^2=0.58$, $p<0.005$, df=12; $u=0.04e^{-0.0013\text{biomass}}$, $R^2=0.53$, $p<0.005$, df=12). During the flood mean *w* increased from 3.6 to 3.9 m, mean *h* decreased from 9 to 8 cm, and *u* increased 5-fold. Once the flood receded, all these parameters returned nearly to pre-flood values.

The Damköhler number ranged between 1.7 and 4.3 (Table 2), and therefore hydraulic parameters estimated with the OTIS model were considered reliable. A_s gradually increased over the leaf fall period (Table 2) and positively correlated with leaf benthic standing stocks ($A_s=0.4e^{0.002\text{biomass}}$, $R^2=0.46$, $p<0.050$, df=8). In contrast, k_1 and k_2 slightly decreased during this period and were negatively correlated with leaf benthic standing stocks ($k_1=0.0004-5\text{x}10^{-5}\ln(\text{biomass})$, $R^2=0.59$, $p=0.010$, df=8 and $k_2=0.0008e^{-0.003\text{biomass}}$, $R^2=0.58$, $p<0.010$, df=8). The flood had opposite effects on the hydraulic parameters (Table 2). After the flood, A_s decreased 2.4-fold and k_1 and k_2 increased 4.2 and 10-fold, respectively. During discharge recession, A_s tended to increase and k_1 and k_2 tended to decrease, but at the end of the sampling period A_s had not fully recovered to pre-flood values and k_1 and k_2 were still 2 times greater than pre-flood values.

Combining all dates, A_s:A and A_s were negatively correlated with Q ($A_s\text{:A}=0.27e^{-0.03Q}$, $R^2=0.63$, $p<0.001$, df=15; $A_s=0.05e^{-0.02Q}$, $R^2=0.36$, $p<0.050$, df=15), while k_1 and k_2 were positively correlated to Q ($k_1= 6.4\text{x}10^{-5}+ 9.1\text{x}10^{-6}Q$, $R^2=0.42$, $p=0.005$, df=15; and

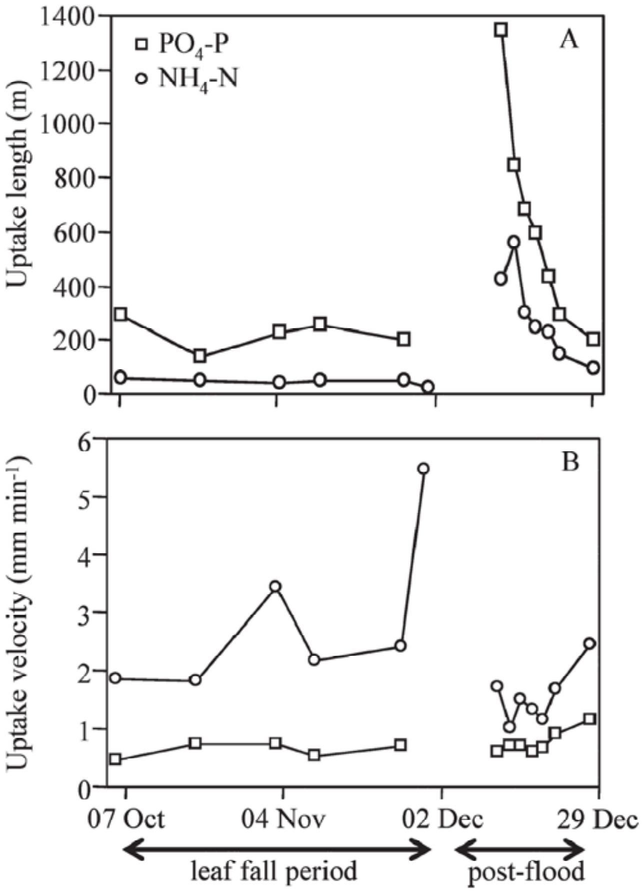

Figure. 4. (A) Temporal variation of uptake length (S_w) and (B) uptake velocity (V_f) over the study period for PO_4-P (squares) and NH_4-N (circles). The abrupt increases in nutrient uptake lengths coincided with the onset of flooding.

$k_2=0.0003e^{0.07Q}$, $R^2=0.69$, $p<0.001$, df=15). No transient water storage parameter was significantly related to litter accumulation when all the sampling dates were combined. Much of the variability in A_s, k_1, and k_2 was explained by the friction factor coefficient, f ($A_s=0.013f^{0.219}$, $R^2=0.74$, $p<0.001$, df=15; $k_1=0.001f^{-0.33}$, $R^2=0.69$, $p<0.001$, df=15; $k_2=0.009f^{-0.48}$, $R^2=0.77$, $p<0.001$, df=15).

3.3. Temporal variation of nutrient retention metrics

During the leaf fall period, S_w did not show a clear temporal trend for either nutrient. On average, S_w-PO_4 and S_w-NH_4 were relatively short (i.e., mean±1 SE were 219±57 m and 49±11 m, respectively). S_w-NH_4 was on average 4 times shorter than S_w-PO_4; thus, the S_w-NH_4:S_w-PO_4 ratio was consistently <1 (Table 1). The flood caused a dramatic increase in S_w for both nutrients (Fig. 4A). This effect was greater for S_w-NH_4 (8.0 times longer than pre-flood values) than for S_w-PO_4 (6.7 times longer than pre-flood values). After the flood, S_w of the two nutrients gradually shortened with stream discharge, almost returning to average pre-flood values by the end of the study. The recovery rate, estimated from post-flood decrease in S_w over time, for S_w-PO_4 (-1.01 d^{-1}) was significantly greater than that for S_w-NH_4 (-0.89 d^{-1}; $p<0.05$, Wilcoxon test for 2 related variables).

Values of V_f-NH_4 were on average 3.0±1.2 times greater than those of V_f-PO_4 (Table 1). V_f tended to gradually increase as leaves accumulated in the channel (Fig. 4B). The flood decreased both V_f-PO_4 and V_f-NH_4 (1.2 times and 1.4 times, respectively). Post-flood, V_f-PO_4 increased gradually, reaching slightly greater values than pre-flood. V_f-NH_4 remained low after the flood, gradually increasing as discharge receded (Fig. 4B). Regression analyses showed that V_f-PO_4 was related to leaf benthic standing stocks and temperature (Table 3). V_f-NH_4 was negatively correlated to DIN concentration and the DIN:P molar ratio (Table 3). No significant relationship was found between V_f and SRP

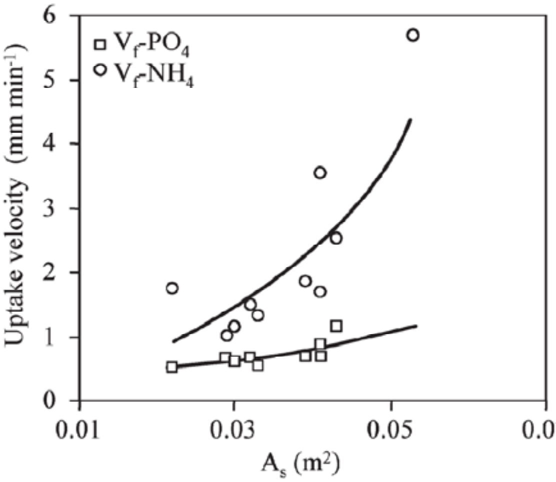

Figure. 5. Relationships between transient storage and nutrient uptake velocities (V_f-$PO_4=0.29e^{25.93As}$, $R^2=0.59$, $p<0.050$, df=7; V_f-$NH_4=0.33e^{48.37As}$, $R^2=0.65$, $p=0.005$, df=8) for PO_4-P (squares) and NH_4-N (circles).

Table 3. Regressions of uptake velocities on environmental variables (leaf biomass as dry weight, water temperature, dissolved inorganic nitrogen concentration (DIN) and DIN:P molar ratio) during the study period. no sign. = no significant relationship.

	V_f -PO_4 (mm min^{-1})	V_f-NH_4 (mm min^{-1})
biomass (g DW m^{-2})	V_f-PO_4=0.42biomass-0.002 R^2=0.55, p<0.010, df=10	no sign.
temperature (ºC)	V_f-PO_4=1.40-0.38ln(temp) R^2=0.47, p<0.050, df=10	no sign.
[DIN] (ppb)	no sign.	V_f-NH_4=14.75-2.73ln[DIN] R^2=0.75, p<0.001, df=11
DIN:P	no sign.	V_f-NH_4=8.82[DIN:P]$^{-0,52}$ R^2=0.37, p<0.050, df=11

concentration. Both V_f-PO_4 and V_f-NH_4 were positively related to A_s (Fig. 5).

4. DISCUSSION

4.1. Influence of leaf litter inputs and the flood on hydraulic parameters

The large quantity of accumulated leaves over the leaf fall period, coinciding with low and stable discharge, affected the relative dominance of habitat type and hydraulic characteristics of the reach. Leaf inputs tended to accumulate at the head of the riffles favoring the formation of small leaf-debris dams, which increased the upstream pool surface area. Shifting proportions of habitat types presumably induced the observed change in hydraulic properties. As leaves accumulated on the stream channel, it became wider and deeper, water velocity decreased, and channel roughness increased. These changes caused a gradual change in transient water storage parameters. The increase in A_s indicates an increased volume of water in transient storage zones, consistent with previous findings on the physical effect of leaf litter accumulation (Hart et al. 1999, Haggard and Storm 2003, Jin and Ward 2005). In addition, the gradual decrease in k_1 and k_2 during the leaf fall period indicates weakened connectivity between the slow and fast flowing hydrologic compartments. Together, these changes in the hydraulic template, mostly driven by litter inputs, increased the water residence time and enhanced the opportunity for nutrient uptake by microbial communities.

Flooding greatly reduced the influence of litter accumulation on channel hydraulics. While changes driven by leaf litter inputs were gradual (weeks), changes due to the flood were abrupt (days). The abrupt increase in discharge significantly increased water velocity, decreased relative transient water storage (i.e., reduced A_s and A_s:A) and increased connectivity between low and high flow hydrologic compartments (i.e., greater k_1 and k_2). These findings are similar to those of Martí et al. (1997) for a Sonoran Desert stream (Arizona, USA). The changes observed in the study stream just after the flood could be the result of both the washout of a high proportion of leaves from the channel, which reduced surface obstructions, as well as an increase in the cross-sectional area (A) of the channel. These changes likely reduced the influence of streambed topography on surface water flow, decreasing the relative importance of A_s. Conversely, following recession, transient water storage parameters returned to pre-flood values faster than discharge. This was probably due to lateral leaf litter inputs from adjacent riparian soil, which created zones of slow moving water in the stream channel. These results suggest that most variation in transient water storage was associated with surface water compartments rather than modification of subchannel flow paths in the hyporheic zone.

Considering all sampling dates, stream discharge accounted for the largest proportion of the variability in the hydraulic parameters. However, when we focus on low flow conditions, our results indicate that leaf standing stocks primarily influenced stream hydraulics. Variation in transient water storage parameters was related to leaf benthic standing stocks only during leaf fall, when discharge was low; however, this relationship was not significant for the whole study period. In contrast, we found a negative relationship between A_s:A and discharge using the complete data set. In a study with similar results, D'Angelo et al. (1993) argued that at high discharge transiently stored water is more quickly incorporated into the main flow, but at low discharge is stored more independently of the main flow. Results from previous studies relating A_s and Q are inconsistent. While no relationship has been found for some streams (e.g., Hart et al. 1999), others agree with the present study (Valett et al. 1996, Martí et al. 1997, and Butturini and Sabater 1999). Similarly, the positive relationship between k_1 and k_2 and discharge found in this study agrees with some previous studies (Hart et al. 1999), but contrasts with others who either found no relationship between exchange coefficient and discharge (Butturini and Sabater 1999, Hall et al. 2002, Jin and Ward 2005) or found an inverse relationship (D'Angelo et al. 1993, Martí et al. 1997).

In order to test if our results obey a general pattern, we compiled data from 17 studies done across several streams worldwide (*n*=187) to see if a significant relation between transient water storage parameters and discharge emerged. Results from this analysis revealed significant relationships between these parameters and streamflow across all the streams considered (Fig. 6) supporting results from our study. Nevertheless, the percentage of variation of transient water storage parameters explained by discharge considering the compiled data was less than that obtained considering only data from the present study, probably reflecting the variable location and nature of the transient water storage zones in each particular stream.

4.2. Combined effects of leaf litter inputs and the flood on nutrient retention

The two nutrient retention metrics (S_w and V_f) helped to determine the relative influence of both leaf inputs and flooding on stream nutrient retention. Leaf litter inputs played an important role in PO_4 and NH_4 retention during stable Q although abrupt flooding and subsequent flood recession largely controlled the overall variability in nutrient retention. To our knowledge, this study is the first of its kind with sufficiently intensive sampling to evaluate stream nutrient retention to gradual and abrupt changes over a short-time scale.

The observed nutrient retention responses (both S_w and V_f) for PO_4 and NH_4 were in line with headwater streams elsewhere (e.g., see Peterson et al. 2001, Hall et al. 2002, Webster et al. 2003). Retention of both nutrients was relatively high during leaf fall. In addition, the S_w-NH_4:S_w-PO_4 ratios, which were consistently <1, indicate greater efficiency in retaining NH_4 than PO_4 regardless of leaf benthic standing stocks or stream discharge. A similar pattern has previously been reported from another stream in the same region (Martí and Sabater, 1996).

In contrast to our expectations, PO_4 and NH_4 retention efficiencies (i.e., S_w) remained relatively constant over the leaf fall period, when discharge was relatively constant and low, despite the clear increase in the additional energy resource from the leaf inputs and in transient water storage size. The lack of relationship between S_w-PO_4 and leaf litter accumulation agrees with D'Angelo et al. (1991), but contrasts with other studies, showing greater P retention efficiency during periods of benthic organic matter accumulation (Mulholland et al. 1985, Haggard and Storm 2003). Differences could be explained in part by the temporal scale of each study. While previous studies were temporally extensive (annual), the present study was temporally intensive (seasonal). Moreover, the relationship between NH_4 retention efficiency and temporal shifts in benthic organic matter has seldom been addressed.

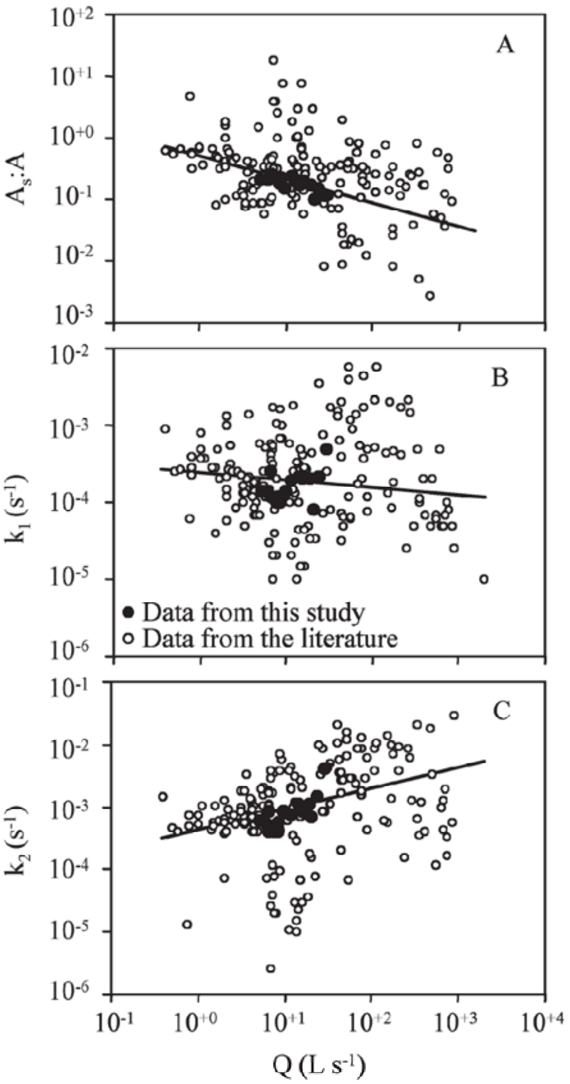

Figure. 6. Relationships between stream discharge and transient storage parameters: (A) transient storage ratio, A_s:A=0.51Q-0.28, R^2=0.14, p<0.001, df=185, (B) exchange ratio from transient storage to main channel, k_1=0.0002$e^{-0.0013Q}$, R^2=0.05, p=0.002, df=175, and (C) exchange ratio from main channel to transient storage, k_2=-0.0003+0.001lnQ, R^2=0.17, p<0.001, df=170. Closed circles are data from the present study and open circles are data from: Bencala and Walters (1983), Bencala et al. (1984), Bencala et al. (1990), Broshears et al. (1993), D'Angelo et al. (1993), Martí et al. (1997), Morrice et al. (1997), Mulholland et al. (1997), Butturini and Sabater (1999), Hart et al. (1999), Hall et al (2002), Haggard and Storm (2003), Harvey et al. (2003), Webster et al. (2003), Ensign and Doyle (2005), Jin and Ward (2005), and Lautz et al. (2006).

Nutrient retention efficiency is affected by both hydromorphologic factors and biogeochemical processes. V_f corrects S_w for depth and velocity (Stream Solute Workshop 1990), and was more sensitive to benthic leaf accumulation and its influence on transient water storage parameters than S_w. The variation in V_f for both nutrients followed the increase in transient water storage (A_s) both before and after the flood. At these two moments transient water storage increased and there was a greater decoupling between fast and slow hydrologic compartments (i.e. reduced k_1 and k_2). These conditions may have favored the interaction between stream communities and available nutrients, and thus increased stream nutrient demand. In addition, because leaf inputs increased the availability of energy-rich substrata, increased nutrient demand could also be directly associated with the development of microbial communities. Ensign and Doyle (2005) similarly observed a greater nutrient demand as superficial transient storage increased after baffles were installed in a channel. Other studies have found no significant relationships between transient storage parameters and V_f (e.g., Hall et al. 2002, Webster et al. 2003). In those studies, however, relationships were compared between different streams; therefore, the lack of relationships could be attributed to differences in the nature of the transient water storage and the associated biogeochemical processes.

The same arguments presented above could also explain the subtle differences in temporal patterns observed between PO_4 and NH_4 uptake velocities. For instance, temporal variation in V_f-NH_4 was influenced by the increase in transient storage volume just like V_f-PO_4, but it was also negatively influenced by the availability of dissolved inorganic nitrogen (mostly in the form of NO_3). Hall et al. (2002) found similar results and suggested that high NO_3 concentration may alleviate microbial demand for NH_4, lowering V_f-NH_4.

The greatest temporal variability in nutrient retention metrics was related to the flood and its subsequent recession. Nutrient retention efficiency of PO_4 and NH_4 was significantly reduced after the flood. The increased discharge would be partly responsible (Martí et al. 1997). The flood also flushed out a high percentage of leaves, removing channel obstructions and exporting part of the microbial community. These effects likely contributed to the decreased post-flood nutrient demand. Therefore, the combination of physical factors (i.e., increase in water depth and velocity, and reduction in transient water storage) and biological consequences (i.e., decrease in nutrient demand) resulted in reduced post-flood retention efficiency. The stream recovered relatively fast from disturbance. Based on the estimated recovery rates, average pre-flood S_w values for PO_4 and NH_4 were reached 22 and 47 days after the flood, respectively. These values are within the range of those reported for S_w-NO_3 in Sycamore Creek (Martí et al. 1997). In that study, the recovery in nutrient retention was attributed to a fast post-flood growth of algal communities. In the present study, S_w recovery could be associated with a significant increase in NH_4 and PO_4 demand as discharge declined and leaves reaccumulated. Return to base flow stream discharge took 64 days, much longer than for S_w. This indicates that biogeochemical processes significantly contributed to the high resiliency (i.e., fast recovery capacity) in nutrient retention, presumably enhanced by the lateral leaf input.

In summary, the seasonal litter input not only modified the physical template of the stream reach, but also increased PO_4 and NH_4 demand, either directly through microbial demand or through increasing transient storage. The flood altered channel hydraulic properties and nutrient retention in the stream. However, once the flood receded, the stream exhibited fast recovery in nutrient retention, especially for PO_4, probably enhanced by the lateral input of riparian leaf litter. Therefore, the net contribution of leaf inputs to headwater stream metabolism over the leaf fall period appears to depend on the timing of floods relative to leaf fall. This has implications in the context of climate change because both the timing of leaf fall and flood regimes are expected to be influenced by climate change (Christensen and Christensen 2004). For example, Peñuelas et al. (2002) have reported that leaf fall has been delayed an average of 13 days relative to 1952 in the study area. Stream hydrologic regimes are also expected to change (McCarthy et al. 2001), with projections for the study region indicating an increase in extreme episodic storms and droughts. These changes may alter the timing of leaf fall and floods, with varying impacts on nutrient retention. Less flooding during the season of leaf fall would increase the residence time of leaves in the stream channel, enhancing nutrient retention. Conversely, an increase in flood frequency during leaf fall would result in leaf litter being periodically flushed from the headwaters and exported downstream to zones of higher flow and lower nutrient retention capacity.

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Temporal Variation of Hydrological Exchange and Hyporheic Biogeochemistry in a Headwater Stream during Fall

Under revision, Journal of the North American Benthological Society

Variació temporal de la connectivitat hidrològica i de la biogeoquímica de la zona hiporreica en un riu de capçalera durant la tardor

Alba Argerich[1], Eugènia Martí[2], Francesc Sabater[1], i Miquel Ribot[2]

[1]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028; [2]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona.

RESUM

Aquest estudi analitza els efectes de l'acumulació de fullaraca durant la tardor i de les variacions de cabal en l'intercanvi d'aigua entre el compartiment superficial i l'hiporreic i les seves possibles conseqüències en la biogeoquímica de la zona hiporreica. Per dur a terme l'estudi, es van instal·lar 15 pous espaiats a intervals de 5 m en un tram de la riera de Santa Fe, dins del Parc Natural del Montseny. Es va mesurar la concentració de nutrients i la saturació d'oxigen (DO) superficials i hiporreics en 23 dates (15 durant el període de caiguda de fulles i 8 després d'una riuada). Els canvis en la connectivitat superfície-hiporreu i en la retenció hiporreica d'amoni (NH_4) i fòsfor (PO_4) es van avaluar mitjançant la coinjecció d'un traçador conservatiu (NaCl) i nutrients.

La variació temporal en la concentració de nutrients i en la DO observada a la zona hiporreica va ser molt similar a l'observada en l'aigua superficial. En comparació amb l'aigua superficial, l'aigua hiporreica contenia menys DO, més PO_4 i nitrat (NO_3), i una concentració similar de NH_4. Pel què fa les diferents zones hiporreiques, els pous d'enfonsament presentaren una major saturació d'oxigen i una major concentració de NH_4 que els pous d'aflorament. L'acumulació de fullaraca disminuí gradualment la connectivitat superfície-hiporreu mentre que la riuada provocà l'efecte contrari. L'augment de la connectivitat va reduir les diferències entre la superfície i el compartiment hiporreic en termes de DO en tots dos tipus de pous i en termes de NO_3 en els pous d'aflorament. La retenció hiporreica de NH_4 i PO_4 va mostrar diferents respostes a aquests canvis. Mentre que la retenció de PO_4 va estar controlada per la concentració hiporreica de PO_4, la qual no es va veure afectada pels canvis en la connectivitat, la retenció de NH_4 va ser afectada indirectament pels canvis en la connectivitat a través de canvis en la disponibilitat de DO. Finalment, encara que no es va injectar NO_3 en el riu, es va observar un augment en la concentració de NO_3 en alguns dels pous fet que assumim a la presència de processos de nitrificació.

Temporal Variation of Hydrological Exchange and Hyporheic Biogeochemistry in a Headwater Stream during Fall

Alba Argerich[1], Eugènia Martí[2], Francesc Sabater[1], and Miquel Ribot[2]

[1]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028, Spain; [2]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona, Spain.

ABSTRACT

The hyporheic zone has become a stream compartment of high interest for stream ecologists since it has been shown its relevance in the stream biogeochemical processing. This study addresses the effects of leaf litter inputs and varying discharge on the surface-hyporheic hydrologic exchange and their possible consequences on the biogeochemistry of the hyporheic zone. The study was conducted during fall in a stream with a well developed deciduous riparian canopy and where we placed 15 wells spaced at 5 m intervals. We measured surface and hyporheic nutrient and dissolved oxygen concentrations (DO) on 23 sampling dates (15 during the leaf fall period and 8 after a flood). Changes in surface-water exchange and on hyporheic ammonium (NH_4-N) and phosphorus (SRP) uptake were assessed through the co-injection of a conservative tracer and nutrients.

Compared to surface water, hyporheic water presented lower DO, higher SRP and nitrate (NO_3-N) concentration, and similar NH_4-N concentration. Among hyporheic water, downwelling wells showed higher DO saturation and higher NH_4-N concentration than at upwelling wells while no significant differences between well types were observed for SRP and NO_3-N concentrations.

Hydrologic connectivity was higher in downwelling wells than in upwelling wells and decreased with leaf litter accumulation in the stream channel and increased with stream discharge. Increased connectivity reduced the differences between surface and hyporheic compartments in terms of DO in upwelling and downwelling wells and in terms of NO_3-N in upwelling wells. N and P uptake showed different responses to these changes. While P uptake was controlled by hyporheic SRP concentration which did not respond to changes in connectivity, N biogeochemistry was indirectly affected by connectivity through changes in DO availability. Finally, although no NO_3-N was injected during the solute additions, we observed an increase in background concentration of NO_3-N in some of the wells during the additions.

1. INTRODUCTION

In temperate streams with well developed riparian forests, massive leaf litter inputs during fall constitute a seasonal event of high ecological relevance for these ecosystems even at annual scale. Leaf litter inputs fuel stream heterotrophic metabolism by providing energy sources to biological communities (Fisher and Likens, 1973; Meyer et al., 1998; Acuña et al., 2004). Higher in-stream uptake efficiency for phosphate during fall has also been associated to the accumulation of leaf litter in the stream channel (Mulholland et al., 1985). In addition, accumulation of leaf litter on the streambed channel can modify the channel hydraulic properties dictated by the geomorphological setting of the stream channel. For instance, previous studies demonstrated that leaf fall inputs increase water transient storage; and thus, the water residence time (Haggard and Storm, 2003; Argerich et al., 2008). This physical effect of leaf litter inputs can have indirect implications on nutrient retention since higher transient storage and residence time is expected to increase the contact between dissolved nutrients and stream microbial communities; thus resulting in higher nutrient uptake as indicated by Argerich et al. (2008). Therefore, in temperate streams, leaf fall can influence nutrient retention through both physical (i.e., increase in water transient storage) and biological (i.e., increase of biological demand) mechanisms. However, massive accumulation of leaves on the streambed may alter the exchange between surface water and the hyporheic zone. This zone is a key stream compartment for biogeochemical nutrient processing (Mulholland and DeAngelis, 2000) and it is also considered to be a major component of the transient storage zone (Harvey and Wagner, 2000). Therefore, the effects of in-channel accumulation of leaves on the hydrologic exchange between stream surface and hyporheic zone may have implications not only for nutrient biogeochemistry within the hyporheic zone, but also for whole-reach nutrient uptake. This latter caused in part to a shift in the relative dominance of different stream compartments contributing to whole-reach water transient storage (e.g., in-channel vs. hyporheic transient storage). However, the effects of leaf litter inputs on the surface-hyporheic hydrologic exchange have been seldom studied. In general, existing studies examining changes in hydraulic parameters in relation to leaf litter accumulation (Haggard and Storm, 2003; Argerich et al., 2008) or coarse organic debris (Nakamura and Swanson, 1993; Lautz et al., 2006) have used models that lump surface and hyporheic transient storage zones. Consequently water exchange coefficients reported in those studies do not express exclusively exchange between the surface channel and the hyporheic zone. In the present study we examined the effect of leaf litter accumulation specifically on the vertical hydrological exchange between the surface stream and the hyporheic zone and the possible effects that variation of this connection have on the hyporheic biogeochemistry.

The hyporheic zone has become a stream compartment of high interest for stream ecologists since it has been shown its relevance in the stream biogeochemical processing (Triska et al., 1989, McKnight et al., 2004) and metabolism (Grimm and Fisher, 1984; Mulholland et al., 1997; Fellows et al., 2001). Biogeochemical conditions in the hyporheic zone may differ greatly from the stream surface waters. In general, hyporheic waters are limited in oxygen availability and less subjected to temporal variation in temperature (Anderson, 2005). Additionally, hyporheic sediments are metabolically active and subjected to higher water residence times than surface waters, which allow a major interaction between solutes and biota, thus enhancing nutrient retention and transformation in these zones (Triska et al., 1989). Physical, chemical and biological conditions within the hyporheic zone support a broad range of biogeochemical processes such as nitrification (Jones et al., 1995a) and denitrification (Baker at al., 2000), which ultimately influence nutrient availability in surface water through different pathways of surface-subsurface hydrologic exchange (Fisher et al., 1998). The size of the hyporheic zone, the exchange fluxes with surface water and the water residence time within it are

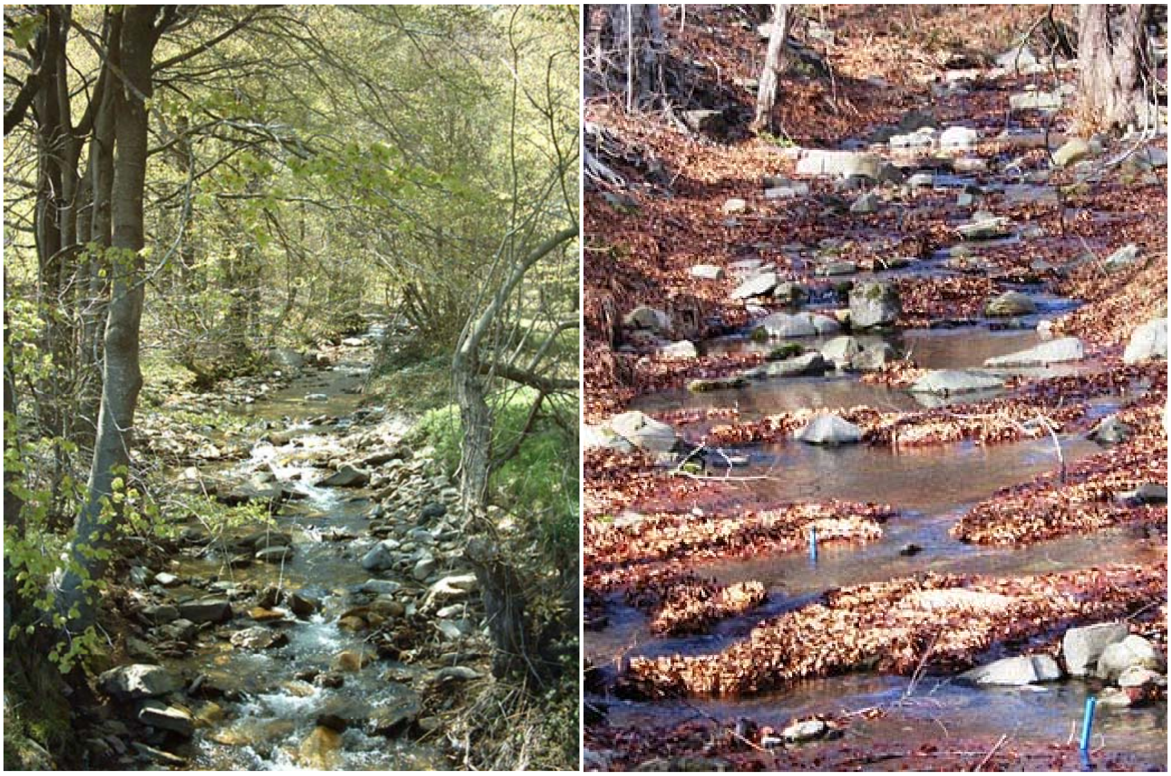

Figure 1. Riera de Santa Fe in late August (left) and in November (right).

determined by bed form configuration (Harvey and Bencala, 1993; Kasahara and Wondzell, 2003), streambed heterogeneity, and stream curvature (Cardenas et al., 2004) among other factors, and are modulated by variation in stream discharge (Tonina et al., 2007).

The surface stream and the hyporheic zone are hydrologically linked at discrete regions of water exchange where hyporheic water discharges vertically to the surface (i.e., upwelling zones) and where surface water reenters the hyporheic zone (downwelling zones). The direction of this linkage (upwelling vs. downwelling) is known to affect nutrient concentrations, biofilm characteristics, and microbial abundances and processes of both surface and subsurface stream habitats (Grimm and Fisher, 1984; Triska et al., 1989, 1990; Hendricks, 1993; Dent et al., 2001; Franken et al., 2001). The strength of these linkages determines the influence that the hyporheic zone exerts on surface stream functioning (Dent et al., 2000) and vice versa. Hyporheic water input can modulate stream metabolic activity through the control on water temperatures over diel and annual cycles (Loheide and Gorelick, 2006). Hyporheic flow has been shown to alter river water alkalinity in a braided gravel bed river (Rodgers et al., 2004). Hyporheic influences on surface biology encompasses effects on microorganisms and microbial processes through changes in nutrient and oxygen availability (Jones et al., 1995b), on the distribution and abundance of primary producers and invertebrates (Pepin and Houer, 2002), and on the distribution and abundance of fish spawning (Baxter and Houer, 2000).

Hydrological exchange between surface and hyporheic zone is known to influence key stream ecosystem services such as nutrient cycling (see review done by Boulton et al., 2010; and Mulholland et al., 2010). Several studies have shown a relationship between surface-hyporheic water exchange and stream nutrient retention through the co-injection of a conservative tracer and nutrients and by comparing plateau concentrations of the two elements between the surface stream and the hyporheic zone. For instance Valett et al. (1996) coinjected Br and NO_3–N into three headwater streams with contrasting geology and hydraulic conductivities and found that NO_3–N uptake increased with increasing surface-hyporheic water exchange. Other studies (Triska et al., 1989, 1990, and 1993; Duff and Triska, 1990) showed that the rate of hydrological exchange determined the hyporheic zone to act as a NO_3-N source (via nitrification) or sink (via denitrification) through the control of NO_3-N supply and redox conditions in the hyporheic zone. Nevertheless, it is less known how these responses vary over time and which are the factors that influence them.

In this study we examined the temporal variation in the exchange of water and solutes between the surface water and the hyporheic zone in a stream with a well developed riparian canopy of deciduous trees during the leaf fall period, which encompassed both baseflow and flood conditions. The specific objectives addressed were a) to examine how the accumulation of leaf litter and the variation in discharge modified surface-hyporheic water exchange, and b) to evaluate possible consequences on the biogeochemistry of the hyporheic zone. We hypothesized that leaf litter accumulated on the streambed will influence the hydrologic exchange and the biogeochemistry of the hyporheic zone. We expected that, during baseflow, surface water-hyporheic hydrologic interaction will decrease as leaves accumulate in the stream channel due to a "clogging" effect, and that this will cause an increase in the chemical differences between the surface stream and the hyporheic zone. Sudden increases in discharge, on the other hand, would reduce the amount of leaf litter accumulated in the stream channel; and thus, we expected that surface-hyporheic water exchange would increase and chemical conditions in the surface stream and the hyporheic zone would become more similar.

2. METHODS

2.1. Study site and sampling strategy

The study was conducted in Riera de Santa Fe, a 2nd order tributary of La Tordera stream located in the natural preserved area of Parc Natural del Montseny in the North East of the Iberian Peninsula (41º 46′ 34″ N, 2º 27′ 40″ E). The selected reach drains a 2.15 km^2 catchment, which is dominated by siliceous geology. The catchment is mostly forested and dominant tree species are European beech (*Fagus sylvatica*) and European Silver Fir (*Abies alba*), which is restricted to the higher elevations. Mean annual precipitation is 1200 mm and most of it occurs during autumn and spring; during coldest months (December, January) snow precipitation is not rare. Stream flow is permanent, with a median discharge of 14.9 Ls^{-1} (value obtained from biweekly data for 2004-2007). However during floods (mainly in autumn) stream discharge can increase more than 2 orders of magnitude.

For this study, we selected a morphologically homogeneous 70 m reach (Fig. 1) characterized by an alternation of pools and riffles, with a slope of 0.072 m/m. The reach substrata type is basically composed of cobbles (47.3%), boulders (25.4%) and pebbles (21.1%) with patches of gravel (5.2%) and sand (1%). The tree canopy along this reach is well developed and is dominated by European beech (*Fagus sylvatica*) with some stems of elder bush (*Sambucus nigra*). Along this reach, we defined 5 surface sampling sites which were evenly distributed. Additionally, we defined 15 channel transects located at 5 m intervals along the reach. In each transect we placed a well at the thalweg of the stream channel. Wells consisted of 2 cm internal diameter PVC pipe with the last 15 cm perforated and introduced in the sediment at 25 cm depth. All wells remained capped between sampling dates. The study was done during the leaf fall period of 2004, which began the first week of October and

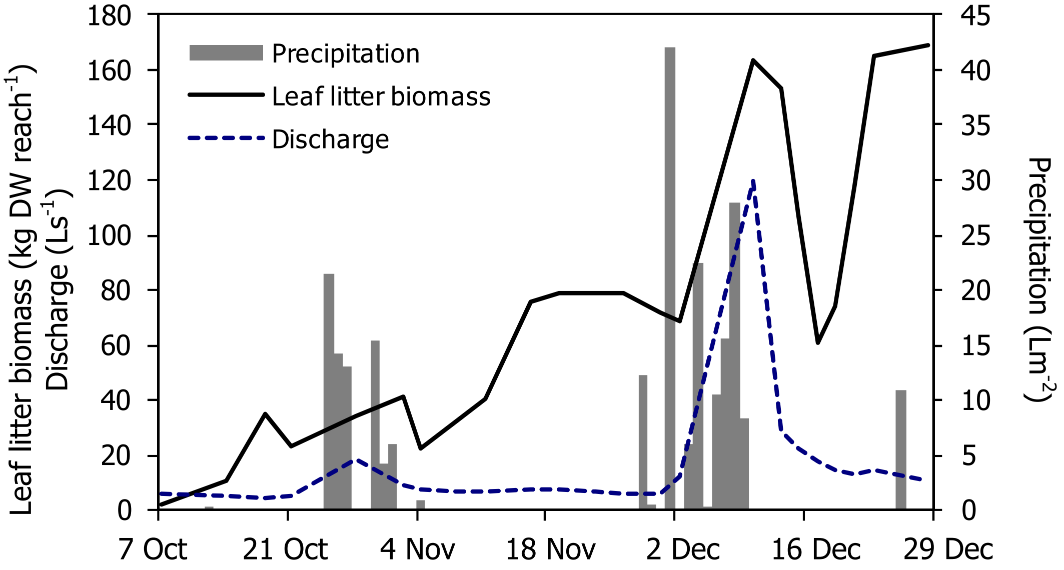

Figure 2. Temporal variation of stream discharge (discontinuous line), standing stocks of leaf litter accumulated in the study reach (black line), and precipitation (grey bars) registered during the study period (from October to December 2004).

lasted until the end of November. During this period we conducted 15 samplings. In the first week of December, just when all the trees had lost their leaves, a major flood occurred which washed out 65 % of the leaf biomass accumulated in the reach. This hydrologic event allowed expanding our initial objectives by examining the effects of a flood on surface-hyporheic water exchange; therefore, we expanded the sampling period until the end of December and intensified the sampling frequency to three times a week to examine the behavior of the stream after the flood. In total, 23 samplings were completed during the study period (Oct-Dec 2004).

2.2. Field sampling

Along the reach, we defined 15 channel transects at 5 m intervals. On each sampling date we measured the channel wetted width (*w*, m) at each transect to calculate the surface area of the reach. Additionally, we visually estimated the percentage of the area left between transects covered by leaves and the percentage occupied by pools and riffles categorized according to flow types following River Habitat Survey guidelines (EA, 2003). Estimated percentages were then used to calculate the total surface area of the reach associated with each habitat type (i.e., pools or riffles) and leave coverage. Leaf standing stock in the reach was determined from twelve 20x20 cm^2 randomly selected samples. Half of the samples were collected in pools and half in riffles to account for differences in leaf accumulation patterns associated with each habitat type. Leaf samples were dried at 60 ºC and weighed to determinate dry weight per unit area (g DW m^{-2}), which was then scaled to kg DW $reach^{-1}$.

On 12 of the 23 sampling dates we injected to the stream a NaCl solution to estimate surface-hyporheic water exchange. On 8 of this 12 injections we also added NH_4-N (as $ClNH_4$), and PO_4-P (as $Na(H_2PO_4)\cdot 2H_2O$), to estimate nutrient uptake in the hyporheic zone. Additions began at approximately 11:00 h and lasted until conductivity reached plateau indicating complete mixing at the bottom of the reach (i.e., 2-4 h). Conductivity was

Table 1. Statistical results of the mixed model analysis of variance to test for differences in dissolved oxygen (DO) saturation and concentrations of SRP, NH_4-N, and NO_3-N between surface and hyporheic water (i.e., stream compartment factor), between the leaf fall and the post-flood periods (i.e., period factor), and for the interaction effect between the two factors.

Dependent variable	Factor	d.f.		F-value	p-value	
		Numerator	Denominator			
DO saturation	Stream compartment	1	37.7	61.7	0.00	**
	Period	1	151.1	2.7	0.10	
	Stream compartment x period	1	151.1	0.0	0.88	
SRP concentration	Stream compartment	1	71.1	45.7	0.00	**
	Period	1	79.3	90.7	0.00	**
	Stream compartment x period	1	79.3	0.7	0.39	
NH_4-N concentration	Stream compartment	1	82.6	2.3	0.13	
	Period	1	91.5	0.2	0.68	
	Stream compartment x period	1	91.5	0.1	0.71	
NO_3-N concentration	Stream compartment	1	65.4	93.8	0.00	**
	Period	1	66.6	394.8	0.00	**
	Stream compartment x period	1	66.6	2.9	0.94	

**denotes significant differences ($p<0.01$)

automatically recorded at the bottom of the reach every 5 seconds using a conductivity meter (WTW, model LF 340) connected to a data logger (Campbell Scientific) to calculate discharge (Q, L s^{-1}) and average water velocity (u, m s^{-1}). Calculation of Q was based on a tracer mass balance approach (Shaw, 1994) and calculation of u was done by dividing the reach length by the time needed to increase the conductivity one half of the plateau (i.e., nominal travel time).

Hyporheic water samples at wells and surface water samples at the 5 surface sampling points were collected on each sampling date for the analysis of nutrient concentrations and chloride. On the dates when we conducted additions, samples were taken just before the addition began (i.e., background concentrations) and when surface water reached plateau conditions at the bottom of the reach. At each point, 3 replicates were collected for background concentrations and 5 for plateau concentrations. All water samples were collected using 50 mL syringes, immediately filtered using Whatman® GF/F fiberglass filters (0.7 µm pore diameter), and refrigerated until analysis. Ammonium (NH_4-N), nitrate (NO_3-N), and soluble reactive phosphorus (SRP) concentrations were analyzed following standard colorimetric methods (APHA, 1998) using a Bran+Luebbe TRAACS 2000 autoanalyzer for NO_3-N, NO_2-N, and SRP, and a Skalar San$^+$ Autoanalyzer for NH_4-N. Chloride concentration was analyzed using an ion chromatograph (Metrohm Compact 761, column 6.1006.520 Metrosep A Supp 5). On 18 sampling dates, dissolved oxygen (DO) concentration and temperature were also recorded at each well and at the adjacent surface water location with a WTW (Weilheim, Germany) 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.

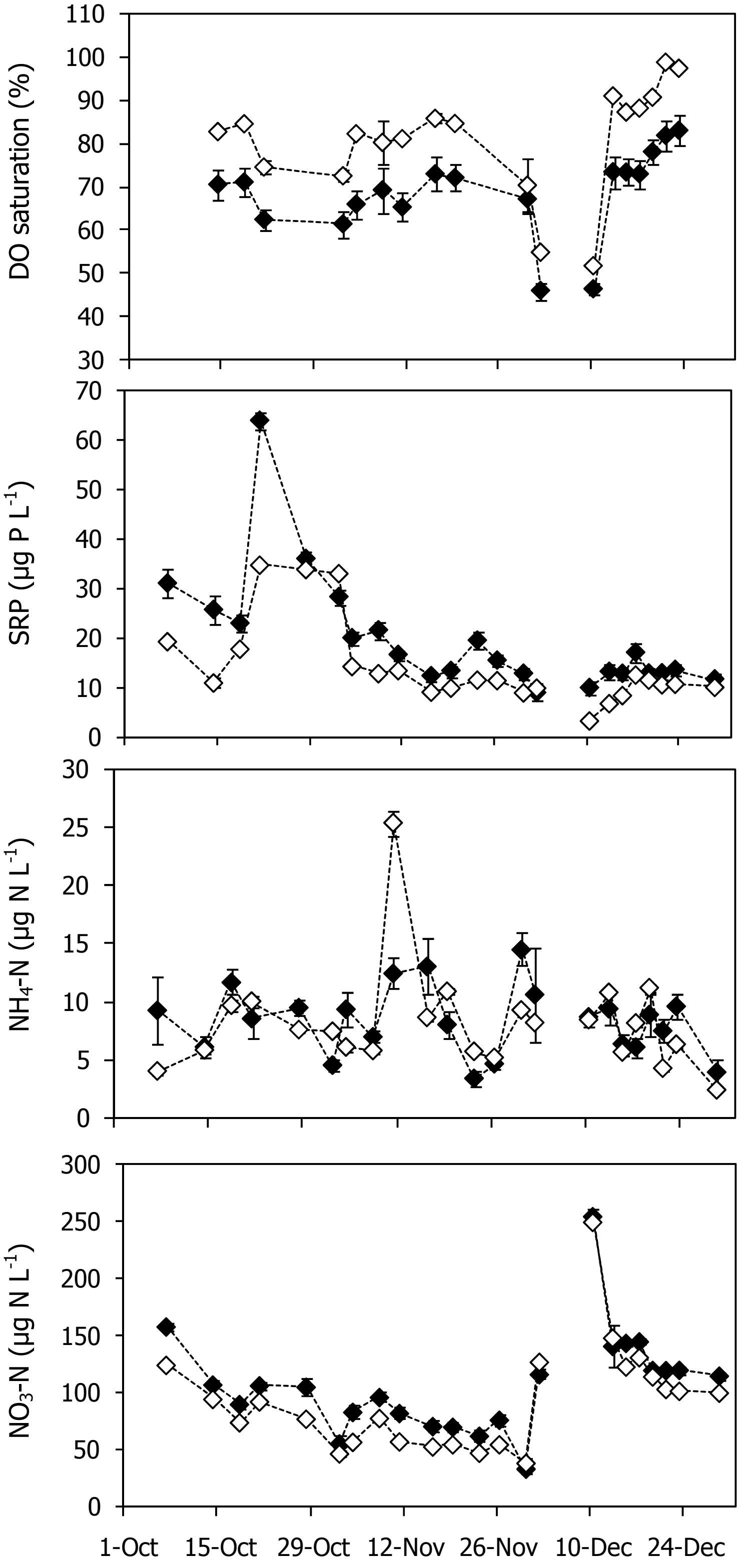

Figure 3. Temporal variation of (A) oxygen saturation, (B) SRP concentration, (C) NH_4–N concentration, and (D) NO_3–N concentration in stream surface water (open diamonds) and in subsurface water (closed diamonds) during the study period (from Oct. to Dec. 2004). Surface values are the average of data from 5 sampling stations along the reach. Subsurface values are the average of data from samples collected at 15 wells along the reach. Error bars represent ± 1SEM. The break between continuous lines indicates the time when the flood occurred.

2.3. Characterization of surface-hyporheic exchange

The direction of water exchange between surface and the hyporheic zone is assessed at each well by measuring the vertical hydraulic gradient (VHG). VHG was calculated following Dahm and Valett (1996), specifically, $VHG = \Delta h / \Delta l$ where Δh is the difference measured between the hydraulic head inside at outside the well (the hydraulic head differential, cm) and Δl is the depth from the substratum surface to the first opening in the piezometer sidewall (the elevation head differential, cm). Thus, VHG is a unit less measure that is positive under upwelling conditions (i.e., groundwater discharge) and negative under downwelling conditions (i.e., groundwater recharge). Two measures of VHG have been done, one during the leaf fall period (on 11 Nov) and the other 1 month after finishing the study (on 3 Feb). On November, 7 wells were placed in upwelling areas, 7 in downwelling areas, and 1 showed a neutral value of VHG. On February, 9 of the wells were in upwelling areas, 5 in downwelling areas and 1 showed a neutral value of VHG. For data analysis comparing upwelling and downwelling zones we only consider the wells that presented a clear upwelling or downwelling tendency in both dates: 5 in upwelling and 4 in downwelling areas.

The magnitude of water exchange between surface water and the hyporheic zone, referred hereafter as *connectivity*, is measured for each well and for each sampling date by calculating the percentage of surface water that exchanges with water in the well. Connectivity is measured using data from the chloride tracer additions by comparing the increase in chloride concentration between background and plateau conditions in wells with increases in chloride in the adjacent surface sampling point following the equation proposed by Wondzell (2006):

$$\text{connectivity} = \left(\frac{Cl^{h}_{plat} - Cl^{h}_{ini}}{Cl^{s}_{plat} - Cl^{s}_{ini}} \right) \times 100 \qquad (1)$$

Where Cl means chloride concentration, subscript *plat* and *ini* indicate concentrations at plateau and at background, respectively, and *h* and *s* indicate water in the well and in the surface stream, respectively.

2.4. Characterization of hyporheic biogeochemistry

The effects of leaf litter accumulation and variation in discharge on the hyporheic biogeochemistry were assessed using two approaches. First we examined changes in the similarities and differences between surface and hyporheic compartments by calculating the ratio between surface and hyporheic nutrient concentrations and DO saturation. Values lower than 1 indicate that surface water is nutrient/DO enriched compared to hyporheic water, values higher than 1 indicate that hyporheic water is nutrient/DO enriched compared to surface water Second, we examined variations in apparent nutrient gain or loss (hereafter referred to as nutrient regeneration and nutrient uptake, respectively) based on data from nutrient and chloride additions. Nutrient uptake is calculated at each well on 9 sampling dates (4 dates corresponding to the leaf fall period and 5 dates on the post-flood period). Calculations are made through the comparison of observed nutrient concentrations at wells when surface plateau was achieved to those predicted assuming hydrological exchange only (i.e., assuming absence of either biotic uptake or regeneration in the hyporheic zone).

Predicted nutrient concentrations are calculated using the following equation:

$$C^{h}_{pred,\,plat} = \left(C^{s}_{obs,\,plat} - C^{s}_{obs,\,ini} \right) \times \left(\frac{connectivity}{100} \right) + C^{h}_{obs,\,ini} \qquad (2)$$

where *C* is nutrient concentration, superscripts *h* and *s* refer to water in the well and in the surface stream, respectively; subscripts *pred* and *obs* refer to predicted and observed values, respectively; subscript *plat* refers to surface plateau conditions and subscript *ini* refers to background conditions;

Table 2. Statistical results of the mixed model analysis of variance to test for differences in dissolved oxygen (DO) saturation, nutrient concentrations, connectivity, nutrient uptake, and increase in hyporheic NO_3-N between background and plateau conditions between upwelling wells and downwelling wells (i.e., well type factor), between the leaf fall and the post-flood periods (i.e., period factor), and for the interaction effect between the two factors.

Dependent variable	Factor	d.f.		F-value	p-value	
		Numerator	Denominator			
Hyporheic DO	Well type	1	18.9	10.12	0.00	**
	Period	1	99.2	1.83	0.18	
	Well type x period	1	99.2	0.13	0.72	
Hyporheic SRP	Well type	1	30.5	0.56	0.46	
	Period	1	35.1	17.87	0.00	**
	Well type x period	1	35.1	0.01	0.93	
Hyporheic NH_4-N	Well type	1	36.0	10.12	0.00	**
	Period	1	39.6	0.01	0.93	
	Well type x period	1	39.6	0.98	0.33	
Hyporheic NO_3-N	Well type	1	40.4	0.51	0.48	
	Period	1	41.6	120.18	0.00	**
	Well type x period	1	41.6	1.97	0.17	
Connectivity	Well type	1	17.4	0.19	0.67	
	Period	1	86.4	19.59	0.00	**
	Well type x period	1	86.4	6.38	0.01	*
SRP uptake	Well type	1	10.9	0.14	0.71	
	Period	1	14.2	5.32	0.04	*
	Well type x period	1	14.2	0.18	0.67	
NH_4-N uptake	Well type	1	12.4	0.85	0.37	
	Period	1	17.3	1.79	0.20	
	Well type x period	1	17.3	0.21	0.62	
Increase in hyporheic NO_3-N	Well type	1	16.0	5.49	0.03	*
	Period	1	16.3	4.62	0.04	*
	Well type x period	1	16.3	2.30	0.15	

*denotes significant relations at $p<0.05$ and **denotes significant relations at $p<0.01$

and connectivity is based on chloride concentration using equation 1. Hyporheic nutrient retention is calculated as the ratio between observed plateau nutrient concentrations and predicted plateau nutrient concentrations for each well. Values <1 indicate nutrient uptake while values >1 indicate nutrient regeneration.

2.5. Statistical analysis

The effect of leaf accumulation and discharge on surface-hyporheic exchange and the implications for hyporheic biogeochemistry are examined separately for wells showing upwelling and downwelling hydrological exchange. Since we only had a measure per sampling date of discharge and leaf litter biomass accumulated into the reach, the relationship between connectivity and leaf litter biomass and discharge is assessed using a linear regression approach of the average values per sampling date.

The existence of significant differences in nutrient and DO concentrations between surface and hyporheic water bodies for upwelling and downwelling wells during the leaf fall period and the post-flood period is tested using a mixed model analysis of variance where "sampling dates" and "wells" are considered random effect factors and "water compartment" (i.e. surface or hyporheic water) or "type of well" (i.e., upwelling or downwelling), and "period" (i.e., leaf fall period or post-flood period) are considered fixed effect factors.

The influence of connectivity on surface-hyporheic differences in DO and nutrient concentration, and the influence of connectivity and hyporheic nutrient concentrations on nutrient uptake is assessed through a linear regression analysis using the data from each well on each sampling date that connectivity and the dependent variables were measured simultaneously (12 sampling dates for nutrient concentrations, 8 sampling dates for DO measurements).

To meet assumptions of normality nutrient concentrations and DO data have been log-transformed, and connectivity and nutrient uptake have been angular-transformed prior to statistical analysis. Statistical analyses were performed using SPSS for Windows (version 17.0, SPSS Inc., Chicago, USA) and levels of significance were set at $\alpha=0.05$ for all statistical tests.

3. RESULTS

3.1. Temporal variation of physical and chemical parameters in surface and hyporheic water

During the entire study period, surface water temperature steadily declined from 12 ºC to 3 ºC. Over the leaf fall period (7 Oct to 2 Dec), leaves gradually accumulated in the stream channel (from 4.0 to 163.4 kg DW reach^{-1}), the wetted area of the reach gradually increased (from 276 m^{2} to 520 m^{2}), and Q remained almost constant around 8 Ls^{-1}, with the exception of a slight increase (18.5 Ls^{-1}) between 25 Oct and 1 Nov due to a small episode of precipitation (Fig. 2). On 10 December, a large storm increased 17.7 times base flow discharge (from 7 to 124 Ls^{-1}, Fig. 2). After the storm, discharge receded rapidly, but at the end of the study it was still twice pre-storm discharge. The wetted area of the reach increased up to 647 m^{2} during the flood and gradually decreased afterwards, measuring 476 m^{2} by the end of the study period. As a consequence of the flood, 62.6% of the leaf benthic standing stock was removed from the reach, decreasing to 61.2 kg DW reach^{-1}. Two weeks after the flood, leaf benthic standing stock in the channel recovered to pre-flood levels.

