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Nitrogen retention and biogeochemical processes in Mediterranean semiarid streams: environmental factors involved in their spatial and temporal variation

Retención de Nitrógeno y procesos biogeoquímicos en ríos Mediterráneos semiáridos: factores medioambientales implicados en su variación espacial y temporal

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RETENCIÓN DE NITRÓGENO Y PROCESOS BIOGEOQUÍMICOS EN RÍOS MEDITERRÁNEOS SEMIÁRIDOS: FACTORES MEDIOAMBIENTALES IMPLICADOS EN SU VARIACIÓN ESPACIAL Y TEMPORAL

NITROGEN RETENTION AND BIOGEOCHEMICAL PROCESSES IN MEDITERRANEAN SEMIARID STREAMS: ENVIRONMENTAL FACTORS INVOLVED IN THEIR SPATIAL AND TEMPORAL VARIATION

Memoria de la Tesis Doctoral presentada por Dña. María Isabel Arce Sánchez para optar al grado de Doctor en Biología por la Universidad de Murcia.

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Dª. Rosa Maria Gómez Cerezo, Profesora Titular de Universidad del Área de Ecología en el Departamento de Ecología e Hidrología, AUTORIZA:

La presentación de la Tesis Doctoral titulada "Retención de Nitrógeno y procesos biogeoquímicos en ríos Mediterraneos semiáridos: factores medioambientales implicados en su variación espacial y temporal ", realizada por Dª. María Isabel Arce Sánchez, bajo mi inmediata dirección y supervisión, y que presenta para la obtención del grado de Doctor por la Universidad de Murcia.

En Murcia, a 27 de Febrero de 2014

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D. Jose Miguel Martínez Paz, Profesor Titular de Universidad del Área de Economia Aplicada y **Presidente Comisión Académica programa doctorado** * Tecnologia, Administración y Gestión del Agua (TAYGA), INFORMA:

Que una vez evaluado, de conformidad con el procedimiento establecido en el artículo 21 del Reglamento de doctorado de la Universidad de Murcia, el expediente completo de la tesis doctoral titulada "Retención de Nitrógeno y procesos biogeoquímicos en ríos Mediterráneos semiáridos: factores medioambientales implicados en su variacion espacial y temporal", realizada por Dª Maria Isabel Arce Sánchez, bajo la inmediata dirección y supervisión de Dª. Rosa Maria Gómez Cerezo, esta Comisión Académica, en sesión celebrada en fecha 28/02/2014, ha dado su autorización para su presentación ante la Comisión General de Doctorado.

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For my family and friends

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General Introduction

General introduction

Nitrogen (N) is a biologically important nutrient fundamental to all organisms for growth, maintenance, and activity. Together with phosphorus (P), N is an essential nutrient that may limit primary production in terrestrial and aquatic ecosystems (Elser et al. 2007). The worldwide population development and the associated activities, have historically altered the natural proportion of N in air, water and land at both regional and global scales (Galloway et al. 2004). With the aim of ameliorating world hunger, "The Green Revolution" of the 1960s involved a series of research, development, and technology improvements that increased agriculture production globally, particularly in the developing countries (Smil 2002; Pingali 2012). Green Revolution literally revolutionized agriculture and a critical result of such success was the advent of synthetic fertilizers, which main component is N.

In additions, all these improvements co-occurred with an unprecedented increase in the human population, which has more than doubled since the Green Revolution began, and it is expected to continue increasing until 2050, at which point there will be approximately between 8.3 and 11 billion people on Earth (UN 2013).

Consequently, the global N budget has shifted from limitation to abundance and many parts of the world now awash in excess N (Vitousek et al. 1997; Galloway et al. 2003).