Average surface DO saturation per sampling date ranged between 70.4 and 85.9% during the leaf fall period, it decreased to 51.8% during the flood, and it gradually increased after the flood reaching the highest values by the end of the study period (Fig. 3A). Average surface DO saturation was negatively correlated with discharge (R=-0.53, n=18, p=0.02) and showed no relationship with leaf litter biomass. Average surface SRP concentration per sampling date ranged between 12.4 and 64.0 µg P L^{-1} during the leaf fall period and tended to decrease with the exception of an increase in concentration between 21 Oct and 2 Nov. SRP concentration decreased after the flood and then gradually increased recovering pre-flood values 5 days after the flood (Fig. 3B). Average surface NH_4-N concentration per sampling date ranged between 3.4 and 14.5 µg N L^{-1} and did not show any clear temporal pattern (mean±SEM was 8.1±0.9 µg N L^{-1}, Fig. 3C). Among the analyzed nutrients, average surface NO_3-N concentration per sampling date showed the largest temporal variation. NO_3-N concentration decreased from 124.6 to 38.8 µg N L^{-1} during leaf fall, increased sharply after the flood (to 249.8 µg N L^{-1}), and decreased again to pre-leaf fall values by the

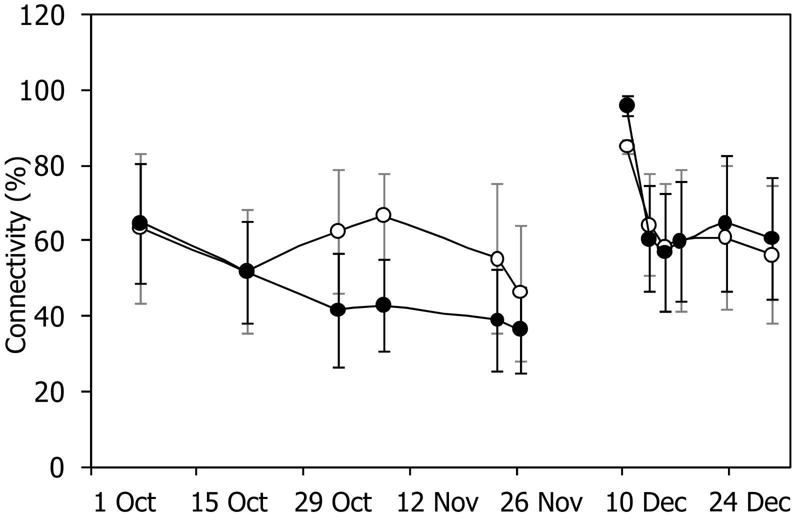

Figure 4. Temporal variation of hydraulic connectivity between surface and hyporheic water, expressed as percentage of surface water found in the wells, during the sampling period (from Oct to Dec 2004). Closed circles indicate data from upwelling wells and open circles indicate data from downwelling wells. Values are the average of data from 5 wells for upwelling and 4 wells for downwelling. Error bars represent ± 1SEM. The break between continuous lines indicates the time when the flood occurred.

end of the study (Fig. 3D). Surface SRP concentration presented a negative correlation with leaf litter biomass (R=-0.56, p<0.01, n=23) and surface NO_3-N concentration a positive correlation to leaf litter biomass (R=0.47, p=0.02, n=23) and to discharge (R=0.83, p=0.00, n=23). No correlations were observed among nutrient concentrations or between NH_4-N concentrations and leaf litter or discharge. Temporal variation in DO and nutrient concentrations observed in surface water was similar to that observed in hyporheic water (Fig. 3A to 3D). Average DO and NO_3-N hyporheic concentrations per sampling date were highly correlated to surface concentrations (R=0.98, p=0.00, n=23 for DO; and R=0.97, p=0.00, n=23 for NO_3-N) while hyporheic NH_4-N and SRP concentrations showed a more moderate correlation with surface values (R=0.52, p<0.01, n=23 for NH_4-N; and R=0.85, p=0.00, n=23 for SRP). Nevertheless, compared to surface water, hyporheic water presented lower DO saturation (67.5±1.1% vs. 82.1±1.0% in surface water), higher SRP concentration (19.7±0.9 µg P L^{-1} vs. 14.3±0.6 µg P L^{-1} in surface water), higher NO_3-N concentration (107.5±3.3 µg N L^{-1} vs. 93.6±3.2 µg N L^{-1} in surface water), and similar NH_4-N concentration (8.4±0.4 vs. 8.1±0.3 in surface water, Table 1). Hyporheic SRP concentration presented a negative correlation with leaf litter biomass (R=-0.58, p=0.00, n=23) and hyporheic NO_3-N concentration a positive correlation with discharge (R=0.80, p=0.00, n=23). No correlations were observed among hyporheic nutrients and DO concentrations or between NH_4-N and DO concentrations and leaf litter or discharge.

Hyporheic water at downwelling wells showed higher DO saturation and higher NH_4-N concentration (70.6±1.5% and 8.9±0.5 µg N L^{-1}, respectively) than at upwelling wells (65.0±1.6% and 7.9±0.5 µg N L^{-1}, respectively); while no significant differences between well types were observed for SRP and NO_3-N concentrations (Table 2).

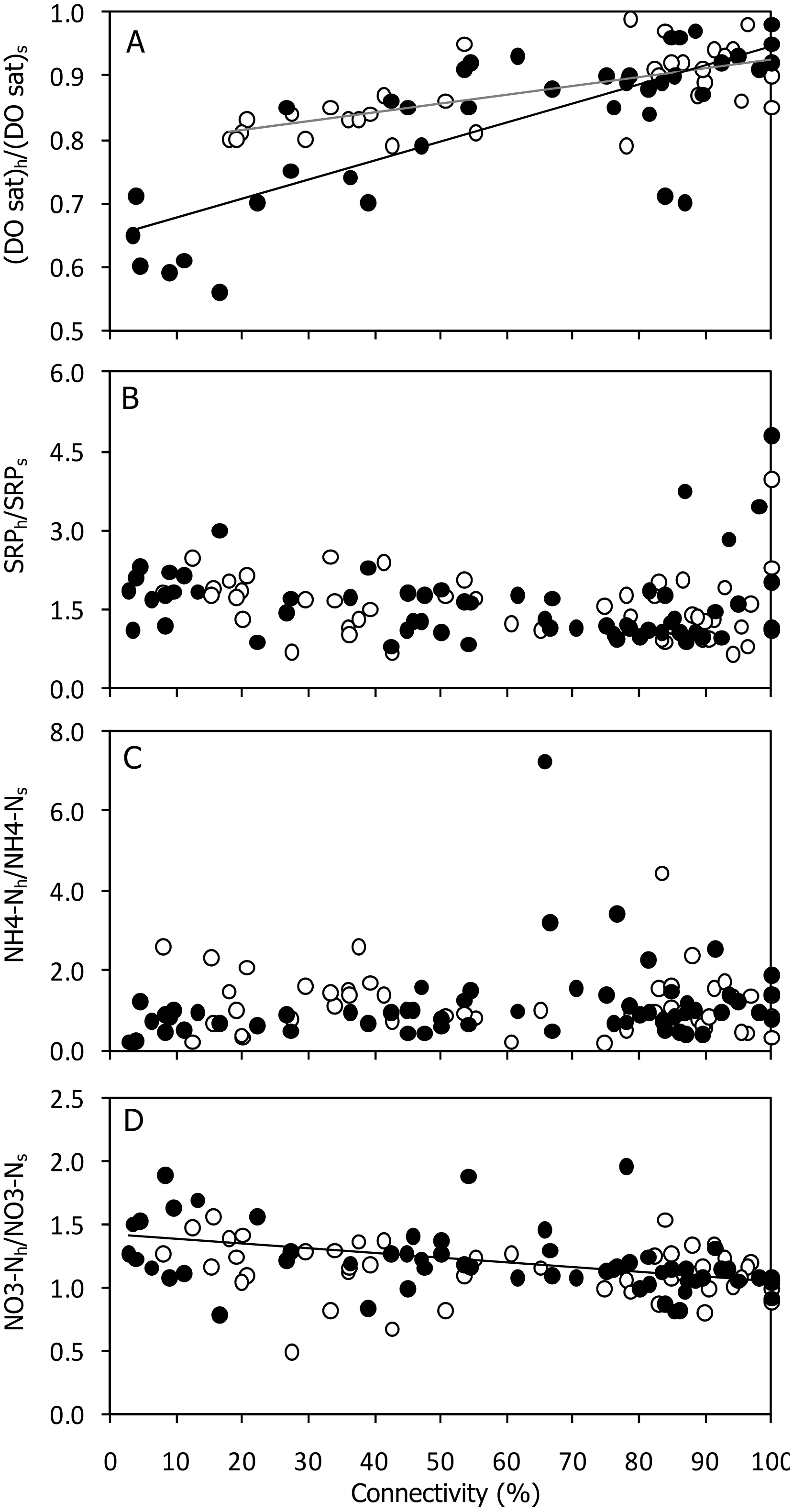

Figure 5. Relationships between hydrologic connectivity and the ratio of hyporheic/surface DO saturation (Fig. A), the ratio of hyporheic/surface SRP concentration (Fig. B), the ratio of hyporheic/surface NH_4-N (Fig. C), and the ratio of hyporheic/surface NO_3-N concentration (Fig. D) for upwelling wells (closed circles) and downwelling wells (open circles). Significant relationships are indicated by solid black lines for upwelling wells and by solid grey lines for downwelling wells. Dots represent well data of all sampling dates that paired measurements were available (n=39 for upwelling wells and n=32 for downwelling wells at plot A; n=59 for upwelling wells and n=48 for downwelling wells at plot B, C and D).

3.2. Effects of leaf litter accumulation and discharge on surface-hyporheic interaction

Chloride from tracer additions reached all wells on all sampling dates during the duration of the injection (2-4 hours) and surface-hyporheic hydrologic connectivity ranged between 2 to 100 %. During the leaf fall period hydrologic connectivity tended to decrease in upwelling wells while in downwelling wells it showed a tendency to decrease during the first two weeks of the experiment, increased after the first episode of precipitation and tended to decrease again until the end of the leaf fall period (Fig. 4). After the flood, connectivity increased by an average of 2.6-fold in upwelling wells and of 1.8-fold in downwelling wells. As flood receded and leaf litter re-accumulated into the channel, connectivity decreased in both upwelling and downwelling wells, but by the end of the study period it was still 1.5-fold higher than before the flood (Fig. 4).

On average, connectivity during the leaf fall period (51.9±3.1%) was lower than after the flood (66.7±3.7%, Table 2). In general, downwelling wells showed higher connectivity than upwelling wells (Fig. 4) although those differences were only significant during the leaf fall period (57.7±3.2% in downwelling wells vs. 46.1±4.3% in upwelling wells; Table 2).

Temporal variability in hydrologic connectivity was related to variability of both discharge and leaf litter accumulation. Connectivity was negatively influenced by the accumulation of leaf litter and positively influenced by increasing discharge in both upwelling wells (R^2=0.65, d.f.=11, p=0.01; Connectivity= 40.9Log(Q)-9.0Log(Leaf litter biomass)+55.3) and downwelling wells (R^2=0.76, d.f.=11, p=0.00; Connectivity=24.7Log(Q)-7.9Log(Leaf litter biomass)+ 71.7).

3.3. Influence of connectivity on hyporheic chemistry

Differences between surface and hyporheic water in terms of DO decreased with increasing connectivity. The ratio between hyporheic and surface DO saturation increased with increasing connectivity in both upwelling (R^2=0.63, n=39, p=0.00) and downwelling wells (R^2=0.46, n=32, p=0.00; Fig. 5A).

In terms of nutrient concentrations, differences in SRP between surface and hyporheic water did not show any relationship with connectivity (Fig. 5B and C), but differences between surface and hyporheic water in terms of NO_3-N concentrations decreased with increasing connectivity in upwelling wells. The ratio between hyporheic and surface NO_3-N concentration in upwelling wells presented values >1 at lower connectivity and tended to values of 1 at higher connectivity (R^2=0.20, n=59, p=0.00; Fig. 5D).

On average, nutrient additions increased hyporheic nutrient background concentrations 2.5±0.2-fold for SRP and 3.7±0.4-fold for NH_4-N although increases in surface concentrations were much higher and variable between sampling dates (7.2±0.3-fold for SRP and 18.1±1.1-fold for NH_4-N). Similar increases in NH_4-N and SRP concentrations were observed in upwelling and downwelling wells (Mixed model analysis, p>0.05). Data from nutrient additions indicate that the hyporheic zone showed SRP and NH_4-N uptake (i.e. values of the ratio between observed and predicted concentrations <1) on all the sampling dates except for 4 wells on 23 Dec that showed values between 1.2 and 1.4 for SRP. However, there were no significant differences in the uptake of the two nutrients between upwelling and downwelling wells (Table 2). On average the observed/predicted ratio for NH_4-N was two times lower than that for SRP, i.e., the hyporheic zone was two-times more effective uptaking NH_4-N than SRP.

SRP uptake was greater during the leaf fall period (SRP_{obs}/SRP_{pred}=0.46±0.05) than during the post-flood period (SRP_{obs}/SRP_{pred}=0.65±0.03, Table 2). Temporal variation in SRP uptake was not significantly related with variation in connectivity or DO, but it increased with increasing hyporheic SRP concentration in both upwelling (R^2=0.19, SRP_{obs}/SRP_{pred}= 0.72·exp(-0.01·SRP_h), p=0.00, n=44)

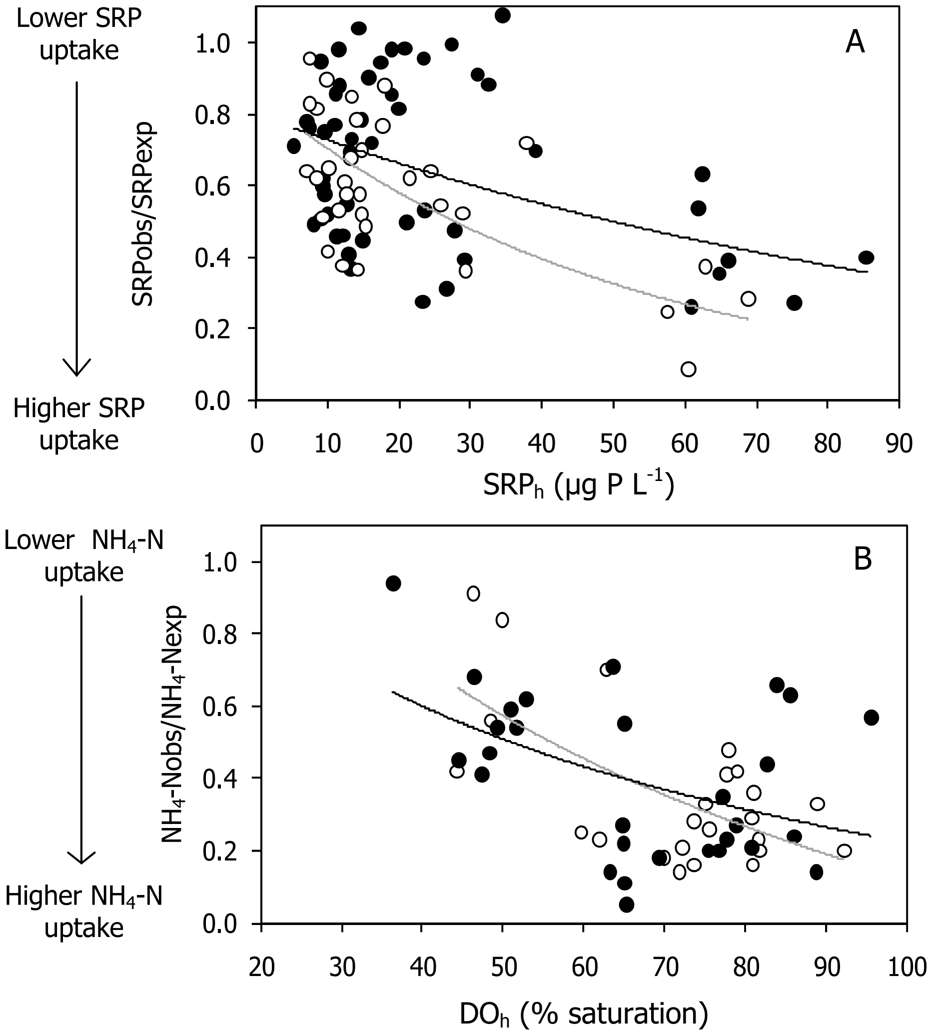

Figure 6. Relationship between (A) SRP uptake (expressed as the ratio between observed and predicted SRP concentration) and hyporheic SRP concentration, and (B) between NH_4-N uptake and hyporheic DO saturation at upwelling wells (closed circles) and at downwelling wells (open circles). Significant relationships are indicated by solid black lines for upwelling wells and solid grey lines for downwelling wells. Dots represent well data of all sampling dates that paired measurements were available (n=44 for upwelling wells and n=36 for downwelling wells at plot A; n=29 for upwelling wells and n=24 for downwelling wells).

and downwelling wells (R^2=0.36, SRP_{obs}/SRP_{pred}= 0.73·exp(-0.02·SRP_h), p=0.00, n=36; Fig. 6A).

NH_4-N uptake was similar between the leaf fall and the post-flood periods (Table 2). Temporal variation in NH_4-N uptake was not significantly related with variation in connectivity, but it increased with higher hyporheic DO saturation in upwelling (R^2=0.21, NH_4-N_{obs}/NH_4-N_{pred}=2.12-0.41Ln(DO_h), p=0.01, n=29) and downwelling wells (R^2=0.42, NH_4-N_{obs}/NH_4-N_{pred}=3.13-0.65Ln(DO_h), p=0.00, n=24; Fig. 6B) and with higher background NO_3-N concentrations in downwelling wells (R^2=0.26, NH_4-N_{obs}/NH_4-N_{pred}=0.51+0.002(NO_3-N_h), p=0.00, n=36).

Finally, although no NO_3-N was injected during the solute additions, we observed an increase in background concentration of NO_3-N in some of the wells during the additions. The increase in NO_3-N concentration was higher in upwelling (25.03±4.37 µg N L^{-1}) than in downwelling wells (17.09±4.21 µg N L^{-1}, Table 2); and was only significant during the post-flood period (35.02±4.61 µg N L^{-1}) and not during the leaf fall period (3.66±1.91 µg N L^{-1}, Table 2). This increase in NO_3-N concentration was not related to any of the independent variables considered, but increases above 25 µg N L^{-1} appeared to be related to hyporheic DO concentrations in upwelling wells ($R^2=0.35$, $\Delta NO_3\text{-}N_h=0.71(DO_h)^{1.1}$, $p<0.01$, $n=19$; Fig. 7A) and to the amount of NH_4-N predicted at wells if no reaction occurred in both upwelling ($R^2=0.36$, $\Delta NO_3\text{-}N_h=40.45 +0.60(NH_4\text{-}N_{pred})$, $p=0.01$, $n=16$) and downwelling wells ($R^2= 0.63$, $\Delta NO_3\text{-}N_h=25.58+0.73(NH_4\text{-}N_{pred})$, $p=0.00$, $n=13$; Fig. 7B).

4. DISCUSSION

4.1. Effects of leaf litter accumulation and discharge on hydrological exchange

Leaf litter inputs during fall in Santa Fe stream modify channel morphology and habitat availability of surface stream, increasing the cross-sectional area of the channel and the number and extension of pools due to the obstruction of the longitudinal flow by the generation of small debris dams (Argerich et al., 2008). Results from this study additionally show that leaf litter inputs can also have an effect on the hyporheic zone of the stream, by reducing the vertical hydrological exchange between surface stream and the hyporheic zone.

During this study, leaf fall inputs coincided with a period of relatively constant base flow discharge, which facilitated the gradual accumulation of leaves in the channel of the study reach. During this period, we found that the percentage of surface water present in the wells tended to decrease as leaf litter accumulated into the stream channel, indicating a decrease in the hydrological connectivity between the surface stream and the hyporheic zone, especially at upwelling locations. Because during the solute injections we sampled at surface plateau conditions and not at hyporheic plateau conditions, the observed decrease could either indicate that less surface water reached the well or that water reached the well more slowly. In any case, these results reflect a weaker hydrologic connection between the surface stream and the hyporheic zone at short-time scales (i.e., scale of few hours) when large amounts of leaves accumulate in the stream channel. However, the leaf litter effect was counterbalanced by the sudden increase in discharge caused by a flood. Under these conditions vertical hydrologic exchange considerably increased (i.e., hydrologic connectivity values up to 85%) both in upwelling and downwelling locations. Because the flood removed a high proportion of the leaves accumulated in the reach, these results reaffirm the relevance of leaf litter inputs on surface-hyporheic hydrological connections during fall. However, the results also indicate that variation in discharge is an important factor influencing hydrological connections. This agrees with results from previous studies. For instance, Butturini and Sabater (1999), found that the percentage of surface water present in wells at a similar depth than those used in this study, varied as a function of discharge from 100% at high discharge to 33-46% at baseflow discharge in a the Riera Major stream (NE Barcelona, Spain). Other studies have found similar effects of discharge when measuring exchange between free flowing water and transient storage at whole-reach scale (Hart et al., 1999), but others reported the opposite effect (Martí et al., 1997). Lack of common effects among studies could be in part explained by the influence of other factors in addition to discharge, such as channel morphology or leaf accumulation as we show in this present study. However assessment of the relevance of the two factors separately is difficult because accumulation of leaves over the study period depends also on stream discharge; and thus, there is a negative interplay between the two factors. This study indicates the relevance of leaf litter inputs as an additional factor to be considered

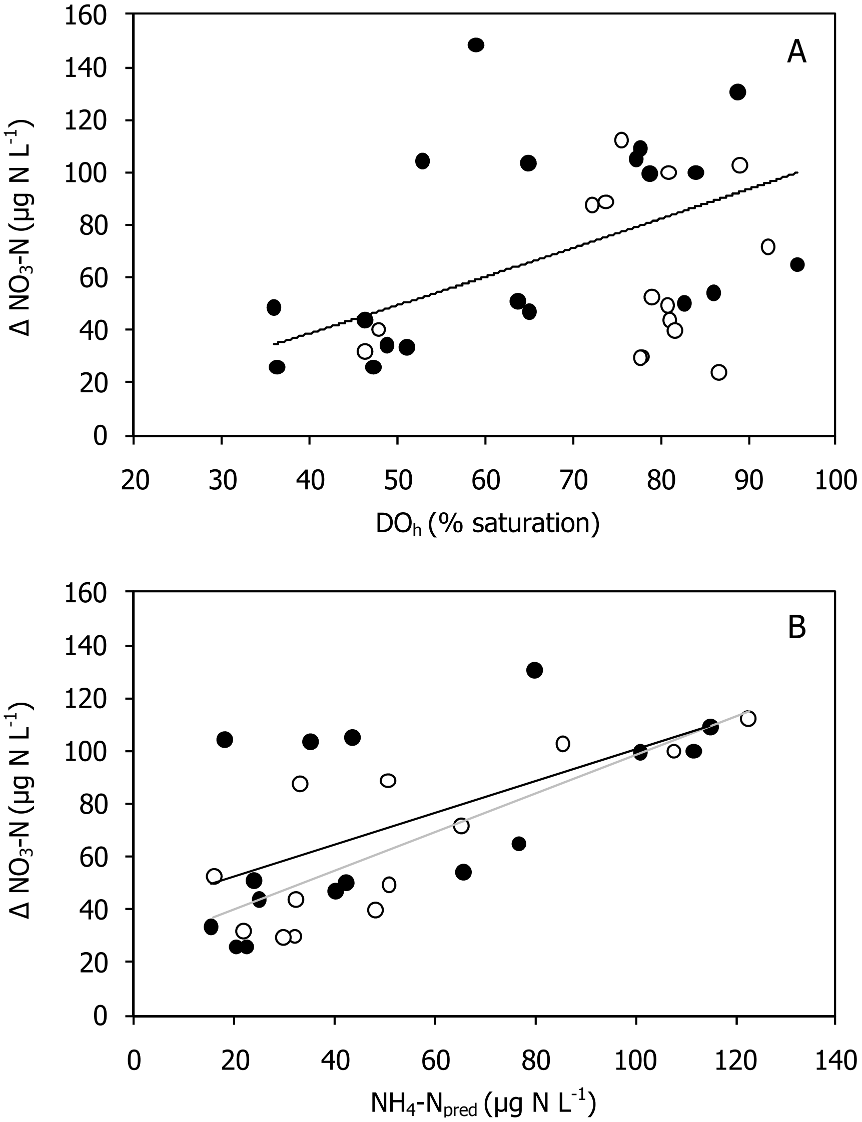

Figure 7. Relationship between increase in hyporheic NO_3-N (measured as the difference between plateau sampling and background sampling) and (A) hyporheic oxygen saturation and (B) predicted hyporheic NH_4-N concentration at plateau in absence of reaction. Closed circles represent paired data for upwelling wells (n=19 in plot A and n=16 in plot B) and open circles represent paired data for downwelling wells (n=14 in plot A and n=13 in plot B). Only increases higher than 25 µg N L^{-1} are considered. Significant relations are expressed as solid black lines for upwelling wells and as solid grey lines for downwelling wells.

to understand solute transport and hydrologic exchange among stream compartments in streams with well forested riparian zones.

In a previous paper (Argerich et al., 2008) we studied the variation in water transient storage size and exchange coefficient in this stream reach during the same study period applying an advection dispersion-transient storage zone model (OTIS; Runkel, 1998) to the surface data. We observed that the coefficients of water exchange between the channel and the transient storage zones decreased during the leaf fall period and increased after the flood. Results from the present study further support these previous findings and provide one of the possible mechanistic explanations of the variation of the exchange coefficient with transient storage observed at whole-reach scale. Taking results from the two studies together, they indicate that the hyporheic zone contributes to some extent to the overall transient storage of the study reach, and thus can be an important compartment influencing whole-reach nutrient uptake.

4.2. Surface and hyporheic biogeochemistry during the study period

Surface SRP and NO_3-N concentrations were within the range reported by von Schiller et al. (2008) in the same reach for a two-year biweekly dataset (2004-2006). NH_4-N concentrations remained very low during all the study period and were in the lower range reported by the same study. Von Schiller et al. (2008) also indicated that surface SRP and NO_3-N concentrations were positively correlated and they were both negatively related with discharge. Results from the present study do not agree with these previous findings. Although the range in nutrient concentrations and discharge was similar to that observed by von Schiller et al. (2008) over two years, temporal variation of NO_3-N and SRP concentrations captured within a single season (i.e., fall) was not correlated and NO_3-N concentration was positively related with discharge. Contrasting results found between the two studies suggests that the relevance of different controlling factors on nutrient concentrations varies at different temporal scales and emphasizes the importance of performing studies at different temporal scales for a full understanding of the biogeochemistry of stream ecosystems.

Temporal variation in DO and nutrient concentrations over the study period was similar between surface and hyporheic water; however, they were chemically distinct. In particular, hyporheic water was depleted in DO concentration and more enriched in nutrient concentrations, especially SRP and NO_3-N, than surface water. Several authors have found similar patterns in different streams (Triska et al., 1989; Valett et al., 1990; Hendricks and White, 1991; Jones et al., 1995) and attributed the differences to distinct biogeochemical processes occurring within each stream compartment. Among these studies, some have included a temporal variation component in the comparison of surface and hyporheic water. For instance, Jones et al. (1995) measured temporal variation in hyporheic chemistry on a monthly basis over 15 months, and Hendricks and White (1991) examined seasonal variation over one year. Our study complements the previous ones by providing information on temporal variability in the hyporheic chemistry and how it compares with the surface stream within a season of high ecological relevance (i.e., fall) for forested headwater streams. It is worth noting that data were obtained at a relatively high frequency (≤ one week) within this season and from wells spaced 5 m apart along the entire reach. Therefore, results provide a very detailed picture of both temporal and spatial variability of hyporheic chemistry. In fact we were also able to capture differences between hyporheic locations of upwelling and downwelling. Concentrations of DO and NH_4-N were higher in downwelling than in upwelling locations, but no differences were found for SRP and NO_3-N concentrations. This indicates that the direction of water exchange, in addition to the strength of this exchange, can contribute to the spatial chemical variability in the hyporheic zone. These results support previous findings observed in other streams (Sycamore Creek, Arizona, Valett et al., 1990; Maple River, Michigan, Hendricks and White, 1995; Speed River, Southern Ontario, Franken et al., 2001; Hunter River, New South Wales, Hancock and Boulton, 2005), which attributed DO differences to the downward flow of oxygenated surface water into the hyporheic zone at downwelling sites. In the case of the difference in NH_4-N concentration, it is possible that high rates of decomposition and mineralization of the large amount of accumulated organic matter during this season may increase the NH_4-N concentration within the leaf packs compared to that of free flowing water. This NH_4-N enriched water exchanges with the hyporheic zone at downwelling locations.

4.3. Effects of varying connectivity on hyporheic biogeochemistry

As indicated in previous studies (Triska et al., 1993b; Findlay, 1995), we found that the supply of dissolved oxygen to the hyporheic compartment was influenced by hydrologic exchange. Assuming that all DO found in hyporheic water has a surface origin; differences between surface and hyporheic DO concentration could be attributable to community respiration and residence time of water in the hyporheic zone. Downwelling wells showed smaller DO differences than upwelling wells, and the DO difference increased with decreasing connectivity, which it was associated with increasing leaf litter accumulated into the channel. This temporal variation in the surface-hyporheic DO difference can be explained by oxygenated water moving slower downward when leaf litter is present causing a longer exposure of water to biota, but also by the occurrence of higher respiration rates in the hyporheic zone enhanced by higher organic matter availability. In fact, probably we are observing the results of the combination of both phenomena since leaf litter inputs are known to be a source of organic carbon that fuels stream metabolism and increase ecosystem respiration rates (Crenshaw et al., 2002; Roberts et al., 2007).

Changes in connectivity did not appear to affect SRP biogeochemistry in the hyporheic zone. In fact, apparent SRP uptake was controlled by hyporheic SRP concentration, which, as introduced above, it was higher in hyporheic than in surface water in agreement with other studies (Grimm and Fisher, 1984; Ford and Naiman, 1989; Valett et al., 1990; Hendricks and White, 1991, 1995; Jones et al., 1995). Hyporheic SRP uptake increased with increasing SRP concentration in the hyporheic zone. Higher SRP concentration was observed at the beginning of the leaf fall period when fresh leaves started to accumulate in the reach. Higher availability of SRP together with the input of fresh organic matter may have enhanced SRP uptake by microbial communities. Mulholland et al. (1985) also found that higher SRP uptake efficiency on an annual scale coincided with higher benthic organic matter in fall, although the sampling frequency of that study did not allow seeing the variability within this season. A similar relationship was found with measurements of whole-reach SRP uptake and surface water SRP concentration in this stream for the same leaf fall period (Argerich et al., 2008). This suggests that similar mechanisms controlling SRP uptake were operating at both whole-reach reach and hyporheic scales. Nevertheless, apparent SRP uptake in the hyporheic zone was two-times lower than that for NH_4-N. This difference in uptake between nutrients is consistent with previous findings in this stream at whole-reach scale (Argerich et al., 2008, von Schiller et al., 2008) and in another Mediterranean stream located close by (Martí and Sabater, 1996; Butturini and Sabater, 1999). This difference may be attributed to the fact that while NH_4-N uptake can be subjected to assimilation, nitrification, and sorption onto sediments or organic substrates, SRP uptake is mostly subjected to assimilation in this stream dominated by siliceous geology.

Connectivity did not appear to directly affect N biogeochemistry in the hyporheic zone, but results indicate an indirect effect through changes in DO availability. Concentration of DO strongly influences nitrogen transformations because of the wide range of available redox conditions for various reactions (Duff and Triska, 2000). In fact, we found that NH_4-N uptake in the hyporheic zone was positively related to DO concentration. NH_4-N uptake in the hyporheic zone could be attributed either to the use of amended NH_4-N as a source of N through assimilation processes or by the aerobic oxidation of NH_4-N as a source of energy through nitrification processes. Although NO_3-N was not used in the solute injections, we observed an increase in hyporheic NO_3-N concentrations during plateau conditions, which suggested that nitrification of injected NH_4-N was taking place in the well-oxygenated hyporheic zone of this stream. This finding is in agreement with previous results in hyporheic zones of different streams. Jones et al. (1995b) presented a conceptual model of hyporheic nitrogen cycling in Sycamore Creek (Arizona) and demonstrated that reduced nitrogen in organic matter can be oxidized to nitrate in subsurface flowpaths. Hyporheic tracer studies at Little Lost Man Creek (Triska et al., 1990), confirmed that NH_4-N dissolved in stream water added directly to an oxygenated hyporheic flowpath could be nitrified based on the increase in NO_3-N concentration (i.e., up to 55 µg N L^{-1}) observed after a 9-day NH_4-N injection. The NH_4-N injections performed in the present study were by far much shorter (few hours), but they resulted in increases in NO_3-N concentration up to 130 µg L^{-1}. Differences in the magnitude of the NO_3-N increases between the two studies can be due to different level of NH_4-N enrichment, but despite this our results indicate a fast and high potential for nitrification in the hyporheic zone of the study stream.

Although NH_4-N enrichment levels from the injections and apparent NH_4-N uptake were similar during all the sampling period, increases in NO_3-N were higher after the flood than during the leaf fall period. This temporal difference could be explained by either a) a lower assimilative demand for NH_4-N by heterotrophic bacteria after the flood, that nitrifiers would take advantage of (Bernhardt et al., 2002), or b) a decrease in denitrification rates due to an increase in DO availability in the hyporheic zone after the flood. We think that the first option is more likely since in this stream, rates of nitrification at whole-reach scale have been measurable, whereas rates of denitrification were almost negligible (von Schiller et al., 2009). Other results from the present provide additional evidences that support the relevance of nitrification as a control of NH_4-N uptake in the hyporheic zone of the study stream during fall. For instance, the relationship found between apparent NH_4-N uptake and background NO_3-N concentrations is consistent with previous findings on the controls on nitrification rates (Bernhardt et al., 2002). In addition, the observed increase in NO_3-N concentration was closely related to the amount of NH_4-N predicted in the wells in absence of transformation (i.e., in the amount of NH_4-N supplied by the addition). A similar relation was also observed by Kemp and Dodds (2002) who found that rates of nitrification in most stream biotic compartments responded very strongly to NH_4-N addition in a suite of experiments.

In summary, leaf litter inputs have an important role in this stream with a well developed riparian canopy, not only introducing organic matter that fuels stream metabolism and shape the morphology of the channel, but also modifying surface-hyporheic water exchange and decreasing the velocity at which surface water arrives to the hyporheic zone. These effects are counterbalanced by the effects of discharge variability, especially under flood conditions which can be common in temperate climates during fall. Increase in discharge leads to an increase in surface-hyporheic water exchange and to a homogenization of upwelling and downwelling wells in terms of connectivity, although this effect can be the result of increasing discharge in itself but also the result of the removal of leaves caused by the flood. Our results also show that together, these hydrologic changes have implications on the temporal variation of hyporheic water chemistry and nutrient uptake. Nevertheless, N and P showed different responses to these changes. While P uptake was controlled by hyporheic SRP concentration which did not respond to changes in connectivity, N biogeochemistry was indirectly affected by connectivity through changes in DO availability.

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Influence of Transient Storage on Stream Nutrient Uptake Based on Substrata Manipulation

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Influència de la zona de retenció temporal d'aigua en la retenció de nutrients, aproximació experimental mitjançant la manipulació de substrats

Alba Argerich[1, 2], Eugènia Martí[3], Francesc Sabater[1], Roy Haggerty[2], i Miquel Ribot[3]

[1]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028; [2]Geosciences Department, 104 Wilkinson Hall, Oregon State University, Corvallis, OR 97331, USA;[3]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona.

RESUM

Les zones de retenció temporal d'aigua (ZRT) incrementen el temps de contacte entre l'aigua i els sediments i per tant, generen oportunitats per a la biota per processar aquests nutrients. No obstant, els estudis publicats en la literatura que relacionen la mida de les ZRT (A_s) i la retenció de nutrients presenten resultats contradictoris. En aquest estudi hipotetitzem que la manca de relacions consistents es deu al fet que, a part de l'A_s, les característiques físiques i biològiques de les estructures que generen aquestes ZRT tenen un rol rellevant en la retenció de nutrients i que poden generar respostes biogeoquímiques diferenciades. En aquest estudi, testem la hipòtesi de manera experimental mitjançant 16 adicions de PO_4, NH_4, i NaCl en 4 trams consecutius d'un canal en els quals es van introduir deflectors construïts amb 3 tipus diferents de substrats (llim, sorra i còdols; tots ells colonitzats biològicament) amb l'objectiu d'augmentar l'A_s. Es va estimar la retenció de NH_4 i PO_4 en el canal principal i en les ZRT fent servir un model de transport de soluts que inclou els processos d'advecció, dispersió, dilució i retenció temporal de l'aigua causada per les ZRT (OTIS-P, Runkel, 1998) i es van comparar els resultats entre trams amb diferents tipus de deflectors. A més, es va fer una injecció de ^{15}N per quantificar l'assimilació biològica dels diferents deflectors. En concret es va quantificar l'assimilació de N en la matèria orgànica bentònica fina (FBOM) i en l'epilíton de cada tipus de deflector.

La introducció dels deflectors va incrementar el temps de residència de l'aigua, va disminuir la velocitat de l'aigua i va incrementar l'A_s de manera homogènia entre tractaments. Tot i que l'increment de A_s no va resultar en un augment de la retenció de nutrients a nivell de tram, els resultats obtinguts en aquest estudi indiquen diferències en el processat de nutrients entre tipus de deflectors, bàsicament associats a la retenció de nutrients que té lloc a les ZRT.

Influence of Transient Storage on Stream Nutrient Uptake Based on Substrata Manipulation

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[1]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028, Spain; [2]Geosciences Department, 104 Wilkinson Hall, Oregon State University, Corvallis, OR 97331, USA;[3]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona, Spain.

ABSTRACT

Quantification of water transient storage (A_s) has become critical in biogeochemical studies of stream ecosystems addressed to examine nutrient retention. It is expected that higher A_s may enhance interaction between nutrients and biota and thus, increase whole-reach nutrient uptake. However, results from literature are controversial. We hypothesize that besides of the size of A_s, the intrinsic physical and biological characteristics of the stream structures that generate it have also a relevant role on whole-reach nutrient uptake. In this study we tested our hypothesis by performing 16 constant rate additions of phosphate, ammonium, and chloride in 4 reaches of a man-made channel where we introduced 3 types of naturally colonized substrata packs (mud, sand and cobbles) to modify A_s. We measured ammonium and phosphate uptake rates in both main channel and transient storage zone using a one-dimensional solute transport model (OTIS-P) and compared the results among reaches with different substrata types. We also performed a ^{15}N addition to characterize the amount of biological uptake in each of the biological compartments (i.e., FBOM and epilithon) of the substrata packs. The introduction of substrata packs increased water residence time, decreased average water velocity, and increased A_s homogeneously among treatments/manipulations. Although increases in transient storage did not result in changes in total uptake of ammonium and phosphate at whole reach scale, our results indicate differences among treatments basically associated with nutrient uptake in the transient storage zone.

1. INTRODUCTION

In recent decades, studies focusing on stream nutrient uptake have gained importance due to global concern about eutrophication of freshwater ecosystems and nutrient delivery to coastal ecosystems (Alexander et al., 2000). Many studies have demonstrated the functional relevance of streams and rivers in modulating the concentrations and the forms of nutrients exported downstream. Up to 50 – 75% of nitrogen (N) load of a watershed can be transformed and retained by in-stream processes (Peterson et al., 2001), and denitrification in streams can account for up to 18% of N loss

(Mulholland et al., 2008). Mulholland (2004) found that 30% of soluble reactive phosphorus (P) annually entering the stream was retained by in-stream processes. Nevertheless, although there is a general understanding of the factors that control variability in stream nutrient uptake response both among streams and within streams over time (Tank et al. 2003; Fellows et al., 2006; Gücker and Pusch, 2006), a detailed understanding of the relation between transient storage zones and nutrient retention is still lacking.

The biogeochemical processing capacity of streams is mostly controlled by biological communities in these ecosystems, but hydrology and physical features of stream channels can modulate the biological response (Battin et al., 2008). Therefore, stream nutrient uptake results from the interaction of hydrologic, chemical, and biological retention (Valett et al., 1996; McClain et al., 2003). Greater hydrologic retention by transient storage is expected to increase nutrient uptake through increases in water residence time and sediment-water interaction that enhance the interaction between nutrients and stream biota. While the influence of stream discharge on nutrient uptake is widely documented the influence of transient water storage on nutrient uptake is less consistent. Several studies have shown that nutrient uptake efficiency decreases as stream discharge increases, with the most plausible explanation being the decrease in the ratio between the stream bioreactive surface and the water volume (Butturini and Sabater, 1998; Peterson et al. 2001; Hall et al., 2002; Martí et al., 2004; Argerich et al., 2008).

Transient storage (Bencala and Walters, 1983) occurs in locations where water moves much slower than the average channel velocity and the residence time is longer than the channel residence time. Surface-to-subsurface water exchange is a major contributor to transient storage; and thus it is usually associated with the hyporheic zone (Triska et al., 1989; Morrice et al., 1997; Butturini and Sabater, 1999). However, surface structures within the channel also contribute to transient storage. For instance, pools, eddies, backwaters and debris dams, but also leaf packs and woody debris, increase water residence time along a stream reach (Lautz et al., 2006; Bukaveckas, 2007; Roberts et al., 2007; Argerich et al., 2008).

It is expected that greater transient storage may enhance interaction between nutrients and microbial communities developed in the different stream habitats and thus, increase whole-reach nutrient uptake. For this reason, quantification of transient storage has become a critical issue in biogeochemical studies of stream ecosystems addressed to examine factors controlling spatial or temporal variation of nutrient uptake. However, results from existing studies on this relationship are controversial. Some studies support the expected relationship between nutrient uptake and transient storage (Valett et al., 1996; Thomas et al., 2003; Ensign and Doyle, 2005; Ryan et al., 2007). Others report only weak relationships (Lautz and Siegel, 2007; Bukaveckas, 2007), or contrasting findings depending on the nutrient considered. For instance, Hall et al. (2002) found a correlation between the relative size of transient storage (A_s/A) and uptake velocity for ammonium (NH_4) but not for phosphate (PO_4). Nonetheless, when we consider studies which compile data from different streams this relation is not as clear. Webster et al. (2003), in a study where they analyzed data collected from 11 streams from different biomes, did not find any relation between transient storage parameters and ammonium uptake. Simon et al. (2005) studied the temporal variation of N and P uptake in two New Zealand streams and concluded that transient storage was not explaining variability in uptake rates. Ensign and Doyle (2006), after analyzing data from 52 published papers found little evidence of a cause-effect relation between transient storage and nutrient uptake. All these studies are mostly based on physical characteristics of the transient storage zones (i.e., A_s/A or water exchange coefficient) to examine relationships with nutrient uptake parameters. However, uptake processes may vary among stream compartments (e.g., pools, algal mats or hyporheic zone) that contribute to transient storage, because communities developed in these compartments are distinct (Fisher et al., 1998). Therefore, the biological differentiation among stream compartments may help explaining the lack of consistent results on the influence of transient storage as a whole on stream nutrient uptake among existing studies. Based on this reasoning, we hypothesize that besides of the size of transient storage zone, the intrinsic physical and biological features of it have also a relevant role on whole-reach nutrient uptake.

The objective of this study was to examine the influence of physical and biological characteristics of stream structures that generate water transient storage on nutrient uptake. To achieve this objective, we experimentally increased the water transient storage of a man-made channel by introducing deflectors, which were made of different naturally-colonized substrata types (mud, sand and cobbles). Deflectors differed in physical (i.e., sediment size and hydraulic conductivity) and biological attributes (i.e., biomass of epilithon and fine benthic organic matter, nitrogen assimilation rate). We measured ammonium and phosphate uptake rates in both main channel and transient storage zone using a one-dimensional solute transport model and compared the results among reaches with different substrata types.

2. METHODS

2.1. Experimental setting

The study was conducted during autumn 2006 in a man-made canal located at the village of Gualba, 60 km north of Barcelona (latitude 41.7332°N, longitude 2.5034°E, northeastern Iberian Peninsula). This canal diverts water from the Santa Fe stream for irrigation. The canal is oriented NW to SE and meanders 2 km parallel to the stream with an average slope of 0.0051. The canal is excavated in the soil, with a width of 61±6 cm (mean±standard deviation) and a water depth of 6.7±1.4 cm during the study period. Along the canal, we selected 4 adjacent straight reaches of 20 m each. Between the bottom of reach 2 and the beginning of reach 3 we left 8 m to avoid a bend in the canal. The natural substratum of the canal was homogeneous and similar among reaches, and it was composed of a 5 – 6 cm layer of sand. Discharge in the canal was regulated by a head gate, which we operated during the experiments to ensure a relatively constant discharge.

To address the objective of this study, we introduced deflectors in the experimental reach. These deflectors measured 25 cm long and 8 cm wide and consisted of nylon mesh bags filled with a single type of substrata. We used 3 substrata types (mud, sand and cobbles) that were introduced in the reaches in discrete packs. Each substrata pack was filled with substrata from the adjacent stream, and thus the substrata packs were naturally colonized. On each sampling date, we placed the substrata packs in three of the selected reaches and the fourth reach was left without any substrata packs to serve as a control. Along each reach, we distributed 40 substrata packs of a single type at 50 cm intervals and with an orientation of 45º relative to the channel margin (Fig.1).

Figure 1. View of the channel with substrata packs.

Each reach contained a different substrata type. The 4 treatments (i.e., control, cobbles, sand, and mud) rotated among reaches on consecutive sampling dates. A total of 6 sampling dates were completed (i.e., n=6 for each treatment). Therefore, each reach was exposed to all the treatments at least once. Substrata packs were removed from the reaches and left in the stream channel between sampling dates. The whole experiment was completed between October 23 and November 29. During this period weather conditions were relatively stable and free of precipitation.

2.2. Characterization of substrata packs

Physical features. Dominant grain size of each type of substrata was measured in the laboratory by sieving the content of 5 packs of the same type. The residence time (T_p, h) of a conservative tracer was measured in each type of substrata pack. We submerged 4 packs of each substrata type in a container with stream water and NaCl for 1 day for cobble and sand packs, and for 1 week for mud packs. We then placed these packs in the canal and we recorded electrical conductivity (EC) upstream and 3 meters downstream of the packs with a conductivity meter (LF 340 WTW, Weilheim, Germany) connected to a data logger (510X Campbell Scientific, Logan, UT, USA). We measured water residence time within the pack as the time needed for the downstream point to reach upstream conductivity values, which indicated that all the interstitial tracer had been released from the packs.

Biological features. Substrata packs were characterized in terms of overall organic matter content, the relative standing stock in the two dominant biotic compartments of the substrata packs (i.e., epilithic biofilm, hereafter epilithon, and fine benthic organic matter - FBOM), the C and N content of these compartments and their N assimilation rate. We used ^{15}N as a tracer to estimate the N assimilation rate of both epilithon and FBOM for each substrata type. A single addition in the canal of a solution of 104.2 mg $^{15}NH_4$ and 104.2 g NaCl mixed in 5 L of ultrapure water, to obtain a water enrichment of 2000‰, was conducted at constant rate during 14 h. The lack of a stable isotope for P prevented us from testing with this element. For this addition we included the 4 reaches together (i.e., the total reach length was 88 m). Substrata packs were arranged as described above; however, substrata types were alternated along the entire reach. The $^{15}NH_4$ addition began at 22:30 and lasted until 12:30 the following day. We collected one 4-L water sample and three substrata packs of each type at 3 locations along the reach (10, 48, and 78 m from the addition point) prior to the $^{15}NH_4$ addition, and at 4 locations (18, 38, 66 and 86 m) just before the addition was stopped. Additional 100 mL water samples were collected at each sampling location for the analysis of nutrient concentrations. Water samples for nutrient concentrations were filtered in the field using Whatman® GF/F fiberglass filters and kept refrigerated until analysis (see details on the section below). The 4-L water samples were processed for ^{15}N content of NH_4^+-N following procedures adapted from Holmes et al. (1998). Samples were filtered through Whatman® GF/F fiberglass filters (0.7 µm pore diameter) and spiked with 300 µL of unlabeled NH_4^+-N to increase NH_4^+-N concentration to the ideal working range for mass-spectrometric measurement. Samples were then amended with 50 g of NaCl, 3.0 g of MgO, and a Teflon filter packet. The filter packet consisted of a precombusted 1-cm glass-fiber filter (Whatman GFD) spiked with 25 µL of 2.5 $molL^{-1}$ $KHSO_4$ and sealed between two pieces of Teflon plumbing tape.. All water samples and substrata packs collected were transported refrigerated to the laboratory for further processing and analysis. Once in the laboratory, we incubated the 4L water samples at 40 ºC for two weeks on a shaker table; we then removed the filter packets and the filters were dried in the oven prior to encapsulation in tins for ^{15}N analysis.

The collected substrata packs were brought to the laboratory on ice and processed to estimate the biomass of FBOM and epilithon, and the carbon (C), N and ^{15}N content of these compartments. All sampled substrata packs were weighted and divided into two parts. One part was used to measure the dry weight (DW) and ash-free dry mass (AFDM) of the whole pack. The other part was submerged and swirled in a known volume of water, and 100 mL of supernatant were filtered through a precombusted Whatman GF/F fiberglass filter. Half of the filter was processed to measure the FBOM biomass content in the pack and the other half was used to measure the content of C and N and the ^{15}N signature of the FBOM fraction. The biomass of epilithon of each substrata pack was measured as the difference between the content of FBOM and the AFDM of the whole pack. To measure the ^{15}N signature and the C and N content of the epilithon, the submerged sub-sample was elutriated several times until de FBOM was totally removed. A known volume of distilled water was then added to the elutriated substrata and this sample was exposed to ultrasonic waves for 10 minutes. After this time, water was filtered through precombusted Whatman GF/F fiberglass filters. We did not characterize the epilithon in the mud packs because it was impossible to elutriate de sediments due to the small size of the particles forming this type of packs. Samples for biomass measurements were weighed, dried at 60ºC for ≥3 days, weighted again, combusted a 500ºC for 5h, and reweighed for calculation of dry mass and AFDM. Filters with epilithon and FBOM samples for the analysis of the content of C and N, and the ^{15}N signature were dried in the oven (60 ºC, ≥3 days) and weighted. We cut 1 cm-diameter disc from each filter, weighted it on a Mettler-Toledo (Greifensee, Swizertland) MX5 microbalance, and encapsulated in tins for analysis.

Encapsulated filters of both water and substrata pack samples were analyzed in the Stable Isotope Facility at the University of California-Davis (Davis, CA, USA). The content (as a percent of dry mass) and the stable isotope ratios of N were measured by continuous flow isotope ratio mass spectrometry (20-20 mass spectrometer; PDZEuropa, Northwich, UK) after sample combustion at 1000ºC in an on-line elemental analyzer (PDZ-Europa ANCA-GSL).

Results of ^{15}N were given in δ^{15}N values (units of ‰) as an expression of the ^{15}N:^{14}N ratio in the sample (R_{sample}) relative to the ^{15}N:^{14}N ratio in air used as the standard ($R_{standard}$=0.003663) according to the following equation:

$$\delta^{15}\mathrm{N} = \left[\left(\frac{\mathrm{R}_{\mathrm{sample}}}{\mathrm{R}_{\mathrm{standard}}}\right) - 1\right] \mathrm{x}\, 1000 \qquad (1)$$

To calculate the N assimilation rates we used δ^{15}N values from the ^{15}N tracer addition. We first estimated the amount of ^{15}N in each compartment (^{15}N$_{\mathrm{compartment}}$, μg ^{15}N pack^{-1}) within the pack of each substrata type using the following equation:

$$^{15}N_{comp} = B_{comp} \times \left(\%N/_{100}\right) \times \left(MF_i - MF_b\right) \qquad (2)$$

where B$_{\mathrm{comp}}$ is the biomass of either epilithon or FBOM compartment in the pack (μg AFDM pack^{-1}), %N is percentage of N of the compartment, MF is the molar fraction of ^{15}N in the compartment at plateau conditions (MF$_{\mathrm{i}}$) and at background conditions (MF$_{\mathrm{b}}$). Molar fractions were calculated as:

$$MF = \frac{^{15}N}{^{15}N + ^{14}N} = \frac{\left(\frac{\delta^{15}N}{1000} + 1\right) * 0.0036765}{1 + \left[\left(\frac{\delta^{15}N}{1000} + 1\right) * 0.0036765\right]} \qquad (3)$$

We then estimated N assimilation rate of each compartment (U$_{\mathrm{comp}}$, μg N h^{-1} pack^{-1}) using the equation adapted from von Schiller et al. (2007):

$$\mathrm{U}_{\mathrm{comp}} = \frac{^{15}\mathrm{N}_{\mathrm{comp}}}{\mathrm{t}_{\mathrm{add}} \times {}^{15}\mathrm{N}_{\mathrm{flux}}/\mathrm{N}_{\mathrm{flux}}} \qquad (4)$$

where t$_{\mathrm{add}}$ is the duration of the ^{15}N addition (h), ^{15}N$_{\mathrm{flux}}$ is the NH$_4^+$-^{15}N flux at plateau conditions (μg ^{15}N s^{-1}) and N$_{\mathrm{flux}}$ is the total NH$_4^+$-N flux (μg N s^{-1}). We also calculated the N assimilation efficiency of each biotic compartment as U$_{\mathrm{comp}}$ divided by B$_{\mathrm{comp}}$. N assimilation rate of the pack was calculated as the weighed sum of U for each compartment within the pack.

2.3. Measurements of whole reach transient storage and nutrient uptake

Solute addition experiments. To quantify the effect of the introduction of substrata packs on transient water storage and nutrient uptake at whole reach scale, we conducted short-term additions of a conservative tracer (Cl^- as NaCl) together with NH_4^+-N (as NH_4Cl) and PO_4^{3-}-P (as $Na(H_2PO_4){\cdot}2H_2O$). On each sampling date we conducted 4 additions; one at each of the 20-m reaches that were exposed either to a different substrata pack type or left as control. Additions started at the most downstream reach and moved consecutively upstream. Duration of each addition varied between 20 and 30 minutes, based on the time needed for the solution to reach plateau conditions at the bottom of the reach. We automatically recorded EC at 10-s intervals at the head and the bottom of the reach using conductivity meters (LF 340 WTW, Weilheim, Germany) connected to data loggers (510X Campbell Scientific, Logan, UT, USA). Water samples for the analysis of NH_4^+-N and soluble reactive phosphorus (SRP) concentration were taken at the bottom of the reach from the beginning of the addition until EC returned to background levels after the addition was stopped. In each addition, sample frequency was accommodated to changes in EC, but in general samples were collected at 10-second intervals during the rise and the fall of the curve and at 30-second intervals during plateau. Samples were collected using 10 mL acid-washed syringes, filtered in situ using Whatman® GF/F fiberglass filters (0.7 µm pore diameter), and kept refrigerated until analysis. Concentrations of NH_4^+-N and SRP were analyzed following standard colorimetric methods (APHA, 1998) using a Bran+Luebbe TRAACS 2000 Autoanalyzer (Norderstedt, Germany). Background concentrations of SRP and NH_4^+-N were low throughout the study and the average (± SEM) was 4.2±0.4 µg P L^{-1} and 9.4±0.8 µg N L^{-1}, respectively. Additions increased EC by 34±2 µS cm^{-1}, SRP concentration by 67.7±6.2 µg P L^{-1}, and NH_4^+-N concentration by 55.6±4.8 µg N L^{-1}. In addition, for each addition we measured the wet width and water depth (at 10, 30, 50, 70, and 90% of the wet width) across transects done every 5 meters along the reach. Slope of the streambed was measured at each reach following the hydrostatic levelling method (Gordon, 1992).

Estimation of hydraulic parameters. Discharge (Q, L s^{-1}) and average water velocity (v, m s^{-1}) were measured using the time-curve EC data recorded at the bottom of the reach. Calculation of Q was based on a tracer mass balance approach (Gordon, 1992). Water velocity was calculated by dividing the reach length by the time needed to increase the EC one half of the plateau (i.e., T_n nominal travel time, s). Cross-section area of transient storage (A_s, cm^2), exchange coefficient between the main channel and the transient storage zone (α, s^{-1}), and cross-section area of the main channel (A, cm^2) were estimated from EC breakthrough curves using the One-dimensional Transport model with Inflow and Storage (OTIS; Runkel, 1998).

This model of downstream solute transport accounts for advection, dispersion, dilution due to lateral water inflow, and exchange with transient storage zones. In addition for non conservative solutes (e.g. nutrients), the model includes decay rates in both the main channel and the transient storage (Fig.2). The model relies on the solution to two equations (equations 5 and 6) that simulates the change in solute concentration over time in the main channel and in the transient storage zone assuming a single timescale of exchange between them:

$$\frac{\partial C}{\partial t} = -\frac{Q}{A}\frac{\partial C}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left(AD\frac{\partial C}{\partial x}\right) + \frac{q_L}{A}\left(C_L - C\right) + \alpha\left(C_s - C\right) - \lambda C \qquad (5)$$

$$\frac{\partial C_s}{\partial t} = \alpha\frac{A}{A_s}\left(C - C_s\right) - \lambda_s C_s \qquad (6)$$

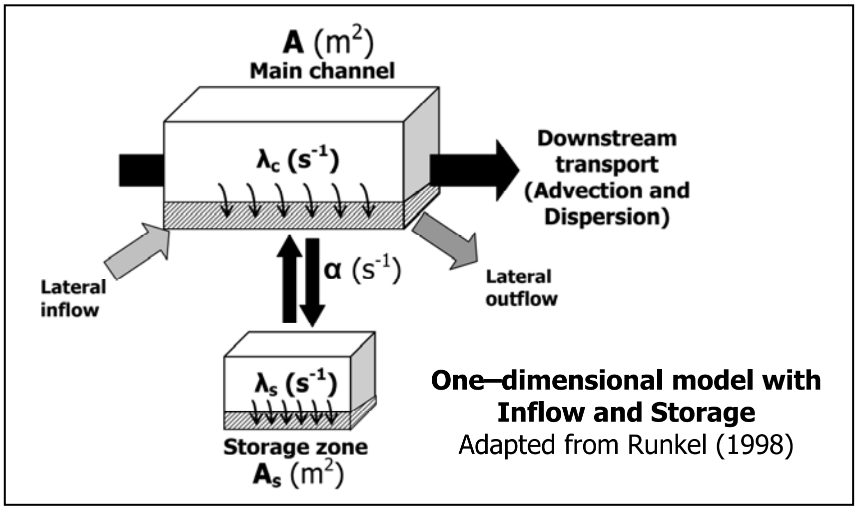

Figure 2. Conceptual model of the transient storage and the main channel compartment. Figure adapted from Runkel (1998).

where C is the solute concentration in the main channel (ML^{-3}), C_s is the solute concentration in the transient storage zone (ML^{-3}), C_L is the solute concentration of the lateral inflow (ML^{-3}), D is the dispersion coefficient (L^2T^{-1}), q_L is the lateral inflow rate on a per length basis ($L^3T^{-1}L^{-1}$), t is time (T), x is distance (L), λ (T^{-1}) is the decay rate of a non-conservative solute in the main channel, and λ_s (T^{-1}) is the decay rate of a non-conservative solute in the transient storage zone. To estimate the hydraulic parameters, λ and λ_s were set to zero (i.e., as conservative solute) in the simulations with the OTIS model. With those estimated parameters as initial conditions we then run OTIS-P, a modified version of OTIS that allows the automated parameter estimation using nonlinear regression techniques to obtain better adjustments to the observed data-set.

Damkölher values (DaI) were calculated with data from each addition to determine whether the length of the reach was suitable for measuring transient storage (Wagner and Harvey, 1997). Values obtained ranged between 1 and 5, and therefore hydraulic parameters estimated with the OTIS model were considered reliable if we assume a single timescale of exchange. The fraction of the median travel time attributable to transient storage (F_{med}^{200}, Runkel, 2002) was calculated for a standardized length of 200 m using the following equation:

$$F_{med}^{200} = \left[1 - e^{-L(\alpha/v)}\right]\frac{A_s}{A + A_s} \qquad (7)$$

where L is reach length.

Estimation of nutrient uptake parameters. Once the OTIS model was parameterized for the hydraulic variables, we performed simulations using the time-curves for nutrient concentrations to estimate λ and λ_s for both NH_4-N and PO_4-P as the descriptors of nutrient uptake at whole reach scale (Runkel, 2007). Total uptake coefficient (k, s^{-1}) for each nutrient was calculated based on Runkel's (2007) "Case II" transport using the following equation:

$$k = \lambda + \frac{\alpha\lambda_s A_s}{\alpha A + \lambda_s A_s} \qquad (8)$$

In addition, we estimated the total percentage of the added nutrient that was taken up in the reach as well as the proportion of it associated with the main channel and the transient storage zone. To estimate these percentages we calculated the mass of nutrient retrieved from the additions at the endpoint of the reach based on OTIS simulations under the following scenarios: (A) conservative transport (λ and λ_s=0), (B) non-conservative transport in both the channel and the transient storage zone (λ and λ_s>0), (C) main channel uptake only (λ>0 and λ_s=0), and (D) storage zone uptake only (λ=0 and λ_s>0). For each simulation, the mass of nutrient retrieved was calculated as the integrated area of each curve. The percentage of nutrient uptake in the main channel (%uptake$_c$) and in the storage zone (%uptake$_{ts}$) was then calculated as:

Table 1. Substrata pack properties. Dominant grain size, water residence time inside the pack (T_p, h), organic matter content (g AFDM), % of weight (in AFDM) for each biotic compartment inside the pack, and C:N mass ratio for each biotic compartment and for each type of substrata pack (n=15 for each treatment). "nm" not measurable.

		Cobbles	Sand	Mud
	grain size	5-10 cm	1-3 mm	< 1 mm
	T_p (h)	0.2	2.3	>16.3
	g AFDM	5.4 ± 0.9	8.4±0.4	56.6 ± 2.9
epilithon	%	98.3 ± 0.3	86.6±2.4	nm
	C:N	9.0 ± 0.4	11.7±0.3	nm
FBOM	%	1.7 ± 0.3	13.4±2.4	100.0 ± 0.0
	C:N	8.0 ± 0.2	11.5±0.2	11.1 ± 0.1

$$\%\text{uptake}_c = \frac{\text{mass}_{simA} - \text{mass}_{simC}}{\text{mass}_{simA} - \text{mass}_{simB}} \times 100 \qquad (9)$$

$$\%\text{uptake}_{ts} = \frac{\text{mass}_{simA} - \text{mass}_{simD}}{\text{mass}_{simA} - \text{mass}_{simB}} \times 100 \qquad (10)$$

The total percentage of nutrient taken up in the reach was calculated as the sum of the percentages estimated in both the main channel and the transient storage after adjusting them proportionately to obtain a sum equal to 100% (Runkel, 2007).

2.4. Statistical analysis

To compare hydraulic and nutrient uptake parameters between control reaches and reaches with substrata packs, and also among substrata pack types we run one-way ANOVA with randomized block design. As a block we considered addition date and as a factor we considered type of substrata pack and control reaches vs. reaches with substrata packs. We used the Shapiro-Wilk test to examine the normality of the variables and the Levene's statistic to test the homogeneity of variances. Some variables (i.e., k and % of nutrient uptake) were log-transformed and others (i.e., λ and λ_s) inverse log-transformed prior to analysis in order to meet assumptions of normality. We conducted analysis of variance (one-way ANOVA) to compare ^{15}N uptake rates among substrata packs. Multiple comparisons were made among treatments following *post-hoc* Tukey HSD procedure in all significant ANOVA tests. Finally, we examined the relationships between transient storage parameters (A_s, α, and F_{med}^{200}) and nutrient uptake parameters for SRP and NH_4^+-N (λ and λ_s, relative proportion of uptake coefficient to total uptake coefficient (λ_s/k) and percentage of nutrient uptake) by using regression analysis. The significance level for the tests was $p<0.05$. All statistical analyses were done using SPSS for Windows (version 12.0, SPSS Inc., Chicago).

3. RESULTS

3.1. Physical and biological characterization of substrata packs

The packs of the different substrata types were comparable in size and volume; however, they differed in grain size, hydraulic conductivity, organic matter content, the relative proportion of epilithon and FBOM, and the C:N ratio of these biotic components (Table 1). Dominant grain size ranged from <1 mm in mud packs to a few cm in cobble

Table 2. Estimated transient storage model parameters, data reported are mean values ± standard error of experiments performed on 6 different dates (n=6).

	Control	Cobbles	Sand	Mud
Q (Ls^{-1})	4.84 ± 1.08	3.95 ± 0.91	4.55 ± 1.21	4.49 ± 0.98
v (ms^{-1})	0.21 ± 0.02	0.15 ± 0.02	0.17 ± 0.03	0.18 ± 0.02
A_s (cm^2)	13.0 ± 2.3	29.2 ± 4.3	28.2 ± 3.2	32.0 ± 1.9
α ($x10^{-3}$ s^{-1})	1.5 ± 0.4	2.7 ± 0.4	3.5 ± 0.5	3.1 ± 0.4
F_{med} (%)	4.5 ± 1.2	10.2 ± 1.4	10.9 ± 2.2	12.0 ± 1.9

packs. Hydraulic conductivity (expressed as water residence time within the packs) followed an inverse relation with grain size. Mud packs had the finest grain size and the highest water residence time inside the pack (i.e., >half day). Water residence time in the sand packs was in the range of few hours and in the cobble packs was in the range of minutes (Table 1). Regarding biological features, organic matter content in mud packs was 10.5 times and 1.6 times higher than in cobble and sand packs, respectively. Almost all organic content was attributable to epilithon in cobble (98.3%) and in sand packs (86.6%) while mud packs were mainly composed of FBOM (Table 1). The C:N ratio was slightly higher in epilithon than in FBOM, and sand packs had higher C:N ratios than cobble and mud packs (Table 1).

Assimilation rates of N estimated from ^{15}N tracer addition were significantly different among types of substrata packs (one-way ANOVA, $F_{(2,23)}$=4.164, p=0.03, Fig. 3A). Cobbles showed the highest N assimilation rate (0.95±0.34 µg N h^{-1}) followed by mud packs (0.57±0.14 µg N h^{-1}), and by sand packs (0.15±0.02 µg N h^{-1}). Most of N uptake in cobbles and sand packs was attributable to the epilithic compartment as the FBOM compartment in this type of packs was small; in mud packs, in contrast, all N uptake was attributable to the FBOM compartment (Fig. 3B). In addition, biotic compartments differed in N assimilation efficiency (i.e., N assimilation rate per unit of AFDM). N assimilation efficiency of FBOM was 3.5±0.5 and 3.7±1.2 times higher than that of epilithon in cobble packs and in sand packs, respectively (Fig. 3B). N assimilation efficiency was highest in cobble packs, intermediate in sand packs and lowest in mud packs (Fig. 3B).

3.2. Effects of substrata packs at the reach scale

Hydraulic parameters. Discharge varied slightly among sampling dates and did not significantly differ between control reaches and reaches with different types of substrata packs. The average (±SEM) discharge for the entire study was 4.5±0.5 L s^{-1} (n=24). Values of v ranged between 0.08 and 0.30 m s^{-1} and were on average 0.8 times lower in reaches with substrata packs than in control reaches (Table 2). Transient storage parameters (i.e., A_s, α, and F_{med}^{200}) increased after introducing substrata packs. Values of A_s were 2.3 times higher in reaches with substrata packs than in control reaches (one-way ANOVA, $F_{(1,17)}$=28.16, p<0.001), with no significant differences among different types of substrata packs (Fig. 4A). Values of α ranged between 0.8 $x10^{-3}$ s^{-1} and 5.0 $x10^{-3}$ s^{-1} and were higher in reaches with substrata packs, especially of sand and mud, than in control reaches (one-way ANOVA, $F_{(1,17)}$= 12.51, p<0.001, Fig. 4B). $F_{med}^{200}$was on average 2.4 times higher in reaches

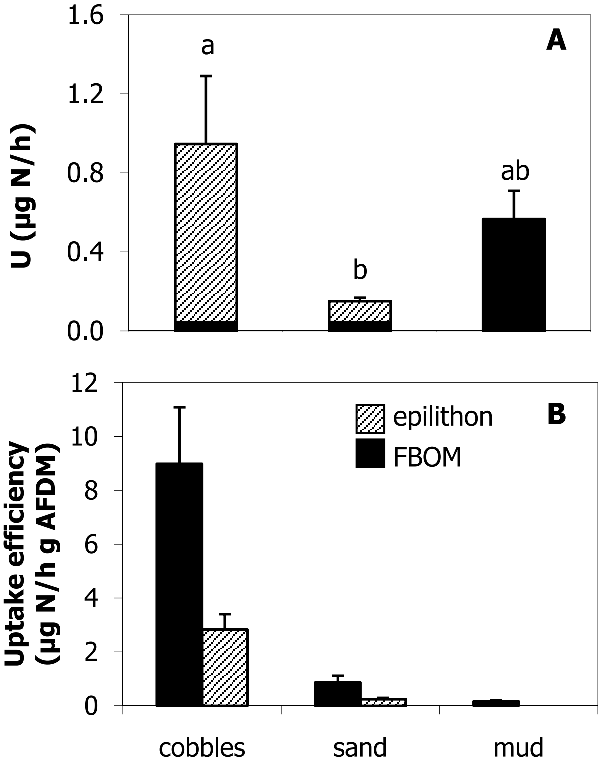

Figure 3. (A) Nitrogen assimilation rates and (B) assimilation efficiencies of each type of substrata packs calculated from ^{15}N addition. Different colors represent the contribution of each compartment of the pack to N uptake. Error bars represent total standard error. Different lower case letters denote significantly different groups based on post hoc Tukey HSD test.

with substrata packs than in control reaches (one-way ANOVA, $F_{(1,17)}=11.77$, $p<0.001$); greatest differences were observed between control reaches and reaches with mud packs (Fig 4C).

Nutrient uptake parameters. Values of k for phosphate ranged from 0.3 x10^{-3} s^{-1} to 6.2 x10^{-3} s^{-1}, and did not significantly differ between control reaches and reaches with different types of substrata packs (Table 3 and Fig. 5A). This k range resulted in an average of 20.8±2.9 % of the phosphate added being taken up in the reaches. Values of λ for phosphate were close to k for this nutrient (Table 3). No significant differences in λ for phosphate were found either between control reaches

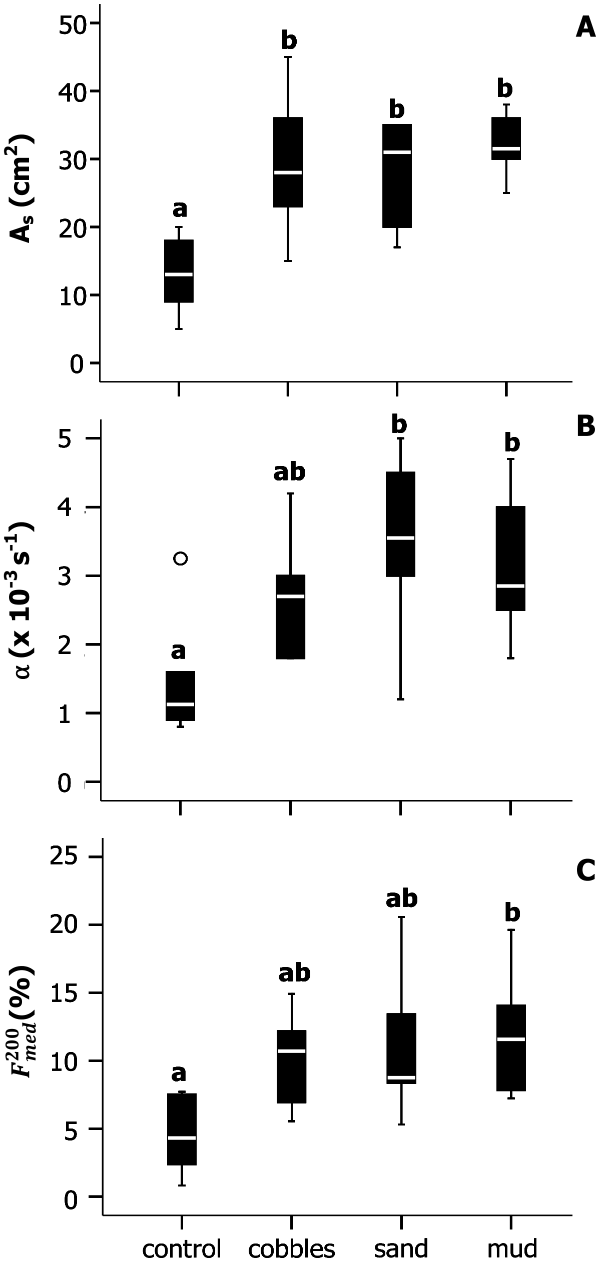

Figure 4. Boxplots of hydraulic parameters for each treatment (n=6 for each treatment), (A) A_s, (B) exchange coefficient between the main channel and the transient storage area (α), and (C) fraction of median travel time due to transient storage for a standardized length of 200 m (F^{200}_{med}). Plots show median (horizontal bars), interquartile ranges (rectangles), and ranges. Different lower case letters denote significantly different groups based on post hoc Tukey HSD test.

Table 3. Nutrient uptake coefficients and percentage of nutrient uptake at each injection experiment. Data reported are mean values ± standard error of experiments performed on 6 different dates (n=6).

	Control	Cobbles	Sand	Mud
phosphate				
k ($x10^{-3}$ s^{-1})	2.1 ± 1.0	2.2 ± 0.8	2.4 ± 0.8	1.6 ± 0.3
% uptake	17.5 ± 7.4	22.8 ± 5.5	24.9 ± 7.2	17.8 ± 3.6
λ ($x10^{-3}$ s^{-1})	2.1 ± 1.0	2.1 ± 0.8	2.4 ± 0.8	1.4 ± 0.3
λ_s ($x10^{-6}$ s^{-1})	0.3 ± 0.2	1327.6 ± 1010.6	60.1 ± 44.8	2827.4 ± 1584.2
ammonium				
k ($x10^{-3}$ s^{-1})	4.9 ± 1.2	3.5 ± 0.7	4.4 ± 0.8	4.2 ± 0.5
% uptake	34.5 ± 6.3	36.2 ± 6.4	42.0 ± 8.2	38.9 ± 6.0
λ ($x10^{-3}$ s^{-1})	4.9 ± 1.2	3.5 ± 0.7	4.4 ± 0.8	3.7 ± 0.6
λ_s ($x10^{-6}$ s^{-1})	0.2 ± 0.1	1.3 ± 0.7	1.1 ± 0.6	4458.9 ± 2614.7

and reaches with substrata packs or among reaches with different substrata pack types. On average, values of λ for phosphate resulted in 92.0±3.5 % of total phosphate uptake, which indicates that most of the total uptake occurred in the main channel. Values of λ_s for phosphate ranged between values close to zero and 7.9 $x10^{-3}$ s^{-1}. In contrast to λ, λ_s for phosphate significantly differed among reaches (one-way ANOVA, $F_{(1,17)}$= 4.49, p=0.02, Table 3 and Fig. 5C). λ_s for phosphate was lowest in control and sand-pack reaches and highest in reaches with mud packs (Fig. 5C). Phosphate uptake occurring in the transient storage accounted between 0.05 and 16.8 % of the percentage of total uptake in the control reaches and in reaches with mud packs, respectively. The ratio between λ_s and k for phosphate was positively related to the transient storage size ($\lambda_s/k= 5.29x10^{-13}e^{0.71A_s}$, $R^2=0.37$, p=0.00; Fig. 6A), the water exchange coefficient ($\lambda_s/k= 5.1\ x10^{-10}\alpha^{13.16}$, $R^2=0.36$, p=0.00), and F_{med}^{200} ($\lambda_s/k= 7.11\ x10^{-10}e^{0.12Fmed}$, $R^2=0.23$, p=0.02).

Values of k for ammonium ranged from 1.1 $x10^{-3}$ s^{-1} to 8.7 $x10^{-3}$ s^{-1} and did not significantly differ between control reaches and reaches with different types of substrata packs (Table 3, Fig. 5B). This k range resulted in an average of 37.9±3.2 % of the ammonium added being taken up in the reaches. Similar to phosphate, values of λ for ammonium were close to k for this nutrient (Table 3). No significant differences in λ for ammonium were found between control reaches and reaches with substrata packs or among reaches with different substrata types. Ammonium λ values accounted for 96.1±2.4% of total uptake for this nutrient, which indicates that most of the ammonium uptake occurred in the main channel. Values of λ_s for ammonium ranged from values close to zero to 16.5 $x10^{-3}$ s^{-1}, and there were significant differences among the different reaches (one-way ANOVA, $F_{(1,17)}$= 7.25, p=0.00). Reaches with mud packs showed the highest λ_s for ammonium (Fig. 5D). While ammonium uptake occurring in the transient storage accounted for 13 % of total ammonium uptake in reaches with mud packs, this percentage was negligible in the rest of the reaches. The ratio between λ_s and k for ammonium was positively related to the transient storage size ($\lambda_s/k= 7.6x\ 10^8e^{0.29As}$, $R^2=0.26$, p=0.01; Fig. 6B), but not to the water exchange coefficient nor to F_{med}^{200}.

4. DISCUSSION

The objective of this study was to examine the influence of water transient storage on nutrient uptake by considering not only the physical features

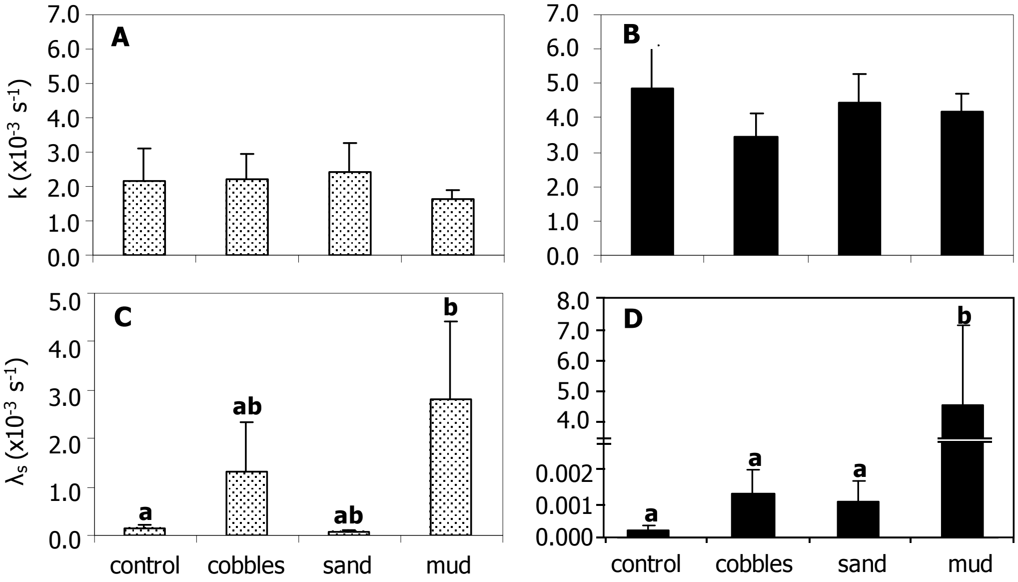

Figure 5. Average values of phosphate uptake coefficients (A and C) and ammonium uptake coefficients (B and D). Error bars represent SE. Significant differences were found for λ_s-PO_4 and λ_s-NH_4 among treatments. Different lower case letters denote significantly different groups based on *post hoc* Tukey HSD test.

of this transient storage (i.e., size of A_s or hydraulic conductivity) but also the biological characteristics (i.e., biomass of epilithon and fine benthic organic matter, nitrogen assimilation rate) associated to this transient storage generated by substrata packs introduced in the streambed.

As expected, the introduction of substrata packs into the channel doubled the size of transient storage (both A_s and A_s/A) and increased water exchange with main channel (α). This increase was similar among the substrata packs considered regardless of differences in hydraulic conductivity. The percentage of participation of transient storage to the overall water median travel time (F_{med}^{200}) also increased as a result of the introduction of substrata packs, especially in the case of mud packs. However, the increases in transient storage did not result in changes in total uptake of ammonium and phosphate at whole reach scale in any of the treatments considered. These results are in contrast with those found in previous studies in which an increase in transient storage similar to our study resulted in concomitant increases in nutrient uptake. For instance, Ensign and Doyle (2005) increased A_s/A and F_{med}^{200} by 1.2 times after the introduction of baffles into the channel and observed a significant increase in both phosphate and ammonium uptake velocity. Similarly, Roberts et al. (2007) observed a significant increase in ammonium uptake when A_s/A and F_{med}^{200} was doubled by introducing coarse woody debris into 4 stream channels. Lack of effect in our study could in part be attributed to the scale of the experimental approach. Our study was conducted in small and relatively short channel (i.e., 20m long by 60 cm wide) with small natural transient storage, in comparison to the reach scale dimensions considered in the studies cited above. The amount of transient storage induced by the introduction of substrata packs easily doubled the initial amount of A_s but it was still low in comparison to the amount of A_s observed in the experiments cited above.

Nevertheless, our results indicate some differences among treatments, which were basically associated with nutrient uptake in the transient storage zone (i.e., in λ_s). In general, λ_s were greater in mud packs

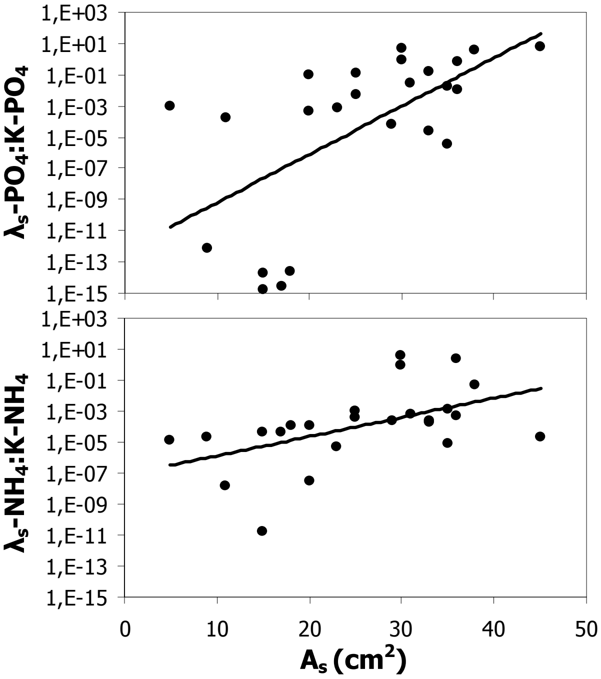

Figure 6. Relationship between transient storage and the relative influence of uptake coefficient at the transient storage zone to the total uptake coefficient for (a) phosphate and (b) ammonium.

followed by cobbles and sand packs. We also observed a change in the relative dominance of uptake processes in transient storage compared to main channel. Reaches with mud substrata packs had higher nutrient uptake in the transient storage than in the main channel in comparison to other substrata types.

In addition, the ratio between λ_s-NH$_4$ and λ_s-PO$_4$ changed among treatments. In mud packs, average λ_s-NH$_4$ was 1.6 times higher than average λ_s-PO$_4$, while in sand and cobble packs this ratio was less than 0.02. Since all treatments presented similar A_s, we suggest that all those differences observed among treatments are explained by differences in biologic communities with differing N:P requirements developed on and into the substrata packs. For instance, mud packs have higher organic matter content, lower hydraulic conductivity, and longer residence time which probably reduced oxygen concentration in the pack in comparison to other treatments. Those characteristics would promote the development of different types of biotic communities in mud packs than in sand or cobbles. Additionally, mud packs were exclusively composed by FBOM while epilithon dominated in cobble and, in minor measure, in sand packs.

Results from the ^{15}N addition evidenced that FBOM was more efficient in assimilating ^{15}N than epilithon. Similar results were observed by Mulholland et al. (2000) and Tank et al. (2000) who examined N assimilation in several stream compartments. Nevertheless, cobble packs presented higher ^{15}N assimilation rate than sand packs, while mud packs did not show significant differences to the rest of the treatments. This higher N assimilation rate observed in cobbles is not reflected on the results of the additions at the whole-reach scale. Data from the short-term additions showed higher rates of ammonium removal at the transient storage zone of reaches with mud packs. While the ^{15}N approach quantifies the amount of N uptake due to biological uptake, with the second approach (i.e., the short-term additions) we measure the amount of N uptake resulting from the combined effects of hydrological, biological, and chemical retention Valett et al. (1996) and McClain et al. (2003). The combination of those results evidences that while cobble and mud packs present the highest biological N uptake, only mud packs provoke a significant hydrological retention and thus, at a reach scale, mud packs are the ones more effective retaining N.

This work is one of the fewer that discriminates whole reach nutrient uptake occurring in the transient storage zone to that occurring in the main channel (but see McKnight et al. (2004) and Runkel (2007)). Conventionally, it has been thought that zones of transient storage are biogeochemical "hotspots" and that they are responsible of an important part of nutrient transformation. In our case, transient storage zones were not contributing significantly to the whole reach nutrient uptake, but we found a good relation between the relative contribution of nutrient uptake in the transient storage zone to total uptake and the size of transient storage zone (A_s). We suggest that the lack of patterns observed in the literature when relating nutrient uptake and transient storage could be caused by a underestimation of the role that different physical and biological features of structures involved in transient storage play in nutrient uptake. In most of the existing studies, specific mechanisms of storage are not identified and transient storage is considered to contribute homogeneously in nutrient uptake processes. Results from this study confirm the hypothesis that nutrient uptake depends not only on the size of transient storage, but also on the physical and biotic features of structures involved in it and that differences among those features will generate differentiated nutrient uptake responses. New tendencies in stream restoration focus on restoring stream functionality. Methodologies developed to achieve this objective include the introduction of deflectors to increase stream habitat heterogeneity. Results obtained in this study suggest that the type of deflectors introduced in the channel would enhance different biotic communities, and thus, will impact not only the overall amount of nutrient uptake but the processes of nutrient uptake affecting the final nutrient balance.

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Development of a “Smart” Tracer for the Assessment of Microbiological Activity and Sediment-Water Interaction in Natural Waters: the Resazurin-Resorufin System

Haggerty et al. (2008) Water Resources Research

Desenvolupament d’un traçador intel·ligent per a l’estudi de l’activitat microbiològica i la interacció entre aigua i sediments en rius: el sistema resazurin-resorufin

Roy Haggerty[1], Alba Argerich[2], i Eugènia Martí[3]

[1] Dept. of Geosciences, Oregon State University, 104 Wilkinson Hall, Corvallis 97331-5506, Oregon, USA; [2]Dept. d’Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028; [3]Limnology group, Centre d’Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona.

RESUM

Un traçador intel·ligent és aquell que a més de proporcionar informació sobre les característiques del transport de soluts en un ambient és capaç de donar-nos informació addicional sobre les propietats del medi pel qual el solut ha estat transportat. En aquest estudi, presentem el compost químic resazurin (Raz) com un traçador intel·ligent capaç de proporcionar informació metabòlica sobre el medi per on viatja. El Raz és un compost fluorescent que experimenta una reducció irreversible a resorufin (Rru), que també és fluorescent, en la presència de microorganismes vius. Per tal d’investigar d’idoneïtat d’aquest sistema de compostos com a traçadors intel·ligents en aquest estudi caracteritzem la degradació, la sorció, la reacció i el transport del Raz i el Rru en varis tipus de sediments i agües en una sèrie d’experiments de laboratori.

El Raz es transforma ràpidament a Rru en sediments colonitzats, a una taxa de 1,41h^{-1} mentre que ho fa a molt més lentament (a una taxa 3 ordres de magnitud menor) en aigua de riu sola indicant que el traçador és sensible a l’activitat biològica associada a la interfície columna d’aigua-sediment. Els compostos poden estar afectats per fenòmens de sorció. En l’experiment portat a terme per verificar aquest fenomen, la isoterma de sorció del resorufin va presentar una K_d de 6,63 mLg^{-1} en un sediment amb un 2,19% de carboni orgànic.

Els compostos són estables durant setmanes en aigua natural, excepte en el cas d’exposició a la llum. En aquest cas s’ha observat una certa degradació fotoquímica però suficientment lenta com perquè no suposi un problema en els estudis de camp convencionals.

Development of a "Smart" Tracer for the Assessment of Microbiological Activity and Sediment-Water Interaction in Natural Waters: the Resazurin-Resorufin System

Roy Haggerty[1], Alba Argerich[2], and Eugènia Martí[3]

[1] Dept. of Geosciences, Oregon State University, 104 Wilkinson Hall, Corvallis 97331-5506, Oregon, USA; [2]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028, Spain; [3]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona, Spain.

ABSTRACT

A "smart" tracer is a tracer that provides, directly or through measurement of its concentration or in combination with another compound, at least one "bit" more of information about the environment through which it travels than a conservative tracer. In this study we propose and present the chemical compound resazurin as a smart tracer to assess the coupling between solute transport and microbiological activity in sediment-water interfaces in freshwaters. Resazurin is a weakly fluorescent redox-sensitive dye that undergoes an irreversible reduction to strongly fluorescent resorufin under mildly reducing conditions – most commonly in the presence of living microorganisms. To investigate the suitability of resazurin as a smart tracer, we characterized the decay, sorption, reaction, and transport behavior of resazurin and resorufin in various waters and sediments using laboratory experiments. Results show that resazurin irreversibly and rapidly reacts to resorufin in colonized sediment with pseudo-first-order behavior and a rate coefficient of 1.41 h-1. This reaction is three orders of magnitude faster than that in stream water alone, indicating the tracer is sensitive to microbiological activity and associated sediment-water interactions. The compounds are affected by significant sorption, with an approximately linear isotherm and a K_d of 6.63 mL/g for resorufin in sediment with 2.19% organic carbon. The compounds are stable over weeks in natural water, except in the presence of strong light where significant photochemical decay may occur more rapidly.

1. INTRODUCTION

Applications of tracers are one of the key techniques used in hydrological studies of surface freshwaters and groundwaters (for a review, see Flury and Wai, 2003). However, the information that conservative tracers provide is largely tied to water transport and arrival time. The processes available for direct study via arrival time are limited to advection, dispersion (spreading of arrival times), and transient storage or mass transfer (tailing of arrival times). In freshwater ecosystems these processes can have a large influence on biogeochemical responses. However, some hydrologic processes may be indistinguishable using arrival times of conservative tracers – for example, advection through low-permeability zones and diffusion into and out of low-permeability zones can yield the same arrival times (Becker and Shapiro, 2000; Sánchez-Vila and Carrera, 2004); and in-stream transient storage can yield similar arrival times to hyporheic transient storage (Gooseff et al., 2005). Furthermore, biogeochemical processes have non-unique responses to arrival times due to dependence on biological community, biogeochemical heterogeneity between flowpaths, and other factors (e.g., Jones et al., 1995; Kirchner, 2003; Nagorski et al., 2003). Given this context, geo/eco-hydrology would be advanced considerably if our community could develop tracers that collect information about the environment through which they traveled – tracers that are, simply put, smarter than conservative tracers. We define a “smart tracer” as a tracer that provides, directly or through measurement of its concentration or in combination with another compound, at least one “bit” more of information about the environment through which it travels than a conservative tracer. In other words, the smart tracer provides direct information about conditions in the hydrologic system - location history, chemical conditions, biological activity, physical interactions - in addition to arrival time. In this study, we present a prototype smart tracer for the measurement of microbiological activity and associated sediment-water interactions in freshwater ecosystems.

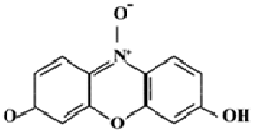
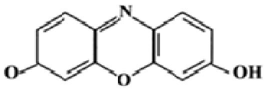
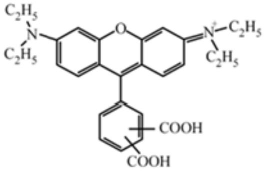
A smart tracer should have characteristics common to all good conservative tracers (Flury and Wai, 2003). That is, the tracer (and, if applicable, its daughter products) should be (1) conservative, with one exception; (2) clearly discernable from the background of the system; (3) of low toxicity; and (4) insensitive to variations in solution or medium chemistry, also with one exception. The one exception is that the smart tracer must undergo an irreversible change in the presence of a process or condition under investigation. This irreversible change must be (1) detectable with a measurement technique or sensor; (2) rapid relative to transport rates; and (3) insensitive to likely chemical or other variations in the solution or medium. Finally, the ideal smart tracer and its measurement technique should be inexpensive, safe to handle and not harmful to the environment.

We are aware of the previous use of at least one smart tracer in hydrology. Gramling et al. (2002) used the irreversible reaction of $CuSO_4$ and $EDTA^{4-}$ to $CuEDTA^{2-}$ and the resulting color change to measure true chemical-scale mixing and distinguish it from hydrodynamic dispersion. This smart tracer allowed direct measurement of the amount of longitudinal molecular scale mixing that occurred in a tank experiment. Smart tracers have been used in other fields; for instance, in fluid mechanics, reacting compounds that change color have been used to measure mixing times in chemical reactors (Melton et al., 2002; Cabaret et al., 2007). Though not strictly a tracer technique, smart radiotracers used in positron emission tomography preferentially lodge in tumors due to unique metabolism found there (e.g., inefficient and higher rate of glucose use). When the radiotracer decays it emits a positron that can be used to tomographically image the tumor (Jewaid and Cheson, 2006).

The objective of this study is to identify and characterize a smart tracer to measure microbiological activity associated with stream water-sediment interaction. Because in lotic (i.e., flowing water) ecosystems, microbial activity is mostly associated with biofilms developed on sediments (Fischer and Pusch, 2001), this assay will be useful for increasing our understanding of the interaction between hydrology and biogeochemistry in these ecosystems, and could also extend to other aquatic ecosystems.

After a search of candidates, we selected resazurin (Raz) as our potential smart tracer. This compound, also known as Alamar Blue (O'Brien et al., 2000), is a redox-sensitive phenoxazine dye (Tratnyek et al., 2001; Bueno et al., 2002) that has been known for more than a century, for example to test the

Figure 1. Properties of the resazurin-resorufin system, with rhodamine WT for comparison. Unless otherwise noted, analyses were conducted on the resazurin-resorufin system near pH 8.

Property	Resazurin	Resorufin	Rhodamine WT
Chemical formula	$NaC_{12}H_6NO_4$[1]	$NaC_{12}H_6NO_3$[1]	$C_{29}H_{29}N_2O_5ClNa_2$[1]
Structure[2]			
CAS #	62758-13-81	34994-50-81	37299-86-8
Molecular weight	251.2 g/mol[1]	235.2 g/mol[1]	567.0 g/mol
Solubility @ 25°C[3]	18.4 g/L	20.0 g/L	180-220 g/L[4]
log(K_{ow})[2, 5]	0.94	1.02	-1.38
Nominal K_d [6]	n/a	6.63 mL/g[7]	33.2 mL/g [8]
Maximum excitation wavelength	602 nm[7]	570 nm[7]	555 nm[9]
Maximum emission wavelength	616 nm[7, 10]	585 nm[7]	580 nm[9]
Quantum efficiency	0,1111	0,7411	n/a but high
Fluorescence temperature correction constant[12]	n/a	-0.0041 ± 0.003 /°C[7]	-0.027 /°C[9]

[1] For sodium salt.
[2] Raz and Rru diagrams from O’Brien et al. (2000). Rhodamine WT from Vasudevan (2001).
[3] Estimated for molecular compound with method of Meylan and Howard (1995) and Meylan et al. (1996) and available at http://www.syrres.com/esc/ (accessed 6/19/2007).
[4] McVoy (1985) reported in Soerens and Sabatini (1994).
[5] K_{ow} = octanol-water partitioning coefficient.
[6] K_d = distribution coefficient.
[7] This study.
[8] Gooseff et al. (2005), reported for sediment 9% organic carbon.
[9] Smart and Laidlaw (1977).
[10] In the case of resazurin, this emission wavelength yielded maximal difference (minimum interference) with the resorufin emission rather than being the maximum absolute value.
[11] Bueno et al. (2002).
[12] Temperature correction constant *n* defined as in Smart and Laidlaw (1997).

hygienic quality of milk (see discussion in Twigg, 1945). In the presence of mildly reducing conditions, Raz loses an oxygen ion irreversibly to become resorufin (Rru). Rru can also undergo a further, reversible reduction to hydroresorufin, but this is not favored in the presence of oxygen. The reduction of Raz to Rru is a well-documented indicator of the presence of living bacteria (Liu, 1983; Peroni and Rossi, 1986; De Fries and Mistuhashi, 1995; O'Brien et al., 2000; Guerin et al., 2001; McNicholl et al., 2006), particularly of aerobic bacteria (Karakashev et al., 2003). The Rru-Raz reaction has been widely used as an indicator of bacterial growth in milk (e.g., Ramsdell et al., 1935; Moyer and Campbell, 1963) and semen quality (e.g., Erb and Ehlers, 1950; Zrimšek et al., 2004). Both Raz (blue) and Rru (red) are intensely colored and fluorescent, but Rru is more fluorescent, as indicated by the quantum efficiency (Bueno et al., 2002) (Table 1). Raz and Rru are not highly toxic, and in fact are used in evaluation of toxicity to count living bacteria (O'Brien et al., 2000). Fung and Miller (1973) did experiments with 10,000 µg/L Raz and found that the growth of most of the species of bacteria considered (23 out of 30) were not inhibited by the dye. Therefore, a priori, this compound fulfilled most of our initial requirements for a smart tracer.

To verify if resazurin could be used as a smart tracer, here we present results from laboratory experiments coupled with application of mathematical models for parameter estimation, which were used to characterize decay, sorption, and reaction of Raz and Rru.

2. METHODOLOGY

2.1. General methods

Raz, Rru, and other reagents were purchased from Panreac Química S.A. (Castellar del Vallès (Barcelona), Spain; www.panreac.com) and used as supplied. The 2007 bulk cost of Raz was slightly less than 1000 USD for 250 g in both the US and Europe (experiments described in this paper required << 5 g). Initial comparison of fluorescence signals showed that Raz was contaminated by 3% Rru. Kangasniemi (2004) showed that the fluorescence of Rru is constant above pH 8, but that it decreases sharply below pH 6.5. Bueno et al. (2002) showed very similar results for the fluorescence of Raz. Consequently, water samples from the experiments were buffered to pH 8 prior to analysis of Raz and Rru. A stock solution of buffer near pH 8.0 was generated by mixing 1 molar $NaH_2PO_4 \cdot H_2O$ with equal parts 1 molar NaOH, which was added to samples at a 1:10 buffer-to-sample ratio. Where noted, filtration of water samples was performed with 0.7 µm glass fiber filter (GF/F, supplied by Whatman, Kent, UK, and Albet, Sant Boi de Llobregat (Barcelona), Spain). All laboratory materials for the experiments described below were triple-washed with tap water, triple-rinsed with deionized water (DIW), and dried if needed.

Fluorescence of Raz and Rru in water samples was measured on a Shimadzu RF spectrofluorometer, with excitation and emission wavelengths given in Table 1. Samples were placed in a quartz cuvette and held within the sample chamber for less than 1 minute to minimize temperature changes. Unless otherwise noted, samples were stored in the dark at room temperature prior to measurement, which was always within 24 h of sampling (and usually within 1 h). Error bars for data were calculated assuming independent contributions of error from sample preparation, sample measurement, and signal measurement (machine error). Sample preparation, sample measurement, and signal measurement were each performed a minimum of 7 times to isolate the targeted error (Clesceri et al., 1999, method 1020). The limit of quantitation (LOQ, approximately 3 times the limit of detection, LOD) for Rru in DIW is 0.012 µg/L (0.05 nmol/L). Because Rru fluoresces more strongly than Raz and because the fluorescence spectra overlap, the LOD and LOQ for Raz depend on Rru concentration. In DIW the LOQ for Raz with 0 µg/L Rru is 0.16 µg/L (0.62 nmol/L), while the LOQ for Raz with 25 µg/L (0.11 nmol/L) Rru rises to 0.96 µg/L (2.2 nmol/L). The LOQ for both compounds in natural water is approximately 5 times larger. The errors reported in this manuscript account for variable Rru concentration.

Change in fluorescence due to variation in temperature in the spectrofluorometer reading cell was measured by placing a sample in the spectrofluorometer and measuring fluorescence immediately at 23.0 °C, turning off the lamp, and then measuring fluorescence again after the sample had equilibrated to the temperature inside the instrument (31.6 °C) (Table 1).

To measure the rates of decay, sorption and reaction of the Raz-Rru chemical system and to examine if reaction rates were susceptible to water transport and biological activity we conducted three sets of experiments in the laboratory using water from two streams ("riera" in Catalan) located in the Tordera catchment (40 km N.E. of Barcelona, Spain) and sediment from one of them. Both streams have near-neutral pH and low total dissolved solids. The

Table 2a. Column properties

Property	Value
Column size	15 cm x 1 cm ID
Temperature, °C	16
Porosity *n*, -	0.422
Fraction organic carbon1, %	2.19
Raz concentration injected, µg/L	200
Dispersivity (conservative), cm	0.107
Dispersivity (sorbing), cm	0.647
Retardation factor *R*, Raz and Rru, -	2.07

2nd-order Riera de Santa Fe de Montseny drains a 2.15 km^2 forested catchment and the 3rd-order Riera de Gualba drains a 13.46 km^2 catchment of mixed forest, agriculture and urban land use. A representative sample of sediment (< 2 mm size fraction) collected from the Riera de Sta. Fe was silicic and consisted of 52.8% fine-grained metasedimentary lithic fragments; 34.8% quartz grains; 6.2% feldspar grains; 3.7% granodiorite lithic fragments; and 2.38 ± 0.09% organic carbon. Inorganic sediment was manually separated by binocular microscope and weighed. Fraction of organic carbon was determined gravimetrically on 3 samples by loss on ignition at 550 °C for 15 h.

Prior to the experiments, the stability of a 25 µg/L solution of Rru in DIW stored in the dark at room temperature was examined. The fluorescence of this solution was measured nearly every work day for approximately 1 month. No degradation in the fluorescence was observed in this time; therefore, this solution was used as a fluorescence standard for all the subsequent experiments.

2.2. Measurement of reaction and decay rates

We measured the reaction, and decay of Raz and Rru in batch experiments using water from the two streams and DIW under different conditions. First, reaction and biochemical or chemical decay in natural water were measured using water from the two streams. Water from Gualba was filtered; Sta. Fe water was used unfiltered. Both were refrigerated and used within 1 week of collection. Solutions of 25 µg/L Raz and Rru were created using these waters and stored in the dark at room temperature (19-23 °C). On an approximately daily schedule for 13-22 days (depending on experiment), samples from each batch were taken, filtered in the case of Sta. Fe water, buffered, and fluorescence was measured. Second, we also measured the reaction and decay of Raz in DIW. The procedure was the same as above except that filtration was not required. No replicates were taken in these experiments.

Third, we examined the effect of light (under laboratory conditions) on the decay of Raz and Rru. For this experiment we used DIW. The procedure was the same as above, except that the solutions were placed in clear glass containers and left on a counter top in the laboratory (19-23 °C) with the lights turned on. Lights were Osram Lumilux daylight 18 W bulbs (Munich, Germany) with a broad spectrum from 400-600 nm and peaks at 427, 478, and 536 nm. The light intensity was 0.624 ± .072 mol/m^2/d, monitored with a photoactive radiation (PAR) sensor (SKP215 Quantum, Skye Instruments, Powys, UK).

2.3. Measurement of sorption

The sorption isotherm for Rru was measured using stream sediment from Sta. Fe. Saturated sediment was collected in a plastic bag, dried at 60 °C for 72 h, then sieved (Retsch AS200, Haan, Germany) to separate the fractions larger and smaller than 2 mm. The size fraction < 2 mm was divided into 17 aliquots of approximately 20 g, which were weighed and placed in 250 mL Pyrex™ jars. Weighed aliquots of approximately 200 g of filtered stream water from Sta. Fe were used to prepare 0.00, 1.00, 4.64, 21.54, and 100.00 µg Rru/L solutions which were then poured into the jars. Four replicates were made of 4.64 and 100.00 µg/L, and 3 replicates were made for other concentrations. The jars were

Table 2b. Experimental conditions

	Low Flow	Medium Flow	High Flow
EC above background, µS/cm	115.3	139.5	131.8
Flow rate, mL/h	3.39	15.5	34.5
O_2 concentration in/out, mg/L	8.4/5.1	9.1/7.2	9.4/8.7
Velocity v, cm/h	13.6	32.9	80.4
Travel time ($\tau = L/v$), h	1.10	0.455	0.187
Sample frequency (approx.), min	130 2	35 2	15
Total experiment time, h	46.9	24.9	6.65

capped, kept in the dark, and manually shaken every 2 h (4 h at night) for 41 h. 15 mL aliquots were extracted from the jars at the end of the incubation, placed into plastic centrifuge tubes, and centrifuged 15 min at 2358 *G*. The supernatant was decanted, filtered, buffered and measured for aqueous Rru concentration. Sorbed Rru was calculated by difference between the initial and final concentration.

The sorption isotherm for Raz was not measured because the compound reduces to Rru in the presence of sediment. However, we may expect that the sorption isotherm for Raz to be similar to Rru because the compounds have very similar octanol-water partitioning coefficients (Table 1) and solubilities in water, and are otherwise chemically similar. Therefore, where needed in the calculations we assumed that the sorption of Raz is the same as Rru.

2.4. Characterization of transport and reaction

Reaction and transport of Raz and Rru were quantified in a series of flow-through column experiments at 3 different flow rates. A 15 cm-long, 1cm-ID, low-pressure glass chromatography column (chromaflex, Kontes Glass Company, New Jersey, USA) was packed underwater in the field with stream sediment from Sta. Fe on March 1, 2007. The majority of sediment (visual estimate) was < 2 mm size fraction with a few pebbles. The column was capped and returned to the laboratory where it was refrigerated in the dark until use (12-21 days later). Column properties are provided in Table 2a. Column experiments were conducted in a temperature-controlled room (16 °C). A solution of Raz and NaCl in filtered Sta. Fe stream water was pumped at constant rate through the column using a high-pressure peristaltic pump (Masterflex L/S pump, Vernon Hills, Illinois, USA). Outflow was delivered to a capped flask on a balance to monitor flow rate. The tracer reservoir was shielded from the light with aluminum foil. The column was shielded from the light except for the slowest-flow experiment (completed first). However, lights were out in the room except when samples were being taken, which for the slow-flow experiment was only a few minutes at a time. Furthermore, the photodecay time for the compounds is at least many 10s of h (see results in section 3.1), so the lack of column shielding on one experiment had no effect. Electrical conductivity (EC) was monitored continuously in a chamber in front of the outflow port using an LF 340 EC meter (WTW, Weilheim, Germany) connected to a CR510 data logger (Campbell Scientific, Logan, Utah, USA). Water samples were extracted manually with a 15-mL Luer lock syringe at an in-line sample port placed in front of the EC chamber. Samples were taken at regular intervals over the duration of each experiment; sample frequency and experimental duration are given in Table 2b. Since samples were taken manually, the 2 slower flow-rate experiments had 10-13 h sample gaps overnight. Samples were filtered immediately, buffered and stored (< 5 h) in the dark at 16 °C in 20-mL clear borosilicate vials prior to fluorescence measurement of Raz and Rru. Oxygen concentration was periodically measured in the inflow carboy and at the EC chamber (Table 2b) with a WTW (Weilheim, Germany) 340i portable oxygen meter.

2.5. Data analysis

In the experiments, Raz and Rru underwent the following processes: reaction of Raz to Rru, decay of both Raz and Rru to other unquantified byproducts; sorption of both Raz and Rru if sediment was present; and in the columns, both advection and dispersion. To estimate the different rates of these processes, we assumed that decay and reaction followed a first-order law and that sorption was at equilibrium. The system of equations for these processes is as follows:

$$\frac{\partial C_{Raz}}{\partial t} = \frac{\alpha_L v}{R}\frac{\partial^2 C_{Raz}}{\partial x^2} - \frac{v}{R}\frac{\partial C_{Raz}}{\partial x} - k_1 C_{Raz} - k_{12} C_{Raz} \tag{1a}$$

$$\frac{\partial C_{Rru}}{\partial t} = \frac{\alpha_L v}{R}\frac{\partial^2 C_{Rru}}{\partial x^2} - \frac{v}{R}\frac{\partial C_{Rru}}{\partial x} - k_2 C_{Rru} + k_{12}\frac{M_{Rru}}{M_{Raz}} C_{Raz} \tag{1b}$$

where C_{Raz} (ML^{-3}) is the concentration of Raz; C_{Rru} (ML^{-3}) is the concentration of Rru; t (T) is time; x (L) is distance from the upstream boundary; α_L (L) is the longitudinal dispersivity; v (LT^{-1}) is the mean pore water velocity; R (-) is the retardation coefficient due to sorption; k_{12} (T^{-1}) is the reaction rate coefficient for Raz to Rru; k_1 (T^{-1}) is the decay rate coefficient for Raz; k_2 (T^{-1}) is the decay rate coefficient for Rru; M_{Rru} (mol M^{-1}) is the molecular weight of Rru; and M_{Raz} (mol M^{-1}) is the molecular weight of Raz. We assume here that molecular diffusion is negligible compared to dispersion and that the dispersion coefficient $D_L = \alpha_L v$. The retardation coefficient is equivalent to $K_d \rho_b / n$, where K_d is the distribution coefficient; ρ_b is the bulk density; and n is the porosity.

For the batch experiments, $v = 0$ m/s, boundary conditions are unnecessary and the initial conditions are as follows:

$$C_{Raz}\,(t = 0) = (1 - F_{Rru}) C_{Raz,0} \tag{2a}$$

$$C_{Rru}\,(t = 0) = F_{Rru}\, C_{Raz,0} \tag{2b}$$

where $C_{Raz,0}$ (ML^{-3}) is the initial concentration of Raz; and F_{Rru} (-) is the mass fraction of Rru contamination in Raz, which we measured as 0.03.