Nowadays, the input of biologically available N worldwide from human activities into the ecosystems is 10 times that of 1890, even prior to the wide spread use of fertilizers that accompanied the Green revolution (Galloway and Cowling 2002). A significant portion of this nitrogen is leached from the landscape and transported by streams and rivers to the ocean (Rabalais 2002; Donner et al. 2004). Excess N that is exported to adjacent and downstream water bodies causes numerous problems. Nitrate (NO_3^-) is a highly mobile form of dissolved N and it can leach to aquifers and shallow groundwater and contaminates drinking water supplies. At high concentrations, NO_3^- causes methemoglobinemia in humans, a condition in which NO_3^- is reduced to nitrite (NO_2^-), leading the conversion of hemoglobin to methemoglobin that limits the oxygen-carrying ability of the blood (Fan and Steinberg 1996). Consumption of excess NO_3^- is also associated with non-Hodgkin's lymphoma (Ward et al. 1996; Camargo and Alonso 2006), and ammonia (NH_3) is toxic to fish and macroinvertebrates (Camargo and Alonso 2006; USEPA 2009). In coastal waters, the N exceeded has led to a massive algal blooms, creating anoxic or hypoxic conditions in the water column resulting in destruction of the marine community (McIsaac et al. 2001; Rabalais 2002) and loss of biodiversity (Carpenter et al. 1998).

Therefore, knowledge of how N is transformed and transported within lotic ecosystems will be essential for mitigating the effects of current and future human activities (Bernot and Dodds 2005).

N cycling in streams: biogeochemical processes

In the context of terrestrial biogeochemistry, streams and rivers have been traditionally considered a unidirectional flow on waters or "pipes", which transport material from the landscape to the ocean simply reflecting biological activity on the terrestrial landscape (Bencala 1993). More recently, studies have shown that lotic ecosystems have the potential to transform and remove a substantial portion of the N entering from terrestrial lands in their own right (Meyer 1990; Alexander et al. 2000; Alexander et al. 2007).

In-stream N retention integrates a set of processes that include assimilatory and dissimilatory mechanisms, that convert N from one form to another (Fig. 1).