The solution of (1) – (2) for $v = 0$ is as follows (e.g., Levine, 1988, p. 522):

$$C_{Raz} = C_{Raz,0}\left(1 - F_{Rru}\right)\exp\left[-k_{1Tot}t\right] \tag{3a}$$

$$C_{Rru} = C_{Raz,0}\left(1 - F_{Rru}\right)\frac{M_{Rru}}{M_{Raz}}\frac{k_{12}}{k_2 - k_{1Tot}} \left\{\exp\left[-k_{1Tot}t\right] - \exp\left(-k_2 t\right)\right\} + C_{Raz,0}F_{Rru}\exp\left(-k_2 t\right) \tag{3b}$$

where k_{1Tot} is the combined reaction and decay rate coefficient for Raz:

$$k_{1Tot} = k_1 + k_{12} \tag{3c}$$

For the column experiments we developed a semi-infinite model, with the following boundary and initial conditions.

$$C_{Raz}\,(x,t=0) = C_{Rru}\,(x,t=0) = 0,\ x \geq 0 \tag{4a}$$

$$C_{Raz}\,(x=0,t) = C_{Raz,0},\ t > 0 \tag{4b}$$

$$C_{Rru}\,(x=0,t) = F_{Rru}\, C_{Raz,0},\ t > 0 \tag{4c}$$

$$C_{Raz}\,(x \to \infty,\ t) = C_{Rru}\,(x \to \infty,\ t) = 0,\ t > 0 \tag{4d}$$

The solution to (1-2) and (4a-d), can be developed from the basic solution to the advection-dispersion equation without reaction using the method of Sun et al. (1999). The solution is as follows:

$$C_{Raz}(x,t)=\frac{C_{Raz,0}(1-F_{Rru})}{2}\Big\{\exp\left[\frac{Pe}{2}(1-B_{Raz})\right]\cdot erfc\left[\frac{1-A_{Raz}t^{1/2}}{2(t/R\tau Pe)^{1/2}}\right]+\exp\left[\frac{Pe}{2}(1+B_{Raz})\right]\cdot erfc\left[\frac{1+A_{Raz}t^{1/2}}{2(t/R\tau Pe)^{1/2}}\right]\Big\} \quad (5a)$$

$$C_{Rru}(x,t)=\frac{C_{Raz,0}}{2}\left[\frac{(1-F_{Rru})y_{12}k_{1Tot}}{k_{1Tot}-k_2}+F_{Rru}\right]\cdot\{\exp\left[\frac{Pe}{2}(1-B_{Rru})\right]erfc\left[\frac{1-A_{Rru}t^{1/2}}{2(t/R\tau Pe)^{1/2}}\right]-\frac{C_{Raz}(x,t)y_{12}k_{1Tot}}{k_{1Tot}-k_2}, \quad (5b)$$

where k_{1Tot} and F_{Rru} are the same as in (2-3), and

$$A_{Raz}=\sqrt{\frac{Pe}{R\tau}+4k_{1Tot}} \quad (5c)$$

$$A_{Rru}=\sqrt{\frac{Pe}{R\tau}+4k_2} \quad (5d)$$

$$B_{Raz}=\sqrt{1+\frac{4k_{1Tot}R\tau}{Pe}} \quad (5e)$$

$$B_{Rru}=\sqrt{1+\frac{4k_2R\tau}{Pe}} \quad (5f)$$

$$y_{12}=\frac{k_{12}}{k_{1Tot}}\frac{M_{Rru}}{M_{Raz}} \quad (5g)$$

$$Pe={}^{x}\!/_{\alpha_L}={}^{vx}\!/_{D_L} \quad (5h)$$

$$\tau={}^{x}\!/_{v} \quad (5i)$$

It is also worth noting the asymptotic values of (5a) and (5b) for an injection of constant concentration:

$$C_{Raz}(x,t\to\infty)=C_{Raz,0}(1-F_{Rru})\exp\left[\frac{1}{2}Pe\left(1-\sqrt{1+\frac{4k_{1Tot}R\tau}{Pe}}\right)\right] \quad (6a)$$

$$C_{Rru}(x,t\to\infty)=C_{Raz,0}\left[\frac{(1-F_{Rru})y_{12}k_{1Tot}}{k_{1Tot}-k_2}+F_{Rru}\right]\cdot\exp\left[\frac{Pe}{2}\left(1-\sqrt{1+\frac{4k_2\tau}{Pe}}\right)\right]-\frac{y_{12}k_{1Tot}}{k_{1Tot}-k_2}C_{Raz}(x,t\to\infty) \quad (6b)$$

Parameters (i.e., decay, sorption, and reaction) were estimated by fitting the appropriate model and minimizing the sum of squared error. In the case of the column experiments, velocities, travel times and a dispersivity for a conservative tracer were estimated from the breakthrough curves (BTCs) of EC. These values were then used in the Raz and Rru models. All Raz and Rru models were used simultaneously to estimate k_1, k_{12}, k_2, and a new value of dispersivity. A different dispersivity was fit to the EC data than to the Raz/Rru data because sorption increases dispersion and because rate limitations were not included in the model, which also increases dispersion (Kučera, 1965; Goltz and Roberts, 1987; Sánchez-Vila and Carrera, 2004). It has been shown that sorbing and nonsorbing tracers should have different dispersivities, even if nonequilibrium effects are properly modeled, due to differences in the boundary conditions at the sediment grain – water interface (Smith, 1983; Shapiro and Brenner, 1988; Grosser et al., 1991; Buckley and Loyalka, 1994).

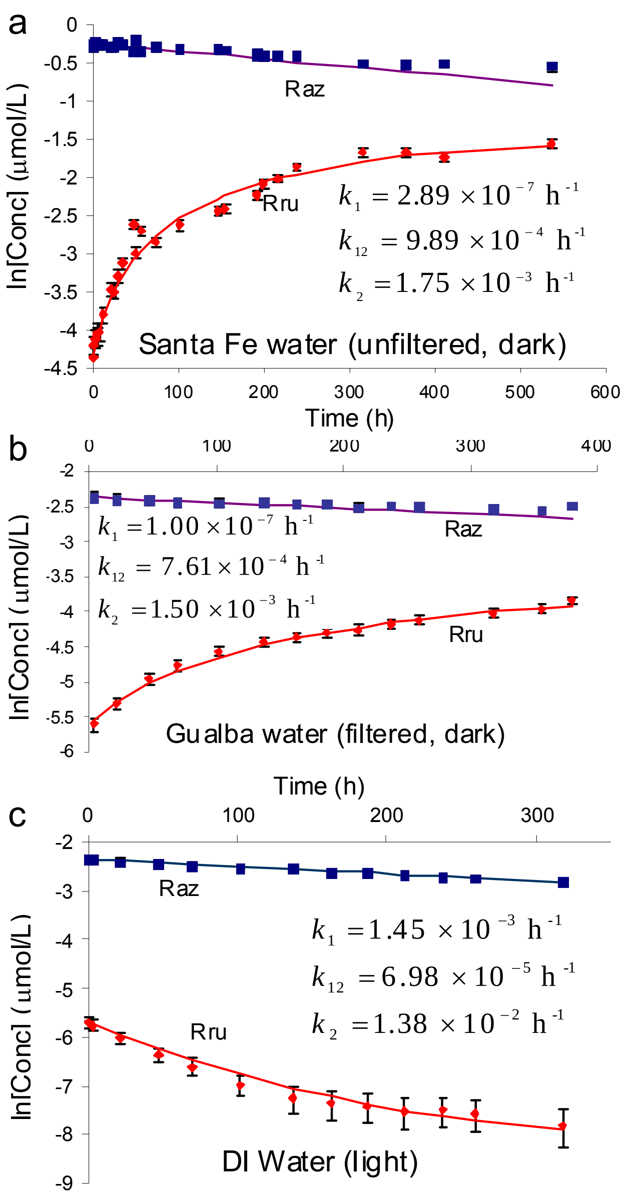


Figure 1. Reaction of resazurin (Raz) to resorufin (Rru) vs. time. (a) Raz in water from Riera de Sta. Fe de Montseny, unfiltered and stored in the dark. (b) Raz in water from Riera de Gualba, filtered and stored in the dark. (c) Raz in deionized water stored under laboratory lights. Data are shown with error bars indicating 95% confidence; some error bars are not visible because they are smaller than the data symbol. Corresponding models from (3a-b) are shown as solid lines.

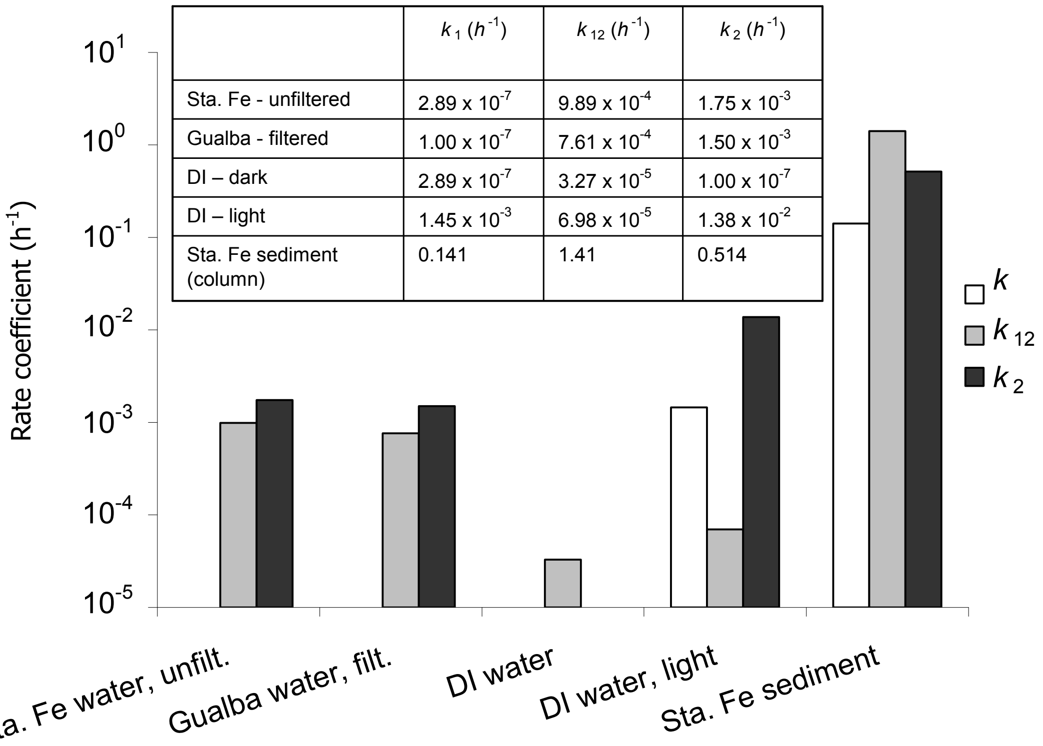
3. RESULTS

3.1. Raz and Rru parameter estimates from batch experiments

Reaction of Raz to Rru and the decay of both Raz and Rru were all well-modeled as first-order processes (3a-b). A representative set of concentration histories of both Raz and Rru from batch reactions are shown in Fig. 1. The reaction and decay processes can all fairly be called pseudo-first-order. The model for Rru generally fit within the data error bars, with the exception of a few data points for water from Sta. Fe, which were our “noisiest” data set, probably due to mistakes in sample preparation. Data for water from Gualba we re not noisy. The model for Raz fit within the error bars, except for data from close to the end of the experiment for both Sta. Fe and Gualba water. This late-time discrepancy appears to be systematic in natural water, but is probably negligible for most lab and field applications because it is only apparent after many days.

A summary of reaction and decay coefficients obtained from all experiments is given in Fig. 2. Raz and Rru have very little decay or reaction in either DIW or water from the two streams. In DIW, all rate coefficients are well below 10^{-4} h^{-1}, indicating that laboratory standards are stable over periods of several weeks if stored in the dark. In the light, Raz and Rru both undergo photochemical decay, within many 10s of hours for Rru and 100s of hours for Raz in our experiments. Biochemical/chemical decay and reaction in the two stream waters was similar. Biochemical/chemical decay of Raz was negligible, while biochemical/chemical decay of Rru was 1.50 – 1.75 x 10^{-3} h^{-1}. In stream water, reaction of Raz to Rru was slower than biochemical/chemical decay, and was slightly below 10^{-3} h^{-1}. Water filtration with a 0.7 μm glass fiber filter made no difference in the results.

The sorption isotherm (Fig. 3) was fit very well by a Freundlich isotherm with $1/n$ = 0.89 ± 0.04 and a Freundlich coefficient (K_f) of 5.15 ± 0.63 mL/g. Since the isotherm is nearly linear, we also fit a



	k_1 (h^{-1})	k_{12} (h^{-1})	k_2 (h^{-1})
Sta. Fe - unfiltered	2.89×10^{-7}	9.89×10^{-4}	1.75×10^{-3}
Gualba - filtered	1.00×10^{-7}	7.61×10^{-4}	1.50×10^{-3}
DI – dark	2.89×10^{-7}	3.27×10^{-5}	1.00×10^{-7}
DI – light	1.45×10^{-3}	6.98×10^{-5}	1.38×10^{-2}
Sta. Fe sediment (column)	0.141	1.41	0.514

Figure 2. Summary of reaction rates (k_{12}), decay rates for Raz (k_1) and decay rates for Rru (k_2) under various conditions. All samples were stored in the dark except "DI water, light". All values were obtained from a batch reactor except "Sta. Fe sediment", which were obtained from a series of column experiments (see text).

linear isotherm model to the data, yielding a distribution coefficient of 6.63 ± 0.40 mL/g.

3.2. Raz and Rru parameter estimates from column experiments

Results from column experiments with stream sediment and water (Fig. 4) show that reaction of Raz to Rru is more than 3 orders of magnitude faster than reaction measured in batch experiments with water alone, with k_{12} = 1.41 h^{-1} (compare all rate coefficients in Fig. 2). A large fraction of Raz was converted to Rru even with water residence times in column as short as 0.19 h (~ 11 min). Nearly all of the Raz was removed from solution in the column with a 1.1 h water residence time, and much of this was converted to Rru.

Travel times cause plateau concentrations to change nonlinearly. Fig. 5 shows that maximum Rru concentration was achieved in the column experiment with intermediate travel time (i.e., 0.46 h for water). Shorter travel time do not allow sufficient time for Raz to Rru reaction. Longer time (i.e., slower flow) allow more conversion of Raz to Rru, but also more time for biochemical/chemical decay and sorption of both Raz and Rru. On the other hand, the loss of oxygen across the column is approximately proportional to the travel time (Table 2), consistent with microbial respiration.

Nonequilibrium sorption, though not modeled, affects column BTCs. The fastest pumping rate (Q = 34.5 mL/h, τ = 0.19 h) has an early breakthrough relative to the equilibrium sorption model while the slow pumping rate (Q = 3.39 mL/h, τ = 1.1 h) has a

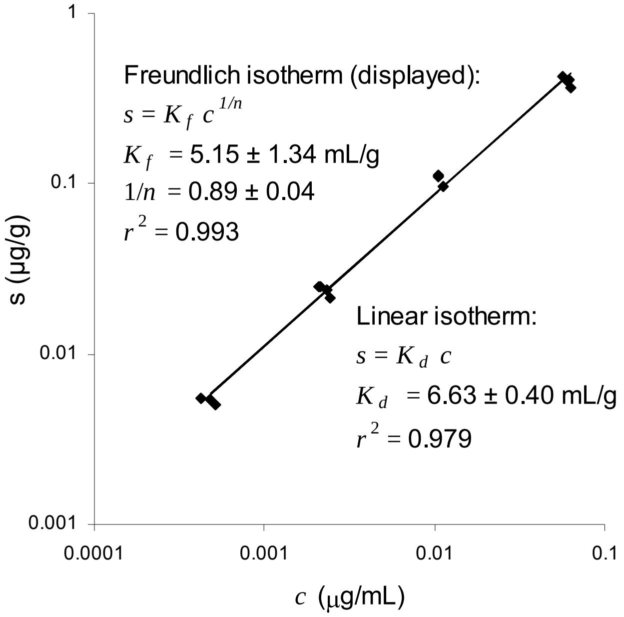


Figure 3. Sorption isotherm for Rru where *s* is sorbed concentration (MM^{-1}) and *c* is aqueous concentration (ML^{-3}).

late breakthrough relative to the equilibrium sorption model. Furthermore, even the conservative tracer BTC displays rate-limited mass transfer for all pumping rates, indicating that the mobile aqueous and immobile sorbed phases are certainly out of equilibrium. We expect that the addition of a first-order or other rate-limited mass transfer mechanism (e.g., Haggerty et al., 1995, 2000) to the model would allow a much better fit to the early-time arrivals, with the expense of a more complicated model and the loss of a fully analytical solution. However, since our primary interest is the data from plateau concentrations, which are not affected by rate limitations in mass transfer, we chose not to add this complexity to the model.

4. DISCUSSION

4.1. Resazurin as a smart tracer

Results from the experiments cautiously support the feasibility of using Raz as a smart tracer, and show that it can provide at least one "bit" more of information than current hydrological tracers in the assessment of the coupling between solute transport and biogeochemical processes in freshwater ecosystems, with particular promise for water-sediment interactions.

Our results indicate that Raz and Rru are relatively stable and nonreactive in water (without sediment) for days to weeks – a period long enough to complete laboratory or field experiments – provided that samples are stored out of the light (Fig. 2). With the exception of long residence times (100s of h) or water with high microbial abundances (e.g., untreated sewage), it appears that significant biochemical/chemical decay and Raz-to-Rru reaction in natural water is not likely. However, sample exposure to intense light (e.g., bright sunlight during field experiments) can result in significant photochemical decay. Therefore, caution should be taken with sample storage, especially in the field.

In contrast, Raz reacts to Rru quickly – in less than an hour – when water interacts with sediment (i.e., in the flow-through sediment columns). The fact that the reaction in sediment is much faster than without sediment makes Raz highly attractive as a smart tracer for water-sediment interaction.

The sorption of Raz and Rru may be of concern when considering this system as a hydrologic tracer. The breakthrough of Raz and Rru in the intermediate flow-rate column was retarded relative to that of a purely conservative tracer (chloride) from travel times of approximately 30 min to travel times of approximately 90 min; the K_d value of 6.63 mL/g (Table 1) for Rru could generate retardation factors at equilibrium as high as 60 in unconsolidated sediment. This K_d is probably maximal for our sediment because we obtained the sorbed concentration by difference from the aqueous. Since we know that Rru decays in addition to sorbs, the actual sorbed concentration is probably overestimated. In any case, other compounds currently used as hydrological tracers, such as rhodamine WT, can have equally strong sorption (Table 1). For example, Gooseff et al. (2005) measured a K_d of 33.2 mL/g for rhodamine WT in sediment with 9% organic carbon (Table 1), and Vasudevan et al. (2001) reported a host of similar or larger values.

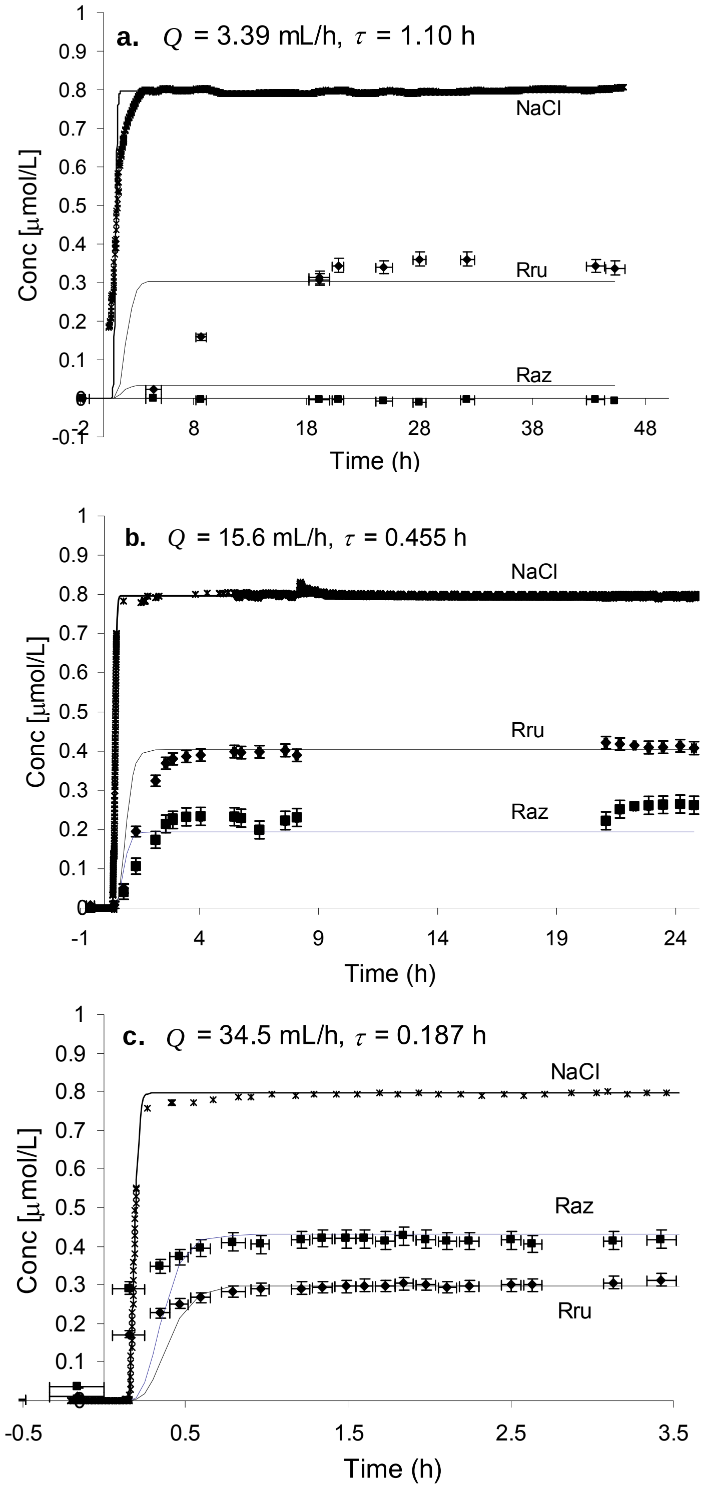


Figure 4. Breakthrough curves for Raz, Rru and NaCl (conservative tracer). Concentrations for NaCl have een rescaled so that plateau concentrations are the same as the injected concentration of Raz. Models are those given in (5a-b). Error bars in time show time needed to extract sample.

We expect that sorption of Raz and Rru will be weak when the fraction of organic carbon in the sediment is relatively small. However, measurements of sorption characteristics of Raz and Rru using a range of sediment composition and organic carbon content will help evaluate the level of sorption for this tracer. This is particularly critical if Raz-Rru is used in groundwater studies.

Raz is a likely smart tracer for microbial activity in freshwater ecosystems. Previous studies have shown that the reduction of Raz to Rru takes place in the presence of living bacteria (Guerin et al., 2001; Karakashev et al., 2003; Liu, 1983; McNicholl et al., 2006; Moyer and Campbell, 1963; O'Brien et al., 2000; Peroni and Rossi, 1986; Twigg, 1945; Zrimšek et al., 2004). This suggests that the Raz to Rru reaction in our columns is probably driven by the redox conditions generated by microbial activity associated with the sediment. This is consistent with metabolic activity in the columns indicated by our oxygen consumption measurements.

Our proposal to use Raz as a smart tracer for water-sediment interaction is supported by our findings of fast reaction in water with sediment and very slow reaction in water alone. Other research suggests that this may be expected in field studies as well. Fischer and Pusch (2001) found that bacterial production was 17-35 times higher in the upper 2 cm of sediment than in the overlying water column in a study of 6th-order River Spree in Germany. If this pattern holds across many streams, then Raz should be viable as a field tracer of water-sediment interaction.

4.2. Potential applications and future needs

Although Raz has been used in previous biological studies (e.g., Ramsdell et al., 1935, Moyer and Campbell, 1963, Erb and Ehlers, 1950; Zrimsĕk et al., 2004, O'Brien et al., 2000), to our knowledge this is the first time it has been assessed for use in hydrology. There is a great potential for application and further development of Raz and Rru as a smart “hydrobiological” tracer in both laboratory and field studies. We foresee contributions using this tracer to help bridge the gap between hydrological and biogeochemical studies in stream ecosystems. In a literature review, Pusch et al. (1998) indicated that respiration in the hyporheic zone could account for 40 to 90 % of total stream ecosystem respiration. This microbial activity is likely to influence nutrient retention at the ecosystem scale if surface-subsurface hydrological exchange is extensive. The use of Raz combined with a conservative tracer could help identify not only the magnitude of water exchange, but also could provide qualitative information on redox conditions (indicative of microbial activity) in the exchange compartments. Raz may be used in hydrologic studies to help differentiate solute transport and transient storage in parts of the system with less microbial activity from those parts with more activity, especially those associated with heterotrophic processes. Interpretation of Raz-Rru dynamics with respect to a conservative tracer (e.g., chloride) could be an indicator of sediment-water interaction in streams because of the generally greater microbial population associated with sediment than with flowing water. Lastly, the Raz-Rru system may prove useful to the assessment of hyporheic processes.

Raz may also be useful as a hydrological tracer for investigation of purely in-channel transport. For example, in-channel dispersion could be isolated with Raz. Because Raz disappears rapidly in the subsurface, the BTC of Raz would represent transport in the channel only.

One advantage of its use in field studies is the fact that Rru is highly fluorescent (quantum efficiency of 0.74) in moderately basic conditions; and thus, it is possible to be measured even at very low concentrations (<< 1 μg/L) on most spectrofluorometers. This characteristic reduces the cost of the experiments and increases the sensitivity of the results.

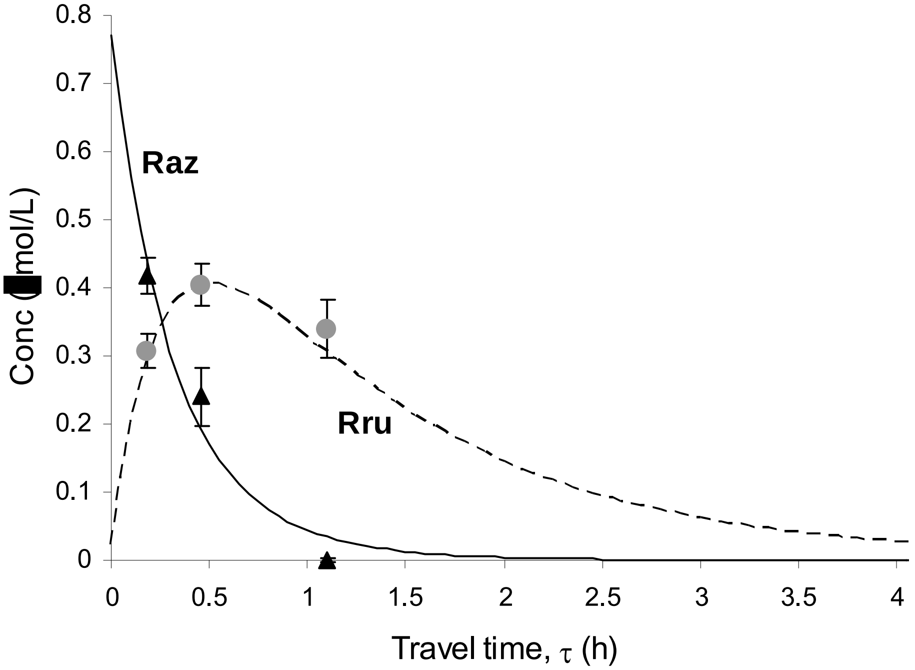


Figure 5. Concentration at plateau (averaged over plateau) vs. travel time for conservative tracer (· = *L/v*) through column as modeled by (6a-b). The model and data shown in Fig. 4, averaged over the plateau, are the same as shown here.

To make the Raz-Rru system truly effective as a smart tracer, further laboratory work is necessary to help refine the properties and to quantitatively correlate the reactions with processes of interest. For instance, we need to quantify the relationship between Raz-to-Rru reaction rates, microbial activity, and sediment surface area. This is likely to be different from one location to another, so we will need to develop a standard and simple methodology for making the correlations. The relative contribution of aerobic and anaerobic respiration to reaction rates should also be quantified. It will be important to understand which organisms are mediating the Raz-Rru reaction and through which mechanisms. As already mentioned, further work on the sorption will be necessary, particularly if it is used in groundwater. Finally, while we know that Raz and Rru are not highly toxic, further work should be done to establish details of toxicity.

We view the development of the Raz-Rru tracer system as a step forward in hydrology, because it provides more "bits" of information than a simple conservative tracer. Although in the present study we present Raz as a smart tracer, we propose that other compounds, nanoparticles, or even "nanobots" (Wood, 2005) could become smart tracers. Other materials may also work well as smart tracers of microbial activity or sediment-water interaction based on their properties (e.g., non-sorbing, less expensive, etc.). Most importantly, while our example may prove useful for characterization of the coupling between solute transport and biological activity or the amount of sediment-water interaction, other smart tracers may be able to be developed to assay other conditions of interest such as temperature, pH, or presence of a nonaqueous phase.

5. CONCLUSIONS

This study shows that Raz is a feasible smart tracer for the assessment of sediment-water interactions and associated biological activity in freshwater ecosystems. Raz reduces rapidly, as a pseudo-first-order process, with a rate coefficient of 1.41 h^{-1} in flow-through experiments with colonized sediment. The reaction in sediment is much faster than in water without sediment and is also a much faster than decay. Both compounds can be measured at low concentrations (<1 g/L) with a standard spectrofluorometer without background interference in natural waters. The compounds are stable over times long enough to complete lab or field work, but care should be taken to avoid samples exposure to intense light. Sorption of both Raz and Rru could be an issue of concern in some settings. Further work is necessary to quantitatively relate the Raz-Rru reaction to sediment-water interaction and microbial activity.

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Quantification of Metabolically Active Transient Storage (MATS) in Two Reaches with Contrasting Transient Storage and Ecosystem Respiration

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Quantificació de les zones de retenció temporals metabòlicament actives (ZRT-MA) en dos trams diferenciats en termes de retenció temporal d'aigua i respiració.

Alba Argerich[1, 2], Roy Haggerty[2], Eugènia Martí[3], Francesc Sabater[1], i Jay Zarnetske[2]

[1]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028; [2]Geosciences Department, 104 Wilkinson Hall, Oregon State University, Corvallis, OR 97331, USA;[3]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona.

RESUM

L'objectiu d'aquest estudi és quantificar les zones de retenció temporal d'aigua metabòlicament actives (ZRT-MA) en dos trams consecutius d'una mateixa riera que difereixen en les característiques hidrològiques i biològiques. L'estudi es va portar a terme en dos trams consecutius de la riera que drena la conca número 3 de l'H. J. Andrews Experimental Forest (Oregon, USA). Els dos trams difereixen en la morfologia: mentre el primer transcorre per roca mare, el segon es caracteritza per tenir una dipòsits al·luvials extensos. L'experiment va consistir en una addició combinada de Raz i NaCl durant 17 hores a la capçalera del tram 1. Addicionalment, es va mesurar la concentració d'oxigen i la temperatura simultàniament al principi i final de cada tram per tal de mesurar la respiració a escala de tram (R).

En el tram de roca mare, les ZRT-MA representaren un 37% del total de les ZRT i tenien una mida de 0,002 m^2 mentre que en el tram al·luvial, les ZRT-MA representaren el 100% de les ZRT i mesuraren 0,291m^2. La taxa efectiva de transformació de Raz en els biofilms presents al llit del tram de roca mare va ser aproximadament 16 vegades més gran que la taxa mesurada al tram al·luvial. Tanmateix, la taxa efectiva volumètrica de transformació de Raz a les ZRT–MA va ser 2,2 vegades menor en el tram de roca mare en comparació amb el tram amb dipòsits al·luvials. Aquesta diferència entre trams va ser similar a la diferència observada en R, que va ser 1,8 vegades inferior al tram 1 en comparació al tram 2. Aquesta similitud en les proporcions suggereix que les ZRT-MA van contribuir de manera significativa en la respiració. Els resultats obtinguts indiquen que la quantificació de les ZRT-MA poden millorar la nostra comprensió del paper que juguen les ZRT en els processos metabòlics. El Resazurin s'ha provat un "traçador intel·ligent" que no tan sols ens proporciona informació a escala de tram sinó que és sensible a l'heterogeneïtat espacial a una escala d'hàbitat.

Quantification of Metabolically Active Transient Storage (MATS) in Two Reaches with Contrasting Transient Storage and Ecosystem Respiration

Alba Argerich[1, 2], Roy Haggerty[2], Eugènia Martí[3], Francesc Sabater[1], and Jay Zarnetske[2]

[1]Dept. d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona 08028, Spain; [2]Geosciences Department, 104 Wilkinson Hall, Oregon State University, Corvallis, OR 97331, USA;[3]Limnology group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes 17300, Girona, Spain.

ABSTRACT

We quantified the fraction of the transient storage zone metabolically active (MATS) in two consecutive reaches with contrasting hydrological and biological characteristics. We conducted combined additions of resazurin (Raz) and Cl to quantify MATS and measured ecosystem respiration in (1) a reach scoured to bedrock where transient storage was associated only with surface compartments; and (2) a reach containing a deep alluvial deposit where transient storage was associated predominantly with hyporheic compartments. The MATS zones measured 0.002 m^2 in the bedrock reach (37% of transient storage) and 0.291 m^2 in the alluvial reach (100% of transient storage). The effective rate of Raz transformation in the MATS of the bedrock reach was approximately 16 times that of the alluvial reach. However, the volumetrically-averaged effective rate of Raz transformation in the MATS zone was 2.2 times lower in the bedrock reach than in the alluvial reach, because the total amount of MATS was much smaller in the bedrock reach than in the alluvial reach. The difference was similar to the difference in ER, which was 1.8 times lower in the bedrock reach than in the alluvial reach, suggesting that the MATS zones were important contributors to ecosystem respiration. Results indicate that the quantification of MATS can improve our understanding of the role that transient storage zones play on stream metabolic processes at a whole-reach scale and demonstrate the utility of Raz as a "smart" tracer that provides new information on metabolic activity at a whole-reach and habitat scale.

Keywords: smart tracer; resazurin; stream; hyporheic zone; respiration; transient storage, metabolism; solute addition.

1. INTRODUCTION

Transient storage in streams occurs at locations where water moves at slower velocity than the average channel velocity, and thus, in these zones, water residence time tends to be longer than in the free-flowing water of the stream channel (Bencala and Walters, 1983). Quantification of these zones has become a critical issue in biogeochemical studies of stream ecosystems, in particular, in those examining spatial or temporal variation in nutrient retention (e.g., Butturini and Sabater, 1999; Bukaveckas, 2007; Argerich et al., 2008) or metabolism (Mulholland et al., 2001). It is expected that larger transient storage may enhance the interaction between dissolved nutrients and microbial communities and, thus, increase nutrient uptake and metabolism at the whole reach scale (Fellows et al., 2006). However, while some studies support a positive relationship between nutrient uptake or metabolism and size of transient storage (Valett et al., 1996; Mulholland et al., 2001; Ensign and Doyle, 2005), others indicate a lack of consistent relationships (Webster et al., 2003; Simon et al., 2005). One of the reasons for this inconsistency may stem from the limitations of conservative hydrologic tracers, which cannot distinguish among the different stream compartments that contribute to transient storage (e.g., hyporheic zone versus surface flow eddy), even though biogeochemical processes may differ among them. Only a few attempts have been made to distinguish among compartments that contribute to transient storage and all of the studies separate the transient storage zones based on their physics and location (e.g., surface vs. hyporheic; Gooseff et al., 2005; Phanikumar et al., 2007; Zarnetske et al., 2007; Briggs et al., 2009), rather than separate them based on their metabolic characteristics.

In this study, we used an alternative philosophical approach to quantify, characterize, and distinguish between transient storage zones. We distinguish metabolically active transient storage (MATS) from a less-active or "metabolically inactive" transient storage (MITS). We used resazurin (hereafter referred to as Raz) as a "smart" tracer, the transformation of which is a proxy for metabolic activity (Haggerty et al., 2008; 2009). Raz is a weakly fluorescent phenoxazine dye that undergoes an irreversible reduction to highly fluorescent resorufin (hereafter referred to as Rru) in the presence of aerobic respiration (Karakashev et al., 2003; McNicholl et al., 2007). Haggerty et al. (2008) characterized the decay, sorption, transformation, and transport of Raz and Rru in water and saturated stream sediment. That study showed that Raz and Rru do not react or degrade in stream water alone, but that Raz does transforms quickly (1.4 h^{-1}) to Rru in colonized stream sediment. Both Raz and Rru photodegrade, but not sufficiently to pose a problem except in the brightest sunlight or over travel times of multiple hours in normal daylight. These results suggested that Raz could be a suitable tracer to help characterize MATS. More recently, Haggerty et al. (2009) tested Raz as a MATS-sensitive tracer under field conditions by conducting an injection in a headwater stream and analyzing Raz and Rru transformation at the reach scale. Results showed that Raz to Rru transformation was proportional to dissolved oxygen (DO) consumption in the hyporheic zone and moderately correlated to whole-reach instantaneous respiration rates. However, Haggerty et al. (2009) worked in a relatively homogeneous reach and made no attempt to quantify the fraction of transient storage that was metabolically active.

Our objective is to quantify the MATS in two reaches that are very different in terms of transient storage and which we hypothesize are different in MATS. Previous studies have shown that the hyporheic zone can contribute a substantial proportion of whole-stream respiration (Grimm and Fisher, 1984; Mulholland et al., 1997; Fellows et al., 2001). Therefore, we hypothesized MATS would be greater in an alluvial reach than in a bedrock reach of the same stream. At the same time, we wanted to know what fraction of the transient storage in these two reaches was metabolically active – i.e., what is the MATS fraction? Furthermore, we wanted to know how the rates of metabolism in the MATS compared between the reaches – e.g., while it was expected that the alluvial reach would have greater metabolism, would the rate of metabolism within zones identified as MATS be larger or smaller in the alluvial reach?

To answer these questions, we needed to develop a parameter estimation method for Raz transformation and MATS. OTIS-P (Runkel, 1998), although well-known among stream ecologists, does not allow the inclusion of several reaction rates and multiple

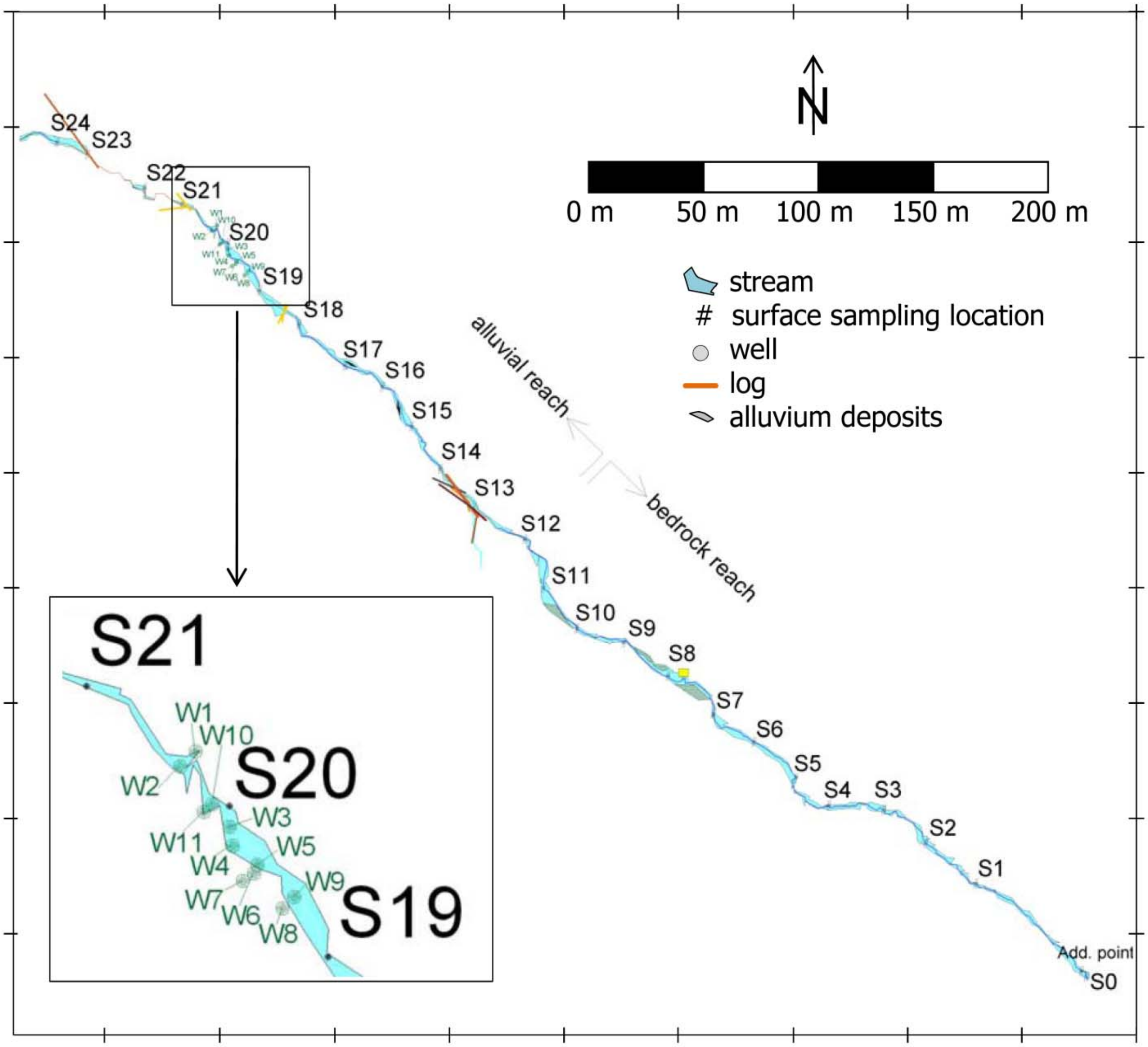

Figure 1. Map of the study section at the watershed 3 of the H. J. Andrews Experimental in the western Cascade Mountains of Oregon, USA (44º10'N, 122º15'W). Data for this map was surveyed on the 24-27th September 2007. The figure indicates the two study reaches and the location of the tributary input. Direction of water flow is from bottom-right to top-left. Point of solute injection and the location of sampling stations for surface water along the reaches are indicated with S0 to S24 symbols. The inset figure provides the detailed location of the 11 wells (W1 to W11).

compounds. In contrast, STAMMT-L (Haggerty and Reeves, 2002) can handle several reaction rates and compounds, and has been applied before in field experiments with Raz (Haggerty et al. 2009). However, STAMMT-L does not handle two sub-reaches with different conditions. Therefore, we decided to estimate the hydraulic parameters with OTIS-P and to use the temporal moments of the breakthrough curves (BTC) to estimate reaction parameters without solving the transport equations directly (Wörman, 2000; Schmid, 2003).

2. STUDY SITE

The study was done in WS03, a steep, 2nd-order stream draining a 1 km^2 catchment forested with ~32-yr-old Douglas fir (*Psudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and western red cedar (*Thuja plicata*) away from the stream and ~11-yr-old red alder (*Alnus rubra Bong*.) and willow (*Salix spp*.) in the riparian zone (Kasahara and Wondzell, 2003), and located in the H.J. Andrews Experimental Forest in the western Cascade Mountains of Oregon, USA (44º10'N, 122º15'W). The climate is characterized by wet, mild winters and dry, cool summers. Mean monthly atmospheric temperature ranges from 1 ºC in January to 18 ºC in July. Precipitation is concentrated in autumn and winter and averages 2300 to 3550 mm per year depending on the elevation. Annual stream low flows occur at the end of the summer dry season, with discharge < 3 L s^{-1}. The Raz experiment was conducted on 17-18 September 2007 and, thus, under low flow conditions.

Two consecutive reaches were considered for this study (Fig. 1). The upstream reach (hereafter referred to as the bedrock reach; SE quadrant of map) was 357.5 m long and was scoured to bedrock by debris flows in the winters of 1964-65 and 1995-96. This reach has a slope of 0.16 mm^{-1}, and cascade-pool morphology with some shallow and sparse alluvium in pools along the last 133 m (downstream of the surface sampling point S7). The downstream reach (hereafter referred to as the alluvial reach; NW quadrant of map) was 205.7 m long and was characterized by a deep alluvium deposit created by the deposition of the debris flows. This reach has a slope of 0.14 mm^{-1} and step-pool morphology. A small tributary joins the WS03 main stem at the downstream end of the bedrock reach. Haggerty et al. (2002), Johnson (2004), Gooseff et al. (2005) and Wondzell (2006), performed tracer and heat experiments in these reaches, showing that transient storage in the bedrock reach is small, rapid and confined to surface water, compared to transient storage in the alluvial reach which is large, slow and in both surface water and an extensive hyporheic zone.

3. METHODS

3.1. Stream tracer injection

The experiment consisted of a constant rate injection of a solution of 36.8 g of Raz and 3.7 kg of NaCl in 139 L of stream water using a Masterflex (Vernon Hills, IL, USA) L/S battery-powered peristaltic pump at a rate of 123 mL min^{-1}. The solution was injected at the head of the bedrock reach starting at 16:00 on 17 September 2007 and lasted for 17.3 h (i.e., until 9:20 the next day). Electrical conductivity (EC), as a surrogate for Cl concentrations, was automatically recorded every 5 minutes at the end of each reach (i.e., S12 and S20 in Fig. 1) using a Hydrolab Water Quality Multiprobe (model MiniSonde 5; Hach Company, Loveland, CO, USA). EC was also measured at 1 minute intervals in 11 wells located in the alluvial reach (Fig. 1) using 11 EC-probes connected via a multiplexor to a CR1000 data logger (Campbell Scientific, Logan, Utah, USA). Wells were located near to the end of the alluvial reach (i.e., surface sampling site S20, Fig. 1) and were installed by Steve Wondzell in the summer of 1997. Wells consisted of 3.18-cm diameter schedule 40 PVC tubes screened over the lower 50 cm (Kasahara and Wondzell, 2003; Wondzell, 2006).

Water samples for the analysis of Raz and Rru concentrations were collected over time at the downstream end of the two reaches (i.e., S12 and S20) using two ISCO autosamplers (Teledyne Isco 3700 portable sampler, Lincoln, Nebraska, USA). Sample frequency was accommodated to changes in EC and lasted until 65 hours after the start of the injection.

To characterize the longitudinal profile of EC, Raz and Rru concentrations, EC measurements and water samples were collected before the injection began (i.e., background concentration) and just

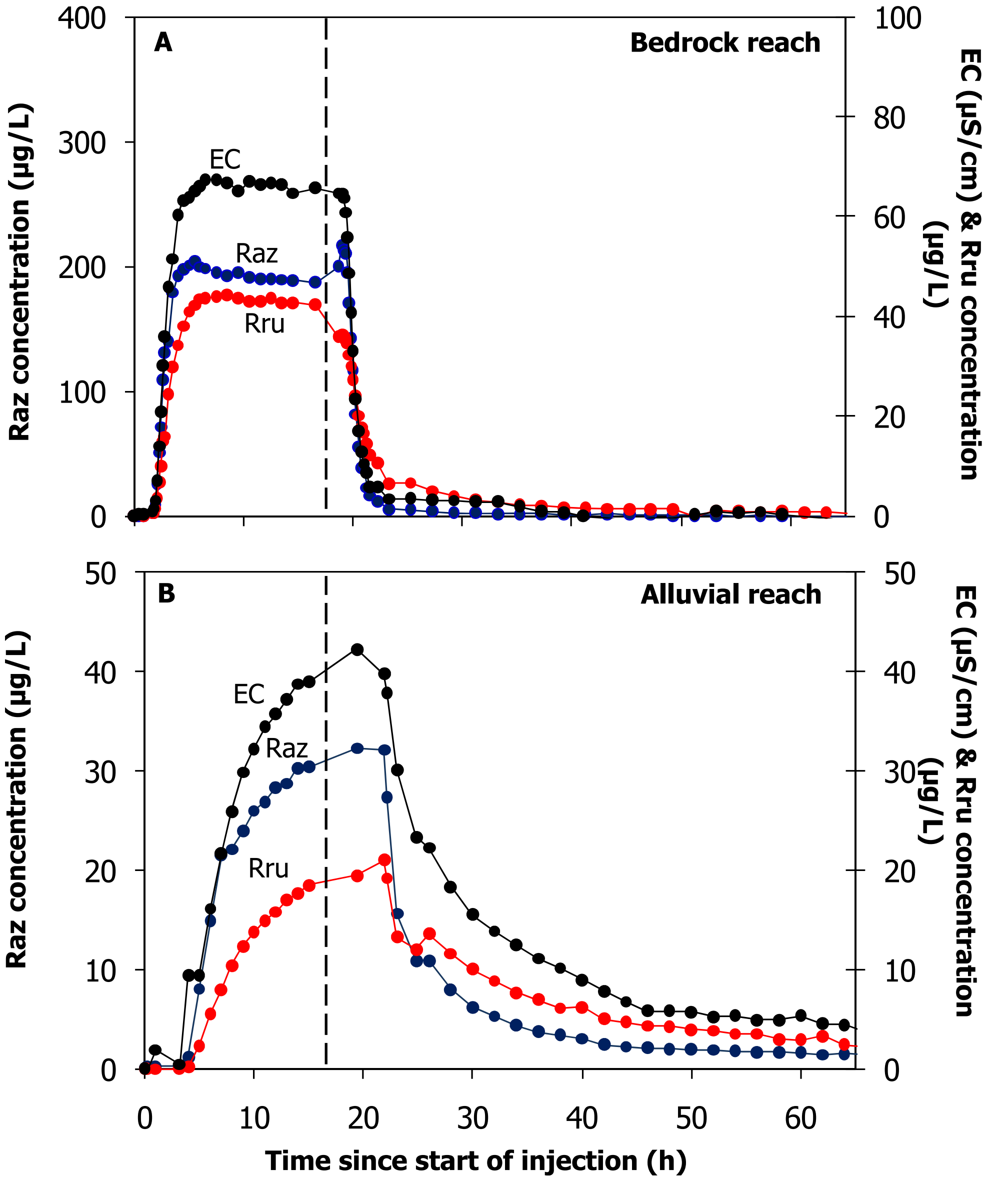

Figure 2. Break-through curves for background-corrected electrical conductivity (EC, asterisks), resazurin concentration (Raz, open circles), and resorufin concentration (Rru, closed circles) measured at the end of the bedrock reach (A) and of the alluvial reach (B). Note the difference in the range of values between Raz and Rru concentrations when comparing Raz and Rru within each reach. Vertical dashed lines show the time when injection was stopped.

before stopping the injection (i.e., plateau concentration) at 20 surface sampling sites spaced approximately 25 m along the thalweg of the reaches (Fig. 1) and at the 11 wells. Water samples were filtered through Whatman (Kent, UK) GF/F glass-fiber filters (0.7 µm pore size), stored in the dark and refrigerated at 4 ºC until analysis. Once in the laboratory, and because Raz and Rru fluorescence is constant above pH 8 but decrease sharply at pH < 6.5, samples were buffered to pH 8 prior to fluorescence analysis and processed following Haggerty et al. (2008).

3.2. Physical characterization of the reaches

The stream topography and surface water elevations were surveyed on 24 to 27 September 2007 using a Topcon GTS 223 total station (Fig. 1). During the topographic survey, the stream discharge was the same as during the tracer experiment. Stream left and right edges were located at every site that differed by more than approximately 5 cm vertically and horizontally from any adjacent site. We recorded the locations of all sampling stations, logs and other elements present in the channel. In total, approximately 500 topographic points were collected to define the banks. Data obtained from the survey was used to calculate the slope and average wet width of the two sub-reaches.

Discharge (Q, L s^{-1}) and average water velocity (u, m s^{-1}) were measured using the time-curve EC data recorded at S12 and S20. Calculation of u was done by dividing the distance between the injection point and the sampling station by the time needed to increase the EC to one half of the plateau (i.e., nominal travel time). Calculation of Q was based on a tracer mass balance approach (Shaw, 1994).

3.3. Measurement of ecosystem respiration

Daily ecosystem respiration (ER, g O_2 m^{-2} d^{-1}) in the two reaches was estimated using the upstream-downstream dissolved oxygen (DO) change technique (Bott, 2006). Concentration of DO and temperature were recorded at 5-min intervals beginning at sunset (7:20 pm) and continuing over night for 6.5 hours (1:35 am) with two Hydrolab Water Quality Multiprobes (model MiniSonde 5; Hach Company, Loveland, Colorado, USA). Measurements were done on the day prior to the injection at the bedrock reach (DO probes located at S0 and S12) and simultaneously with the injection experiment at the alluvial reach (DO probes located at S12 and S20). Percent DO saturation was estimated using DO concentration and temperature data together with a standard altitude-air pressure algorithm to correct for site altitude. The reaeration rate (K_{oxy}) at each reach was estimated based on the nighttime regression method (Young and Huryn, 1996). Exchange of DO with the atmosphere (i.e., reaeration flux) was calculated based on the average DO saturation deficit in the reach, the reaeration rate, the travel time between the two stations, and the stream discharge. Daily rates of ER were calculated as the average of instantaneous net oxygen change rates corrected by the reaeration flux measured at night-time and extrapolated to 24 h. Daily ER rates were expressed per unit of reach surface area (i.e., distance between the two stations times the average wetted width).

3.4. Surface vs. hyporheic Raz transformation

In the alluvial reach, surface water did not reach plateau conditions; and, thus, water that entered the hyporheic zone (only in the alluvial reach) had different concentrations of Raz and Rru over time. Therefore, to examine if hyporheic Raz and Rru concentrations were significantly different than those in surface water, we compared Raz and Rru concentrations in the wells at time t to those measured at the nearest surface sampling point (i.e., S20) at the time when the water entered the subsurface, $t - t_m^{well} + t_m^{surf}$, where t_m^{well} is the minimum travel time between the head of the alluvial reach and the well, t_m^{surf} the minimum travel time between the head of the alluvial reach and S20. The equation is:

$$\Delta C_{well,t}^{exp} = \Delta C_{S20}^{obs}\left(t - t_m^{well} + t_m^{surf}\right) \times SW \qquad (1)$$

where $\Delta C_{well,t}^{exp}$ is the expected concentration in the well (assuming no transformation), $\Delta C_{S20}^{obs}\left(t - t_m^{well} + t_m^{surf}\right)$ is the observed concentration in surface water and SW is the percentage of surface water that reached the well at the time of plateau sampling (t). SW was calculated as the increase in EC at wells between time t and t=0 (i.e., measured EC at pre-injection conditions) relative to the increase observed at surface water at the beginning of the alluvial reach between the time that water potentially entered to the hyporheic zone (i.e., time= $t - t_m^{well}$) and t=0.

$$SW = \frac{EC_{well}(t) - EC_{well}(t=0)}{EC_{S12}(t - t_m^{well}) - EC_{S12}(t=0)} \tag{2}$$

t_m^{well} was calculated from the first arrival time (Triska et al., 1989).

3.5. Governing equations

The mass balance equation for transport of a conservative tracer with advection, dispersion, and transient storage is (Bencala and Walters, 1983):

$$\frac{\partial C}{\partial t} = -\frac{Q}{A}\frac{\partial C}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left(AD\frac{\partial C}{\partial x}\right) - \frac{A_s}{A}\alpha_2(C - S) \tag{3}$$

$$\frac{\partial S}{\partial t} = \alpha_2(C - S) \tag{4}$$

where C and S are solute concentrations (µgL^{-1}) in the main channel and in the storage zone, respectively; A and A_s(m^2) are the cross-sectional areas of the main channel and the transient storage zone, respectively; D is the dispersion coefficient (m^{2}s^{-1}), Q is discharge (Ls^{-1}), t is time (s), x is distance (m), and α_2 (s^{-1}) is the inverse of mean residence time in transient storage. The parameter α_2 equals $\alpha A/A_s$ where α is the storage zone exchange coefficient (s^{-1}) of Bencala and Walters (1983) and many others (e.g., Runkel, 1998).

For reactive solutes, such as Raz and Rru, we need to add decay and transformation in the metabolically active transient storage (MATS) fraction (f). The equations, following Haggerty et al. (2009) and including the MATS fraction (f), are:

$$\begin{aligned}\frac{\partial C_{Raz}}{\partial t} = -\frac{Q}{A}\frac{\partial C_{Raz}}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left(AD\frac{\partial C_{Raz}}{\partial x}\right) \\ -\frac{A_s}{A}\alpha_2\big[C_{Raz} - fS_{Raz}^{\mathrm{a}} \\ -(1-f)S_{Raz}^{i}\big] - \lambda_1^{i}C_{Raz}\end{aligned} \tag{5}$$

$$\frac{\partial S_{Raz}^{\mathrm{a}}}{\partial t} = \alpha_2(C_{Raz} - S_{Raz}^{\mathrm{a}}) - \lambda_1^{\mathrm{a}}S_{Raz}^{\mathrm{a}} \tag{6}$$

$$\frac{\partial S_{Raz}^{i}}{\partial t} = \alpha_2\left(C_{Raz} - S_{Raz}^{i}\right) - \lambda_1^{i}S_{Raz}^{i} \tag{7}$$

$$\begin{aligned}\frac{\partial C_{Rru}}{\partial t} = -\frac{Q}{A}\frac{\partial C_{Rru}}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left(AD\frac{\partial C_{Rru}}{\partial x}\right) \\ -\frac{A_s}{A}\alpha_2\big[C_{Rru} - fS_{Rru}^{\mathrm{a}} \\ -(1-f)S_{Rru}^{i}\big] - \lambda_2^{i}C_{Rru} \\ +\lambda_1^{i}y^{i}C_{Raz}\end{aligned} \tag{8}$$

$$\begin{aligned}\frac{\partial S_{Rru}^{\mathrm{a}}}{\partial t} = \alpha_2(C_{Rru} - S_{Rru}^{\mathrm{a}}) - \lambda_2^{\mathrm{a}}S_{Rru}^{\mathrm{a}} \\ +\lambda_1^{\mathrm{a}}y^{\mathrm{a}}S_{Raz}^{\mathrm{a}}\end{aligned} \tag{9}$$

$$\begin{aligned}\frac{\partial S_{Rru}^{i}}{\partial t} = \alpha_2\left(C_{Rru} - S_{Rru}^{i}\right) - \lambda_2^{i}S_{Rru}^{i} \\ +\lambda_1^{i}y^{i}S_{Raz}^{i}\end{aligned} \tag{10}$$

where superscript "a" refers to the metabolically active transient storage (MATS) zone, superscript "i" refers to the metabolically inactive transient storage zone, λ_1 is the sum of Raz decay rate and Raz to Rru transformation rate (h^{-1}), λ_2 is Rru decay rate (h^{-1}), f is the MATS fraction, and y^{a} and y^{i} are conversions factors (see notation section). Other variables and parameters are defined in the notation section.

3.6. Parameter estimation

We used OTIS-P (Runkel, 1998) to estimate the dispersion coefficient (D), channel cross-sectional area (A), channel storage zone cross-sectional area (A_s/A), and storage zone exchange coefficient (α) from the BTC of EC.

To estimate the other parameters we compared the temporal moments of the breakthrough data to the moments calculated with the model. The model moments can be derived with the Laplace transform of the model (eq. 3 through to 10) and can be calculated without having to solve the equations themselves (e.g., Aris, 1958; Kučera, 1965; Goltz and Roberts, 1987; Schmid, 2003; Wörman and Wachniew, 2007). Temporal moments are defined as:

$$m_n = \int_0^\infty t^n C(x,t)dt \tag{11}$$

where m_n is the n^{th}temporal moment at a location x and at a time t. The zeroth moment is the area under the breakthrough curve and the first moment is the zeroth moment multiplied by the mean arrival time. Several properties of the moments are very useful and commonly used in hydrology. The most important property is that the zeroth moment of a conservative solute is independent of exchange with the transient storage zone, while the zeroth moment of a reacting solute is dependent on both exchange and reaction rates. Another property is that the mean arrival time of a conservative solute depends on the ratio A_s/A (expressed as β below to save space), but not on the rate of exchange with the transient storage zone (Harvey and Gorelick, 1995). To keep the equations for reactive solutes reasonably simple, we set D=0. While D does not have a large influence on the zeroth or first moments, it has a significant influence on the second moment. Therefore, the analyses that follow are based only on the zeroth and first moments. We then compared the moments from the breakthrough data to the theoretical moments predicted using the model.

The mean travel time (τ, h) between any upstream (superscript u) and any downstream (superscript d) sampling point can be calculated from the temporal moments at the upstream and downstream sampling points as (Sardin and Schweich, 1991):

$$\tau = \frac{m_{1,EC}^{d}}{m_{0,EC}^{d}} - \frac{m_{1,EC}^{u}}{m_{0,EC}^{u}} = t_n(1+\beta) \tag{12}$$

The mean travel time of a reacting solute is dependent on exchange and reaction rates. Therefore, for a reactive tracer such as Raz, the mean travel time is:

$$\tau_{Raz} = \frac{m_{1,Raz}^{d}}{m_{0,Raz}^{d}} - \frac{m_{1,Raz}^{u}}{m_{0,Raz}^{u}} = t_n\left(1 + (1-f)\beta + \frac{f\alpha_2^2\beta}{(\alpha_2+\lambda_1^a)^2}\right) \tag{13}$$

The relationship between upstream and downstream temporal moments for EC are:

$$m_{0,EC}^{d} = m_{0,EC}^{u} \tag{14}$$

$$m_{1,EC}^{d} = m_{1,EC}^{u} + m_{0,EC}^{u}(1+\beta)t_n \tag{15}$$

The relationship between upstream and downstream temporal moments for Raz are:

$$m_{0,Raz}^{d} = m_{0,Raz}^{u} e^{-L/S_{w-Raz}} \tag{16}$$

$$m_{1,Raz}^{d} = \left[m_{1,Raz}^{u} + m_{0,Raz}^{u} t_n \left(1 + \frac{\alpha_2^2\beta}{\left(\alpha_2+\lambda_1^a+\lambda_1^i\right)^2}\right)\right] e^{-L/S_{w-Raz}} \tag{17}$$

The uptake length (S_w, m), defined as the average distance travelled by a reactive molecule before decay or transformation, can be considered as a measure of the reach efficiency in processing reactive solutes (Stream Solute Workshop, 1990). There are several ways to calculate S_w, depending on the complexity of the system. The simplest one assumes that solutes are transported by advection only (mixing due to dispersion – i.e., D = 0, and transient storage does not occur) and uptake takes place only in the main channel (Stream Solute Workshop, 1990). In this study, transient storage plays an important role in reactive solute processing so we included it in the S_w for Raz. This case, which is consistent with, but expanded from, the S_w type II of Runkel, 2007 (who also assumed D = 0), yields:

$$S_{w-Raz} = \frac{Q}{A}\left(\lambda_1^i + \beta f \frac{\alpha_2 \lambda_1^{\mathrm{a}}}{\alpha_2 + \lambda_1^{\mathrm{a}}} + \beta(1-f)\frac{\alpha_2 \lambda_1^i}{\alpha_2 + \lambda_1^i}\right)^{-1} \tag{18a}$$

Note that there are three terms that contribute to the transformation of Raz, which are, from left to right within the outer parenthesis: (1) transformation in the channel; (2) transformation in MATS; and (3) transformation in MITS. We can define an effective reaction rate for MATS,

$$\lambda_e^{\mathrm{a}} = \frac{\alpha_2 \lambda_1^{\mathrm{a}}}{\alpha_2 + \lambda_1^{\mathrm{a}}} \tag{18b}$$

and a volume-weighted effective reaction rate, $\beta f \lambda_e^{\mathrm{a}}$. MITS also has an effective reaction rate and a volume-weighted effective reaction rate of the same form.

Equation (18a) can be re-written with the effective reaction rates:

$$S_{w-Raz} = \frac{Q}{A}\left(\lambda_1^i + \beta f \lambda_e^{\mathrm{a}} + \beta(1-f)\lambda_e^i\right)^{-1} \tag{19a}$$

where λ_e^{a} is the effective reaction rate in the MATS [h^{-1}], and λ_i^{a} is the effective reaction rate in the MITS [h^{-1}]. In most circumstances (where transformation in the water column is slow relative to transformation in the benthos and hyporheos), we expect the following simplification for Raz to be valid:

$$S_{w-Raz} = \frac{Q}{A\beta f \lambda_e^{\mathrm{a}}} = \frac{u}{\beta f \lambda_e^{\mathrm{a}}} \tag{19b}$$

In words, the uptake length is the velocity divided by the volume-weighted effective reaction rate of the MATS zone.

An important point is that the effective reaction rate λ_e^{a} encompasses the combined consequences of hydrology and biology on Raz transformation (which is a proxy for metabolism). When exchange is very fast relative to reaction, $\lambda_e^{\mathrm{a}} \cong \lambda_1^{\mathrm{a}}$, then stream metabolism is controlled by biology (which, in turn, could be controlled by other factors such as nutrients, grazing pressure, or temperature). However, when exchange is very slow relative to reaction, $\lambda_e^{\mathrm{a}} \cong \alpha_2$, then stream metabolism is controlled by hydrology.

The uptake length can be calculated from the data from a short injection (non-plateau) using the upstream and downstream temporal moments (16), which was implicitly conjectured by Tank et al. (2008) in the use of pulse injections to measure uptake length, and which can be proven using temporal moments:

$$S_{w-Raz} = -\frac{L}{ln\left(m_{0,Raz}^{d} \big/ m_{0,Raz}^{u} \right)} \tag{20}$$

Uptake velocity, (the average velocity at which reactive solutes are transferred to the benthos; V_f, mm/min) can then be calculated by dividing u times h by S_w (Stream Solute Workshop, 1990).

In order to calculate decay and reaction rates in the MATS zone we assumed decay and reaction rates in the main channel and in the MITS to be equal to those obtained experimentally by Haggerty et al. (2008) in stream water. Decay and reaction rates were measured in stream water (See Notation section) and found to be very low in comparison to values observed in sediments. We used a nonlinear least squares fitting algorithm to find the values of λ_1^a and f that minimized the error between the observed and the theoretical zeroth and first temporal moments for Raz.

4. RESULTS

4.1. Physical and biological characterization of the reaches

Discharge was 1.25 Ls^{-1} at the bottom of the bedrock reach and increased to 1.34 Ls^{-1} at the bottom of the alluvial reach, mainly due to the inflow from the small tributary. The bedrock reach

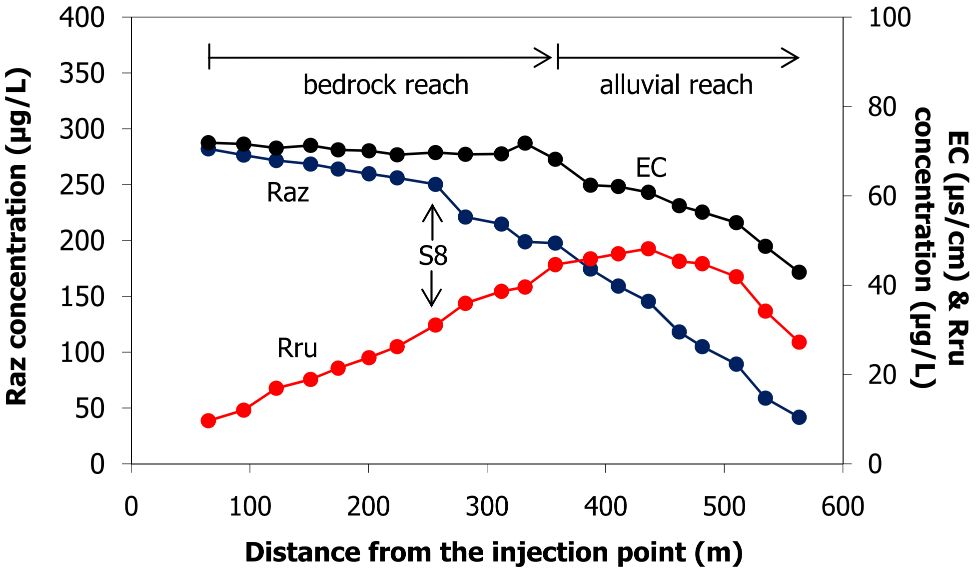

Fig. 3. Variation of background-corrected electrical conductivity (EC, asterisks), resazurin concentration (Raz, open circles), and resorufin concentration (Rru, closed circles) in surface water along the two reaches. Raz and Rru concentrations have been multiplied by the dilution factor (i.e., by EC at the injection site/EC at the sampling site). Samples were collected before the injection was stopped (i.e., after 17 hours of solute injection). The arrow highlights a change/break in Raz and Rru longitudinal profile near the end of the bedrock reach (station S8).

had a narrower and shallower wetted channel with 2.7 x faster water velocity than the alluvial reach (Table 1). Although the bedrock reach was 1.7 x longer than the alluvial reach, the nominal travel time was only 0.6 x that of the alluvial reach, and the mean travel time was 0.23 x that of the alluvial reach (Table 2).

Average water temperature during night was similar in both reaches and averaged ca. 12 ºC (Table 1). During this period, DO saturation was relatively high in both reaches, but was 2.2% higher in the bedrock reach than in the alluvial reach (Table 1; Wilcoxon text, *Z*=-7.57, *d.f.*=76, *p*<0.001).

Whole-reach daily ER was 1.8 x higher in the alluvial reach than in the bedrock reach (Table 1). Regression methods for estimating k_{oxy} gave significant slopes and intercepts (*p*<0.001) and very low k_{oxy} values (<0.001 min^{-1}) in both reaches. In the bedrock reach, instantaneous respiration was stable during the measurement period (0.066 ± 0.000 mg O_2 m^{-2} min^{-1}). In the alluvial reach instantaneous respiration was relatively constant until two hours after the sunset (0.118 ± 0.001 mg O_2 m^{-2} min^{-1}) and then gradually increased, reaching its maximum (0.145 mg O_2 m^{-2} min^{-1}) by the end of the measurement period.

4.2. Surface results of solute tracers

4.2.1 Analysis of the breakthrough curves (BTC): The shapes of the EC profiles over time indicate clear differences between the hydraulic characteristics of the two reaches. The bedrock reach showed a BTC for EC typical of a reach with moderate A_s/A, with a sharp rise, a shoulder on the rising limb, a sharp decline, and a low-concentration tail (Fig. 2A). In contrast, the alluvial reach showed a BTC for EC typical of a reach with large A_s/A, with a slow rise and a slow fall with an extended tail (Fig.

Table 1. Physical characteristics and ecosystem respiration rates of the two reaches.

	bedrock reach (S0-S12)	alluvial reach (S12-S20)
Reach length (m)	357.5	205.7
Slope (mm^{-1})	0.16	0.14
Discharge (Ls^{-1})	1.25	1.34
Water velocity (cm s^{-1})	3.68	1.35
Average wetted width (m)	1.91	2.04
Average depth (cm)	1.78	4.85
Temperature (ºC)[a,b]	12.0 ± 0.0	11.9 ± 0.1
DO saturation (%)[a,b]	94.54 ± 0.01	92.34 ± 0.02
Ecosystem respiration (g O_2 m^{-2} d^{-1})[a]	0.24	0.44

[a]Temperature, DO saturation, and ecosystem respiration were measured during 6 hours at night beginning at sunset. Average ecosystem respiration rate from this period was extrapolated to 24 h to estimate a daily rate.

[b]Average ± SE.

2B). A_s/A was 0.19 in the bedrock reach and 2.45 in the alluvial reach (Table 2). The bedrock reach arrived at plateau 6.5 h after the injection began and recovered to pre-addition values 23 h after the injection was stopped. The alluvial reach did not arrive at plateau during the injection and did not recover to pre-injection values by the end of the measurements (49 h after the injection was stopped).

In the bedrock reach, Raz was higher than Rru (Fig. 2A). During plateau, Raz and Rru slightly decreased over time. This decrease was not explained by variation in the solute injection rate because it was not observed for EC. Additionally, Raz concentration showed a sharp increase and Rru concentration showed a decrease 2 hours after the stop of the injection. By the end of the experiment we recovered 100% of the mass of Cl injected, but only 90% of the sum of Raz and Rru injected. In the alluvial reach, Raz was also higher than Rru (Fig. 2B) but the difference between Raz and Rru concentrations was smaller than in the bedrock reach. Raz and Rru concentrations gradually increased until 2 hours after the end of the injection and then gradually decreased. We recovered 95% of the mass of Cl injected. Accounting for the loss of Cl, we only recovered 37.2% of the mass of Raz and Rru that entered the alluvial reach.

4.2.2 Longitudinal profile: Along the bedrock reach, the longitudinal EC profile was relatively steady. Raz concentration decreased uniformly until S8 where a sharp decline was observed (Fig. 3). In contrast, Rru concentration gradually increased along the bedrock reach. Along the alluvial reach, EC decreased with distance because we did not achieve plateau conditions at the most downstream points. As in the bedrock reach, Raz concentration gradually decreased along the reach. However, Rru concentration increased with distance in the first 3 sampling sites (i.e., from S13 to S15) and then gradually decreased towards the downstream end of the reach (Fig. 3).

4.2.3: Transport and transformation of Raz: The uptake length for Raz was 7.8 times longer in the bedrock reach than in the alluvial reach (Table 2). The uptake velocity ($V_{f\text{-}Raz}$) was 0.13 as fast in the bedrock reach as in the alluvial reach. The fraction of MATS was 0.37 in the bedrock reach but was 1.0 in the alluvial reach. In the alluvial reach, we

Table 2. Hydraulic parameters, transport of Resazurin (Raz), and size of the MATS of the two reaches[a].

	Bedrock reach (S0-S12)	alluvial reach (S12-S20)
Relative size of transient storage zone (A_s/A, -)	0.19	2.45
Nominal travel time (t_n, h)	2.7	4.2
Mean travel time of EC (τ, h)	3.5	15.3
Raz uptake length ($S_{w\text{-}Raz}$, m)	1085	140
Raz uptake velocity ($V_{f\text{-}Raz}$, mm min^{-1})	0.036	0.281
MATS fraction in transient storage zone (f, -)	0.37	1.00
Size of the MATS zone (m^2)	0.002	0.291
Effective reaction rate of MATS zone (λ_e^a, h^{-1})	1.88	0.12
Volume-weighted effective reaction rate of MATS zone ($\beta f \lambda_e^a$, h^{-1})	0.13	0.29

[a]EC= electrical conductivity.

measured whole-reach ecosystem respiration simultaneously with the injection; and thus, we were able to examine the relationship between the Rru:Raz concentration ratio and the instantaneous respiration rates which was positively correlated (r^2=0.75, n=7, p=0.01, Fig. 4).

While the whole-stream measurements indicate that the bedrock reach is much slower at transforming Raz, the reaction rate within the MATS in the bedrock reach is high. The effective reaction rate λ_e^a for Raz was 16 x larger in the bedrock reach than in the alluvial reach. At the same time, the volume-weighted effective reaction rate for Raz was 2.2 x larger in the alluvial reach than in the bedrock reach, a factor which is similar in magnitude to the difference in respiration (1.8 x). We emphasize these fascinating results and will discuss them further below.

4.3. Hyporheic results

All wells except W5 and W11 showed an increase in EC within 26 h after the solute injection started, but only 3 of them reached a clear plateau before the end of the injection. The first arrival time ranged between 2.8 and 13.5 h among wells, and the degree of connection with surface water ranged between 10.6 and 65.5% (Table 3). Raz and Rru concentrations showed great variability among wells ranging between 0.00 and 26.39 µg Raz/L and 0.00 and 17.49 µg Rru/L. Raz and Rru concentrations at wells were lower than at the adjacent surface sampling point indicating a loss of both solutes in the hyporheic zone (Table 4). We further explore if these differences were due to higher transformation in hyporheic zone or were caused by hydrological factors. We compared the transformation of Raz to Rru in the hyporheic zone relative to that in the surface zone. Well 7 was excluded from the analysis since we did not observe Raz nor Rru. On average, corrected Rru:Raz concentration ratios were significantly higher in the hyporheic zone than at surface (Wilcoxon test, Z=-2.38, p=0.02, n=8).

5. DISCUSSION

5.1. WS03 dynamics and metabolism

The reaches differed in terms of both hydraulic parameters and metabolic activity. The relative size of the transient storage zone was 12.7 times larger and the mean water residence time was 4.3 longer in the alluvial reach than in the bedrock reach. The relative sizes of the transient storage zones agree with other previous studies conducted in these reaches (Gooseff et al., 2005; Wondzell, 2006). The value of A_s/A measured in the bedrock reach by Gooseff et al. (0.14) was in the same range as the present study; however, their A_s/A measured in the alluvial reach (105) was much larger than this study. Most of this difference was probably due to the different tracers used in the two studies. NaCl is much less sensitive to long residence times than rhodamine WT, and rhodamine WT can sorb to sediments while NaCl does not. In our opinion, the value measured by NaCl in this study is probably more accurate.

Metabolic activity measured by changes in DO, was higher in the alluvial than in the bedrock reach. Despite differences between reaches, daily rates of ecosystem respiration were in the range of other streams of similar size from the same region. For instance, Bott et al. (1985) measured ecosystem respiration seasonally at 4 reaches of increasing size at the H.J. Andrews Experimental Forest and the obtained values were 0.36±0.19 g O_2 m^{-2} d^{-1} in a first-order stream and 0.42±0.22 g O_2 m^{-2} d^{-1} in a second-order stream. Naiman and Sedell (1980) also found respiration values ranging between 0.2 and 0.7 g O_2 m^{-2} d^{-1} in streams from or close to the H.J. Andrews Experimental Forest. In general, respiration in this region is in the lower range of reported values for streams worldwide (Mulholland et al., 2001; Acuña et al., 2004; Ortiz-Zayas et al., 2006).

Differences in ecosystem respiration between the two study reaches were consistent with what we expected from the literature based on differences in transient storage size. Several authors have shown a positive relationship between size of transient storage zones and respiration. For instance, Mulholland et al. (2001) compared community metabolism in 8 streams draining watersheds in different biomes to determine the physical and biological factors most strongly related to metabolism and concluded that ecosystem respiration was significantly correlated with inorganic phosphorus concentration and size of the transient storage zone and, together, these factors explained 73% of the variation in ecosystem respiration. Similarly, Fellows et al. (2001) demonstrated the importance of transient storage on respiration in two montane streams from New Mexico (USA). Finally, Mulholland et al. (1997) showed that metabolism and nutrient uptake rates were substantially higher in a stream with a large transient storage zone than in a stream with a small transient storage zone.

5.2. Resazurin as a metabolic tracer

The use of Raz as a metabolic tracer is supported by (1) the difference in the whole-reach Raz processing efficiency (expressed as the uptake length and velocity of Raz, and as the volume-weighted effective Raz reaction rate) observed between reaches; and (2) the positive relationship observed between Rru:Raz ratios and instantaneous respiration rates. This relationship was also observed in a lab and field study by Haggerty et al. (2008, 2009) in which they injected Raz and measured ecosystem respiration in a Mediterranean headwater stream. In that case, the reach had higher ecosystem respiration (1.30 g O_2 m^{-2} d^{-1}) and higher Raz processing efficiency (i.e., shorter Raz uptake length, S_{w-Raz} = 674 m and faster uptake

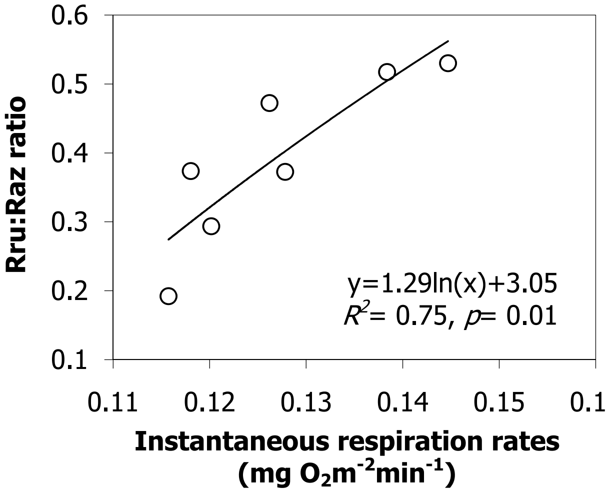

Figure 4. Relationship between instantaneous respiration rates and the resorufin:resazurin ratio estimated from the break-through curves data. Data is from the alluvial reach where the two measurements were done simultaneously.

Table 3. Transport and surface water exchange characteristics for each of the wells[a]

Well	First arrival time (h)	% SW	[Raz] (µg/L)	[Rru] (µg/L)
W1	13.5	60.5	9.92	7.23
W2	4.9	63.9	15.21	11.07
W3	7.1	65.5	26.39	17.49
W4	6.9	10.6	1.61	0.92
W6	5.0	14.1	0.25	0.18
W7	6.8	15.2	0.00	0.00
W8	2.8	48.9	4.64	3.68
W9	12.3	53.7	19.36	13.50
W10	12.2	30.3	1.33	0.99
S20	2.0	100	32.26	19.90

[a] Wells (W) were located near surface station S20 (Fig. 1). Raz and Rru are the peak concentrations scaled by %SW. For comparison, data from surface sampling station S20 are also included. Data from wells W5 and W11 are not shown because these wells did not show breakthrough.

velocity, $V_{f\text{-}Raz}$ = 0.69 mm min^{-1}) than what we observed in WS03.

The use of Raz also allowed us to observe spatial heterogeneity in metabolic activity through the analysis of the longitudinal profiles i.e., through changes in the ratios of Rru to Raz along the reach. Unfortunately, because plateau conditions were not achieved in the alluvial reach, we could only examine these changes in the bedrock reach. The longitudinal profile of Rru to Raz ratios in the bedrock reach showed a sharp increase over the last 133 m, which corresponded to a segment that, although scoured to bedrock, presented some alluvial deposits and some vegetation on the banks. Thus, this downstream portion of the bedrock reach was susceptible to contain more MATS than the upstream segment. Additionally, the great variability in Rru:Raz ratios observed at wells indicated heterogeneity in metabolic activity among flowpaths in the hyporheic zone.

The longitudinal changes seen in the bedrock reach suggest that Raz is sensitive to changes in metabolic activity at a whole-reach scale but also at a habitat scale and opens new perspectives and possibilities in the study of ecosystem metabolism. Our results support the idea of using Raz as a metabolic tracer to infer rates of ecosystem respiration *in situ* without the need to estimate reaeration rates, which is proved to be a critical source of error in metabolism measurements (McCutchan et al. 1998), and without the need to use incubation chambers which are known to be not spatially representative of ecosystem metabolism under natural conditions (Grimm and Fisher, 1984; Bott, 1996). Since the Raz to Rru reaction is irreversible, and since this reaction only happens in the presence of living organisms (McNicholl et al., 2007), this transformation could, in the future, be used as a measure of the consumption of DO in the reach.

Some open questions remain such as the loss of mass at the end of the alluvial reach, something which was also observed by Haggerty et al. (2009). There is evidence of Rru photodegradation in laboratory experiments (Haggerty et al., 2008), and thus, some of the observed loss could be explained by the exposure of the compound to sunlight during the study. In addition, we hypothesize that a fraction of the mass loss could be due to (1) a transformation of both Raz and Rru to unknown compounds and (2) to sorption to sediments and organic matter, some of which is probably irreversible on the timescale of the experiment. These issues will need to have some resolution and a quantitative relationship between Raz transformation and aerobic respiration will need development for Raz to be used to measure respiration in streams quantitatively.

5.3. Parameter estimation methods

The method of temporal moments used to calculate the model parameters is encouraging because it provided reasonable estimates of the Raz uptake length and the size of the MATS that were consistent with what we observed from the comparison of Raz and Rru concentrations at wells and in the adjacent surface zone. This method is easy to apply with the use of a spreadsheet application, although it has some limitations. As observed in Wörman and Wachniew (2007) the method of temporal moments requires a solute mass-recovery of the conservative tracer higher than c.a. 70% for a reliable estimate of the storage parameters because it gives a comparatively large weight to the tail of the breakthrough curve. In our case, this did not represent a problem because we obtained a mass recovery of the conservative tracer close to 100% at the bottom of the two reaches.

5.4. Surface vs. hyporheic MATS

Metabolic activity, measured both by changes in DO and Raz transformation, was higher in the alluvial reach than in the bedrock reach. While our metabolism results agree with previous findings (i.e., higher respiration in the reach with larger transient storage), the use of Raz and the concept of MATS allow us to further understand important differences between a reach dominated by surface transient storage (the bedrock reach) and a reach dominated by hyporheic transient storage (the alluvial reach). While the transient storage size was ca. 13 times higher in the alluvial reach, ecosystem respiration was only 2 times higher. This suggests a differential metabolic contribution of the transient storage zones in the two reaches, with the MATS in the alluvial reach proportionally less active than that in the bedrock reach. In fact, reaction rates within MATS (the effective reaction rate, λ_e^a) are ca. 16 times faster in the bedrock reach (surface MATS) than in the alluvial reach (hyporheic MATS). This is an important observation to make, because it indicates that while whole-stream respiration is lower in the bedrock reach than in the alluvial reach, the MATS in the bedrock reach has higher rates of metabolism. However, the higher rate in the bedrock MATS was offset by the MATS measuring only 0.002 m^2 in the bedrock reach in comparison to 0.291 m^2 in the alluvial reach.

The effective reaction rate for Raz (λ_e^a) in the bedrock reach was 1.9 h^{-1}, a result similar to the biological reaction rate (λ_1^a) for Raz of 1.55 h^{-1} measured in colonized hyporheic sediments by Haggerty et al. (2008). However, the effective reaction rate in the alluvial reach was only 0.12 h^{-1}. The reason for the difference can be explained by examining equation 18b. The effective reaction rates result from the interaction between hydrology (i.e., α_2, the water exchange rate between free-flowing water and transient storage zones) and biology (i.e., biological reaction rates at MATS; λ_1^a). The bedrock reach exhibits a rapid exchange between the channel and the transient storage (α_2= 1.69 h^{-1}). However, to obtain an effective rate of 1.9 h^{-1}, the biological reaction rate must also be fast (>1.9 h^{-1}), a conjecture supported by the data of Haggerty et al. (2008) as well as other similar rates we have collected for Oregon streams. Therefore, we conclude that in the bedrock reach, both hydrology and biology control the whole-stream metabolic activity. In contrast, in the alluvial reach, the exchange rate between the channel and hyporheic transient storage is much slower (α_2= 0.09 h^{-1}). The effective reaction rate (λ_e^a) is 0.12 h^{-1}. It is reasonable to assume that the biological reaction rate of Raz is > 1 h^{-1}, as we have seen elsewhere. Therefore, we conclude that hydrology (specifically, the advection rate into the hyporheic zone) limits metabolic activity. In summary, surface MATS metabolism in WS03 is controlled both by hydrology and biology while hyporheic MATS metabolism is limited by the hydrology, which limits the supply rate of reactive solutes.

Findlay (1995) proposed a conceptual model in which the functional significance of the hyporheic zone from a stream ecosystem perspective was determined by surface-subsurface hydrological exchange and rates of hyporheic biogeochemical processes. Results from Battin (1999, 2000) present hydrodynamics as a template that drives microbial and biogeochemical processes on the sediment and reach scales. Results presented in this study provide evidence supporting these conceptual models and give insight into previously contradictory results in studies comparing metabolic activity in surface and hyporheic transient storage zones. For example, Romaní et al. (1998), and Gücker and Boëchat (2007) found higher metabolic activity in the surface benthic zone than in the hyporheic zone, while Grimm and Fisher (1984) and Mulholland et al. (1997) found that hyporheic transient storage was more important for metabolism and nutrient cycling. It is clear that under different conditions either surface or hyporheic transient storage can have greater metabolic activity. For example, we would hypothesize that most of the metabolism would be located in surface transient storage in a stream with large quantity of shallow surface transient storage and rapid exchange. However, hyporheic transient storage would host most of the metabolism in a stream with large quantity of rapid hyporheic exchange and without much surface transient storage (or surface transient storage that has very slow exchange or deep pools).

6. CONCLUSIONS

Results from this study provide evidence that resazurin is a sensitive tracer to quantify metabolically active transient storage. Raz to Rru ratios are shown here to be a function of respiration rates. In addition, Raz is shown to be sensitive to differences in channel configuration and morphology at the reach scale as well as to heterogeneity in spatial habitat within reach. Therefore, Raz as a "smart" tracer can overcome the limitations of conventional respiration measurement methods. For example, the method of using incubation chambers leads to difficulties in upscaling results to a whole-reach level. While the upstream-downstream dissolved oxygen change technique measures respiration at whole-reach level but does not provide information about spatial heterogeneity in metabolic rates. Since Raz to Rru conversion is an irreversible process which occurs in the presence of microbial metabolism, the Raz method could be developed to measure "in situ" stream respiration. Further, Raz provides previously unavailable information on the efficiency of stream reaches in transforming solutes via metabolic activity, and on the separation of that metabolic activity between different stream compartments.

In summary, the methodology presented in this paper allowed us to make comparisons of metabolism patterns in WS03 simultaneously at a reach and at a habitat scale. Further, the Raz tracer method is affordable (Raz used in this experiment cost less than USD $200) and easy to apply (it only requires the use of a spectrofluorometer to analyze the samples). Lastly, the simultaneous injection of a conservative tracer and Raz allows us to quantify MATS and to advance the understanding of the role that transient storage zones play on stream metabolic processes at a whole-reach scale.

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7. NOTATION SECTION:

Variable	Units	Definition
A	m^2	stream cross-sectional area
A_s	m^2	cross-sectional area of sum of storage zones
C	μg L^{-1}	concentration in stream
D	m h^{-2}	dispersion coefficient
f	-	fraction of storage zone volume that is metabolically active
L	m	reach length
h	m	water depth
M_{Raz}	g mol^{-1}	molecular weight of Raz
M_{Rru}	g mol^{-1}	molecular weight of Rru
$m_{0,EC}^{d}$	μS cm^{-1} h	zeroth moment at the downstream location for EC
$m_{0,Raz}^{d}$	μgL^{-1} h	zeroth moment at the downstream location for Raz
$m_{1,EC}^{d}$	μS cm^{-1} h^2	first moment at the downstream location for EC
$m_{1,Raz}^{d}$	μgL^{-1} h^2	first moment at the downstream location for Raz
$m_{0,EC}^{u}$	μS cm^{-1} h	zeroth moment at the upstream location for EC
$m_{0,Raz}^{u}$	μgL^{-1} h	zeroth moment at the upstream location for Raz
$m_{1,EC}^{u}$	μS cm^{-1} h^2	first moment at the upstream location for EC
$m_{1,Raz}^{u}$	μgL^{-1} h^2	first moment at the upstream location for Raz
Q	m^{3}h^{-1}	stream discharge
S^{a}	μgL^{-1}	solute concentration in metabolically active storage zone
S^{i}	μgL^{-1}	solute concentration in metabolically inactive storage zone
S_{w-Raz}	m	Raz uptake length
t	h	time
t_n	h	nominal travel time between the upstream and the downstream sampling point for free flowing water in main channel.
u	mh^{-1}	water velocity
V_{f-Raz}	mm min^{-1}	Raz uptake velocity
x	m	longitudinal coordinate
y^{a}	-	conversion factor, $y^a = \lambda_{12}^a \frac{M_{Rru}}{M_{Raz}}$
y^{i}	-	conversion factor, $y^i = \lambda_{12}^i \frac{M_{Rru}}{M_{Raz}}$
α	h^{-1}	exchange rate coefficient in the transient storage zone
α_2	h^{-1}	$\alpha_2 = \alpha A / A_S$
β	-	(A_s/A)
τ	h	mean travel time between the upstream and the downstream sampling point including time in the transient storage zone
λ_1^a	h^{-1}	Sum of Raz to Rru transformation rate and Raz decay rate in MATS

λ_{12}^{a}	h^{-1}	Raz to Rru transformation rate in MATS
λ_{2}^{a}	h^{-1}	Rru decay rate at MATS
λ_{1}^{i}	h^{-1}	Sum of Raz to Rru transformation rate and Raz decay rate in MITS (obtained experimentally from *Haggerty et al.* (2008), equivalent to k_1+k_{12}= 2.89 x 10^{-7} + 9.89 x 10^{-4} h^{-1})
λ_{12}^{i}	h^{-1}	Raz to Rru transformation rate in MITS (obtained experimentally from *Haggerty et al.* (2008), equivalent to k_{12}= 9.89 x 10^{-4} h^{-1})
λ_{2}^{i}	h^{-1}	Rru decay rate in MITS (obtained experimentally from *Haggerty et al.* (2008), equivalent to k_2= 1.75 x 10^{-3} h^{-1})

7. REFERENCE LIST

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GENERAL DISCUSSION

General Discussion

1. FACTORS CONTROLLING HYDRAULIC PARAMETERS

Several authors have pointed out the geomorphology of the stream as a major determinant of the size of the transient storage zones (Harvey and Bencala, 1993; Kasahara and Wondzell, 2003) implicitly recognizing hyporheic zone as the major contributor to transient storage. Results from the study done in WS03 at the H.J. Andrews Experimental Forest confirm this idea being the transient storage zone of the reach with alluvial deposits 49 times higher than that of the bedrock reach. However, this Ph D. research shows that the presence of in-channel flow obstacles can also modify the size of the transient storage. For instance, the experimental introduction of naturally-colonized deflectors in the in the man-made canal at Gualba multiplied transient storage by 2.3 times. In addition, we found that leaf litter accumulation in the stream channel at Santa Fe caused an increase in the size of transient storage from 0.036 m^2 to 0.056 m^2 (x 1.5 times). Nevertheless, factors controlling temporal variation of in-channel and hyporheic transient storage probably differ due to the specific properties and the hydrologic exchange with free flowing water of the two compartments.

1.1. Temporal variation of hydraulic parameters in Santa Fe stream during fall

Results presented in this dissertation indicate that temporal variation of transient storage in Santa Fe under constant baseflow conditions during fall was driven by leaf litter accumulation in the stream channel. During the leaf fall period, we observed an increase in transient storage, which we attributed to an increase in the number and extension of pools (i.e., to an increase in in-channel transient storage) generated by the accumulation of leaves in the stream channel. This finding agrees with results from previous studies examining the effect of in-stream litter accumulation on stream transient storage (Hart et al. 1999, Jin and Ward 2005, Lautz et al. 2006). Additionally, we observed a decrease in the water exchange coefficient and a loss of connectivity between the surface channel and the hyporheic zone. Although water exchange coefficient and connectivity do not represent exactly the same -the first measures a water exchange rate between the main channel and the transient storage zone (both hyporheic and in-channel), while connectivity measures the exchange of water between the surface stream and the hyporheic zone-, both parameters indicated a decrease in water exchange between the fast-flowing and slow-flowing stream compartments as leaves accumulated in the stream channel. In fact, when we combine the results from the two studies done in Santa

Fe stream, we observe a negative correlation between the water residence time in the transient storage zone, calculated as the inverse of k_2, and the average connectivity per sampling date estimated using data from upwelling and downwelling wells together (R=-0.76, p=0.03, n=8).

While temporal variation of transient storage under baseflow conditions during leaf fall was mostly associated with leaf litter accumulation, it highly depended on stream discharge under post-flood conditions. The flood had opposite effects on the stream channel hydraulics to those observed for the leaf litter inputs. The abrupt increase in discharge increased water velocity, water exchange coefficients between slow and high flow hydrological compartments (i.e., higher k_1 and k_2) and hydrologic connectivity between surface water and hyporheic zone; and it decreased the relative water transient storage size (i.e., lower A_s and A_s/A). Those results point at discharge as a key factor on the relevance of transient storage zones in relation to the cross-sectional area of the stream. Nevertheless, results on the relation between hydraulic parameters and discharge found in previous published studies are controversial. For instance, Hart et al. (1999) did not find a significant relationship between transient storage and discharge in a study conducted in the West Fork of Walter Branch (Tennessee, USA). In contrast, Valett et al. (1996), Martí et al. (1997), and Butturini and Sabater (1999) found significant relationships between discharge and the relative size of the transient storage. However, in the analysis of the data compiled from a wide range of published studies we demonstrate that k_2 is positively related and k_1 and A_s/A are negatively related with discharge (chapter 1), although the percentage of variance of the exchange coefficients explained by discharge is lower than that for transient storage size.

1.2. Temporal variation of hydraulic parameters in Santa Fe stream at different temporal scales

The range of variation in the size of transient storage and discharge measured during the study period (i.e., fall 2004) is within the range measured in a monthly basis during a two hydrological-year period (Fig. 1, unpublished data). During this period, the greater size of transient storage was observed during summer and fall, coinciding with low flow and leaf litter inputs into the channel, whereas the smallest size of the transient storage was observed during highest flows, which in Dec 2004 and Feb-March 2006. Overall, variation in discharge explains 74% of variation in the relative size of the transient storage zone ($R^2=0.74$, $A_s/A= 0.82\ Q^{-0.53}$, p=0.000, n=24) a slightly greater percentage than that obtained with data from the leaf fall period study (63% of the variation in A_s/A explained by Q).

Additionally, when we consider data from the two year period, water exchange coefficients present a negative relation with discharge ($R^2=48$, $k_1= 7.91E\text{-}05\ Q^{0.39}$, p=0.000, n=24; and $R^2=0.90$, $k_2=7.67E\text{-}05\ Q^{0.96}$, p=0.000, n=24), which was similar to what we observed in during the fall period.

The high A_s/A observed during summer, which cannot be attributable to an input of leaf litter and which occurred at slightly higher discharge and water velocity than the maximum A_s/A observed during fall 2004, suggests a seasonal shift in the relative relevance of different compartments to whole-reach transient storage. Therefore, while variation in water residence time during fall was mainly attributed to an increase in in-channel transient storage, during summers it is probably more influenced by variations in the size of the hyporheic zone.

In summary, the controls of hydraulic parameters are primarily determined by the geomorphology of the stream since it physically determines the potential size of the hyporheic zone. However, our results show that

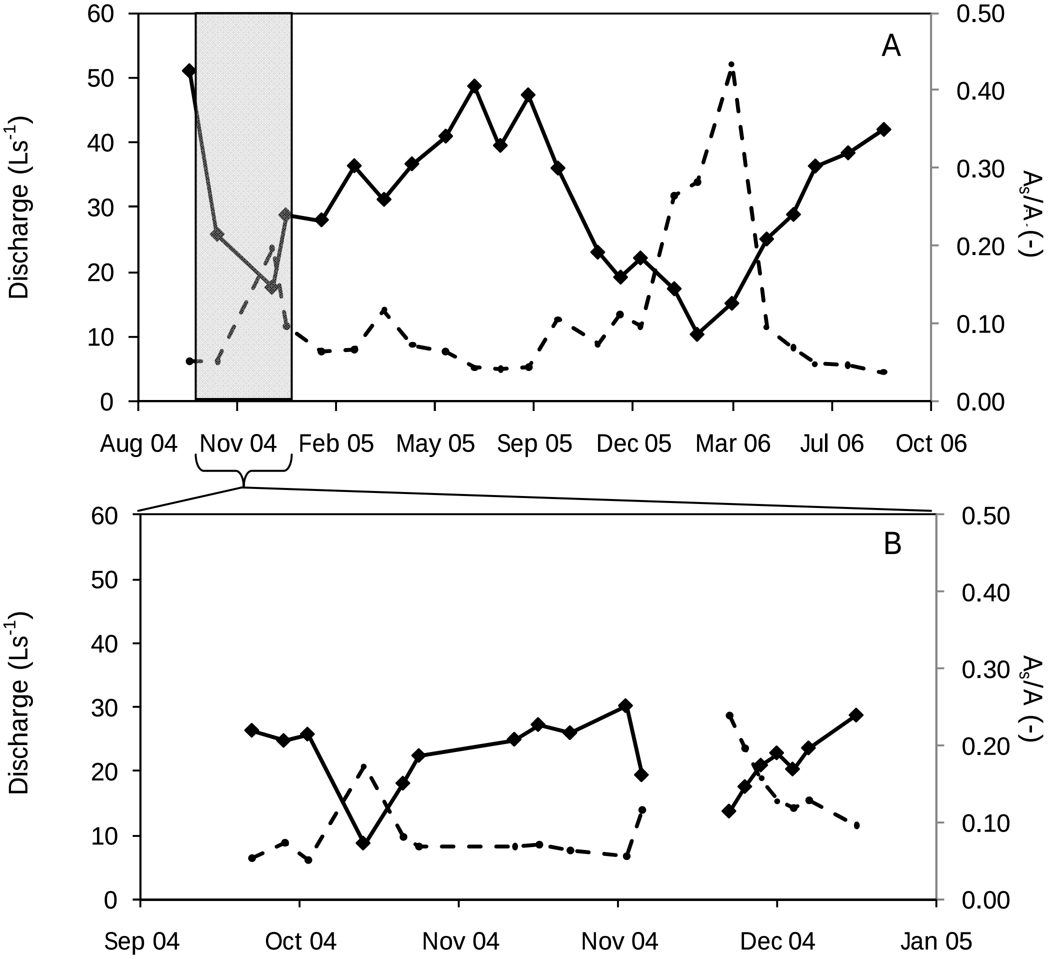

Figure 1. Temporal variation of the relative size of the transient storage respect to the cross-sectional area of the main channel (A_s/A, solid line) and discharge (discontinuous line) in Santa Fe stream. (A) Temporal variation during the 2004-2006 hydrological year period and (B) temporal variation observed during the 2004 leaf fall period. The discontinuity in the plot B reflects the occurrence of the 10 Dec flood.

discharge can modulate the hydraulic parameters both among streams and within a given stream among time. Higher discharge increases the channel cross-sectional area (A) and reduces the influence of streambed topography on surface flow; consequently, it decreases the relative importance of transient storage size (A_s). Results from Santa Fe stream also indicate that the relative contribution of different stream compartments to transient storage can vary seasonally as a result of the interaction between discharge and the input of organic debris. In this stream, with a well developed riparian canopy, temporal changes in transient storage during the leaf fall period under constant baseflow conditions are attributable to an increase in in-channel transient storage while the hyporheic zone probably determines variability in transient storage during summer periods.

2. TRANSIENT STORAGE AND NUTRIENT UPTAKE

Several authors have demonstrated the importance of hydraulic parameters (e.g., size of the transient storage, water exchange coefficients between the main channel and the storage zone) as controls of metabolic processes in streams. Results presented in this dissertation contribute to support and expand previous knowledge on this subject. The studies performed at Santa Fe and at H. J. Andrews show that stream metabolic processing capacity is strongly linked to the size of transient storage and to the water exchange between the main channel and the transient storage zone. In addition to the hydraulic control, environmental variables such as nutrient concentrations or water temperature affect biologic processing rates; and thus, may ultimately determine stream metabolic processes.

2.1. Nutrient uptake controls in Santa Fe stream during fall

In this dissertation we estimated nutrient uptake at both the whole-reach scale (chapter 1) and the hyporheic zone (chapter 2) in Santa Fe stream. The different uptake metrics used in the two studies are not directly comparable quantitatively, but qualitatively the two studies indicated that SRP uptake was higher and faster during the leaf fall period than after the flood. While SRP ambient concentration appeared to play a major control on hyporheic SRP uptake, leaf litter biomass and water temperature were key factors controlling SRP uptake at whole-reach scale. Discrepancies between the two scales of observation also appeared when examining controls of NH_4-N uptake. For instance, whole-reach NH_4-N uptake was higher during the leaf fall period than after the flood while no significant differences between periods were observed at the hyporheic level. Ambient NO_3-N concentration negatively influenced NH_4-N uptake at both, whole-reach and hyporheic zone scales. Finally, DO concentration was a determinant factor for NH_4-N uptake in the hyporheic zone (i.e., uptake increased at higher DO concentration).

Regarding the influence of hydraulic parameters on nutrient uptake, size of the transient storage was positively related to whole-reach SRP and NH_4-N uptake velocities and to NH_4-N hyporheic uptake (R^2=0.74, $NH_4\text{-}N_{obs}/NH_4\text{-}N_{exp}=-0.45-0.23\ln(A_s)$, n=6, p=0.03), but there was no influence of these parameters on hyporheic SRP uptake.

Whole-reach nutrient uptake measurements and hyporheic SRP uptake did not show any significant relationship with water residence time in the transient storage zone (i.e. the inverse of k_2 in minutes) while hyporheic NH_4-N uptake increased at longer residence times (R^2=0.91, $NH_4\text{-}N_{obs}/NH_4\text{-}N_{exp}=0.56-0.09\ln(1/k_2)$, n=6, p=0.00).

2.2. Nutrient uptake controls in Santa Fe stream at different temporal scales

Nutrient uptake lengths and uptake velocities observed in Santa Fe stream during the fall of 2004 are within the range of those measured in this stream on a monthly basis during a two-year period (von Schiller et al., 2008). The two studies registered higher demand for NH_4-N than for SRP (4.9-fold in the two-year dataset; and 3-fold during fall).

Our results indicate that the size of the transient storage explained 59% and 65% of the variability in uptake velocities of SRP and NH_4-N, respectively, during fall. In contrast, a regression analysis between nutrient uptake velocities and transient storage parameters using data the two-year data set (from von Schiller et al. 2008 and unpublished transient storage data) indicated no significant relationships between these parameters. The contrast between results including variation only from fall period and those including two-year variation suggest that relative relevance of transient storage on nutrient uptake may vary seasonally. While transient storage is determinant during the leaf fall period, at annual scale it loses its relevance in front of other environmental variables.

2.3. Differences between NH_4-N and SRP uptake

We observed a higher demand for NH_4-N than for SRP at whole-reach scale and in the hyporheic zone during the fall period (2.0-fold). This was also observed in the experiments with naturally-colonized substrata packs conducted in Gualba (chapter 3). However, in the Gualba experimental study, the quotient between the uptake coefficients of NH_4-N and SRP ranged between 1.6 and 2.6-fold depending of the type of substrata pack introduced in the channel. Other studies performed in nearby streams with siliceous geology have found similar results in the NH_4-N:SRP uptake ratio (Riera Major, Martí and Sabater, 1996; Fuirosos, von Schiller et al., 2008). Results on this ratio from studies done in other stream types are controversial. Some studies report findings similar to our study (Simon et al., 2005), others indicate a higher retention of SRP than of NH_4-N (Hanafi et al., 2006), and others did not find any difference between the two nutrients (Hoellein et al., 2007). These contrasting findings could be explained by differences in geology, substrate types, or nutrient limitation status among streams. In addition, results from Gualba indicate that differences in biologic communities with different nutrient requirements may also explain the contrasting findings among stream sites.

Overall, results presented in this dissertation indicate different controls of NH_4-N and SRP uptake probably reflecting differences in the locations where those processes are occurring. In Santa Fe stream, SRP uptake is more influenced by environmental factors affecting the surface compartments of transient storage and NH_4-N uptake is more influenced by factors affecting the hyporheic zone. Numerous studies have shown that the hyporheic zone plays an important role in the N cycling of stream ecosystems (e.g., Jones et al., 1995; Duff and Triska, 2000). The wide range of redox conditions potentially present in this zone favors a wide variety of biogeochemical reactions involved in N cycling, such as nitrification (observed in Santa Fe), denitrification and biotic assimilation. On the other hand, SRP uptake is mainly associated with biotic assimilation (Reddy et al., 1999) and thus it is expected to increase following the availability of energy-rich substrata. For instance, the leaf litter input in Santa Fe stream; which also contribute to increase in-channel transient storage.

3. METABOLICALLY ACTIVE TRANSIENT STORAGE

Results from the study done in Santa Fe stream and the experiment in Gualba show that the compartments contributing to transient storage zones may differentially influence stream biogeochemical processing due not only to their different influence on water residence time, but also to differences in the biological and chemical characteristics of each particular compartment. Common hydrologic tracer techniques do not allow characterizing the biological communities developed on the different compartments contributing to transient storage or the metabolic reactivity associated to these communities. Therefore, empirical relationships between transient storage and ecosystem processes found in the literature are surprisingly weak or even contradictory (e.g., Hall et al., 2002; Webster et al. 2003; Bukaveckas, 2007).

The use of the resazurin tracer presented in this dissertation aimed to overcome this methodological limitation by providing information of the transient storage zones from a functional perspective, in addition to that from a hydraulic perspective. In this dissertation (chapter 4) we show that resazurin is useful to a) quantify metabolically active storage zones, despite they may be physically dissimilar (e.g., pools and parts of the hyporheic zone with high respiration), and b) separate these zones from other transient storage zones with less metabolic activity. This perspective follows calls for a functional definition of transient storage based on combined hydrologic and biogeochemical characteristics (Findlay, 1995; Boulton et al., 1998, Fisher et al. 1998, Kemp et al. 2000).

The field experiment performed in WS03 H.J. Andrews Experimental Forest (chapter 5) proved the feasibility of the use of resazurin at a whole-reach scale and also showed that this tracer provides relevant information about the metabolic characteristics of the stream transient storage both at reach scale and also at smaller spatial scales. We also show that this tracer is sensitive to differentiate reaches with contrasting channel morphologies. More interestingly field results obtained with this novel technique indicated the relevance of water exchange between the surface and the hyporheic zone as a factor controlling stream metabolism. Additionally, with this dissertation we also provided a mathematically development of the metrics to quantify the relative proportion of metabolically active transient storage. It is worth note that this quantification does not involve the need to solve the equations of the transport model; and thus, making this estimation relatively easy to be broadly applied.

4. FURTHER IMPLICATIONS

Streams and their associated riparian zones provide important ecosystem goods and services through their biodiversity and their role in the cycling of water, energy, and materials (Palmer et al., 2004). The retention of nutrients can be viewed as an ecosystem process that provides a key service to human society (Postel and Carpenter, 1997) by reducing the export of inorganic nutrients to downstream ecosystems, which otherwise can generate problems associated to eutrophication. Consequently, nutrient uptake can be a significant measure of stream health or integrity (Meyer 1997, Bunn et al. 1999).

Although urbanized areas cover only approximately 1% to 6% of Earth’s surface, their footprint have extraordinary large ecological consequences and complex, strong, and often indirect effects on ecosystems (Alberti et al., 2003). According to the United Nations, all expected population growth from 2000 to 2030 (approximately 2 billion people) will be mostly concentrated in urban areas. By 2030, more than 60% (4.9 billion) of the estimated world population (8.1 billion) will live in cities (Alberti et al., 2003).

Urbanization’s increase in impervious surface area affects both geomorphological and hydrological processes and it changes fluxes of water, nutrients, and sediment into the streams (Paul and Meyer, 2001), which clearly alter nutrient retention processes (Grimm et al., 2005; Groffman et al. 2005, Meyer et al. 2005). These impacts have been referred collectively to as the Urban Stream Syndrome (Paul and Meyer 2001; Walsh et al. 2005), a term which recognizes the fact that all urban streams experience similar severe hydrologic, geomorphic and chemical alterations. Under this scenario, several rehabilitation efforts have been carried to restore stream ecosystems to their natural state which is determined by a set of reference conditions.

Results from this dissertation highlight the importance of stream geomorphology and its associated water transient storage. It also indicates that different transient storage zones can result in differences in nutrient uptake. The results also provide insights on different factors controlling temporal variation in water transient storage and highlight the relevance of intensive studies over time to capture factors that cannot be observed with studies at larger spatial or temporal scales. Additionally, results from the experimental manipulation performed at Gualba demonstrates that the use of deflectors, at least at this dimension, does not significantly increase whole-reach nutrient retention but that the use of different materials affects distinctively N and P uptake at the transient storage zone. Finally, the resazurin tracer can be a useful tool for understanding stream metabolic processes in unaltered streams (and therefore to help determining reference conditions), but also for assessing the success of restoration projects for the rehabilitation of stream functionality. Therefore, collectively, results from this dissertation can help understanding the alteration of the nutrient uptake processes associated to urban-altered streams and also provide insights on key controls of stream transient storage that can enhance the nutrient processing capacity of these ecosystems. Finally, we propose a different approach to characterize transient storage from a functional perspective, which expands the view of transient storage zones from being mere hydrological storage zones of the streams to being the metabolically active zones of these ecosystems.

CONCLUSIONS

Conclusions

Conclusions of the first chapter, "**Combined effects of leaf litter inputs and a flood on nutrient retention in a Mediterranean mountain stream during fall**":

The seasonal litter input not only modified the physical template of the reach, but also increased PO_4 and NH_4 demand, either directly through microbial demand or through increasing transient storage. The flood altered channel hydraulic properties and nutrient retention in the stream. However, once the flood receded, the stream exhibited fast recovery in nutrient retention, especially for PO_4, probably enhanced by the lateral input of riparian leaf litter. Therefore, the net contribution of leaf inputs to the stream metabolism during the leaf fall period appears to depend on the timing of floods relative to leaf fall.

Conclusions of the second chapter, "**Effects of leaf litter accumulation and discharge on hydrological exchange and hyporheic biogeochemistry in a Mediterranean headwater stream**":

Leaf litter inputs have an important role in this stream with a well developed riparian canopy, not only introducing organic matter that fuels stream metabolism and shape the morphology of the channel, but also modifying surface-hyporheic water exchange and decreasing the velocity at which surface water arrives to the hyporheic zone. These effects are counterbalanced by the effects of discharge variability, especially under flood conditions which can be common in temperate climates during fall. Increase in discharge leads to an increase in surface-hyporheic water exchange and to a homogenization of upwelling and downwelling wells in terms of connectivity. Together, these hydrologic changes have implications on the temporal variation of hyporheic water chemistry and nutrient uptake. Nevertheless, N and P showed different responses to these changes. While P uptake was controlled by hyporheic SRP concentration which did not respond to changes in connectivity, N biogeochemistry was indirectly affected by connectivity through changes in DO availability.

Conclusions of the third chapter, "**Influence of transient storage on stream nutrient uptake based on substrata manipulation**":

Results from this study confirm the hypothesis that nutrient uptake depends not only on the size of transient storage, but also on the physical and biotic features of structures involved in it and that differences among those features will generate differentiated nutrient uptake responses. The introduction of substrata packs increased water residence time, decreased average water velocity, and increased A_s homogeneously among treatments/manipulations. Although increases in transient storage did not result in changes in total uptake of ammonium and phosphate at whole reach scale, our results indicate differences among treatments basically associated with nutrient uptake in the transient storage zone.

Conclusions of the fourth chapter, "**Development of a "smart" tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: The resazurin-resorufin system"**:

This study shows that Raz is a feasible smart tracer for the assessment of sediment-water interactions and associated biological activity in freshwater ecosystems. Raz reduces rapidly, as a pseudo-first-order process, with a rate coefficient of 1.41 h^{-1} in flow-through experiments with colonized sediment. The reaction in sediment is much faster than in water without sediment and is also a much faster than decay. Both compounds can be measured at low concentrations (<1 g/L) with a standard spectrofluorometer without background interference in natural waters. The compounds are stable over times long enough to complete lab or field work, but care should be taken to avoid samples exposure to intense light. Sorption of both Raz and Rru could be an issue of concern in some settings. Further work is necessary to quantitatively relate the Raz-Rru reaction to sediment-water interaction and microbial activity.

Conclusions of the fifth chapter, "**Quantification of metabolically active transient storage (MATS) in two reaches with contrasting transient storage and ecosystem respiration"**:

Results from this study provide evidence that resazurin is a sensitive tracer to quantify metabolically active transient storage. Raz to Rru ratios are shown here to be a function of respiration rates. In addition, Raz is shown to be sensitive to differences in channel configuration and morphology at the reach scale as well as to heterogeneity in spatial habitat within reach. Therefore, Raz as a "smart" tracer can overcome the limitations of conventional respiration measurement methods. Since Raz to Rru conversion is an irreversible process which occurs in the presence of microbial metabolism, the Raz method could be developed to measure "in situ" stream respiration. Further, Raz provides previously unavailable information on the efficiency of stream reaches in transforming solutes via metabolic activity, and on the separation of that metabolic activity between different stream compartments. Lastly, the simultaneous injection of a conservative tracer and Raz allows us to quantify MATS and to advance the understanding of the role that transient storage zones play on stream metabolic processes at a whole-reach scale.

INFORME DELS DIRECTORS DE TESI

Informe dels directors de la Tesi Doctoral referent a la contribució de la doctorand en cadascun dels articles publicats

La Dra. Eugènia Martí Roca del Centre d'Estudis Avançats de Blanes (CSIC) i el Dr. Francesc Sabater i Comas del Departament d'Ecologia (UB), directors de la Tesi Doctoral elaborada per la Sra. Alba Argerich Terradas, amb el títol "Hydrological and geomorphological controls on stream nutrient retention" (Controls hidrològics i geomorfològics sobre la retenció de nutrients en rius)

INFORMEN

Que els treballs de recerca portats a terme per la Sra. Alba Argerich Terradas com a part de la seva formació predoctoral i inclosos a la seva Tesi Doctoral han donat lloc a dues publicacions, a dos manuscrits que estan essent revisats en revistes ISI d'àmbit internacional, i a un manuscrits que està a punt de ser enviat a una revista ISI d'àmbit internacional. A continuació es detalla la llista d'aquests articles:

1. Argerich, A., E. Martí, F. Sabater, M. Ribot, D. von Schiller, and J.L. Riera. 2008. Combined effects of leaf litter inputs and a flood on nutrient retention in a Mediterranean mountain stream during fall. *Limnology and Oceanography.* 53:631-641.
2. Haggerty, R, A. Argerich, and E. Martí. 2008. Development of a "smart" tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: the resazurin-resorufin system. *Water Resources Research.* 44, W00D01, doi:10.1029/2007WR006670
3. Argerich, A., E. Martí, F. Sabater, M. Ribot, 2010.Temporal variation in hydrologic exchange and hyporheic biogeochemistry in a headwater stream during leaf fall period. *Journal of the North American Benthological Society* (enviat el 3 de juny del 2010).
4. Argerich, A., R. Haggerty, E. Martí, F. Sabater, J. Zarnetske. 2010. Quantification of metabolically active transient storage (MATS) in two reaches with contrasting transient storage and ecosystem respiration. *Journal of Geophysical Research-Biogeosciences*. (enviat el 3 d'abril 2010).
5. Argerich, A., E. Martí, F. Sabater, R. Haggerty, M. Ribot. 2010. Influence of transient storage on stream nutrient uptake based on substrata manipulation. A punt de ser enviat a *Aquatic Sciences*.

Alhora, CERTIFIQUEN

Que la Sra. Alba Argerich Terradas 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 un projecte finançat pel Plan Nacional I+D del Ministerio de Ciencia e Innovación (NICON) i en un projecte integrat finançat pel 6è programa marc de la Comissió Europea (EUROLIMPACS).
- Desenvolupament de la part experimental de camp i de laboratori, així com la posada a punt de les metodologies de camp i de laboratori associades a cadascun dels experiments. Cal dir que el treball de posada a punt del mètode del traçador "resazurin" al laboratori el va desenvolupar en estreta

col·laboració amb el Dr. Roy Haggerty durant l'estada en règim de sabàtic d'aquest al CEAB-CSIC dins del grup de la Dra. E. Martí. Alhora, el treball d'aplicació al camp del traçador "resazurin" el va realitzar a l'estació experimental de H.J. Andrews Experimental Forest durant la seva estada a Oregon State University (Corvallis, Oregon, USA) amb el grup del Dr. Roy Haggerty per l'aprenentatge de metodologies de camp i el desenvolupament dels models matemàtics per descriure el transport i els processos de transformació del resazurin així com definir els paràmetres de quantificació de les zones d'emmagatzematge transitori d'aigua que són metabòlicament actives.

- Realització dels diversos experiments inclosos a cadascun dels articles.
- Processat i anàlisis 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 co-autors dels articles abans esmentats i que formen part de la Tesi Doctoral de la Sra. Alba Argerich Terradas ha utilitzat o bé té present utilitzar implícita o explícitament aquests treballs per a l'elaboració d'una altra Tesi Doctoral.

Atentament,

Eugènia Martí Roca　　　　Francesc Sabater i Comas

Blanes, 3 de juny del 2010

Informe dels directors de la Tesi Doctoral referent al factor d'impacte dels articles publicats

La Dra. Eugènia Martí Roca del Centre d'Estudis Avançats de Blanes (CSIC) i el Dr. Francesc Sabater i Comas del Departament d'Ecologia (UB), directors de la Tesi Doctoral elaborada per la Sra. Alba Argerich Terradas, amb el títol "Hydrological and geomorphological controls on stream nutrient retention" (Controls hidrològics i geomorfològics sobre la retenció de nutrients en rius).

INFORMEN

Que els treballs de recerca portats a terme per la Sra. Alba Argerich Terradas com a part de la seva formació predoctoral i inclosos a la seva Tesi Doctoral han donat lloc a dues publicacions, a dos manuscrits que estan essent revisats en revistes ISI d'àmbit internacional, i a un manuscrits que està a punt de ser enviat a una revista ISI d'àmbit internacional. A continuació es detalla la llista d'articles publicats i enviats així com els índexs d'impacte (segons el SCI de la ISI Web of Knowledge) de les revistes on han estat publicats els treballs i el nombre de citacions que han tingut fins al moment (segons el SCI de la ISI Web of Knowledge).

1. Argerich, A., E. Martí, F. Sabater, M. Ribot, D. von Schiller, and J.L. Riera. 2008. Combined effects of leaf litter inputs and a flood on nutrient retention in a Mediterranean mountain stream during fall. *Limnology and Oceanography.* 53:631-641. Citacions: 6

   L'índex d'impacte de la revista *Limnology and Oceanography* a l'any de publicació de l'article va ser de 3.663. Aquesta revista està inclosa a la categoria "Limnology". Aquesta categoria té una mediana d'índex d'impacte de 1.055 i inclou un total de 19 revistes. Tenint en compte l'índex d'impacte de *Limnology and Oceanography, aquesta* ocupa el 1^{é} lloc de la seva categoria.

2. Haggerty, R, A. Argerich, and E. Martí. 2008. Development of a "smart" tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: the resazurin-resorufin system. *Water Resources Research.* 44, W00D01, doi:10.1029/2007WR006670. Citacions: 3

   L'índex d'impacte de la revista *Water Resources Research* a l'any de publicació de l'article va ser de 2.398. Aquesta revista està inclosa a la categoria "Limnology". Aquesta categoria té una mediana d'índex d'impacte de 1.055 i inclou un total de 19 revistes. Tenint en compte l'índex d'impacte de *Water Resources Research, aquesta* ocupa el 2^{on} lloc de la seva categoria.

3. Argerich, A., E. Martí, F. Sabater, M. Ribot, 2010.Temporal variation in hydrologic exchange and hyporheic biogeochemistry in a headwater stream during leaf fall period. *Journal of the North American Benthological Society* (enviat el 3 de juny del 2010).

   L'índex d'impacte de la revista *Journal of the North American Benthological Society* al 2008 va ser de 2.360. Aquesta revista està inclosa a la categoria "Marine and Freshwater Biology". Aquesta categoria té una mediana d'índex d'impacte de 1.739 i inclou un total de 87 revistes. Tenint en compte l'índex d'impacte de *Journal of the North American Benthological Society, aquesta* ocupa el 14^{é} lloc de la seva categoria.

4. Argerich, A., R. Haggerty, E. Martí, F. Sabater, J. Zarnetske. 2010. Quantification of metabolically active transient storage (MATS) in two reaches with contrasting transient storage and ecosystem respiration. *Journal of Geophysical Research-Biogeosciences*. (enviat el 3 d'abril 2010)

   L'índex d'impacte de la revista *Journal of Geophysical Research-Biogeosciences*. al 2008 va ser de 3.147. Aquesta revista està inclosa a la categoria "Geosciences, multidisciplinary". Aquesta categoria té una mediana d'índex d'impacte de 1.223 i inclou un total de 144 revistes. Tenint en compte l'índex d'impacte de *Journal of Geophysical Research-Biogeosciences*., *aquesta* ocupa el 11é lloc de la seva categoria.

5. Argerich, A., E. Martí, F. Sabater, R. Haggerty, M. Ribot. 2010. Influence of transient storage on stream nutrient uptake based on substrata manipulation. A punt de ser enviat a *Aquatic Sciences*

   L'índex d'impacte de la revista *Aquatic Sciences* al 2008 va ser de 1.915. Aquesta revista està inclosa a la categoria "Limnology". Aquesta categoria té una mediana d'índex d'impacte de 1.055 i inclou un total de 19 revistes. Tenint en compte l'índex d'impacte de *Aquatic Sciences, aquesta* ocupa el 4rt lloc de la seva categoria.

Atentament,

Eugènia Martí Roca

Francesc Sabater i Comas

Blanes, 3 de juny del 2010

RESUM

Controls geomorfològics i hidrològics sobre la retenció de nutrients en els rius

1. INTRODUCCIÓ

El nitrogen i el fòsfor són nutrients essencials que limiten la producció primària en els ecosistemes terrestres i aquàtics. El cicle natural d'aquest dos nutrients s'ha vist greument alterat en els últims 60 anys per la mà de l'home, principalment a causa d'un canvi en les pràctiques agràries (amb un ús extensiu de fertilitzants), però també a causa de l'ús de combustibles fòssils. S'estima que l'acumulació de fòsfor (P) en els sistemes terrestres s'ha quasi quadruplicat des de l'inici de les pràctiques mineres i agrícoles a gran escala (de 1-6 Tg/any a 10,5-15,5 Tg/any, Bennet, 2001) i l'acumulació de nitrogen(N) es mou en uns rangs semblants (de ~15 Tg N yr^{-1} el 1860 a ~187 Tg N yr^{-1} el 2005; Galloway et al., 2008). L'alteració del cicle natural del P i el N ha comportat seriosos desequilibris en el funcionament dels ecosistemes (Smith, 2003), com ara l'eutrofització i la conseqüent manca d'oxigen en els ambients aquàtics. Per exemple, els episodis recurrents d'hipòxia estacionals observats al golf de Mèxic, amb greus repercussions econòmiques en les pesqueries, s'atribueixen a la càrrega de nutrients del riu Mississipi (Justic et al. 2002).

Els rius i les rieres tenen un paper fonamental en la modulació de les concentracions i les formes dels nutrients exportats aigües avall arribant a retentir fins el 50 - 75% de la càrrega de N (Alexander et al. 2000; Seitzinger et al. 2002; Peterson et al., 2001) i fins a un 30% de la càrrega de P d'una conca (Mulholland, 2004). Així doncs, i a causa de la preocupació global sobre les repercussions de l'eutrofització en els sistemes aquàtics, els estudis centrats en els factors que controlen la retenció de nutrients en els rius són crítics. En aquest sentit, aquesta tesi doctoral pretén contribuir en aquest camp, concretament, en com la morfologia i la hidrologia afecten la capacitat de transformació del N i el P dels rius.

1.2. El transport de soluts en rius

El coneixement dels mecanismes de transport de soluts en rius prové del món de la enginyeria, associat a la recerca desenvolupada per proporcionar aigua neta als nuclis urbans. En els inicis, el transport de soluts en rius es descrivia mitjançant models matemàtics que només incloïen l'efecte de l'advecció i la dispersió. Aquest models es van aplicar amb fortuna en grans rius com el Tàmesi (Whitehead i Williams, 1982), però no descrivien acuradament el moviment dels soluts en rius de capçalera. Bencala i Walters (1983) va proposar incloure a les equacions del model un terme que representés una "retenció temporal" de l'aigua. És a dir, incloure l'efecte generat per zones on l'aigua no es mou seguint el corrent principal com ara darrere de grans troncs o roques, molt freqüents en rius de dimensions petites. Les assumpcions lligades a aquest model de retenció temporal són a) que l'advecció, la dispersió i l'entrada i sortida lateral d'aigua no actuen en les zones de retenció temporal (ZRT), i b) que l'intercanvi de soluts entre el canal principal i les ZRT és proporcional a la diferència de concentració entre els dos compartiments, és a dir, que el transport des de i cap a les ZRT està governat només per processos de difusió. Els mateixos autors reconeixien que aquestes assumpcions rarament es compleixen en condicions naturals però consideraven que l'ús de les equacions usades tradicionalment per descriure els processos de difusió són una bona aproximació per descriure el retard que pateixen els soluts a mesura que són transportats aigües avall. La utilitat del model de Bencala i Walters s'ha comprovat en nombrosos estudis empírics (Legrand-Marcq i Laudelout, 1985; Bencala et al., 1990; D'Angelo et al., 1993; Hart et al., 1999) i les ZRT s'han incorporat en diferents codis computacionals com l'OTIS (Runkel, 1998) i l'STAMMT-L (Haggerty i Reeves, 2002) per modelar el transport de soluts en petits rius.

1.3. La retenció de nutrients

Abans dels anys 70, el transport de nutrients en rius s'estudiava sense tenir gaire en consideració la transformació dels nutrients que ocorria dins dels rius a mesura que aquests eren transportat aigües avall (Mulholland i Webster, 2010). No va ser fins a finals dels anys 70 que Webster i Pattern van introduir el concepte de l'espiral de nutrients (Webstern i Pattern, 1979). Aquesta teoria descriu el cicle dels nutrients en els rius a mesura que es transporten aigües avall com una gran espiral, on els nutrients són successivament transportats en forma inorgànica en la columna d'aigua, assimilats per la biota, retinguts temporalment i retornats una altra vegada a la columna d'aigua. El Stream Solute Workshop (1990) va marcar un punt d'inflexió en l'estudi de la retenció de nutrients; en aquest article, resultat de l'aportació de nombrosos científics, es van establir les bases metodològiques per a l'estudi del transport de soluts reactius mitjançant la presentació d'un model de transport que integrava processos físics, químics i biològics. L'article també va introduir 4 mètriques fonamentals per a l'estudi de la retenció de nutrients: la distància de captació (S_w, m; la distància que recorre una molècula de nutrient al llarg del riu abans de ser retinguda), la velocitat de captació (V_f, mm/s; la velocitat vertical en que una molècula de solut migra cap a la interfície aigua/sediment), la taxa de captació (U, mg $m^{-2}h^{-1}$; el flux total de nutrient transferit de la columna d'aigua a la interfície aigua-sediment) i el coeficient de captació (k_c, s^{-1}); i finalment, la relació matemàtica entre totes aquestes mètriques. Actualment la comunitat científica considera la retenció de nutrients com el resultat d'una retenció química i biològica modulada per una retenció hidrològica, que en últim terme, està determinada per la morfologia del riu i el cabal.

1.4. Les zones de retenció temporal d'aigua (ZRT)

La retenció hidrològica ocorre en les ZRT. Aquestes es localitzen en zones d'aigües estancades, que no estan sotmeses al transport aigües avall (Bencala i Walters 1983), o bé que hi estan sotmeses però on l'aigua presenta una velocitat molt menor en comparació amb la del canal principal. La mida de les ZRT usualment es reporta com una secció transversal (A_s, m^2) o bé com la seva importància en relació a la secció transversal del canal principal (A_s/A). Un dels principals contribuents de les ZRT és l'intercanvi d'aigua superficial amb la zona hiporreica (Triska et al., 1989; Morrice et al., 1997; Butturini i Sabater, 1999). Es considera zona hiporreica o hiporreu el compartiment que interacciona hidrològicament amb l'aigua superficial, és a dir, totes aquelles zones que reben aigües superficials i les retornen al canal superficial després d'un determinat temps de residència (Blanc 1993). Aquesta definició separa la zona hiporreica de la zona d'aigües subterrànies en el sentit que la zona subterrània presenta aigua que encara no ha estat influenciada pels processos del canal superficial. A part de la zona hiporreica, les ZRT també es poden generar al canal superficial a les zones d'aigua estancada, rabeigs o darrere de petites preses generades per l'acumulació de restes orgàniques (Hall et al., 2002; Lautz et al., 2006; Bukaveckas, 2007; Roberts et al., 2007; Jin et al., 2009).

La mida de les ZRT està determinada per la geomorfologia del riu i de la llera (Harvey i Bencala, 1993; Kasahara i Wondzell, 2003; Cardenas et al. 2004;Gooseff et al., 2005) i la conductivitat hidràulica del sediment (Valett et al. 1996, Morrice et al. 1997) ja que aquest controla el flux d'intercanvi d'aigua entre la superfície i les zona hiporreica. A més, la mida de les ZRT està influenciada per la complexitat del canal (Gücker i

Boëchat, 2004; Gooseff et al., 2007) i la presència d'obstacles al flux d'aigua com ara vegetació (Salehin et al., 2003, Harvey et al., 2003) o acumulació de restes orgàniques (Haggard i Storm, 2003; Lautz et al. 2006; Roberts et al. 2007). De totes maneres, l'efecte d'aquests factors està modulat pel cabal (Tonina et al., 2007). Generalment s'ha trobat que la relació entre cabal i mida de les ZRT és negativa, és a dir, que a major cabal la mida de les ZRT decreix (D'Angelo et al., 1993; Martí et al 1997; Morrice et al., 1997; Butturini i Sabater, 1999; Hall et al., 2002; Zarnetske et al. 2007) tot i que alguns autors han trobat relacions contradictòries (Legrand-Marcq i Laudelout, 1985; Hart et al., 1999).

La mida de les ZRT se sol mesurar mitjançant l'ús de traçadors (Harvey i Wagner, 2000). De manera molt similar a la quantificació de la distància d'assimilació de nutrients, el mètode consisteix en la injecció d'un traçador conservatiu en el riu (ex. Cl-) i en examinar-ne l'arribada al llarg del temps en un o varis punts aigües avall. Normalment, se sol ajustar la corba observada a una corba obtinguda mitjançant l'aplicació d'un model de transport de soluts que conté paràmetres relatius a la mida de les ZRT. La solució numèrica d'aquest model es pot obtenir mitjançant l'ús de codis computacionals com l'OTIS (One-Dimensional Transport with Inflow i Storage, Runkel, 1998) o el STAMMT-L (Haggerty i Reeves, 2002) tots dos utilitzats en algun dels estudis presentats en aquesta tesi.

Una de les limitacions més importants d'aquest mètode és que els traçadors convencionals no diferencien entre els compartiments del riu que creen les ZRT. Els paràmetres estimats amb els models com ara la secció transversal de les ZRT (A_s) o els coeficients d'intercanvi d'aigua entre les ZRT i el canal principal (k_1 o α, k_2 o α_2 –la notació varia entre estudis) s'interpreten com paràmetres agregats que representen un espectre de ZRT que ocorren simultàniament al llarg del tram. Així doncs, ZRT superficials poden generar temps d'arribada del traçador similars a les ZRT hiporreiques (Gooseff et al., 2005). Per altra banda, els processos biogeoquímics i les taxes de transformació que ocorren en distintes ZRT poden ser molt diferents entre ells (per ex. assimilació de nutrients en una bassa amb un extens biofilm vs. una bassa sense biofilm; o vs. desnitrificació a les ZRT hiporreiques) i per tant aquesta limitació és particularment crítica per entendre els mecanismes que governen la retenció de nutrients en rius. En aquesta tesi presentem un nou traçador que intenta superar aquesta limitació metodològica ja que dóna informació de les ZRT des d'un punt de vista funcional.

1.5. Relació entre les ZRT i la retenció de nutrients

Les ZRT incrementen el temps de contacte entre l'aigua i els sediments i per tant, generen més oportunitats per a la biota per processar aquests nutrients. Així doncs, les ZRT es consideren "punts calents" metabòlics. Per exemple, Triska et al. (1989) trobà que les ZRT retenien fins a un 10% del nitrat (NO_3-N) afegit durant 10 dies a Little Lost Man Creek, California. Valett et al. (1996) trobà que la S_w-NO_3 estava inversament relacionada a A_s/A en 3 rius del sud-oest americà. Mulholland et al. (1997) van trobar que les ZRT eren responsables del 43% de la retenció de P a Hugh White Creek (North Carolina), però que tenien poc efecte a Walker Branch (Tennessee). Lautz i Siegel (2007) Altres estudis no han trobat una relació directa entre la retenció de nutrients i la mida de les ZRT (Martí et al., 1997; Webster et al., 2003), una relació ambigua (positiva per alguns nutrients i negativa per altres, o diferent en funció de la mètrica utilitzada, Ensign i Doyle, 2006) o fins i tot una relació negativa (Valett et al., 2002). Una de les possibles causes d'aquesta manca d'acord en els resultats es pot atribuir a la dominació de la variabilitat en els factors ambientals per sobre de la variabilitat hidràulica en la retenció de nutrients o bé que les diferents ZTR estudiades contribueixen de manera diferent al processat de nutrients. Els estudis presentats en aquesta tesi tractaran aquestes qüestions.

2. OBJECTIUS

L'objectiu principal d'aquesta tesi és estudiar la influència que la geomorfologia i la hidrologia tenen sobre la retenció de nutrients en els rius. Tal com ja s'ha explicat a la introducció, la retenció de nutrients en els rius resulta de la combinació entre les taxes de transport de nutrients (determinada per la morfologia del canal i la hidrologia) i de les taxes de processat biològiques (determinades per les comunitats biològiques que habiten en el riu). Dins d'aquest context, la combinació de la morfologia i la hidrologia determina les "oportunitats" perquè la retenció de nutrients tingui lloc, mentre que l'activitat biòtica regularà la "capacitat" de processat. El balanç entre aquests dos components, en última instància, determinarà el flux d'exportació de nutrients aigües avall (Battin et al. 2008). Des d'aquesta perspectiva, la hipòtesi central d'aquesta tesi és que les zones de retenció temporal d'aigua tenen un paper important en el cicle de nutrients en rius, ja que augmenten el temps d'interacció entre els nutrients i la biota, i per tant, augmenten les oportunitats de la biota per processar aquests nutrients.

Aquesta tesi s'articula en dues parts i conté cinc capítols. La primera part consta de tres capítols i examina la influència de diferents factors en les ZRT i com la variació d'aquestes ZRT afecta la retenció de nutrients. El primer capítol se centra en els processos superficials i a nivell de tram mentre que el segon capítol se centra en els processos hiporreics. El tercer capítol és una manipulació experimental i se centra en la contribució que diferents tipus de substrats tenen en la retenció de nutrients i en les ZRT. La segona part de la tesi presenta el desenvolupament i l'aplicació d'un nou traçador capaç de caracteritzar les ZRT des d'una perspectiva metabòlica. Aquesta part inclou dos capítols. El primer capítol descriu els experiments de laboratori portats a terme per caracteritzar el traçador i el segon descriu l'ús del traçador en un experiment de camp i de les mètriques per quantificar les ZRT metabòlicament actives.

Els objectius detallats de cada capítol són:

El primer capítol, **"Efectes combinats de l'acumulació de fullaraca i una riuada en la retenció de nutrients en un riu mediterrani durant la tardor**", té com a objectiu estudiar la variabilitat temporal de retenció de nutrients en relació amb els paràmetres hidràulics en condicions naturals. Els objectius específics d'aquest estudi són:

> a) examinar com l'acumulació de fullaraca a la tardor modifica les propietats hidràuliques del canal, incloent la mida de les ZRT,
>
> b) avaluar la resposta de retenció de nutrients durant aquest període,
>
> c) examinar la relació entre la retenció de nutrients i l'acumulació de fulles i les propietats hidràuliques.

El segon capítol, **"Variació temporal de la connectivitat hidrològica i de la biogeoquímica de la zona hiporreica en un riu de capçalera durant la tardor**", té com a objectiu estudiar els efectes que genera l'acumulació de fullaraca en l'intercanvi d'aigua entre el compartiment superficial i l'hiporreic, i si aquestes possibles modificacions en l'intercanvi hidràulic afecten les concentracions de nutrients i oxigen, així com la retenció de nutrients a la zona hiporreica. Els objectius específics d'aquest estudi són:

> a) examinar si l'acumulació de fullaraca i les variacions en el cabal modifiquen l'intercanvi d'aigua entre el compartiment superficial i l'hiporreic,
> b) avaluar les possibles conseqüències d'aquestes modificacions en la biogeoquímica de la zona hiporreica.

El tercer capítol "**Influència de la zona de retenció temporal d'aigua en la retenció de nutrients basada en manipulacions dels substrats**", estudia la relació entre la retenció de nutrients i la mida de les

ZRT generades experimentalment mitjançant deflectors creats amb diferents tipus de substrats. L'objectiu específic d'aquest estudi és:

a) examinar la influència de les característiques físiques i biològiques dels substrats que generen ZRT en la retenció de nutrients.

El quart capítol, **"Desenvolupament d'un traçador intel·ligent per a l'estudi de l'activitat microbiològica i la interacció entre aigua i sediments en rius: el sistema resazurin-resorufin"** presenta un nou traçador que caracteritza les ZRT des d'una perspectiva metabòlica. L'objectiu específic d'aquest estudi és:

a) identificar i caracteritzar un traçador intel·ligent capaç de mesurar l'activitat microbiològica associada amb la interacció entre la columna d'aigua i el sediment en rius.

El cinquè capítol, **"Quantificació de les zones de retenció temporals metabòlicament actives en dos trams diferenciats en termes de retenció temporal d'aigua i respiració**", té com a objectiu aplicar el traçador desenvolupat en l'anterior capítol per quantificar les ZRT metabòlicament actives. L'objectiu específic d'aquest estudi és:

a) quantificar les ZRT metabòlicament actives en dos trams d'un mateix riu que difereixen en la quantitat de ZRT i presumiblement, en activitat metabòlica.

3. RESUM DE LES PUBLICACIONS

3.1. Efectes combinats de l'acumulació de fullaraca i una riuada en la retenció de nutrients en un riu mediterrani durant la tardor

Aquest estudi examina l'efecte de l'acumulació de fullaraca en la retenció hidràulica i de nutrients en un riu de capçalera mediterrani. Una riuada al final del període de caiguda de fulles creà la oportunitat per estudiar l'efecte del "rentat" sobtat de la major part de la biomassa de fulles que s'havia acumulat a la llera durant la tardor. L'estudi es va portar a terme a la riera de Santa Fe, dins del Parc Natural del Montseny entre l'octubre i el desembre del 2004. En total, es van realitzar vint-i-una addicions de clorurs per mesurar els paràmetres hidràulics i en 13 d'aquestes, també es va afegir amoni i fosfat per calcular la distància i la velocitat de captació de nutrients. Els paràmetres hidràulics del tram d'estudi es van estimar mitjançant l'OTIS, un codi computacional que permet resoldre les equacions de transport de soluts considerant processos d'advecció, dispersió, dilució i zones de retenció temporal d'aigua (ZRT).

Durant el període de caiguda de fulles (entre l'octubre i el novembre) les fulles s'acumularen gradualment en el canal de 9,3 a 217 g PS m^{-2} (unitats en grams de pes sec per metre quadrat). L'entrada de fulles causà modificacions en els paràmetres hidràulics del riu i en la disponibilitat d'hàbitat. El cabal durant aquest període es mantingué constant, al voltant dels 8 Ls^{-1}. La riuada del 10 de desembre (124 Ls^{-1}) provocà el rentat d'un 65% de les fulles que s'havien acumulat en el canal durant el període de caiguda de fulles disminuint els estocs a 77,1 g PS m^{-2}. Dues setmanes després de la pertorbació, la biomassa de fulles dins del canal havia recuperat els valors pre-riuada.

L'acumulació de fulles provocà un increment gradual de la mida de les ZRT (A_s) i una disminució dels coeficients d'intercanvi d'aigua entre el canal principal i les ZRT. La riuada generà els efectes contraris a l'acumulació de fulles. Just després de la riuada l'A_s va disminuir x2,4 vegades i k_1 i k_2 incrementaren x4,2 i x10 vegades, respectivament. Durant la recessió de la riuada A_s tendí a incrementar i k_1 i k_2 tendiren a disminuir, però al final de període de mostreig A_s encara era menor i k_1 i k_2 eren més grans que les observades durant el període de caiguda de fulles. Considerant totes les dates de mostreig, A_s:A i A_s presentaren una relació negativa amb el cabal (Q) (A_s:A=0,27$e^{-0,03Q}$, R^2=0,63, p<0,001, g.ll.=15; A_s=0,05$e^{-0,02Q}$, R^2=0,36, p<0.050, g.ll. =15), mentre que k_1 i k_2 es relacionaren positivament amb Q (k_1=6,4x10^{-5}+9,1x10^{-6}Q, R^2=0,42, p=0,005, g.ll.=15).

Durant el període de caiguda de fulles, les S_w no van mostrar cap tendència temporal clara per cap dels nutrients. En general, S_W-PO_4 i S_W-NH_4 es van mostrar relativament curtes (219 ± 57 m i 49 ± 11 m, respectivament) i la S_W-NH_4 va ser en mitjana 4 vegades més curta que la S_W-PO_4. La riuada va causar un augment dramàtic en les S_w dels dos nutrients que es van anar recuperant gradualment gairebé tornant als valors mitjans de pre-riuada al final de l'estudi. Els valors de la V_f-NH_4 van ser de mitjana 3,0 ± 1,2 vegades més grans que els de la V_f-PO_4. Les V_f tendiren a augmentar gradualment a mesura que les fulles s'acumularen en el canal. La riuada va disminuir tant la V_f-PO_4 com la V_f-NH_4 (1,2 vegades i 1,4 vegades, respectivament). En el període post-riuada la V_f-PO_4 va augmentar gradualment, arribant a valors lleugerament més alts que abans de la riuada, mentre que V_f-NH_4 es va mantenir baixa tot i que amb certa tendència cap a la recuperació dels valors pre-riuada. Les anàlisis de regressió van mostrar una relació positiva entre la V_f-PO_4 i la biomassa de fullaraca i entre la V_f-PO_4 i la temperatura mentre que la V_f-NH_4 es va relacionar negativament amb la concentració de DIN (nitrogen inorgànic dissolt) i la relació molar DIN:P. Ambdues V_f es van mostrar positivament relacionades amb A_s.

3.2. Variació temporal de la connectivitat hidrològica i de la biogeoquímica de la zona hiporreica en un riu de capçalera durant la tardor

Aquest estudi analitza els efectes de l'acumulació de fullaraca i de les variacions de cabal en l'intercanvi d'aigua entre el compartiment superficial i l'hiporreic i les seves possibles conseqüències en la biogeoquímica de la zona hiporreica. Per dur a terme l'estudi, es van instal·lar 15 pous espaiats a intervals de 5 m en un tram de la riera de Santa Fe, dins del Parc Natural del Montseny. L'estudi es va fer durant la tardor del 2004 (entre el mes d'octubre i el mes de desembre). Es va mesurar la concentració de nutrients i la saturació d'oxigen (DO) superficials i hiporreics en 23 dates (15 durant el període de caiguda de fulles i 8 després d'una riuada). Els canvis en la connectivitat superfície-hiporreu i en la retenció hiporreica d'amoni (NH_4) i fòsfor (PO_4) es van avaluar mitjançant la coinjecció d'un traçador conservatiu (NaCl) i nutrients. La connectivitat superfície-hiporreu es va mesurar comparant el percentatge de traçador conservatiu que va arribar al pou en comparació amb el que es trobava a la superfície en el moment del mostreig. La retenció de nutrients es va mesurar com el quocient entre la concentració observada en els pous i la concentració esperada en aquests pous suposant que els nutrients afegits al riu es comportessin com soluts conservatius, és a dir, com soluts no lliures de processos de retenció.

Durant el període de caiguda de les fulles (entre el 7 d'octubre i el 2 de desembre), el cabal (Q) es va mantenir gairebé constant al voltant de 8 Ls^{-1} i la biomassa de fullaraca en el tram va passar de 4,0 a 163,4 kg PS. Entre el 2 i el 10 de desembre, el cabal va augmentar 17,7 vegades (fins a 124 Ls^{-1}) i va provocar el "rentat" del 65% de les fulles acumulades en el canal. Després de la riuada, Q va anar disminuint gradualment, però al final de l'estudi, encara era el doble del mesurat just abans de la riuada mentre que la biomassa de fullaraca ja havia recuperat els valors pre-riuada.

La variació temporal en la concentració de nutrients i en la DO observada a la zona hiporreica va ser molt similar a l'observada en l'aigua superficial (correlacions entre valors de superfície i hiporreics: R=0,98 pel DO;

R=0,97 pel NO_3; R=0,52 pel NH_4; i R=0,85 pel PO_4). En comparació amb l'aigua superficial, l'aigua hiporreica contenia menys DO, més PO_4 i nitrat (NO_3), i una concentració similar de NH_4. Pel què fa les diferents zones hiporreiques, aquells pous que mostraven una tendència a fluir de la superfície cap a l'hiporreu (pous d'enfonsament) presentaren una major saturació d'oxigen i una major concentració de NH_4 que els pous que presentaven una tendència en el flux de sentit contrari (és a dir, una tendència a fluir des de l'hiporreu cap a la superfície, a partir d'ara anomenats pous d'aflorament). La concentració de PO_4 i NO_3 no va presentar diferències significatives entre els dos tipus de pous.

L'acumulació de fullaraca disminuí gradualment la connectivitat superfície-hiporreu mentre que la riuada provocà l'efecte contrari. En general, la variació de la connectivitat observada al llarg del període d'estudi responia als efectes conjunts de l'acumulació de fulles i variació en Q tant en els pous d'aflorament (R^2=0,65, g.ll.=11, p=0,01; Connectivitat= 40,9Log(Q)-9,0Log(Biomassa de fullaraca)+55,3) com en els pous d'enfonsament (R^2=0,76, g.ll.=11, p=0,00; Connectivitat=24,7Log(Q)-7,9Log(Biomassa de fullaraca)+ 71,7).

L'augment de la connectivitat va reduir les diferències entre la superfície i el compartiment hiporreic en termes de DO en tots dos tipus de pous i en termes de NO_3 en els pous d'aflorament. La retenció hiporreica de NH_4 i PO_4 va mostrar diferents respostes a aquests canvis. Mentre que la retenció de PO_4 va estar controlada per la concentració hiporreica de PO_4, la qual no es va veure afectada pels canvis en la connectivitat, la retenció de NH_4 va ser afectada indirectament pels canvis en la connectivitat a través de canvis en la disponibilitat de DO. Finalment, encara que no es va injectar NO_3 en el riu, es va observar un augment en la concentració de NO_3 en alguns dels pous fet que assumim a la presència de processos de nitrificació.

3.3. Influència de la zona de retenció temporal d'aigua en la retenció de nutrients, aproximació experimental mitjançant la manipulació de substrats

La quantificació de la mida de les zones de retenció temporal d'aigua (A_s) ha esdevingut crítica en els estudis biogeoquímics adreçats a entendre els processos de retenció de nutrients. Les ZRT incrementen el temps de contacte entre l'aigua i els sediments i per tant, generen més oportunitats per a la biota per processar aquests nutrients. Així doncs, les ZRT es consideren "punts calents" metabòlics i s'espera observar major retenció de nutrients a mesura que l'A_s augmenta. De totes maneres els estudis publicats en la literatura presenten resultats contradictoris. En aquest estudi hipotetitzem que la manca de relacions consistents es deu al fet que, a part de la mida de les ZRT, les característiques físiques i biològiques de les estructures que generen aquestes ZRT tenen un rol rellevant en la retenció de nutrients i que poden generar respostes biogeoquímiques diferenciades. En aquest estudi, testem la nostra hipòtesi de manera experimental mitjançant 16 adicions de PO_4, NH_4, i NaCl en 4 trams consecutius d'un canal d'irrigació situat a Gualba, i que rep aigua de la riera de Santa Fe. En els trams, vam introduir deflectors fets amb 3 tipus diferents de substrats (llim, sorra i còdols; tots ells colonitzats biològicament) amb l'objectiu d'augmentar l'A_s. Es va estimar la retenció de NH_4 i PO_4 en el canal principal i en les ZRT fent servir un model de transport de soluts que inclou els processos d'advecció, dispersió, dilució i retenció temporal de l'aigua causada per les ZRT (OTIS-P, Runkel, 1998) i es van comparar els resultats entre trams amb diferents tipus de deflectors. A més, es va fer una injecció de ^{15}N per quantificar l'assimilació biològica dels diferents deflectors. En concret es va quantificar l'assimilació de N en la matèria orgànica bentònica fina (FBOM) i en l'epilíton de cada tipus de deflector.

Els deflectors eren comparables en mida i volum, però diferien en la mida de gra (entre <1mm en els deflectors de llim fins a diversos cm en els deflectors fets de còdols), en la conductivitat hidràulica (de minuts a més de 16,3 hores), en el contingut en matèria orgànica (entre 5,4 i 56,6 g AFDM –pes lliure de cendres-), en la proporció relativa d'epilíton i FBOM (quasi un 100% de FBOM en els deflectors fets de llim enfront d'un 1,7% en els deflectors fets de còdols) i en la ratio C:N (més gran als deflectors fets de sorra).

Les taxes d'assimilació de N eren diferents entre els tipus de deflectors (ANOVA d'un factor, $F_{(2,23)}$=4,164, p=0,03). Els deflectors fets de sorra van presentar les taxes d'assimilació de N més altes (0,95±0,34 µg N h^{-1}) seguits pels deflectors de llim (0,57±0,14 µg N h^{-1}), i finalment pels deflectors fets amb sorra (0,15±0,02 µg N h^{-1}). La major part del N assimilat pels deflectors de còdols i sorra es va atribuir a l'epilíton. A part, l'epilíton i el FBOM diferien en l'eficiència d'assimilació de nutrients (taxa d'assimilació per unitat de AFDM). La eficiència d'assimilació va ser 3,5±0,5 i 3,7±1,2 vegades més alta al FBOM que a l'epilíton als deflectors fets de còdols i sorra, respectivament. En global, l'eficiència d'assimilació de N va ser més alta en els deflectors de còdols, mitjana en els deflectors de sorra i baixa en els deflectors de llim.

La introducció dels deflectors va incrementar el temps de residència de l'aigua, va disminuir la velocitat de l'aigua i va incrementar l'A_s de manera homogènia entre tractaments.

Tot i que l'increment de A_s no va resultar en un augment de la retenció de nutrients a nivell de tram, probablement perquè els deflectors eren massa petits per augmentar prou les ZRT com per afectar el processat de nutrients, els resultats obtinguts en aquest estudi indiquen diferències en el processat de nutrients entre tipus de deflectors, bàsicament associats a la retenció de nutrients que té lloc a les ZRT.

3.4. Desenvolupament d'un traçador intel·ligent per a l'estudi de l'activitat microbiològica i la interacció entre aigua i sediments en rius: el sistema resazurin-resorufin

Un traçador intel·ligent és aquell que a més de proporcionar informació sobre les característiques del transport de soluts en un ambient és capaç de donar-nos informació addicional sobre les propietats del medi pel qual el solut ha estat transportat. En aquest estudi, presentem el compost químic resazurin (Raz) com un traçador intel·ligent capaç de proporcionar informació metabòlica sobre el medi per on viatja. El Raz és un compost fluorescent que experimenta una reducció irreversible a resorufin (Rru), que també és fluorescent, en la presència de microorganismes vius. Per tal d'investigar d'idoneïtat d'aquest sistema de compostos com a traçadors intel·ligents en aquest estudi caracteritzem la degradació, la sorció, la reacció i el transport del Raz i el Rru en varis tipus de sediments i agües en una sèrie d'experiments de laboratori.

El Raz es transforma ràpidament a Rru en sediments colonitzats, a una taxa de 1,41h^{-1} mentre que ho fa a molt més lentament (a una taxa 3 ordres de magnitud menor) en aigua de riu sola indicant que el traçador és sensible a l'activitat biològica associada a la interfície columna d'aigua-sediment. Els compostos poden estar afectats per fenòmens de sorció. En l'experiment portat a terme per verificar aquest fenomen, la isoterma de sorció del resorufin va presentar una K_d de 6,63 mLg^{-1} en un sediment amb un 2,19% de carboni orgànic.

Els compostos són estables durant setmanes en aigua natural, excepte en el cas d'exposició a la llum. En aquest cas s'ha observat una certa degradació fotoquímica però suficientment lenta com perquè no suposi un problema en els estudis de camp convencionals.

3.5. Quantificació de les zones de retenció temporals metabòlicament actives (ZRT-MA) en dos trams diferenciats en termes de retenció temporal d’aigua i respiració.

La quantificació de les zones de retenció temporal d’aigua (ZRT) s'ha convertit en una qüestió crítica en els estudis biogeoquímics dels ecosistemes fluvials que tenen per objectiu examinar els factors que controlen la variació espacial o temporal de la retenció de nutrients i el metabolisme. S'espera que les ZRT augmentin la interacció entre els nutrients dissolts i les comunitats microbianes, i per tant, que augmentin la retenció de nutrients i el metabolisme. El mètode comunament utilitzat per mesurar les ZRT no permet distingir entre els diferents compartiments que contribueixen aquesta retenció hidràulica (per exemple, la zona hiporreica en comparació amb una zona d’aigua estancada superficial), encara que els processos biogeoquímics siguin molt diferents en cada compartiment. En aquest estudi utilitzem un mètode alternatiu, l'ús de resazurin (Raz) com a traçador “intel·ligent" que en combinació amb un traçador conservatiu ens permet diferenciar les ZRT que presenten activitat metabòlica del total de ZRT. L'objectiu d'aquest estudi és quantificar les ZRT metabòlicament actives en dos trams consecutius d’una mateixa riera que difereixen en les característiques hidrològiques i biològiques.

L’estudi es va portar a terme en dos trams consecutius de la riera que drena la conca número 3 de l’H. J. Andrews Experimental Forest (Oregon, USA). Els dos trams difereixen en la morfologia: mentre el primer transcorre per roca mare (a partir d’ara anomenat “tram de roca mare”, i per tant no té zona hiporreica, el segon es caracteritza per tenir una dipòsits al·luvials extensos (a partir d’ara anomenat “tram al·luvial”). L’experiment va consistir en una addició combinada de Raz i NaCl durant 17 hores a la capçalera del tram 1. Es van establir un total de 20 punts de mostreig superficials (12 corresponents al tram 1 i 8 al tram 2) separats aproximadament uns 25 metres. A més a més es van establir 11 punts de mostreig hiporreics. En tots els punts de mostreig es van recollir mostres en el temps per tal de mesurar les taxes de reacció del Raz a Rru i estimar els paràmetres hidràulics. Addicionalment, es va mesurar la concentració d’oxigen i la temperatura simultàniament al principi i final de cada tram per tal de mesurar la respiració a escala de tram (R).

En el tram de roca mare, les ZRT-MA representaren un 37% del total de les ZRT i tenien una mida de 0,002 m^2 mentre que en el tram al·luvial, les ZRT-MA representaren el 100% de les ZRT i mesuraren 0,291m^2. La taxa efectiva de transformació de Raz en els biofilms presents al llit del tram de roca mare va ser aproximadament 16 vegades més gran que la taxa mesurada al tram al·luvial. Tanmateix, la taxa efectiva volumètrica de transformació de Raz a les ZRT–MA va ser 2,2 vegades menor en el tram de roca mare en comparació amb el tram amb dipòsits al·luvials. Aquesta diferència entre trams va ser similar a la diferència observada en R, que va ser 1,8 vegades inferior al tram 1 en comparació al tram 2. Aquesta similitud en les proporcions suggereix que les ZRT-MA van contribuir de manera significativa en la respiració. Els resultats obtinguts indiquen que la quantificació de les ZRT-MA poden millorar la nostra comprensió del paper que juguen les ZRT en els processos metabòlics. El Resazurin s’ha provat un “traçador intel·ligent” que no tan sols ens proporciona informació a escala de tram sinó que és sensible a l’heterogeneïtat espacial a una escala d’hàbitat.

4. DISCUSSIÓ I CONCLUSIONS

Dels resultats del primer capítol podem concloure que l’entrada de fullaraca no només modifica la morfologia del riu sinó que en aquest cas, també provoca un augment en la demanda de PO_4 i NH_4, ja sigui directament a través d’un augment en la demanda microbiana o bé per un augment el la retenció hidràulica lligada a les ZRT. La riuada va alterar les propietats hidràuliques i la retenció de nutrients a la riera. No obstant això, una vegada que el cabal va disminuir, el riu va mostrar una recuperació ràpida en la capacitat de retenció de nutrients, especialment pel PO_4. Per tant, la contribució de l'entrada de fullaraca en el metabolisme del riu durant la tardor sembla dependre de les dates de les riuades en relació amb la caiguda de fulles. Això té implicacions en el context del canvi climàtic ja que es preveu una alteració tant el temps de caiguda de les fulles com en el règim hidrològic que marca la freqüència i la intensitat de les riuades (Christensen i Christensen, 2004). Per exemple, Peñuelas et al. (2002) han observat un endarreriment en la caiguda de fulles de 13 dies respecte al 1952 en el Montseny. Per altra banda, les projeccions de canvi climàtic a la zona d'estudi preveuen un augment de les tempestes extremes i episodis de sequera recurrents (McCarthy et al. 2001). En conjunt, aquests canvis poden alterar el temps de caiguda de les fulles i les inundacions, amb impactes incerts en la retenció de nutrients. Menys inundacions durant la tardor incrementaria el temps de residència de les fulles en el llit del riu, augmentant la retenció de nutrients. Per contra, un augment en la freqüència d'inundacions durant el període fulles es traduiria en un rentat periòdic de la fullaraca aigües avall, a zones de major cabal i usualment amb menor capacitat de retenció de nutrients.

Per altra banda, l'entrada de fullaraca durant la tardor modifica les connexions entre la superfície i l'hiporreu provocant una disminució de la velocitat amb què l'aigua arriba a la zona hiporreica. Aquests efectes es veuen contraposats als de l'augment de cabal, que incrementa la connectivitat superfície-hiporreu i homogeneïtza els pous d'aflorament i d'enfonsament pel què fa al seu grau de connexió amb l'aigua superficial. Aquest efecte, però, pot ser degut a l'increment de cabal *per se* però també al "rentat" de fulles causat per la riuada. Els resultats d'aquest treball mostren que, en global, els canvis hidrològics poden tenir repercussions en la variació temporal de la química de la zona hiporreica i en la retenció de nutrients en aquest compartiment. Tot i així, la resposta del N i del P és diferent, mentre que la retenció de P respon a canvis no lligats a la variació en la connectivitat (depèn de la concentració ambiental de P), el N es veu afectat per canvis en la connectivitat de forma indirecta, a través de canvis en la disponibilitat d'oxigen.

Per altra banda, comparant els resultats obtinguts dels dos treballs podem deduir que, mentre que les ZRT del canal, és a dir, aquelles zones d'aigües estancades creades per acumulacions de fulles, juguen un paper molt important en el processat de P durant la tardor mentre que el processat de N depèn, en gran mesura, dels processos que tenen lloc a la zona hiporreica.

4.1. Factors de control dels paràmetres hidràulics

Diversos autors han assenyalat la geomorfologia del canal com a un factor determinant de la mida de les ZRT (Harvey i Bencala, 1993; Kasahara i Wondzell, 2003) fet que reconeix implícitament la zona hiporreica com la principal zona contribuent a l'emmagatzematge transitori d'aigua. Els resultats de l'estudi realitzat al WS03 a l' H. J. Andrews Experimental Forest confirmen aquesta idea. En aquest riu, la ZRT del tram amb dipòsits al·luvials és 49 vegades superior a la del tram que flueix per roca mare. No obstant això, aquesta tesi doctoral demostra que la presència d'obstacles en el canal també pot modificar la mida de la ZRT. Per exemple, la introducció experimental de deflectors en el canal de Gualba va multiplicar la ZRT en 2,3 vegades i l'acumulació de fullaraca a la riera de Santa Fe va incrementar la mida de la ZRT de 0,036 m^2 a 0,056 m^2 (1,5 x vegades). No obstant això, els factors que controlen la variació temporal de la ZRT localitzada al canal probablement difereixen d'aquells que controlen la variació temporal de la ZRT hiporreica ja que aquests dos compartiments es veuen afectats de manera diferent pels processos de transport.

4.1.1. La variació dels paràmetres hidràulics a Santa Fe durant la tardor

Els resultats presentats en aquesta tesi indiquen que la variació temporal en la mida de la ZRT en condicions de cabal basal a Santa Fe durant la tardor va estar controlada per l'acumulació de fullaraca en el llit del riu. Durant el període de caiguda de les fulles, es va observar un augment de la ZRT atribuït a un augment en el nombre i l'extensió de les zones estancades dins del corrent generades per l'acumulació de fulles en el curs d'aigua (és a dir, a un augment de la ZRT localitzada al canal). Aquesta troballa està d'acord amb els resultats observats per altres autors sobre l'efecte de l'acumulació en el canal de troncs, fulles i vegetació, en els paràmetres hidràulics (Hart et al. 1999, Jin i Ward, 2005, Lautz et al. 2006). A més, es va observar una disminució en el coeficient d'intercanvi d'aigua i una pèrdua de connectivitat entre la superfície i la zona hiporreica. De totes maneres, el coeficient d'intercanvi d'aigua i la connectivitat no representen exactament el mateix –el primer mesura la rapidesa en l'intercanvi d'aigua entre el canal principal i la ZRT (englobant la ZRT hiporreica amb la localitzada al canal), mentre que la connectivitat mesura l'intercanvi d'aigua entre la superfície i la zona hiporreica–. Tot i axí, tots dos paràmetres van indicar una disminució en l'intercanvi d'aigua entre els compartiments de flux ràpid i estancat com a resposta a l'acumulació de fullaraca. De fet, quan es combinen els resultats dels dos estudis realitzats a Santa Fe, s'observa una correlació negativa entre el temps de residència de l'aigua a la ZRT, calculat com l'invers de k_2, i la connectivitat mitjana per data de mostreig estimada utilitzant les dades de pous d'aflorament i d'enfonsament conjuntament (R =- 0,76, p = 0,03, n = 8).

Mentre que la variació temporal de la ZRT en condicions de cabal base durant el període de caiguda de fulles estava associat principalment a l'acumulació de fullaraca, l'increment de cabal tingué els efectes oposats (ja sigui perquè la riuada va eliminar part de les fulles acumulades com per l'efecte del cabal *per se*). L'augment brusc de cabal va augmentar la velocitat de l'aigua, els coeficients d'intercanvi d'aigua entre el canal i la ZRT (és a dir, va augmentar k_1 i k_2) i la connectivitat hidrològica entre les aigües superficials i la zona hiporreica, i va disminuir la mida de la ZRT i la seva proporció relativa respecte al canal (és a dir, va disminuir A_s i la A_s/A). Aquests resultats indiquen que a la riera de Santa Fe el cabal és un factor clau que determina la rellevància de les ZRT en relació a la secció transversal del riu. No obstant això, els resultats sobre la relació entre els paràmetres hidràulics i el cabal en estudis publicats prèviament són controvertits. Per exemple, Hart et al. (1999) no va trobar una relació significativa entre la ZRT i el cabal en un estudi realitzat a Walter Brank (Tennessee, EUA). Per contra, Valett et al. (1996), Martí et al. (1997), i Butturini i Sabater (1999) van trobar relacions significatives entre el cabal i la mida relativa de la ZRT. No obstant això, en un anàlisi de regressió entre les dades hidràuliques i el cabal publicades en 18 estudis, el cabal apareix com un factor de control positiu de la k_1 i la k_2 i de control negatiu de l'A_s/A (capítol 1), encara que el percentatge de variància dels coeficients d'intercanvi s'explica perquè la descàrrega és menor que la mida d'emmagatzematge transitori.

4.1.2. La variació dels paràmetres hidràulics a Santa Fe a diferents escales temporals

El rang de variació en la mida de la ZRT i el cabal mesurat durant el període d'estudi (és a dir, tardor de 2004) es troba dins del rang dels mesures observades en una base mensual durant un període de dos anys hidrològics (dades no publicades). Durant aquest període, el màxim de ZRT es va observar durant l'estiu i la tardor, coincidint amb cabals baixos i com a mínim durant la tardor, amb acumulació de fullaraca al canal, mentre que el mínim va coincidir amb cabals alts, durant el desembre del 2004 i el febrer-març del 2006. En general, la variació en el cabal explica el 74% de la variació en la mida relativa de la ZRT (R^2=0,74, A_s/A= 0,82Q-0,53, p=0,000, n=24), un percentatge lleugerament superior que l'obtingut amb les dades del la tardor del 2004 (variació d'A_s/A explicada per variacions en el cabal= 63%). Pel que fa als coeficients d'intercanvi d'aigua entre el canal i les ZRT, aquests presenten una relació negativa amb el cabal en l'anàlisi de les dades relatives als 2 anys d'estudi (R^2=48, k_1=7.91E-05$Q^{0.39}$, p=0,000, n=24, i R^2=0,90, k_2=7.67E-05$Q^{0.96}$, p=0,000, n=24), que va ser similar a l'observat en el període tardor.

Els valors alts d'A_s/A observats durant l'estiu no es poden atribuir a una entrada de fullaraca. Per altra banda, es van produir en un moment de cabal i velocitat de l'aigua lleugerament superiors als observats durant el màxim d'A_s/A de la tardor de 2004. En conjunt, aquests resultats suggereixen l'existència d'un canvi estacional en la importància relativa dels diferents compartiments que composen la ZRT. Mentre que la variació en la ZRT durant la tardor es pot atribuir a un canvi el la ZRT dins del canal, durant l'estiu la variació en la ZRT probablement ve controlada per canvis que afecten la mida de la zona hiporreica.

En resum, els paràmetres hidràulics estan controlats primàriament per la geomorfologia del riu, ja que determina físicament la mida potencial de la zona hiporreica. No obstant això, els nostres resultats mostren que el cabal pot modular els paràmetres hidràulics, tant en comparacions entre rius com dins d'un mateix riu al llarg del temps. A cabals més grans, la secció transversal del riu augmenta i per tant es redueix la influència de la topografia del llit del riu en el transport superficial d'aigua i nutrients. En conseqüència, disminueix la importància relativa de la mida de la ZRT (A_s). Els resultats de Santa Fe també indiquen que la contribució relativa dels diferents compartiments que generen ZRT pot variar segons l'estació i que estan determinats per la interacció entre el cabal i l'entrada de fullaraca. En aquest riu, amb una entrada de matèria orgànica molt important durant la tardor, els canvis temporals en la ZRT en condicions de cabal basal durant el període de la caiguda de fulles són atribuïbles a un augment en la ZRT dins del canal mentre que canvis en la ZRT hiporreica dominen durant l'estiu.

4.2. Zones de retenció temporal d'aigua i retenció de nutrients

Diversos autors han demostrat la importància dels paràmetres hidràulics (per exemple, la mida de les ZRT, els coeficients d'intercanvi d'aigua entre el canal principal i la ZRT) com a controls dels processos metabòlics fluvials. Aquesta tesi contribueix a ampliar els coneixements anteriors sobre el tema i corrobora aquesta teoria. Els estudis realitzats a Santa Fe i a l'H. J. Andrews demostren que la capacitat de metabòlica dels rius (ja sigui en termes de retenció de nutrients a Santa Fe, com d'activitat metabòlica mesurada com a respiració a l'H. J. Andrews) està fortament lligada a la mida de les ZRT i a l'intercanvi d'aigua entre aquestes i el canal principal. També hem demostrat que, a part del control hidràulic, variables ambientals com ara la concentració de nutrients o la temperatura de l'aigua afecten les taxes de processament biològic, i per tant, en última instància també controlen els processos metabòlics.

4.2.1. Retenció de nutrients a Santa Fe durant la tardor

En aquesta tesi, la retenció de nutrients a la riera de Santa Fe s'ha mesurat tant a escala de tram (capítol 1) com a la zona hiporreica (capítol 2). Les mètriques de retenció utilitzades en els dos estudis no són directament comparables quantitativament, però si qualitativament. Els dos estudis van indicar una major retenció de PO_4 durant el període de caiguda de les fulles que després de la riuada. Si bé la concentració de ambiental de PO_4 sembla haver jugat un major control sobre la retenció hiporreica de PO_4, la biomassa de fullaraca i la temperatura de l'aigua són factors clau en el control de la retenció de PO_4 a nivell de tram. Les discrepàncies entre les dues escales d'observació també van aparèixer a l'hora d'examinar els controls de retenció de NH_4. Per exemple, a escala de tram la retenció de NH_4 va ser més gran durant el període de caiguda de les fulles que després de la riuada, mentre que no es van observar diferencies significatives entre períodes a escala hiporreica. La concentració ambiental de NO_3 va exercir un control negatiu en la retenció de

NH_4 (és a dir, concentracions més altes de NO_3 es van veure acompanyades per valors de retenció de NH_4 més baixos) tant a escala de tram com a la zona hiporreica. Finalment, la concentració d'oxigen ha resultat un factor determinant en la retenció d'amoni hiporreic (major retenció de NH_4 a major concentració d'oxigen).

Pel que fa a la influència dels paràmetres hidràulics en la retenció de nutrients, la mida de la ZRT es relaciona positivament amb les velocitats d'assimilació de PO_4 i NH_4 a escala de tram i amb la retenció de NH_4 a la zona hiporreica ($R^2=0{,}74$; $NH_4\text{-}N_{obs}/NH_4\text{-}N_{exp}=-0{,}45-0{,}23\ln(A_s)$, $n=6$, $p=0{,}03$), mentre que no es va observar una relació significativa entre A_s i la retenció hiporreica de PO_4.

4.2.2. Controls de la retenció de nutrients a Santa Fe a diferents escales temporals

Les distancies i velocitats de captació de nutrients (S_w i V_f, respectivament) mesurades a la riera de Santa Fe durant la tardor del 2004 estan dins del rang de les mesurades mensualment en aquest riu per von Schiller et al. (2008) durant un període de dos anys hidrològics (2004-2006). Els dos estudis van registrar una major demanda de NH_4 que de PO_4 (4,9 vegades més altes en el conjunt de dades de dos anys, i 3 vegades durant la tardor).

Els nostres resultats indiquen que la mida de les ZRT, va explicar el 59% de la variabilitat en les velocitats de captació de PO_4 i el 65% de les de NH_4 durant la tardor. En contrast, una anàlisi de regressió entre les velocitats de captació de nutrients i els paràmetres de ZRT utilitzant les dades de von Schiller et al. (2008) i dades no publicades de ZRT van indicar que no hi havia relacions significatives entre aquests paràmetres. La diferència de resultats entre els estudis fets a diferent escala temporal suggereixen que la importància relativa de les ZRT en la retenció de nutrients varien temporalment. Mentre que la ZRT és determinant durant el període de caiguda de les fulles, a escala anual perd rellevància davant d'altres variables ambientals.

4.2.3. Diferències entre la retenció d'amoni i de fosfat

La demanda de NH_4 va ser més alta que la de PO_4 tant a escala de tram com de zona hiporreica durant el període de caiguda de fulles (2 vegades més alta). Aquest fenòmen també es va observar en l'experiment amb deflectors de Gualba. No obstant això, en l'estudi experimental de Gualba, el quocient entre el coeficient de captació de NH_4 i PO_4 va oscil·lar entre 1,6 i 2,6 vegades, en funció del tipus substrat utilitzat per construir el deflector. Altres estudis realitzats en rius silícics propers han trobat resultats similars, és a dir, una major preferència de captació pel NH_4 que pel PO_4 (Riera Major, Martí i Sabater, 1996; Fuirosos, von Schiller et al., 2008). Altres estudis, però, indiquen una major retenció de PO_4 que de NH_4 (Hanafi et al., 2006), i altres no han trobat cap diferència entre la velocitat de captació dels dos nutrients (Hoellein et al., 2007). La disparitat en els resultats publicats s'explicaria per les diferències en la geologia, en el tipus de substrat, o en l'estat de limitació de nutrients entre els rius estudiats. Per altra banda, els resultats de l'experiment portat a terme a Gualba indiquen com a una explicació plausible, la presència de comunitats biològiques amb diferents necessitats de nutrients.

En resum, els resultats presentats en aquesta tesi indiquen que la retenció de NH_4 i PO_4 responen a controls diferents, i que aquests controls probablement reflecteixen les diferents condicions en els compartiments on la retenció de cada nutrient té lloc. A la riera de Santa Fe, la retenció de PO_4 està més influenciada per factors ambientals que afecten les ZRT superficials mentre que la retenció de NH_4 respon a canvis que afecten la zona hiporreica. Nombrosos estudis han demostrat que la zona hiporreica juga un paper important en el cicle del N en rius (per exemple, Jones et al., 1995; Duff i Triska, 2000). L'àmplia gamma de condicions redox present en aquesta zona afavoreix una àmplia varietat de reaccions biogeoquímiques que participen en el cicle del N, com ara la nitrificació (com s'observa a Santa Fe), la desnitrificació i l'assimilació biòtica. D'altra banda, la retenció de PO_4 en rius silícics s'associa principalment amb l'assimilació biòtica (Reddy et al., 1999) i per tant, s'espera que augmenti amb l'entrada de substrats rics en energia, per exemple l'acumulació de fulles a la riera de Santa Fe que a més contribueix a l'augment de la ZRT.

4.3. Zones de retenció temporal metabòlicament actives

Els resultats dels estudis realitzats a la riera de Santa Fe i la manipulació experimental a Gualba mostren que els compartiments que contribueixen a les ZRT poden influir de forma substancial en els processos biogeoquímics, ja sigui per diferències en el temps de residència de l'aigua o per les diferències químiques i biològiques de cada ZRT. Els traçadors comunament utilitzats per estimar els paràmetres hidràulics no permeten caracteritzar les particularitats metabòliques dels diversos compartiments que contribueixen a la ZRT. Per tant, les relacions empíriques entre les ZRT i la capacitat de processat de nutrients que es troben a la literatura són sorprenentment febles o fins i tot contradictòries entre si (per exemple, Hall et al., 2002; Webster et al. 2003; Bukaveckas, 2007).

L'ús del traçador resazurin (Raz) presentat en aquesta tesi pretén superar aquesta limitació metodològica, proporcionant informació de les ZRT des d'una perspectiva funcional. En aquesta tesi (capítol 4) es demostra que a) el Raz proporciona informació de les diferents zones de ZRT metabòlicament actives tot i que poden ser físicament diferents (per exemple, zones embassades del canal amb una comunitat microbiològica activa, o parts de la zona hiporreica amb una respiració elevada), i b) que pot separar aquestes zones d'altres ZRT amb menor activitat metabòlica. Aquesta aproximació respon a la demanda feta per diversos autors (Findlay, 1995; Boulton et al., 1998, Fisher et al. 1998, Kemp et al. 2000) d'una caracterització funcional de les ZRT.

L'experiment realitzat al WS03 de l'H. J. Andrews Experimental Forest (capítol 5) va demostrar la viabilitat de l'ús del Raz en experiments de camp. Addicionalment, en aquest estudi es va desenvolupar el mètode matemàtic per quantificar la proporció de les ZRT que són metabòlicament actives sense la necessitat de resoldre les equacions de transport i reacció de soluts, és a dir, utilitzant un mètode relativament fàcil d'aplicar amb l'objectiu que arribi a una audiència àmplia. Aquest traçador és sensible a l'heterogeneïtat espacial tant a escala de tram com a escala d'hàbitat i proporciona informació rellevant sobre les característiques metabòliques de les ZRT. En aquest estudi es demostra de manera quantitativa que l'intercanvi d'aigua entre les zones metabòliques i el canal principal poden limitar l'activitat biològica i que per tant els paràmetres hidràulics (controlats per la geomorfologia i la hidrologia) són claus a l'hora de determinar els processos metabòlics en sistemes fluvials.

4.6. Implicacions

Els sistemes fluvials i les seves zones riberenques proporcionen béns i serveis importants a escala global a través del seu paper en el cicle de l'aigua, l'energia, i la matèria (Palmer et al., 2004). La retenció de nutrients es pot considerar un procés d'ecosistema que proporciona un servei clau per a la societat (Postel i Carpenter, 1997) ja que disminueix l'exportació de nutrients aigües avall, i que en cas contrari pot generar problemes d'eutrofització. Encara que en les zones urbanitzades només cobreixen entre un 1% i un 6% de la superfície de la Terra, la seva empremta té conseqüències ecològiques complexes amb una afectació a gran escala (Alberti et al., 2003). Segons les Nacions Unides, el creixement demogràfic previst entre l'any 2000 i el 2030 (aproximadament un increment en 2 milions de persones) es concentrarà sobretot a les zones urbanes. En el 2030, més del 60% de la població mundial estimada (4,9 milions de persones sobre 8,1 milions) viurà en una ciutat (Alberti et al., 2003). L’increment en superfície urbanitzada augmenta la superfície impermeable i afecta els ecosistemes fluvials tant en la geomorfologia com en els processos hidrològics provocant canvis en els fluxos d'aigua, de nutrients i de sediments (Paul i Meyer, 2001), que clarament modifiquen els processos de retenció de nutrients (Grimm et al., 2005; Groffman et al. 2005, Meyer et al. 2005). Aquests impactes han estat referits en conjunt com a síndrome del riu urbà (Urban Stream Syndrome; Paul i Meyer, 2001; Walsh et al. 2005), un terme que reconeix el fet que tots els corrents d’aigua urbans experimenten severes alteracions hidrològiques, geomorfològiques i químiques. Davant d’aquest escenari, en els últims anys s’han incrementat els esforços per rehabilitar els ecosistemes fluvials al seu estat natural, aquest últim determinat per un conjunt de condicions de referència.

Els resultats d'aquesta tesi posen en relleu la importància de la geomorfologia i les ZRT en el processat de nutrients. També indiquen que ZRT localitzades en diferents compartiments i amb diferents característiques biològiques afecten de manera diferenciada la retenció de nutrients. Els resultats també proporcionen informació sobre els diferents factors que controlen la variació temporal de les ZRT i posen en relleu la pertinença d'estudis intensius en el temps per capturar la influència de factors que no poden ser observats amb els estudis a més gran escala espacial o temporal. Addicionalment, els resultats de la manipulació experimental realitzada a Gualba demostren que l'ús de deflectors, almenys d’aquestes dimensions, no comporta un increment de la retenció de nutrients a escala de tram, però que diferents tipus de materials provoquen una resposta diferenciada en l’assimilació de N i P a la ZRT. Finalment, es demostra que el traçador resazurin pot ser una eina útil per avaluar els processos metabòlics en sistemes fluvials que podria ser utilitzat tant per determinar les condicions metabòliques en rius inalterats i així proporcionar les condicions de referència necessàries en els processos de rehabilitació, com per avaluar l'èxit dels projectes de rehabilitació encaminats a millorar la funcionalitat dels corrents d’aigua.

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ANNEX

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Combined effects of leaf litter inputs and a flood on nutrient retention in a Mediterranean mountain stream during fall

Alba Argerich[1]
Department d'Ecologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain

Eugènia Martí
Limnology Group, Centre d'Estudis Avançats de Blanes (CSIC), Accés a la Cala St. Francesc 14, 17300 Blanes, Spain

Francesc Sabater
Department d'Ecologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain

Miquel Ribot and Daniel von Schiller
Limnology Group, Centre d'Estudis Avançats de Blanes (CSIC), Accés a la Cala St. Francesc 14, 17300 Blanes, Spain

Joan L. Riera
Department d'Ecologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain

Abstract

This study examined the effect of increasing in-channel leaf standing stocks on hydrologic transient storage and nutrient retention in a Mediterranean mountain stream. A flood at the end of the leaf fall period provided the opportunity to examine the effect of abrupt removal of much of the leaf material. Twenty-one chloride additions were performed from October to December 2004. In 13 of these, we also added ammonium and phosphate to estimate nutrient uptake lengths and uptake velocities to assess nutrient retention. The one-dimensional transport with inflow and storage (OTIS) model was used to estimate transient water storage parameters. Although discharge remained constant during leaf fall, water residence time increased because of in-channel litter accumulation, as did nutrient uptake velocity. Flooding reduced leaf benthic standing stocks by 65% and dramatically altered hydraulic and nutrient retention properties of the channel. After recession, the stream rapidly recovered in terms of nutrient retention, especially for phosphate. Abrupt changes in discharge under flood conditions largely determined the variability in stream nutrient retention. However, leaf litter inputs played an important role in nutrient dynamics during constant flow. Because both the flood regime and the timing of leaf fall are being regionally altered by climate change, our results have implications for stream nutrient dynamics under climate change scenarios.

Intense leaf fall from deciduous riparian vegetation is of major importance for both the community structure (Wallace et al. 1997) and metabolism (Crenshaw et al. 2002) of streams. Leaf litter inputs provide large quantities of energy to headwater streams that typically exhibit low levels of primary productivity (Fisher and Likens 1973). The ecological relevance of these inputs is well recognized, especially in temperate regions (Wallace et al. 1997). Streams in Mediterranean regions, in contrast to those from more humid climates, are characterized by high interannual variability in the intensity and frequency of floods as well as by periods of intermittent flow. In addition, in Northern Hemisphere Mediterranean regions higher leaf litter input coincides with periods of high flood frequency, low temperature, and low autotrophic activity (Gasith and Resh 1999). Therefore, the residence time of the allochthonous matter in Mediterranean streams may be much shorter than in temperate streams because it enters shortly before or in conjunction with flooding. In consequence, the ecological relevance of this allochthonous energy source may depend on the timing of flood events relative to leaf inputs.

Mediterranean regions are among the most vulnerable to climate change (Schröter et al. 2005). In these regions, both a change in the frequency and intensity of rains and an increase in temperatures are expected (Christensen and Christensen 2004). Under this scenario, it is likely that alterations in the regimes of autumnal leaf inputs and flooding will have implications for stream metabolism and

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[1] Corresponding author (alba@ceab.csic.es).

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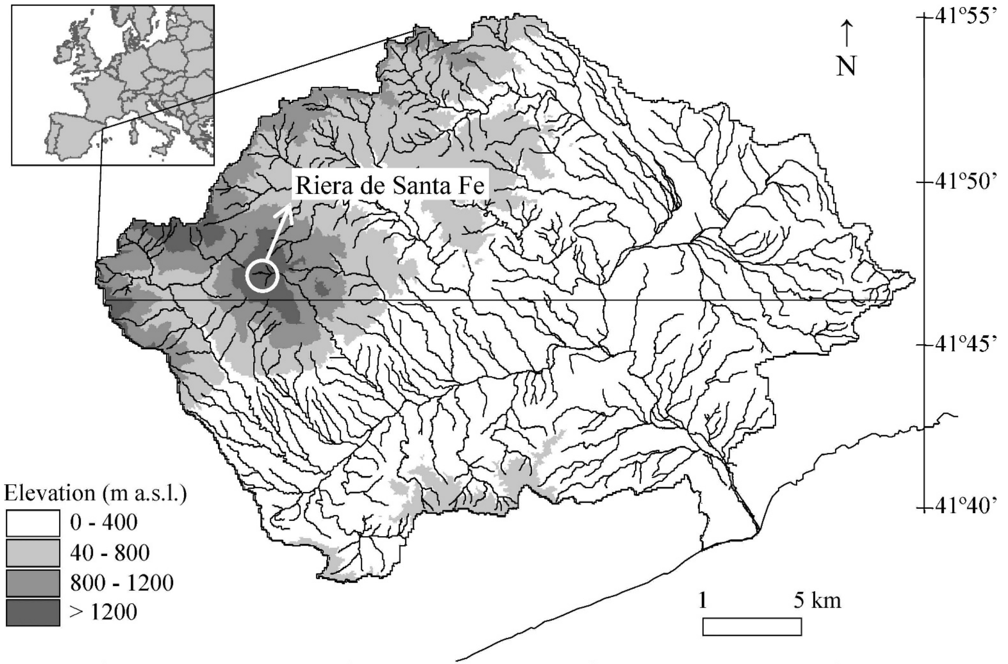

Fig. 1. Map showing general location of Riera de Santa Fe in Europe and in the La Tordera catchment.

biogeochemistry. The effects of autumnal leaf litter inputs on stream biota (Maamri et al. 1997) and metabolism (Acuña et al. 2004) have been studied previously in Mediterranean streams, but little is known about their effect on nutrient retention. In addition, methodological constraints on measuring nutrient retention under flood conditions, together with the unpredictability and high rate of change of these episodic events, have restricted the number of studies assessing flood effects on nutrient retention (but *see* Martí et al. 1997).

Nutrient retention in stream ecosystems is a combination of hydrologic, biologic, and chemical retention (Valett et al. 1996). Hydrologic retention is influenced by discharge and the hydraulic and morphologic properties of the stream channel, which determine the extent of the transient water storage (i.e., water moving at slower velocity than the average velocity in the channel, relative to the free flowing water). The magnitude of the transient water storage can be relevant for nutrient retention, because the interaction between stream biota and available nutrients is greater in transient storage zones than in the free flowing water (Triska et al. 1989). Therefore, it has been hypothesized that the influence of in-stream processes on stream nutrient concentrations increases with transient water storage. However, literature results conflict (Mulholland et al. 1997; Hall et al. 2002; Ensign and Doyle 2005), possibly because transient water storage can originate in a variety of channel structures that delay water transport (e.g., eddies, back waters, side channels, streambed irregularities, pools, mats of filamentous green algae, hyporheic zones, and organic debris dams), which may contribute differently to nutrient retention across streams or within streams over time.

The presence of in-channel vegetation (Harvey et al. 2003), debris dams (Lautz et al. 2006), flow obstacles (Ensign and Doyle 2005), and complex stream morphology (Gücker and Boëchat 2004) enlarges transient storage zones relative to main channel cross-sectional area. In temperate streams with well developed riparian forests, autumnal litter inputs may naturally modify channel hydraulic properties, increasing transient water storage (Haggard and Storm 2003). Ecologically, leaf litter constitutes an external input of energy and resources to stream communities and provides new colonization substrata for microbial communities. Therefore, an increase in transient water storage coupled to enhanced microbial colonization during leaf fall can result in a hot nutrient retention moment (sensu McClain et al. 2003). Mulholland et al. (1985) attributed temporal variation in phosphate retention efficiency to the availability of benthic organic matter in streams, showing that it was greatest during leaf fall. Nevertheless, several studies have also shown that discharge is a key factor in controlling stream nutrient retention efficiency. In general, rising discharge lowers nutrient retention, which may override or counterbalance other effects such as the accumulation of benthic organic matter. This pattern is consistent both for variation across streams (Peterson et al. 2001; Hall et al. 2002; Martí et al. 2004) and for temporal variation within a stream (Butturini and Sabater 1998). Floods not only suddenly increase discharge, but also dramatically affect stream biota and decrease stream nutrient retention (Martí et al. 1997). Little is currently known about the subsequent recovery of streams from high discharge events.

In this study we present insights into the combined effects of leaf litter inputs and flooding on in-stream nutrient retention. The aims of this study were (1) to examine how autumnal accumulation of leaf litter modifies the hydraulic properties of the channel, including transient water storage, (2) to evaluate nutrient retention response during this period, and (3) to examine its relationship with leaf accumulation and hydraulic properties. The occurrence of a flood just after all trees had completely lost their leaves enabled us to examine flood effects and subsequent responses of both hydraulic properties and in-stream nutrient retention to the abrupt removal of much of the accumulated leaf litter.

Methods

Study site—The study was conducted in Riera de Santa Fe, a second-order tributary of La Tordera stream in the natural protected area of Parc Natural del Montseny in the northeastern Iberian Peninsula (50 km north of Barcelona, Spain; Fig. 1). At the study site (1,180 m a.s.l.) the stream drains a 2.15 km^2 catchment dominated by siliceous geology and forested primarily with *Fagus sylvatica* at lower elevations and *Abies alba* at higher elevations. Mean annual precipitation is 1,200 mm and occurs mostly during autumn and spring. Precipitation occasionally falls as snow during the coldest months (December and January). Stream flow is permanent, with a mean discharge of 16.2 L s^{-1} (biweekly 2004–2005). During floods, which occur mostly during spring and autumn, stream discharge can increase by more than two orders of magnitude.

We selected a morphologically homogeneous 140-m reach, dominated by pools and riffles and 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 (5%) and sand (1%). The riparian vegetation was well developed and dominated by trees (*Fagus sylvatica*) with some shrubs (*Sambucus nigra*).

Sampling strategy—Between October and December 2004 we assessed the temporal variation in hydraulic and nutrient retention parameters relative to variation in leaf benthic standing stocks and stream discharge. The leaf fall period began in the first week of October and lasted through November. We sampled benthic litter weekly in October, then at least twice weekly through November until all the trees had lost their leaves. In the first week of December, a major flood washed out 65% of the leaf biomass. To assess the temporal variation in hydraulic and nutrient retention parameters as the flood receded, we then sampled three times a week through December. In total, 21 samplings were completed during the study period (Oct–Dec 2004).

On each sampling date, we conducted a short-term conservative tracer (Cl$^-$ as NaCl) addition at constant rate to estimate transient water storage parameters. In 13 additions we also added ammonium (NH_4-N) (as NH_4Cl) and PO_4-P (as $Na(H_2PO_4)\cdot 2H_2O$) to estimate nutrient retention metrics. Additions began at approximately 11:00 h and lasted until conductivity reached a plateau indicating complete mixing at the bottom of the reach (i.e., 2–3 h). Conductivity was automatically recorded every 5 seconds using a conductivity meter (WTW, model LF 340) connected to a data logger (Campbell Scientific) placed at the bottom of the reach. On the dates of nutrient addition, we measured conductivity and collected water samples at eight locations along the reach before the addition and once conductivity reached a plateau. Water samples for chemical analyses were collected using 50-mL syringes. At each location, two replicates were collected for ambient concentrations and five for plateau concentrations. Water samples were filtered in the field using Whatman GF/F fiberglass filters (0.7 μm pore diameter) and refrigerated until analysis. NH_4-N, nitrate (NO_3-N), nitrite (NO_2-N), and soluble reactive phosphorus (SRP) concentrations were analyzed following standard colori-

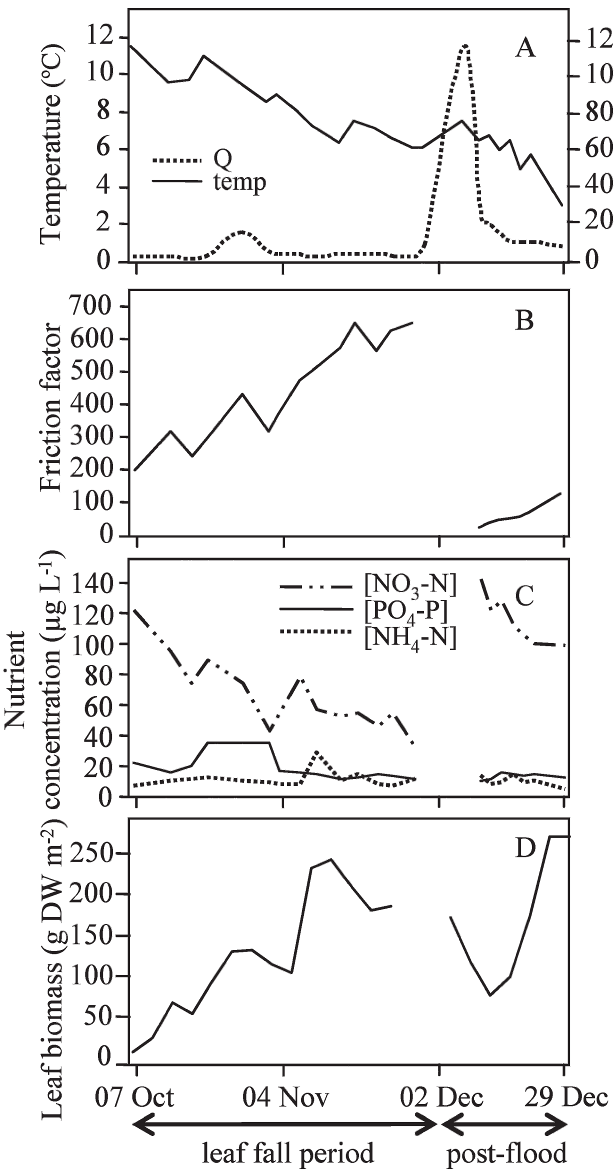

Fig. 2. Temporal variation of (A) stream discharge and water temperature, (B) Darcy–Weisbach friction factor, (C) ambient nutrient concentrations, and (D) leaf standing stocks in the stream channel during the study period. The break in the lines corresponds to the onset of flooding.

Table 1. Temporal variation in nutrient uptake length (S_w), uptake length ratio (S_w NH_4-N : S_w PO_4-P), nutrient uptake velocity (V_f), uptake velocity ratio (V_f NH_4-N : V_f PO_4-P), and DIN : P molar ratio during the study period.

Date	S_w (m)		S_w ratio	V_f (mm min^{-1})		V_f ratio	DIN : P molar ratio
	PO_4-P	NH_4-N		PO_4-P	NH_4-N		
Leaf fall period							
07 Oct 04	287.3	60.5	0.21	0.40	1.89	4.75	14.8
21 Oct 04	135.8	51.3	0.38	0.71	1.87	2.65	6.6
04 Nov 04	223.1	44.4	0.20	0.71	3.56	5.03	9.3
11 Nov 04	251.0	55.7	0.22	0.49	2.22	4.51	14.9
26 Nov 04	196.6	51.9	0.26	0.66	2.49	3.79	11.5
30 Nov 04	—	30.0	—	—	5.70	—	10.3
Post-flood period							
13 Dec 04	1312.2	416.4	0.32	0.55	1.73	3.15	45.4
15 Dec 04	825.2	551.5	0.67	0.68	1.01	1.50	32.9
17 Dec 04	665.6	299.9	0.45	0.68	1.50	2.22	24.0
19 Dec 04	585.2	240.8	0.41	0.55	1.34	2.43	24.0
21 Dec 04	429.3	227.6	0.53	0.61	1.15	1.89	22.8
23 Dec 04	287.2	148.6	0.52	0.88	1.70	1.93	21.0
29 Dec 04	202.5	92.5	0.46	1.16	2.54	2.19	22.9

metric methods (APHA 1998) using Bran+Luebbe autoanalyzers (TRAACS for NO_3-N, NO_2-N, and SRP, and Technicon for NH_4-N). On average, the nutrient additions increased NH_4-N and SRP concentrations by 14.4 ± 7.6 and 6.8 ± 2.7 fold above ambient concentrations, respectively. Despite this variability in enrichment levels, we did not find a significant relationship between the degree of enrichment and uptake lengths or uptake velocities.

Water temperature at each sampling station was determined several times over the course of each addition, and values were averaged. Discharge (Q, L s^{-1}) and average water velocity (u, m s^{-1}) were measured using the time–curve conductivity data recorded at the bottom of the reach. Calculation of u was done by dividing the reach length by the time needed to increase the conductivity to one half of the plateau (i.e., nominal travel time). Q calculation was based on a tracer mass balance approach.

Along the reach, we defined 29 channel transects at 5-m intervals. On each sampling date at each transect we measured the channel wetted width (w, m) and a cross-sectional profile (every 20 cm) of water depth (h, m) and estimated the percentage coverage by different kinds of substrata. A measure of channel roughness was calculated by solving the Darcy–Weisbach equation

$$f = (8ghs)/u^2 \qquad (1)$$

where f is the friction factor coefficient, g is the gravitational acceleration (9.8 m s^{-2}), and s (m m^{-1}) is the streambed slope. This coefficient was used as an integrative measure of the temporal changes in both discharge and leaf standing stocks.

Finally, on each sampling date we visually estimated the percentage of stream surface that was covered by leaves and the percentage occupied by pools and riffles. These estimates were made for each stream segment between every consecutive transect. Pools and riffles were categorized according to flow types following River Habitat Survey guidelines (EA 2003). Estimated percentages for each stream segment were then used to calculate the surface area for each habitat type (i.e., pools or riffles). Leaf standing stock was determined by sampling twenty-four 20 cm × 20 cm quadrats on each sampling date. Half of the samples were collected in pools and half in riffles to account for differences in leaf accumulation patterns associated with each habitat type. Leaf samples were dried and weighed to determine dry weight (g DW m^{-2}).

Estimation of transient water storage parameters—The magnitude of transient water storage zones was quantified using the one-dimensional transport model with inflow and storage (OTIS, Runkel 1998) to obtain two parameters: (2) the transient water storage zone (A_s, m^2) and (2) the water exchange coefficient (k_1, s^{-1}) between the free flowing water and the transient storage zone.

The cross-sectional area of the stream channel (A) was calculated by dividing Q by u and was used to obtain the ratio between the cross section of the transient water storage zone and that of the surface stream channel (A_s : A). This was used to estimate k_2 (the water exchange coefficient between the transient storage zone and the free flowing water) as

$$\frac{A_s}{A} = \frac{k_1}{k_2} \qquad (2)$$

Before applying the OTIS model to the conductivity time-series data, we calculated the Damkohler number (DaI) to evaluate whether the selected reach length (L) was appropriate (Wagner and Harvey 1997). DaI was estimated as

$$\mathrm{DaI} = ((k_1 + k_2)L)/u \qquad (3)$$

Although estimated hydraulic uncertainties are lowest at DaI values ~1.0, values of 0.5 to 5 have been considered acceptable (Hall et al. 2002; Ensign and Doyle 2005). If the reach length is too short ($\mathrm{DaI} < 0.5$), not enough water enters the transient storage zone to estimate transient water storage parameters properly. Conversely, DaI values >5 indicate that dispersion dominates the shape of the solute concentration curve.

Relationships between hydraulic parameters (i.e., *w*, *h*, *u*, A_s, $A_s:A$, k_1, and k_2) and discharge or leaf benthic standing stocks were examined using univariate regression analyses. The level of significance considered was $p < 0.05$.

Calculation of nutrient retention metrics—Two nutrient retention metrics were estimated from the short-term nutrient additions at constant rate: nutrient uptake length (S_w, m) and nutrient uptake velocity (V_f, m s^{-1}), also referred to as mass-transfer coefficient (Stream Solute Workshop 1990). S_w, the average distance traveled by a nutrient molecule before being removed from the water column, was calculated based on the variation in tracer nutrient concentrations along the reach at plateau conditions (corrected for ambient levels) and by the variation in conductivity using the following equation:

$$N_x = N_0 e^{-bx} \tag{4}$$

where N is the nutrient concentration at the first sampling station (N_0) and at the sampling station located x m downstream (N_x), and b is the nutrient retention coefficient (m^{-1}). S_w is the inverse of this coefficient (i.e., $S_w = -b^{-1}$) and is inversely proportional to stream nutrient retention efficiency. Simple linear regression analysis was used to determine the degree of significance of the S_w values (accepted significance level was $p < 0.05$). The ratio between S_w for ammonium and phosphate (i.e., S_w-$NH_4:S_w$-PO_4, Martí and Sabater 1996) was calculated to compare the relative retention efficiency of the two nutrients among sampling dates.

We calculated V_f by dividing *u* times *h* by S_w. V_f indicates the rate at which a molecule of a nutrient is removed from the water column and is a good indicator of the stream nutrient demand across variable flows (Hall et al. 2002).

Relationships between nutrient retention metrics (i.e., S_w and V_f) and hydrologic and hydraulic parameters or leaf benthic standing stocks were examined using univariate regression analyses. The level of significance considered was $p < 0.05$. All statistical analyses were done using SPSS for Windows (version 12.0, SPSS, Chicago).

Results

Environmental characterization during the study period—During the leaf fall period (07 October to 02 December), Q remained almost constant at around 8 L s^{-1}. However, just after defoliation was complete on 10 December, a large storm increased Q from 7 L s^{-1} to 124 L s^{-1} (Fig. 2A). After the flood, Q receded rapidly, but at the end of the study the stream was still twice its pre-storm discharge. During the study, water temperature steadily declined from 12°C to 3°C (Fig. 2A) and was unaffected by changes in discharge. The values of the friction factor *f* increased three-fold during the leaf fall period from 205 to 657 (Fig. 2B), dramatically decreased just after the flood, and gradually increased afterward. By the end of the study, *f* was still five-fold less than the pre-flood values. Concentrations of SRP and NH_4-N remained low throughout the study (mean ± 1 SE were 14.4 ± 8.2 μg P L^{-1} and 8.3 ±

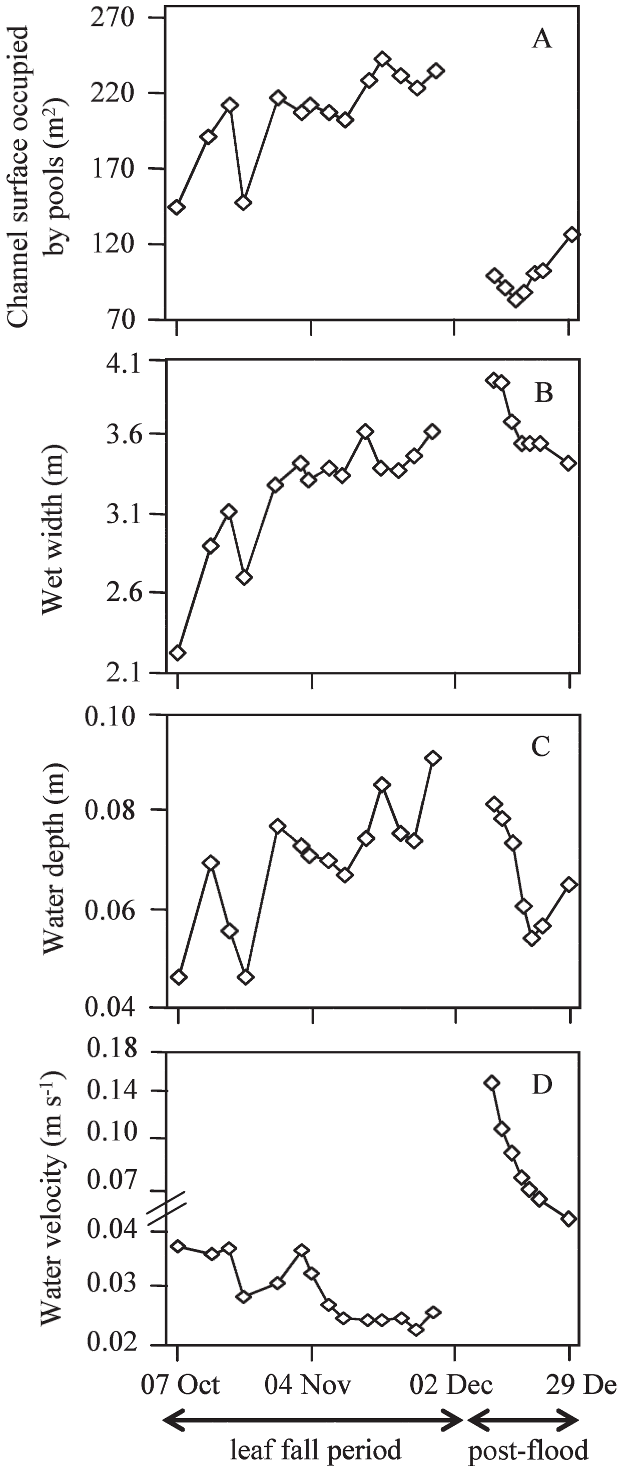

Fig. 3. (A) Surface area of the stream channel dominated by pools, (B) average channel wetted width, (C) average water depth, and (D) average water velocity during the study period. The break in the lines corresponds to the onset of flooding.

Table 2. Temporal variation of hydraulic parameters during the study period. Discharge (Q), dispersion (D), cross-sectional area of the transient storage (A_s), transient storage exchange rates (k_1, from channel to transient storage, and k_2, from transient storage to main channel), ratio of the cross-sectional area of the transient storage zone to the cross-sectional area of the stream ($A_s:A$), and Damkohler number (DaI).

Date	Q (L s^{-1})	D (m^2 s^{-1})	A_s (m^2)	$k_1 \times 10^{-3}$ (s^{-1})	$k_2 \times 10^{-3}$ (s^{-1})	$A_s:A$	DaI
Leaf fall period							
14 Oct 04	6.53	0.07	0.036	0.25	0.88	0.29	3.96
18 Oct 04	8.90	0.08	0.045	0.14	0.61	0.23	2.58
21 Oct 04	6.19	0.09	0.039	0.14	0.54	0.26	2.79
28 Oct 04	20.76	0.14	0.042	0.08	0.54	0.07	2.28
02 Nov 04	9.70	0.08	0.036	0.14	0.82	0.17	3.33
04 Nov 04	8.18	0.10	0.041	0.12	0.56	0.22	2.55
16 Nov 04	8.34	0.08	0.050	0.10	0.43	0.23	2.44
19 Nov 04	8.55	0.07	0.063	0.12	0.42	0.29	2.44
23 Nov 04	7.76	0.08	0.054	0.10	0.39	0.26	2.20
30 Nov 04	6.69	0.07	0.053	0.12	0.40	0.30	2.31
Post-flood period							
13 Dec 04	28.72	0.22	0.022	0.50	4.05	0.12	4.27
15 Dec 04	23.65	0.23	0.029	0.22	1.48	0.15	2.00
17 Dec 04	18.94	0.20	0.032	0.20	1.08	0.18	1.74
19 Dec 04	15.34	0.18	0.033	0.21	1.02	0.21	1.95
21 Dec 04	14.25	0.17	0.030	0.21	1.11	0.19	2.30
23 Dec 04	15.47	0.17	0.041	0.20	0.93	0.22	2.13
29 Dec 04	11.61	0.15	0.043	0.19	0.75	0.25	2.18

5.0 μg N L^{-1}, respectively; Fig. 2C). In contrast, NO_3-N concentration decreased from 122 μg N L^{-1} to 30 μg N L^{-1} during leaf fall, increased sharply after the flood (to 252 μg N L^{-1}), and then decreased again. Due to the variation in NO_3-N concentration, the dissolved inorganic nitrogen to phosphorus concentration molar ratio (DIN:P) varied from a mean of 11.3 ± 4.0 before the flood to 45.4 just after the flood, after which it decreased gradually to 22.9 by the end of the study (Table 1). Leaves accumulated in the stream channel during the leaf fall period from 9.3 g DW m^{-2} to 217.5 g DW m^{-2} (Fig. 2D). After the flood, 65% of leaf benthic standing stocks were washed from the reach, decreasing stocks to 77.1 g DW m^{-2}. Two weeks after the disturbance, in-channel leaf standing stocks had recovered to pre-flood levels (Fig. 2D).

Temporal variation of hydraulic parameters—During leaf fall, the percentage of reach surface area dominated by pools increased from 51% to 67% (141 m^2 to 339 m^2; Fig. 3A). The decrease of leaf standing stocks after the flood coincided with a sharp decrease in the proportion of reach surface area dominated by pools. Once the flood receded, pool area increased again, but by the end of the study was still less than before (Fig. 3A).

Because Q was relatively constant during the leaf fall period, the observed changes in the relative proportion of habitat types resulted in a gradual increase of the average channel w and h (Fig. 3B,C) and a concomitant decrease in average u (Fig. 3D). During this period, the variation in these parameters was significantly related to the total biomass (g DW m^{-2}) of leaves accumulated in the stream channel ($w = 1.7\text{biomass}^{0.14}$, $R^2 = 0.87$, $p < 0.001$, df = 12; $h = 0.03\text{biomass}^{0.17}$, $R^2 = 0.58$, $p < 0.005$, df = 12; $u = 0.04e^{-0.0013\text{biomass}}$, $R^2 = 0.53$, $p < 0.005$, df = 12). During the flood, mean w increased from 3.6 m to 3.9 m, mean h decreased from 9 cm to 8 cm, and u increased five-fold. Once the flood receded, all of these parameters returned nearly to pre-flood values.

The Damkohler number ranged between 1.7 and 4.3 (Table 2), and therefore hydraulic parameters estimated with the OTIS model were considered reliable. A_s gradually increased over the leaf fall period (Table 2) and positively correlated with leaf benthic standing stocks ($A_s = 0.4e^{0.002\text{biomass}}$, $R^2 = 0.46$, $p < 0.050$, df = 8). In contrast, k_1 and k_2 slightly decreased during this period and were negatively correlated with leaf benthic standing stocks ($k_1 = 0.0004\text{-}0.0001\ln(\text{biomass})$, $R^2 = 0.59$, $p = 0.010$, df = 8 and $k_2 = 0.0008e^{-0.003\text{biomass}}$, $R^2 = 0.58$, $p < 0.010$, df = 8). The flood had opposite effects on the hydraulic parameters (Table 2). After the flood, A_s decreased 2.4-fold and k_1 and k_2 increased 4.2-fold and 10-fold, respectively. During discharge recession, A_s tended to increase and k_1 and k_2 tended to decrease, but at the end of the sampling period A_s had not fully recovered to pre-flood values and k_1 and k_2 were still two times greater than pre-flood values.

Combining all dates, $A_s:A$ and A_s were negatively correlated with Q ($A_s:A = 0.27e^{-0.03Q}$, $R^2 = 0.63$, $p < 0.001$, df = 15; $A_s = 0.05e^{-0.02Q}$, $R^2 = 0.36$, $p < 0.050$, df = 15), whereasa k_1 and k_2 were positively correlated to Q ($k_1 = 6.4 \times 10^{-5} + 9.1 \times 10^{-6}Q$, $R^2 = 0.42$, $p = 0.005$, df = 15; and $k_2 = 0.0003e^{0.07Q}$, $R^2 = 0.69$, $p < 0.001$, df = 15). No transient water storage parameter was significantly related to litter accumulation when all the sampling dates were combined. Much of the variability in A_s, k_1, and k_2 was explained by the friction factor coefficient, f ($A_s = 0.013f^{0.219}$, $R^2 = 0.74$, $p < 0.001$, df = 15; $k_1 = 0.001f^{-0.33}$, $R^2 = 0.69$, $p < 0.001$, df = 15; $k_2 = 0.009f^{-0.48}$, $R^2 = 0.77$, $p < 0.001$, df = 15).

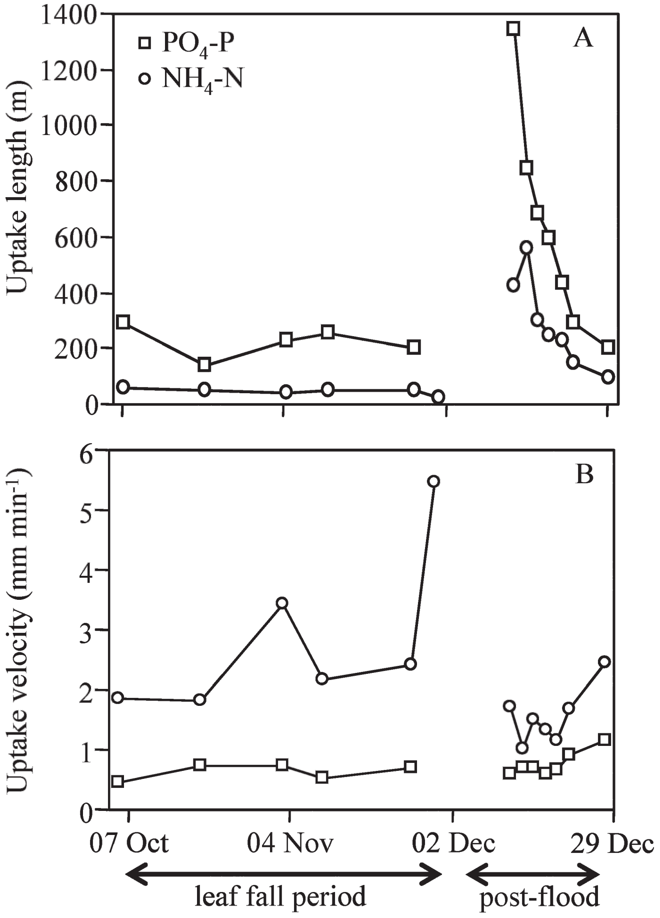

Fig. 4. (A) Temporal variation of uptake length (S_w) and (B) uptake velocity (V_f) during the study period for PO_4-P (squares) and NH_4-N (circles). The abrupt increases in nutrient uptake lengths coincided with the onset of flooding.

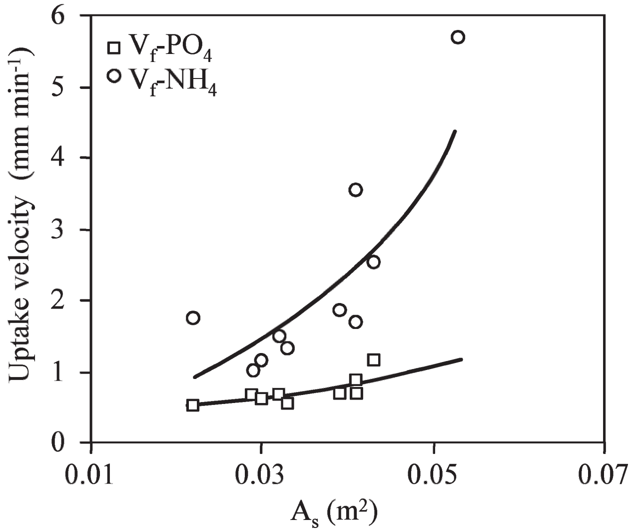

Fig. 5. Relationships between transient storage and nutrient uptake velocities (V_f-PO_4 = $0.29e^{25.93A_s}$, $R^2 = 0.59$, $p < 0.050$, df = 7; V_f-NH_4 = $0.33e^{48.37A_s}$, $R^2 = 0.65$, $p = 0.005$, df = 8) for PO_4-P (squares) and NH_4-N (circles).

Temporal variation of nutrient retention metrics—During the leaf fall period, S_w did not show a clear temporal trend for either nutrient. On average, S_w-PO_4 and S_w-NH_4 were relatively short (i.e., mean ± 1 SE were 219 ± 57 m and 49 ± 11 m, respectively). S_w-NH_4 was on average four times shorter than S_w-PO_4; thus, the S_w-NH_4 : S_w-PO_4 ratio was consistently <1 (Table 1). The flood caused a dramatic increase in S_w for both nutrients (Fig. 4A). This effect was greater for S_w-NH_4 (eight times longer than pre-flood values) than for S_w-PO_4 (6.7 times longer than pre-flood values). After the flood, S_w of the two nutrients gradually shortened with stream discharge, almost returning to average pre-flood values by the end of the study. The recovery rate, estimated from a post-flood decrease in S_w with time, for S_w-PO_4 (-1.01 d^{-1}) was significantly greater than that for S_w-NH_4 (-0.89 d^{-1}; $p < 0.05$, Wilcoxon test for two related variables).

Values of V_f-NH_4 were on average 3.0 ± 1.2 times greater than those of V_f-PO_4 (Table 1). V_f tended to gradually increase as leaves accumulated in the channel (Fig. 4B). The flood decreased both V_f-PO_4 and V_f-NH_4 (1.2 times and 1.4 times, respectively). V_f-PO_4 increased gradually post-flood, reaching slightly greater values than pre-flood. V_f-NH_4 remained low after the flood, gradually increasing as discharge receded (Fig. 4B). Regression analyses showed that V_f-PO_4 was related to leaf benthic standing stocks and temperature (Table 3). V_f-NH_4 was negatively correlated to DIN concentration and the DIN : P molar ratio (Table 3). No significant relationship was found between V_f and SRP concentration. Both V_f-PO_4 and V_f-NH_4 were positively related to A_s (Fig. 5).

Discussion

Influence of leaf litter inputs and the flood on hydraulic parameters—The large quantity of accumulated leaves during the leaf fall period, coinciding with low and stable discharge, affected the relative dominance of habitat type and hydraulic characteristics of the reach. Leaf inputs tended to accumulate at the head of the riffles favoring the formation of small leaf-debris dams, which increased the upstream pool surface area. Shifting proportions of habitat types presumably induced the observed change in hydraulic properties. As leaves accumulated on the stream channel, it became wider and deeper, water velocity decreased, and channel roughness increased. These changes caused a gradual change in transient water storage parameters. The increase in A_s indicates an increased volume of water in transient storage zones, consistent with previous findings on the physical effect of leaf litter accumulation (Hart et al. 1999; Haggard and Storm 2003; Jin and Ward 2005). In addition, the gradual decrease in k_1 and k_2 during the leaf fall period indicates weakened connectivity between the slow and fast flowing hydrologic compartments. Together, these changes in the hydraulic template, mostly driven by litter inputs, increased the water residence time and enhanced the opportunity for nutrient uptake by microbial communities.

Flooding greatly reduced the influence of litter accumulation on channel hydraulics. Whereas changes driven by leaf litter inputs were gradual (weeks), changes due to the

Table 3. Regressions of uptake velocities on environmental variables (leaf biomass as dry weight, water temperature, dissolved inorganic nitrogen concentration [DIN] and DIN : P molar ratio) during the study period.

	V_f-PO_4 (mm min^{-1})	V_f-NH_4 (mm min^{-1})
Biomass (g DW m^{-2})	V_f-PO_4=0.42biomass-0.002 $R^2=0.55$, $p<0.010$, df=10	no sign.
Temperature (°C)	V_f-PO_4=1.40-0.38ln(temp) $R^2=0.47$, $p<0.050$, df=10	no sign.
[DIN] (ppb)	no sign	V_f-NH_4=14.75-2.73ln[DIN] $R^2=0.75$, $p<0.001$, df=11
DIN : P	no sign.	V_f-NH_4=8.82[DIN : P]$^{-0,52}$ $R^2=0.37$, $p<0.050$, df=11

No sign. no significant relationship.

flood were abrupt (days). The abrupt increase in discharge significantly increased water velocity, decreased relative transient water storage (i.e., reduced A_s and A_s : A) and increased connectivity between low and high flow hydrologic compartments (i.e., greater k_1 and k_2). These findings are similar to those of Martí et al. (1997) for a Sonoran Desert stream (Arizona). The changes observed in the study stream just after the flood could be the result of both the washout of a high proportion of leaves from the channel, which reduced surface obstructions, and an increase in the cross-sectional area (A) of the channel. These changes likely reduced the influence of streambed topography on surface-water flow, decreasing the relative importance of A_s. Conversely, after recession, transient water storage parameters returned to pre-flood values faster than discharge. This was probably because of lateral leaf litter inputs from adjacent riparian soil, which created zones of slow moving water in the stream channel. These results suggest that most variation in transient water storage was associated with surface-water compartments rather than modification of subchannel flow paths in the hyporheic zone.

Considering all sampling dates, stream discharge accounted for the largest proportion of the variability in the hydraulic parameters. However, when we focus on low flow conditions, our results indicate that leaf standing stocks primarily influenced stream hydraulics. Variation in transient water storage parameters was related to leaf benthic standing stocks only during leaf fall, when discharge was low; however, this relationship was not significant for the entire study period. In contrast, we found a negative relationship between A_s : A and discharge using the complete data set. In a study with similar results, D'Angelo et al. (1993) argued that at high discharge transiently stored water is more quickly incorporated into the main flow, but at low discharge is stored more independently of the main flow. Results from previous studies relating A_s and Q are inconsistent. Whereas no relationship has been found for some streams (e.g., Hart et al. 1999), others agree with the present study (Valett et al. 1996; Martí et al. 1997; Butturini and Sabater 1999). Similarly, the positive relationship between k_1 and k_2 and discharge found in this study agrees with some previous studies (Hart et al. 1999), but contrasts with others who either found no relationship between exchange coefficient and discharge (Butturini and Sabater 1999; Hall et al. 2002; Jin and Ward 2005) or found an inverse relationship (D'Angelo et al. 1993; Martí et al. 1997).

In order to test if our results obey a general pattern, we compiled data from 17 studies done across several streams worldwide (n = 187) to see if a significant relation between transient water storage parameters and discharge emerged. Results from this analysis revealed significant relationships between these parameters and streamflow across all the streams considered (Fig. 6), supporting results from our study. Nevertheless, the percentage of variation of transient water storage parameters explained by discharge considering the compiled data was less than that obtained considering only data from the present study, probably reflecting the variable location and nature of the transient water storage zones in each particular stream.

Combined effects of leaf litter inputs and the flood on nutrient retention—The two nutrient retention metrics (S_w and V_f) helped to determine the relative influence of both leaf inputs and flooding on stream nutrient retention. Leaf litter inputs played an important role in PO_4 and NH_4 retention during stable Q although abrupt flooding and subsequent flood recession largely controlled the overall variability in nutrient retention. To our knowledge, this study is the first of its kind with sufficiently intensive sampling to evaluate stream nutrient retention to gradual and abrupt changes over a short time scale.

The observed nutrient retention responses (both S_w and V_f) for PO_4 and NH_4 were in line with headwater streams elsewhere (e.g., *see* Peterson et al. 2001; Hall et al. 2002; Webster et al. 2003). Retention of both nutrients was relatively high during leaf fall. In addition, the S_w-NH_4 : S_w-PO_4 ratios, which were consistently <1, indicate greater efficiency in retaining NH_4 than PO_4 regardless of leaf benthic standing stocks or stream discharge. A similar pattern was previously reported from another stream in the same region (Martí and Sabater 1996).

In contrast to our expectations, PO_4 and NH_4 retention efficiencies (i.e., S_w) remained relatively constant during the leaf fall period, when discharge was relatively constant and low, despite the clear increase in the additional energy resource from the leaf inputs and in transient water storage size. The lack of relationship between S_w-PO_4 and leaf litter accumulation agrees with D'Angelo et al. (1991), but

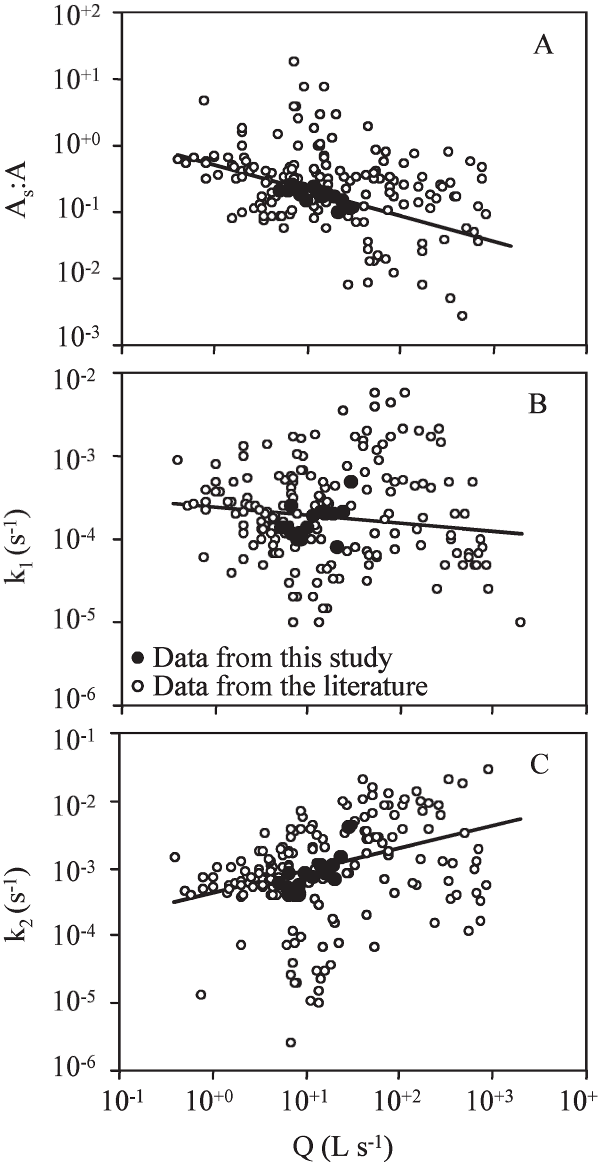

Fig. 6. Relationships between stream discharge and transient storage parameters: (A) transient storage ratio, $A_s : A = 0.51Q$-0.28, $R^2 = 0.14$, $p < 0.001$, df = 185, (B) exchange ratio from transient storage to main channel, $k_1 = 0.0002e^{-0.0013Q}$, $R^2 = 0.05$, $p = 0.002$, df = 175, and (C) exchange ratio from main channel to transient storage, $k_2 = -0.0003 + 0.001 \ln Q$, $R^2 = 0.17$, $p < 0.001$, df = 170. Closed circles are data from the present study and open circles are data from Bencala and Walters (1983), Bencala et al. (1984), Bencala et al. (1990), Broshears et al. (1993), D'Angelo et al. (1993), Martí et al. (1997), Morrice et al. (1997), Mulholland et al. (1997), Butturini and Sabater (1999), Hart et al. (1999), Hall et al. (2002), Haggard and Storm (2003), Harvey et al. (2003), Webster et al. (2003), Ensign and Doyle (2005), Jin and Ward (2005), and Lautz et al. (2006).

contrasts with other studies, showing greater P retention efficiency during periods of benthic organic matter accumulation (Mulholland et al. 1985; Haggard and Storm 2003). Differences could be explained in part by the temporal scale of each study. Whereas previous studies were temporally extensive (annual), the present study was temporally intensive (seasonal). Moreover, the relationship between NH_4 retention efficiency and temporal shifts in benthic organic matter has seldom been addressed.

Nutrient retention efficiency is affected by both hydromorphologic factors and biogeochemical processes. V_f corrects S_w for depth and velocity (Stream Solute Workshop 1990), and was more sensitive to benthic leaf accumulation and its influence on transient water storage parameters than S_w. The variation in V_f for both nutrients followed the increase in transient water storage (A_s) both before and after the flood. At these two moments, transient water storage increased and there was a greater decoupling between fast and slow hydrologic compartments (i.e., reduced k_1 and k_2). These conditions may have favored the interaction between stream communities and available nutrients and thus increased stream nutrient demand. In addition, because leaf inputs increased the availability of energy-rich substrata, increased nutrient demand could also be directly associated with the development of microbial communities. Ensign and Doyle (2005) similarly observed a greater nutrient demand as superficial transient storage increased after baffles were installed in a channel. Other studies have found no significant relationships between transient storage parameters and V_f (e.g., Hall et al. 2002; Webster et al. 2003). In those studies, however, relationships were compared between different streams; therefore, the lack of relationships could be attributed to differences in the nature of the transient water storage and the associated biogeochemical processes.

The same arguments presented above could also explain the subtle differences in temporal patterns observed between PO_4 and NH_4 uptake velocities. For instance, temporal variation in V_f-NH_4 was influenced by the increase in transient storage volume just like V_f-PO_4, but it was also negatively influenced by the availability of dissolved inorganic nitrogen (mostly in the form of NO_3). Hall et al. (2002) found similar results and suggested that high NO_3 concentration may alleviate microbial demand for NH_4, lowering V_f-NH_4.

The greatest temporal variability in nutrient retention metrics was related to the flood and its subsequent recession. Nutrient retention efficiency of PO_4 and NH_4 was significantly reduced after the flood. The increased discharge would be partly responsible (Martí et al. 1997). The flood also flushed out a high percentage of leaves, removing channel obstructions and exporting part of the microbial community. These effects likely contributed to the decreased post-flood nutrient demand. Therefore, the combination of physical factors (i.e., increase in water depth and velocity and reduction in transient water storage) and biological consequences (i.e., decrease in nutrient demand) resulted in reduced post-flood retention efficiency. The stream recovered relatively fast from disturbance. Based on the estimated recovery rates, average pre-flood S_w values for PO_4 and NH_4 were reached 22 days and 47 days after the flood, respectively. Theses values are within the range of those reported for S_w-NO_3 in Sycamore Creek (Martí et al. 1997). In that study, the recovery in nutrient retention was attributed to a fast post-flood growth of algal communities. In the present study, S_w recovery could be associated with a significant increase in NH_4 and PO_4 demand as discharge declined and leaves reaccumulated. Return to base flow stream discharge took 64 days, much longer than for S_w. This indicates that biogeochemical processes significantly contributed to the high resiliency (i.e., fast recovery capacity) in nutrient retention, presumably enhanced by the lateral leaf input.

In summary, the seasonal litter input not only modified the physical template of the stream reach, but also increased PO_4 and NH_4 demand, either directly through microbial demand or through increasing transient storage. The flood altered channel hydraulic properties and nutrient retention in the stream. However, once the flood receded, the stream exhibited fast recovery in nutrient retention, especially for PO_4, probably enhanced by the lateral input of riparian leaf litter. Therefore, the net contribution of leaf inputs to headwater stream metabolism over the leaf fall period appears to depend on the timing of floods relative to leaf fall. This has implications in the context of climate change because both the timing of leaf fall and flood regimes are expected to be influenced by climate change (Christensen and Christensen 2004). For example, Peñuelas et al. (2002) have reported that leaf fall has been delayed an average of 13 days relative to 1952 in the study area. Stream hydrologic regimes are also expected to change (McCarthy et al. 2001), with projections for the study region indicating an increase in extreme episodic storms and droughts. These changes may alter the timing of leaf fall and floods, with varying impacts on nutrient retention. Less flooding during the season of leaf fall would increase the residence time of leaves in the stream channel, enhancing nutrient retention. Conversely, an increase in flood frequency during leaf fall would result in leaf litter being periodically flushed from the headwaters and exported downstream to zones of higher flow and lower nutrient retention capacity.

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Development of a "smart" tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: The resazurin-resorufin system

Roy Haggerty,[1] Alba Argerich,[2] and Eugènia Martí[3]

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[1] A "smart" tracer is a tracer that provides, directly or through measurement of its concentration or in combination with another compound, at least one "bit" more of information about the environment through which it travels than a conservative tracer. In this study we propose and present the chemical compound resazurin as a smart tracer to assess the coupling between solute transport and microbiological activity in sediment-water interfaces in freshwaters. Resazurin is a weakly fluorescent redox-sensitive dye that undergoes an irreversible reduction to strongly fluorescent resorufin under mildly reducing conditions, most commonly in the presence of living microorganisms. To investigate the suitability of resazurin as a smart tracer, we characterized the decay, sorption, reaction, and transport behavior of resazurin and resorufin in various waters and sediments using laboratory experiments. Results show that resazurin irreversibly and rapidly reacts to resorufin in colonized sediment with pseudo-first-order behavior and a rate coefficient of 1.41 h^{-1}. This reaction is 3 orders of magnitude faster than that in stream water alone, indicating the tracer is sensitive to microbiological activity and associated sediment-water interactions. The compounds are affected by significant sorption, with an approximately linear isotherm and a K_d of 6.63 mL/g for resorufin in sediment with 2.19% organic carbon. The compounds are stable over weeks in natural water, except in the presence of strong light where significant photochemical decay may occur more rapidly.

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1. Introduction

[2] Applications of tracers are one of the key techniques used in hydrological studies of surface freshwaters and groundwaters (for a review, see *Flury and Wai* [2003]). However, the information that conservative tracers provide is largely tied to water transport and arrival time. The processes available for direct study via arrival time are limited to advection, dispersion (spreading of arrival times), and transient storage or mass transfer (tailing of arrival times). In freshwater ecosystems these processes can have a large influence on biogeochemical responses. However, some hydrologic processes may be indistinguishable using arrival times of conservative tracers; for example, advection through low-permeability zones and diffusion into and out of low-permeability zones can yield the same arrival times [*Becker and Shapiro*, 2000; *Sánchez-Vila and Carrera*, 2004]; and in-stream transient storage can yield similar arrival times to hyporheic transient storage [*Gooseff et al.*, 2005]. Furthermore, biogeochemical processes have non-unique responses to arrival times due to dependence on biological community, biogeochemical heterogeneity between flow paths, and other factors [e.g., *Jones et al.*, 1995; *Kirchner*, 2003; *Nagorski et al.*, 2003]. Given this context, geo/eco-hydrology would be advanced considerably if our community could develop tracers that collect information about the environment through which they traveled, tracers that are, simply put, smarter than conservative tracers. We define a "smart tracer" as a tracer that provides, directly or through measurement of its concentration or in combination with another compound, at least one "bit" more of information about the environment through which it travels than a conservative tracer. In other words, the smart tracer provides direct information about conditions in the hydrologic system (location history, chemical conditions, biological activity, physical interactions) in addition to arrival time. In this study, we present a prototype smart tracer for the measurement of microbiological activity and associated sediment-water interactions in freshwater ecosystems.

[3] A smart tracer should have characteristics common to all good conservative tracers [*Flury and Wai*, 2003]. That is, the tracer (and, if applicable, its daughter products) should be (1) conservative, with one exception; (2) clearly discernable from the background of the system; (3) of low toxicity; and

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[1] Department of Geosciences, Oregon State University, Corvallis, Oregon, USA.

[2] Departament d'Ecologia, Universitat de Barcelona, Barcelona, Spain.

[3] Limnology Group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Blanes, Spain.

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(4) insensitive to variations in solution or medium chemistry, also with one exception. The one exception is that the smart tracer must undergo an irreversible change in the presence of a process or condition under investigation. This irreversible change must be (1) detectable with a measurement technique or sensor; (2) rapid relative to transport rates; and (3) insensitive to likely chemical or other variations in the solution or medium. Finally, the ideal smart tracer and its measurement technique should be inexpensive, safe to handle and not harmful to the environment.

[4] We are aware of the previous use of at least one smart tracer in hydrology. *Gramling et al.* [2002] used the irreversible reaction of $CuSO_4$ and $EDTA^{4-}$ to $CuEDTA^{2-}$ and the resulting color change to measure true chemical-scale mixing and distinguish it from hydrodynamic dispersion. This smart tracer allowed direct measurement of the amount of longitudinal molecular-scale mixing that occurred in a tank experiment. Smart tracers have been used in other fields; for instance, in fluid mechanics, reacting compounds that change color have been used to measure mixing times in chemical reactors [*Melton et al.*, 2002; *Cabaret et al.*, 2007]. Though not strictly a tracer technique, smart radiotracers used in positron emission tomography preferentially lodge in tumors owing to unique metabolism found there (e.g., inefficient and higher rate of glucose use). When the radiotracer decays it emits a positron that can be used to tomographically image the tumor [*Juweid and Cheson*, 2006].

[5] The objective of this study is to identify and characterize a smart tracer to measure microbiological activity associated with stream water-sediment interaction. Because in lotic (i.e., flowing water) ecosystems, microbial activity is mostly associated with biofilms developed on sediments [*Fischer and Pusch*, 2001], this assay will be useful for increasing our understanding of the interaction between hydrology and biogeochemistry in these ecosystems, and could also extend to other aquatic ecosystems.

[6] After a search of candidates, we selected resazurin (Raz) as our potential smart tracer. This compound, also known as Alamar Blue [*O'Brien et al.*, 2000], is a redox-sensitive phenoxazine dye [*Tratnyek et al.*, 2001; *Bueno et al.*, 2002] that has been known for more than a century, for example to test the hygienic quality of milk (see discussion by *Twigg* [1945]). In the presence of mildly reducing conditions, Raz loses an oxygen ion irreversibly to become resorufin (Rru). Rru can also undergo a further, reversible reduction to hydroresorufin, but this is not favored in the presence of oxygen. The reduction of Raz to Rru is a well-documented indicator of the presence of living bacteria [*Liu*, 1983; *Peroni and Rossi*, 1986; *De Fries and Mistuhashi*, 1995; *O'Brien et al.*, 2000; *Guerin et al.*, 2001; *McNicholl et al.*, 2007], particularly of aerobic bacteria [*Karakashev et al.*, 2003]. The Rru-Raz reaction has been widely used as an indicator of bacterial growth in milk [e.g., *Ramsdell et al.*, 1935; *Moyer and Campbell*, 1963] and semen quality [e.g., *Erb and Ehlers*, 1950; *Zrimšek et al.*, 2004]. Both Raz (blue) and Rru (red) are intensely colored and fluorescent, but Rru is more fluorescent, as indicated by the quantum efficiency [*Bueno et al.*, 2002] (Figure 1). Raz and Rru are not highly toxic, and in fact are used in evaluation of toxicity to count living bacteria [*O'Brien et al.*, 2000]. *Fung and Miller* [1973] did experiments with 10,000 μg/L Raz and found that the growth of most of the species of bacteria considered (23 out of 30) were not inhibited by the dye. Therefore, a priori, this compound fulfilled most of our initial requirements for a smart tracer.

[7] To verify if resazurin could be used as a smart tracer, here we present results from laboratory experiments coupled with application of mathematical models for parameter estimation, which were used to characterize decay, sorption, and reaction of Raz and Rru.

2. Methodology

2.1. General Methods

[8] Raz, Rru, and other reagents were purchased from Panreac Química S.A. (Castellar del Vallès (Barcelona), Spain; www.panreac.com) and used as supplied. The 2007 bulk cost of Raz was slightly less than 1000 USD for 250 g in both the U.S. and Europe (experiments described in this paper required $\ll$5 g). Initial comparison of fluorescence signals showed that Raz was contaminated by 3% Rru. *Kangasniemi* [2004] showed that the fluorescence of Rru is constant above pH 8, but that it decreases sharply below pH 6.5. *Bueno et al.* [2002] showed very similar results for the fluorescence of Raz. Consequently, water samples from the experiments were buffered to pH 8 prior to analysis of Raz and Rru. A stock solution of buffer near pH 8.0 was generated by mixing 1 molar $NaH_2PO_4{\cdot}H_2O$ with equal parts 1 molar NaOH, which was added to samples at a 1:10 buffer-to-sample ratio. Where noted, filtration of water samples was performed with 0.7 μm glass fiber filter (GF/F, supplied by Whatman, Kent, UK, and Albet, Sant Boi de Llobregat (Barcelona, Spain). All laboratory materials for the experiments described below were triple-washed with tap water, triple-rinsed with deionized water (DIW), and dried if needed.

[9] Fluorescence of Raz and Rru in water samples was measured on a Shimadzu RF spectrofluorometer, with excitation and emission wavelengths given in Figure 1. Samples were placed in a quartz cuvette and held within the sample chamber for less than 1 min to minimize temperature changes. Unless otherwise noted, samples were stored in the dark at room temperature prior to measurement, which was always within 24 h of sampling (and usually within 1 h). Error bars for data were calculated assuming independent contributions of error from sample preparation, sample measurement, and signal measurement (machine error). Sample preparation, sample measurement, and signal measurement were each performed a minimum of 7 times to isolate the targeted error (*Clesceri et al.* [1999], method 1020). The limit of quantitation (LOQ, approximately 3 times the limit of detection, LOD) for Rru in DIW is 0.012 μg/L (0.05 nmol/L). Because Rru fluoresces more strongly than Raz and because the fluorescence spectra overlap, the LOD and LOQ for Raz depend on Rru concentration. In DIW the LOQ for Raz with 0 μg/L Rru is 0.16 μg/L (0.62 nmol/L), while the LOQ for Raz with 25 μg/L (0.11 nmol/L) Rru rises to 0.96 μg/L (2.2 nmol/L). The LOQ for both compounds in natural water is approximately 5 times larger. The errors reported in this manuscript account for variable Rru concentration.

[10] Change in fluorescence due to variation in temperature in the spectrofluorometer reading cell was measured by placing a sample in the spectrofluorometer and measuring

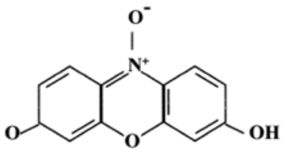
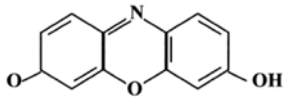
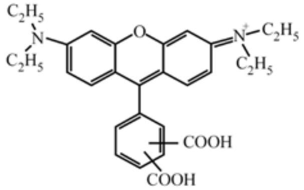
Property	Resazurin	Resorufin	Rhodamine WT
Chemical formula[1]	$NaC_{12}H_6NO_4$	$NaC_{12}H_6NO_3$	$C_{29}H_{29}N_2O_5ClNa_2$
Structure[2]			
CAS #[1]	62758-13-8	34994-50-8	37299-86-8
Molecular weight[1]	251.2 g/mol	235.2 g/mol	567.0 g/mol
Solubility @ 25°C[1]	18.4 g/L[3]	20.0 g/L[3]	180-220 g/L[4]
$\log(K_{ow})$[3,5]	0.94	1.02	-1.3
Nominal K_d[6]	n/a	6.63 mL/g[7]	33.2 mL/g[8]
Maximum excitation wavelength	602 nm[7]	570 nm[7]	555 nm[9]
Maximum emission wavelength	616 nm[7, 10]	585 nm[7]	580 nm[9]
Quantum efficiency	0.11[11]	0.74[11]	n/a but high
Fluorescence temperature correction constant[12]	n/a	-0.0041 ± 0.003 /°C[7]	-0.027 /°C[9]

Figure 1. Properties of the resazurin-resorufin system, with rhodamine WT for comparison. Unless otherwise noted, analyses were conducted on the resazurin-resorufin system near pH 8. 1, for sodium salt; 2, Raz and Rru diagrams from *O'Brien et al.* [2000], with rhodamine WT after *Vasudevan et al.* [2001] 3, estimated for molecular compound with method of *Meylan and Howard* [1995] and *Meylan et al.* [1996] and available at http://www.syrres.com/esc/ (accessed 19 June 2007); 4, *McVoy* [1985] reported by *Soerens and Sabatini* [1994]; 5, K_{ow} = octanol-water partitioning coefficient, with rhodamine WT value from *Smart* [1984]; 6, K_d = distribution coefficient; 7, this study; 8, *Gooseff et al.* [2005], reported for sediment containing 9% organic carbon; 9, *Smart and Laidlaw* [1977]; 10, in the case of resazurin, this emission wavelength yielded maximal difference (minimum interference) with the resorufin emission rather than being the maximum absolute value; 11, *Bueno et al.* [2002]; 12, temperature correction constant defined as by *Smart and Laidlaw* [1977].

fluorescence immediately at 23.0°C, turning off the lamp, and then measuring fluorescence again after the sample had equilibrated to the temperature inside the instrument (31.6°C) (Figure 1).

[11] To measure the rates of decay, sorption and reaction of the Raz-Rru chemical system and to examine if reaction rates were susceptible to water transport and biological activity we conducted three sets of experiments in the laboratory using water from two streams ("riera" in Catalan) located in the Tordera catchment (40 km NE of Barcelona, Spain) and sediment from one of them. Both streams have near-neutral pH and low total dissolved solids. The second-order Riera de Santa Fe de Montseny drains a 2.15 km^2 forested catchment and the third-order Riera de Gualba drains a 13.46 km^2 catchment of mixed forest, agriculture and urban land use. A representative sample of sediment (<2 mm size fraction) collected from the Riera de Sta. Fe was silicic and consisted of 52.8% fine-grained metasedimentary lithic fragments; 34.8% quartz grains; 6.2% feldspar grains; 3.7% granodiorite lithic fragments; and 2.38 ± 0.09% organic carbon. Inorganic sediment was manually separated by binocular microscope and weighed. Fraction of organic carbon was determined gravimetrically on 3 samples by loss on ignition at 550°C for 15 h.

[12] Prior to the experiments, the stability of a 25 μg/L solution of Rru in DIW stored in the dark at room temperature was examined. The fluorescence of this solution was measured nearly every work day for approximately 1 month. No degradation in the fluorescence was observed in this time; therefore, this solution was used as a fluorescence standard for all the subsequent experiments.

2.2. Measurement of Reaction and Decay Rates

[13] We measured the reaction, and decay of Raz and Rru in batch experiments using water from the two streams and DIW under different conditions. First, reaction and biochemical or chemical decay in natural water were measured using water from the two streams. Water from Gualba was filtered; Sta. Fe water was used unfiltered. Both were refrigerated and used within 1 week of collection. Solutions of 25 μg/L Raz and Rru were created using these waters and stored in the dark at room temperature (19°–23°C). On an approximately daily schedule for 13–22 days (depending on experiment), samples from each batch were taken, filtered in the case of Sta. Fe water, buffered, and fluorescence was measured. Second, we also measured the reaction and decay of Raz in DIW. The procedure was the same as above except that filtration was not required. No replicates were taken in these experiments.

[14] Third, we examined the effect of light (under laboratory conditions) on the decay of Raz and Rru. For this experiment we used DIW. The procedure was the same as above, except that the solutions were placed in clear glass containers and left on a counter top in the laboratory (19°–

Table 1a. Column Properties

Property	Value
Column size	15 cm × 1 cm ID
Temperature, °C	16
Porosity *n*, -	0.422
Fraction organic carbon, %	2.19
Raz concentration injected, μg/L	200
Dispersivity (conservative), cm	0.107
Dispersivity (sorbing), cm	0.647
Retardation factor *R*, Raz and Rru, -	2.07

23°C) with the lights turned on. Lights were Osram Lumilux daylight 18 W bulbs (Munich, Germany) with a broad spectrum from 400 to 600 nm and peaks at 427, 478, and 536 nm. The light intensity was 0.624 ± 0.072 mol/m^2/d, monitored with a photoactive radiation (PAR) sensor (SKP215 Quantum, Skye Instruments, Powys, U.K.).

2.3. Measurement of Sorption

[15] The sorption isotherm for Rru was measured using stream sediment from Sta. Fe. Saturated sediment was collected in a plastic bag, dried at 60°C for 72 h, then sieved (Retsch AS200, Haan, Germany) to separate the fractions larger and smaller than 2 mm. The size fraction <2 mm was divided into 17 aliquots of approximately 20 g, which were weighed and placed in 250 mL Pyrex™ jars. Weighed aliquots of approximately 200 g of filtered stream water from Sta. Fe were used to prepare 0.00, 1.00, 4.64, 21.54, and 100.00 μg Rru/L solutions which were then poured into the jars. Four replicates were made of 4.64 and 100.00 μg/L, and 3 replicates were made for other concentrations. The jars were capped, kept in the dark, and manually shaken every 2 h (4 h at night) for 41 h. Fifteen mL aliquots were extracted from the jars at the end of the incubation, placed into plastic centrifuge tubes, and centrifuged 15 min at 2358 *G*. The supernatant was decanted, filtered, buffered and measured for aqueous Rru concentration. Sorbed Rru was calculated by difference between the initial and final concentration.

[16] The sorption isotherm for Raz was not measured because the compound reduces to Rru in the presence of sediment. However, we may expect that the sorption isotherm for Raz to be similar to Rru because the compounds have very similar octanol-water partitioning coefficients (Figure 1) and solubilities in water, and are otherwise chemically similar. Therefore, where needed in the calculations we assumed that the sorption of Raz is the same as Rru.

2.4. Characterization of Transport and Reaction

[17] Reaction and transport of Raz and Rru were quantified in a series of flow-through column experiments at 3 different flow rates. A 15-cm-long, 1-cm-ID, low-pressure glass chromatography column (chromaflex, Kontes Glass Company, New Jersey, USA) was packed underwater in the field with stream sediment from Sta. Fe on 1 March 2007. The majority of sediment (visual estimate) was <2 mm size fraction with a few pebbles. The column was capped and returned to the laboratory where it was refrigerated in the dark until use (12–21 days later). Column properties are provided in Table 1a. Column experiments were conducted in a temperature-controlled room (16°C). A solution of Raz and NaCl in filtered Sta. Fe stream water was pumped at constant rate through the column using a high-pressure peristaltic pump (Masterflex L/S pump, Vernon Hills, Illinois, USA). Outflow was delivered to a capped flask on a balance to monitor flow rate. The tracer reservoir was shielded from the light with aluminum foil. The column was shielded from the light except for the slowest-flow experiment (completed first). However, lights were out in the room except when samples were being taken, which for the slow-flow experiment was only a few minutes at a time. Furthermore, the photodecay time for the compounds is at least many 10s of h (see results in section 3.1), so the lack of column shielding on one experiment had no effect. Electrical conductivity (EC) was monitored continuously in a chamber in front of the outflow port using an LF 340 EC meter (WTW, Weilheim, Germany) connected to a CR510 data logger (Campbell Scientific, Logan, Utah, USA). Water samples were extracted manually with a 15-mL Luer lock syringe at an in-line sample port placed in front of the EC chamber. Samples were taken at regular intervals over the duration of each experiment; sample frequency and experimental duration are given in Table 1b. Since samples were taken manually, the 2 slower flow rate experiments had 10–13 h sample gaps overnight. Samples were filtered immediately, buffered and stored (<5 h) in the dark at 16°C in 20-mL clear borosilicate vials prior to fluorescence measurement of Raz and Rru. Oxygen concentration was periodically measured in the inflow carboy and at the EC chamber (Table 1b) with a WTW (Weilheim, Germany) 340i portable oxygen meter.

2.5. Data Analysis

[18] In the experiments, Raz and Rru underwent the following processes: reaction of Raz to Rru, decay of both Raz and Rru to other unquantified byproducts; sorption of both Raz and Rru if sediment was present; and in the columns, both advection and dispersion. To estimate the different rates of these processes, we assumed that decay and reaction followed a first-order law and that sorption was at equilibrium. The system of equations for these processes is as follows:

$$\frac{\partial C_{Raz}}{\partial t} = \frac{\alpha_L v}{R}\frac{\partial^2 C_{Raz}}{\partial x^2} - \frac{v}{R}\frac{\partial C_{Raz}}{\partial x} - k_1 C_{Raz} - k_{12} C_{Raz} \tag{1a}$$

$$\frac{\partial C_{Rru}}{\partial t} = \frac{\alpha_L v}{R}\frac{\partial^2 C_{Rru}}{\partial x^2} - \frac{v}{R}\frac{\partial C_{Rru}}{\partial x} - k_2 C_{Rru} + k_{12}\frac{M_{Rru}}{M_{Raz}} C_{Raz}, \tag{1b}$$

where C_{Raz} [ML^{-3}] is the concentration of Raz; C_{Rru} [ML^{-3}] is the concentration of Rru; t [T] is time; x [L] is distance

Table 1b. Experimental Conditions

Condition	Low Flow	Medium Flow	High Flow
EC above background, μS/cm	115.3	139.5	131.8
Flow rate, mL/h	3.39	15.5	34.5
O_2 concentration in/out, mg/L	8.4/5.1	9.1/7.2	9.4/8.7
Velocity *v*, cm/h	13.6	32.9	80.4
Travel time (τ = *L*/*v*), h	1.10	0.455	0.187
Sample frequency (approx.), min	130	35	15
Total experiment time, h	46.9	24.9	6.65

from the upstream boundary; α_L [L] is the longitudinal dispersivity; v [LT^{-1}] is the mean pore water velocity; R [-] is the retardation coefficient due to sorption; k_{12} [T^{-1}] is the reaction rate coefficient for Raz to Rru; k_1 [T^{-1}] is the decay rate coefficient for Raz; k_2 [T^{-1}] is the decay rate coefficient for Rru; M_{Rru} [mol M^{-1}] is the molecular weight of Rru; and M_{Raz} [mol M^{-1}] is the molecular weight of Raz. We assume here that molecular diffusion is negligible compared to dispersion and that the dispersion coefficient $D_L = \alpha_L v$. The retardation coefficient is equivalent to $K_d\rho_b/n$, where K_d is the distribution coefficient; ρ_b is the bulk density; and n is the porosity.

[19] For the batch experiments, v = 0 m/s, boundary conditions are unnecessary and the initial conditions are as follows:

$$C_{Raz}(t=0) = (1-F_{Rru})C_{Raz,0} \tag{2a}$$

$$C_{Rru}(t=0) = F_{Rru}C_{Raz,0}, \tag{2b}$$

where $C_{Raz,0}$ [ML^{-3}] is the initial concentration of Raz; and F_{Rru} [-] is the mass fraction of Rru contamination in Raz, which we measured as 0.03.

[20] The solution of (1)–(2) for v = 0 is as follows [e.g., *Levine*, 1988, p. 522]:

$$C_{Raz} = C_{Raz,0}(1-F_{Rru})\exp[-k_{1Tot}t] \tag{3a}$$

$$\begin{aligned} C_{Rru} = {} & C_{Raz,0}(1-F_{Rru})\frac{M_{Rru}}{M_{Raz}}\frac{k_{12}}{k_2-k_{1Tot}}\{\exp[-k_{1Tot}t]-\exp(-k_2t)\} \\ & + C_{Raz,0}F_{Rru}\exp(-k_2t), \end{aligned} \tag{3b}$$

where k_{1Tot} is the combined reaction and decay rate coefficient for Raz,

$$k_{1Tot} = k_1 + k_{12}. \tag{3c}$$

[21] For the column experiments we developed a semi-infinite model, with the following boundary and initial conditions.

$$C_{Raz}(\mathrm{x}, t=0) = C_{Rru}(\mathrm{x}, t=0) = 0, \mathrm{x} \geq 0, \tag{4a}$$

$$C_{Raz}(x=0,t) = C_{Raz,0}, t>0, \tag{4b}$$

$$C_{Rru}(x=0,t) = F_{Rru}C_{Raz,0}, t>0, \tag{4c}$$

$$C_{Raz}(x\to\infty,t) = C_{Rru}(x\to\infty,t) = 0, t>0. \tag{4d}$$

[22] The solution to (1)–(2) and (4a)–(4d) can be developed from the basic solution to the advection-dispersion equation without reaction using the method of *Sun et al.* [1999]. The solution is as follows:

$$\begin{aligned} C_{Raz}(x,t) = {} & \frac{C_{Raz,0}(1-F_{Rru})}{2}\left\{\exp\left[\frac{Pe}{2}(1-B_{Raz})\right]\right. \\ & \cdot erfc\left[\frac{1-A_{Raz}t^{1/2}}{2(t/R\tau Pe)^{1/2}}\right] + \exp\left[\frac{Pe}{2}(1+B_{Raz})\right] \\ & \left.\cdot erfc\left[\frac{1+A_{Raz}t^{1/2}}{2(t/R\tau Pe)^{1/2}}\right]\right\} \end{aligned} \tag{5a}$$

$$\begin{aligned} C_{Rru}(x,t) = {} & \frac{C_{Raz,0}}{2}\left[\frac{(1-F_{Rru})y_{12}k_{1Tot}}{k_{1Tot}-k_2} + F_{Rru}\right] \\ & \cdot\left\{\exp\left[\frac{Pe}{2}(1-B_{Rru})\right]erfc\left[\frac{1-A_{Rru}t^{1/2}}{2(t/R\tau Pe)^{1/2}}\right]\right. \\ & \left. + \exp\left[\frac{Pe}{2}(1+B_{Rru})\right]erfc\left[\frac{1+A_{Raz}t^{1/2}}{2(t/R\tau Pe)^{1/2}}\right]\right\} \\ & - \frac{c_{Raz}(x,t)y_{12}k_{1Tot}}{k_{1Tot}-k_2}, \end{aligned} \tag{5b}$$

where k_{1Tot} and F_{Rru} are the same as in (2–3), and

$$A_{Raz} = \sqrt{\frac{Pe}{R\tau} + 4k_{1Tot}}, \tag{5c}$$

$$A_{Rru} = \sqrt{\frac{Pe}{R\tau} + 4k_2}, \tag{5d}$$

$$B_{Raz} = \sqrt{1 + \frac{4k_{1Tot}R\tau}{Pe}}, \tag{5e}$$

$$B_{Rru} = \sqrt{1 + \frac{4k_2R\tau}{Pe}}, \tag{5f}$$

$$y_{12} = \frac{k_{12}}{k_{1Tot}}\frac{M_{Rru}}{M_{Raz}}, \tag{5g}$$

$$Pe = x/\alpha_{\mathrm{L}} = vx/D_L, \tag{5h}$$

$$\tau = x/v. \tag{5i}$$

[23] It is also worth noting the asymptotic values of (5a) and (5b) for an injection of constant concentration,

$$\begin{aligned} C_{Raz}(x, t\to\infty) = {} & C_{Raz,0}(1-F_{Rru}) \\ & \cdot\exp\left[\frac{1}{2}Pe\left(1-\sqrt{1+\frac{4k_{1Tot}R\tau}{Pe}}\right)\right] \end{aligned} \tag{6a}$$

$$\begin{aligned} C_{Rru}(x, t\to\infty) = {} & C_{Raz,0}\left[\frac{(1-F_{Rru})y_{12}k_{1Tot}}{k_{1Tot}-k_2} + F_{Rru}\right] \\ & \cdot\exp\left[\frac{Pe}{2}\left(1-\sqrt{1+\frac{4k_2\tau}{Pe}}\right)\right] \\ & - \frac{y_{12}k_{1Tot}}{k_{1Tot}-k_2}C_{Raz}(x, t\to\infty) \end{aligned} \tag{6b}$$

[24] Parameters (i.e., decay, sorption, and reaction) were estimated by fitting the appropriate model and minimizing the sum of squared error. In the case of the column experiments, velocities, travel times and a dispersivity for a conservative tracer were estimated from the breakthrough curves (BTCs) of EC. These values were then used in the Raz and Rru models. All Raz and Rru models were used simultaneously to estimate k_1, k_{12}, k_2, and a new value of dispersivity. A different dispersivity was fit to the EC data than to the Raz/Rru data because sorption increases dispersion and because rate limitations were not included in the model, which also increases dispersion [*Kučera*, 1965; *Goltz and Roberts*, 1987; *Sánchez-Vila and Carrera*, 2004]. It has been shown that sorbing and nonsorbing tracers should have different dispersivities, even if nonequilibrium effects are properly modeled, owing to differ-

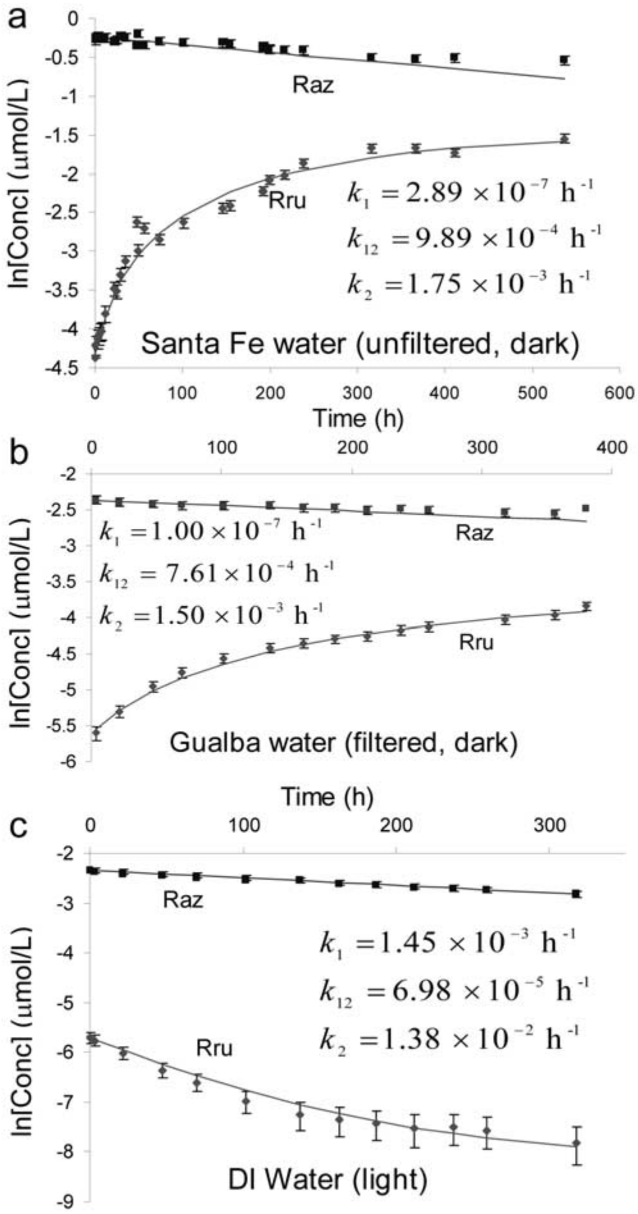


Figure 2. Reaction of resazurin (Raz) to resorufin (Rru) versus time. (a) Raz in water from Riera de Sta. Fe de Montseny, unfiltered and stored in the dark. (b) Raz in water from Riera de Gualba, filtered and stored in the dark. (c) Raz in deionized water stored under laboratory lights. Data are shown with error bars indicating 95% confidence; some error bars are not visible because they are smaller than the data symbol. Corresponding models from equations (3a) and (3b) are shown as solid lines.

ences in the boundary conditions at the sediment grain–water interface [*Smith*, 1983; *Shapiro and Brenner*, 1988; *Grosser et al.*, 1991; *Buckley and Loyalka*, 1994].

3. Results

3.1. Raz and Rru Parameter Estimates From Batch Experiments

[25] Reaction of Raz to Rru and the decay of both Raz and Rru were all well-modeled as first-order processes (3a) and (3b). A representative set of concentration histories of both Raz and Rru from batch reactions are shown in Figure 2. The reaction and decay processes can all fairly be called pseudo-first-order. The model for Rru generally fit within the data error bars, with the exception of a few data points for water from Sta. Fe, which were our "noisiest" data set, probably due to mistakes in sample preparation. Data for water from Gualba were not noisy. The model for Raz fit within the error bars, except for data from close to the end of the experiment for both Sta. Fe and Gualba water. This late-time discrepancy appears to be systematic in natural water, but is probably negligible for most lab and field applications because it is only apparent after many days.

[26] A summary of reaction and decay coefficients obtained from all experiments is given in Figure 3. Raz and Rru have very little decay or reaction in either DIW or water from the two streams. In DIW, all rate coefficients are well below 10^{-4} h^{-1}, indicating that laboratory standards are stable over periods of several weeks if stored in the dark. In the light, Raz and Rru both undergo photochemical decay, within many tens of hours for Rru and hundreds of hours for Raz in our experiments. Biochemical/chemical decay and reaction in the two stream waters was similar. Biochemical/chemical decay of Raz was negligible, while biochemical/chemical decay of Rru was $1.50–1.75 \times 10^{-3}$ h^{-1}. In stream water, reaction of Raz to Rru was slower than biochemical/chemical decay, and was slightly below 10^{-3} h^{-1}. Water filtration with a 0.7 μm glass fiber filter made no difference in the results.

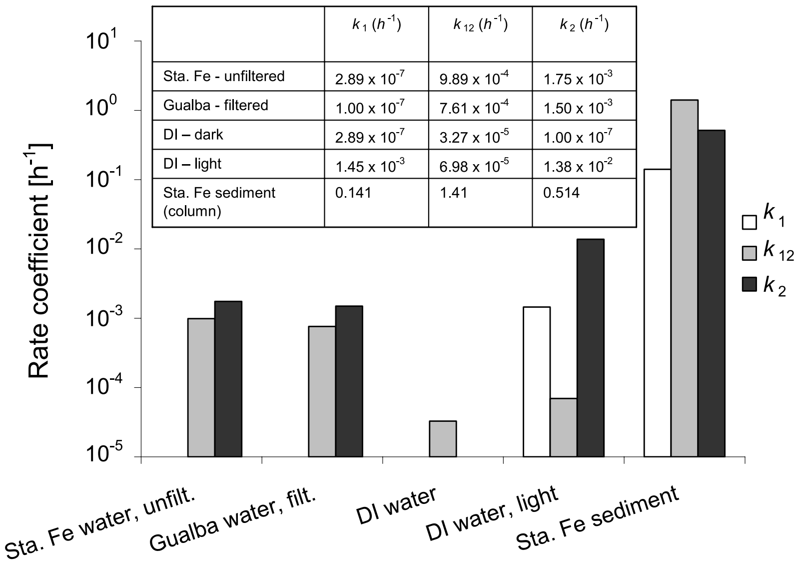
[27] The sorption isotherm (Figure 4) was fit very well by a Freundlich isotherm with $1/n = 0.89 \pm 0.04$ and a Freundlich coefficient (K_f) of 5.15 ± 0.63 mL/g. Since the isotherm is nearly linear, we also fit a linear isotherm model to the data, yielding a distribution coefficient of 6.63 ± 0.40 mL/g.

3.2. Raz and Rru Parameter Estimates From Column Experiments

[28] Results from column experiments with stream sediment and water (Figure 5) show that reaction of Raz to Rru is more than 3 orders of magnitude faster than reaction measured in batch experiments with water alone, with $k_{12} = 1.41$ h^{-1} (compare all rate coefficients in Figure 3). A large fraction of Raz was converted to Rru even with water residence times in column as short as 0.19 h ($\sim$11 min). Nearly all of the Raz was removed from solution in the column with a 1.1 h water residence time, and much of this was converted to Rru.

[29] Travel times cause plateau concentrations to change nonlinearly. Figure 6 shows that maximum Rru concentration was achieved in the column experiment with intermediate travel time (i.e., 0.46 h for water). Shorter travel time do not allow sufficient time for Raz to Rru reaction. Longer time (i.e., slower flow) allows more conversion of Raz to Rru, but also more time for biochemical/chemical decay and sorption of both Raz and Rru. On the other hand, the loss of oxygen across the column is approximately proportional to the travel time (Table 1b), consistent with microbial respiration.

[30] Nonequilibrium sorption, though not modeled, affects column BTCs. The fastest pumping rate (Q = 34.5 mL/h, τ = 0.19 h) has an early breakthrough relative to the



	k_1 (h^{-1})	k_{12} (h^{-1})	k_2 (h^{-1})
Sta. Fe - unfiltered	2.89×10^{-7}	9.89×10^{-4}	1.75×10^{-3}
Gualba - filtered	1.00×10^{-7}	7.61×10^{-4}	1.50×10^{-3}
DI – dark	2.89×10^{-7}	3.27×10^{-5}	1.00×10^{-7}
DI – light	1.45×10^{-3}	6.98×10^{-5}	1.38×10^{-2}
Sta. Fe sediment (column)	0.141	1.41	0.514

Figure 3. Summary of reaction rates (k_{12}), decay rates for Raz (k_1), and decay rates for Rru (k_2) under various conditions. All samples were stored in the dark except "DI water, light." All values were obtained from a batch reactor except "Sta. Fe sediment," which were obtained from a series of column experiments (see text).

equilibrium sorption model while the slow pumping rate (Q = 3.39 mL/h, τ = 1.1 h) has a late breakthrough relative to the equilibrium sorption model. Furthermore, even the conservative tracer BTC displays rate-limited mass transfer for all pumping rates, indicating that the mobile aqueous and immobile sorbed phases are certainly out of equilibrium. We expect that the addition of a first-order or other rate-limited mass transfer mechanism [e.g., *Haggerty and Gorelick*, 1995; *Haggerty et al.*, 2000] to the model would allow a much better fit to the early time arrivals, with the expense of a more complicated model and the loss of a fully analytical solution. However, since our primary interest is the data from plateau concentrations, which are not affected by rate limitations in mass transfer, we chose not to add this complexity to the model.

4. Discussion

4.1. Resazurin as a Smart Tracer

[31] Results from the experiments cautiously support the feasibility of using Raz as a smart tracer, and show that it can provide at least one "bit" more of information than current hydrological tracers in the assessment of the coupling between solute transport and biogeochemical processes in freshwater ecosystems, with particular promise for water-sediment interactions.

[32] Our results indicate that Raz and Rru are relatively stable and nonreactive in water (without sediment) for days to weeks, a period long enough to complete laboratory or field experiments, provided that samples are stored out of the light (Figure 3). With the exception of long residence times (hundreds of hours) or water with high microbial abundances (e.g., untreated sewage), it appears that significant biochemical/chemical decay and Raz-to-Rru reaction in natural water is not likely. However, sample exposure to intense light (e.g., bright sunlight during field experiments) can result in significant photochemical decay. Therefore, caution should be taken with sample storage, especially in the field.

[33] In contrast, Raz reacts to Rru quickly, in less than an hour, when water interacts with sediment (i.e., in the flow-through sediment columns). The fact that the reaction in sediment is much faster than without sediment makes Raz highly attractive as a smart tracer for water-sediment interaction.

[34] The sorption of Raz and Rru may be of concern when considering this system as a hydrologic tracer. The breakthrough of Raz and Rru in the intermediate flow rate

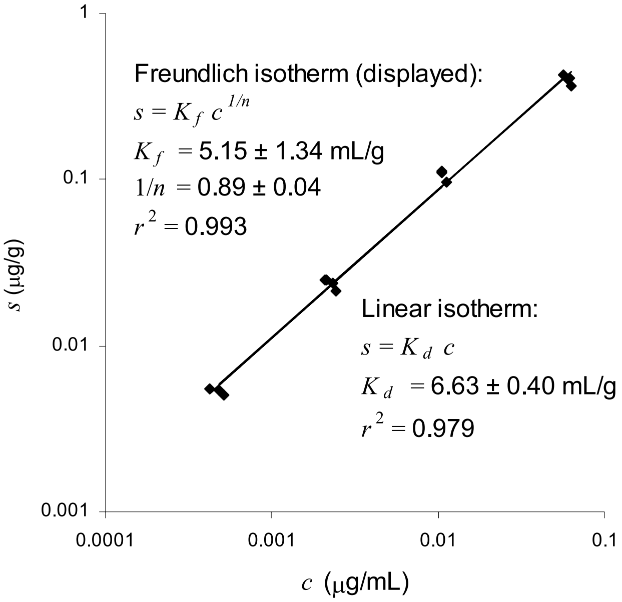


Figure 4. Sorption isotherm for Rru where s is sorbed concentration (MM^{-1}) and c is aqueous concentration (ML^{-3}).

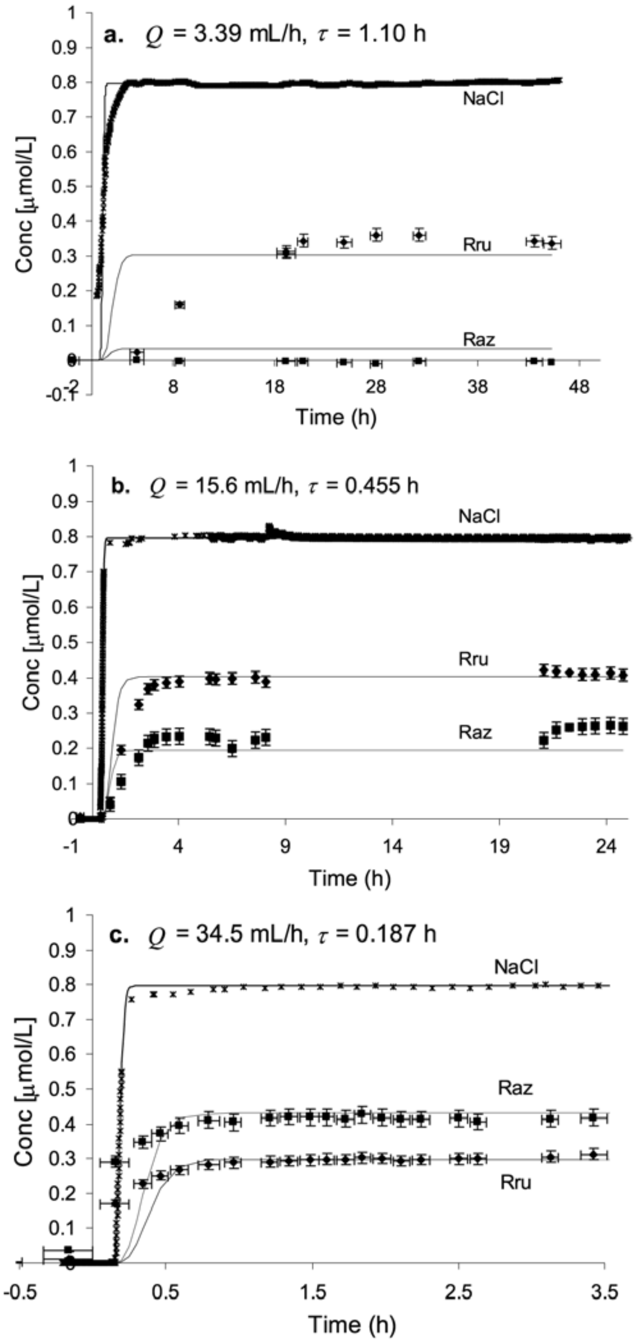


Figure 5. Breakthrough curves for Raz, Rru and NaCl (conservative tracer). Concentrations for NaCl have been rescaled so that plateau concentrations are the same as the injected concentration of Raz. Models are those given in equations (5a) and (5b). Error bars in time show time needed to extract sample.

column was retarded relative to that of a purely conservative tracer (chloride) from travel times of approximately 30 min to travel times of approximately 90 min; the K_d value of 6.63 mL/g (Figure 1) for Rru could generate retardation factors at equilibrium as high as 60 in unconsolidated sediment. This K_d is probably maximal for our sediment because we obtained the sorbed concentration by difference from the aqueous. Since we know that Rru decays in addition to sorbs, the actual sorbed concentration is probably overestimated. In any case, other compounds currently used as hydrological tracers, such as rhodamine WT, can have equally strong sorption (Figure 1). For example, *Gooseff et al.* [2005] measured a K_d of 33.2 mL/g for rhodamine WT in sediment with 9% organic carbon (Figure 1), and *Vasudevan et al.* [2001] reported a host of similar or larger values. We expect that sorption of Raz and Rru will be weak when the fraction of organic carbon in the sediment is relatively small. However, measurements of sorption characteristics of Raz and Rru using a range of sediment composition and organic carbon content will help evaluate the level of sorption for this tracer. This is particularly critical if Raz-Rru is used in groundwater studies.

[35] Raz is a likely smart tracer for microbial activity in freshwater ecosystems. Previous studies have shown that the reduction of Raz to Rru takes place in the presence of living bacteria [*Guerin et al.*, 2001; *Karakashev et al.*, 2003; *Liu*, 1983; *McNicholl et al.*, 2007; *Moyer and Campbell*, 1963; *O'Brien et al.*, 2000; *Peroni and Rossi*, 1986; *Twigg*, 1945; *Zrimšek et al.*, 2004]. This suggests that the Raz to Rru reaction in our columns is probably driven by the redox conditions generated by microbial activity associated with the sediment. This is consistent with metabolic activity in the columns indicated by our oxygen consumption measurements.

[36] Our proposal to use Raz as a smart tracer for water-sediment interaction is supported by our findings of fast reaction in water with sediment and very slow reaction in water alone. Other research suggests that this may be expected in field studies as well. *Fischer and Pusch* [2001] found that bacterial production was 17–35 times higher in the upper 2 cm of sediment than in the overlying water column in a study of sixth-order River Spree in Germany. If this pattern holds across many streams, then Raz should be viable as a field tracer of water-sediment interaction.

4.2. Potential Applications and Future Needs

[37] Although Raz has been used in previous biological studies [e.g., *Ramsdell et al.*, 1935; *Moyer and Campbell*, 1963; *Erb and Ehlers*, 1950; *Zrimšek et al.*, 2004; *O'Brien et*

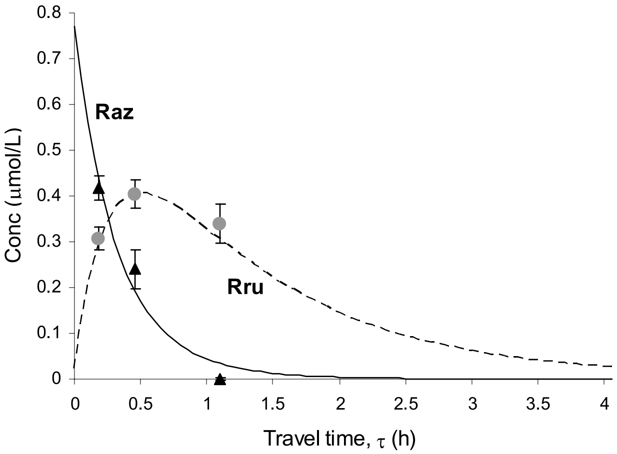


Figure 6. Concentration at plateau (averaged over plateau) versus travel time for conservative tracer ($\tau = L/v$) through column as modeled by equations (6a) and (6b). The model and data shown in Figure 5, averaged over the plateau, are the same as shown here.

al., 2000], to our knowledge this is the first time it has been assessed for use in hydrology. There is a great potential for application and further development of Raz and Rru as a smart "hydrobiological" tracer in both laboratory and field studies. We foresee contributions using this tracer to help bridge the gap between hydrological and biogeochemical studies in stream ecosystems. In a literature review, *Pusch et al.* [1998] indicated that respiration in the hyporheic zone could account for 40 to 90% of total stream ecosystem respiration. This microbial activity is likely to influence nutrient retention at the ecosystem scale if surface-subsurface hydrological exchange is extensive. The use of Raz combined with a conservative tracer could help identify not only the magnitude of water exchange, but also could provide qualitative information on redox conditions (indicative of microbial activity) in the exchange compartments. Raz may be used in hydrologic studies to help differentiate solute transport and transient storage in parts of the system with less microbial activity from those parts with more activity, especially those associated with heterotrophic processes. Interpretation of Raz-Rru dynamics with respect to a conservative tracer (e.g., chloride) could be an indicator of sediment-water interaction in streams because of the generally greater microbial population associated with sediment than with flowing water. Last, the Raz-Rru system may prove useful to the assessment of hyporheic processes.

[38] Raz may also be useful as a hydrological tracer for investigation of purely in-channel transport. For example, in-channel dispersion could be isolated with Raz. Because Raz disappears rapidly in the subsurface, the BTC of Raz would represent transport in the channel only.

[39] One advantage of its use in field studies is the fact that Rru is highly fluorescent (quantum efficiency of 0.74) in moderately basic conditions; and thus, it is possible to be measured even at very low concentrations ($\ll 1$ μg/L) on most spectrofluorometers. This characteristic reduces the cost of the experiments and increases the sensitivity of the results.

[40] To make the Raz-Rru system truly effective as a smart tracer, further laboratory work is necessary to help refine the properties and to quantitatively correlate the reactions with processes of interest. For instance, we need to quantify the relationship between Raz-to-Rru reaction rates, microbial activity, and sediment surface area. This is likely to be different from one location to another, so we will need to develop a standard and simple methodology for making the correlations. The relative contribution of aerobic and anaerobic respiration to reaction rates should also be quantified. It will be important to understand which organisms are mediating the Raz-Rru reaction and through which mechanisms. As already mentioned, further work on the sorption will be necessary, particularly if it is used in groundwater. Finally, while we know that Raz and Rru are not highly toxic, further work should be done to establish details of toxicity.

[41] We view the development of the Raz-Rru tracer system as a step forward in hydrology, because it provides more "bits" of information than a simple conservative tracer. Although in the present study we present Raz as a smart tracer, we propose that other compounds, nanoparticles, or even "nanobots" [*Wood*, 2005] could become smart tracers. Other materials may also work well as smart tracers of microbial activity or sediment-water interaction on the basis of their properties (e.g., nonsorbing, less expensive, etc.). Most importantly, while our example may prove useful for characterization of the coupling between solute transport and biological activity or the amount of sediment-water interaction, other smart tracers may be able to be developed to assay other conditions of interest such as temperature, pH, or presence of a nonaqueous phase.

5. Conclusions

[42] This study shows that Raz is a feasible smart tracer for the assessment of sediment-water interactions and associated biological activity in freshwater ecosystems. Raz reduces rapidly, as a pseudo-first-order process, with a rate coefficient of 1.41 h^{-1} in flow-through experiments with colonized sediment. The reaction in sediment is much faster than in water without sediment and is also a much faster than decay. Both compounds can be measured at low concentrations (<1 μg/L) with a standard spectrofluorometer without background interference in natural waters. The compounds are stable over times long enough to complete lab or field work, but care should be taken to avoid samples exposure to intense light. Sorption of both Raz and Rru could be an issue of concern in some settings. Further work is necessary to quantitatively relate the Raz-Rru reaction to sediment-water interaction and microbial activity.

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A. Argerich, Departament d'Ecologia, Universitat de Barcelona, Diagonal 645, Barcelona E-08028, Spain. (alba@ceab.csic.es)

R. Haggerty, Dept. of Geosciences, 104 Wilkinson Hall, Oregon State University, Corvallis, OR 97331-5506, USA. (haggertr@geo.oregonstate.edu)

E. Martí, Limnology Group, Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas, Accés a la Cala St. Francesc 14, Blanes, Girona E-17300, Spain. (eugenia@ceab.csic.es)