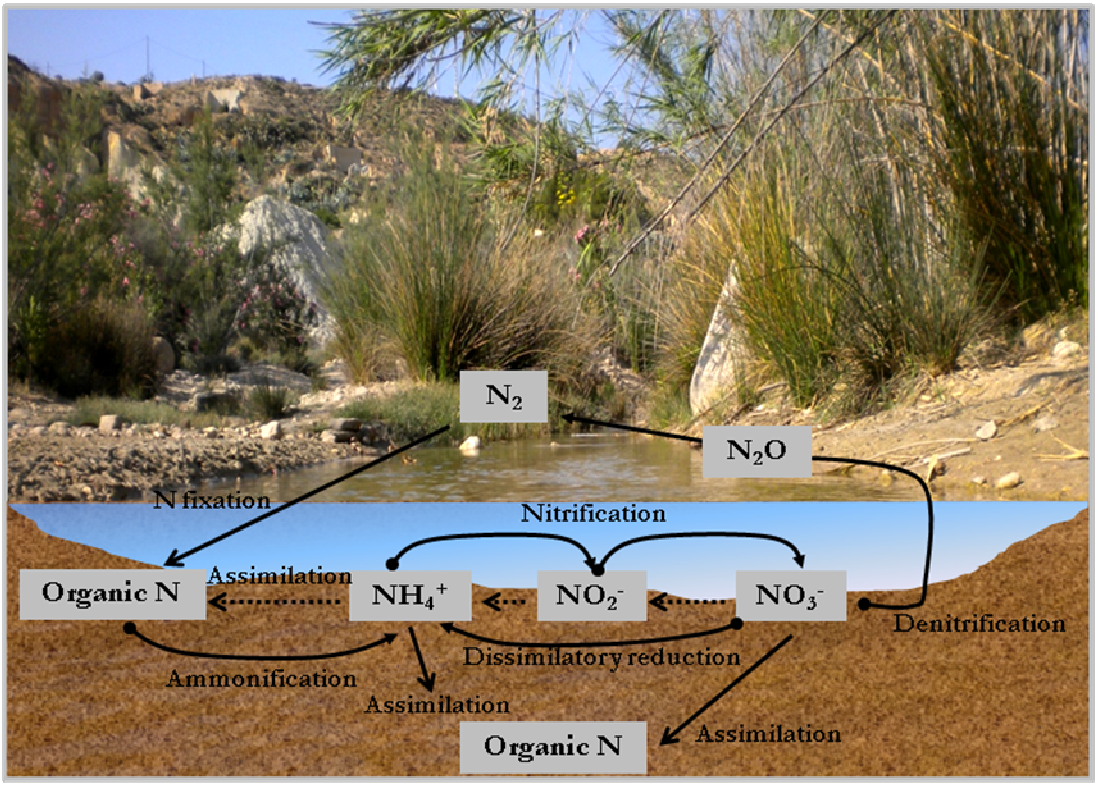

Figure 1. The nitrogen (N) cycle showing transformations between different forms of N in streams. Modified from Bernot and Dodds (2005).

The ***assimilation*** of N by autotrophic (primarily algae) and heterotrophic (bacteria and fungi) organisms are important on transforming N (Hall and Tank 2003). Their effectiveness at retaining N can be very high as nutrients are intensively recycled within benthic communities (Burns 1998). Yet, factors such as nutrients, light, grazing, and scouring caused by increased flow, ultimately determinate the amount of total N that can be retained in biofilms regardless of whether they are net autotrophic or heterotrophic (Bernot and Dodds 2005).

However, the influence of prolonged exposure to elevated N conditions is not fully understood. In many instances, the growths of heterotrophic and autotrophic organisms are limited or co-limited by N and P (Tank and Dodds 2003; Niyogi et al. 2004). Conversely, Kemp and Dodds (2002a) demonstrated that uptake of N in prairie stream substrata may often saturate in response to added NO_3^- and ammonium (NH_4^+).

Heterotrophic microbial community, in particular, release nutrients as a product of their consumption of detritus in a process named ***mineralization***. Mineralization is recognized within N processing, as a critical process in any nutrient cycle is the conversion of organic forms of nutrients in dead biomass (detritus) into simpler, soluble forms that can be taken up again by plants and other microbes. Mineralization is the conversion of organic-N to NH_4^+ primarily by the heterotrophs- aerobes, anaerobes, fungi and bacteria (Robertson and Groffman 2007). This process is not only influenced by environmental conditions such as temperature and dissolved nutrients (Tank and Webster 1998). The rate of mineralization relative to respiration is highly dependent of detritus characteristics and it is expected to increase as organic matter quality improves (e.g. lower carbon (C):N ratios) with greater N availability (Robertson and Groffman 2007). If organic detritus is poor in N, microorganisms must scavenge additional N from their surroundings, thereby, assimilating or, so-called, immobilizing N in their biomass. It is important to highlights that mineralization and immobilization can simultaneously occur at small scales. While one group of microbes is consuming or mineralizing some detritus rich in both C and N, another group, might be consuming a piece of detritus rich in C but low in N. Thus, the first group is mineralizing N while the second is immobilizing it.

Assimilatory processes, mediated both by autotrophic or heterotrophic organisms, do not represent permanent removal; rather, they slow downstream transport of inorganic dissolved N (DIN), which, unless it is permanently removed, may ultimately be exported down direction either in the inorganic form or organic form, or remineralized (Arango et al. 2008).

Nitrification and ***denitrification***, two microbially-mediated reactions, are the primary dissimilatory N processes in streams, thus is, contrary to assimilatory processes, they do not incorporate N into cellular constituents.

Although nitrification is mainly associated to autotrophic bacteria, heterotrophic microbes can also nitrify. However, autotrophic nitrification appears to be the more described process reported in streams ecosystems. As same as nitrification, albeit *vice versa*, denitrification can be also mediated by autotrophic microbes, yet most information derived from rivers and streams dealing with denitrification refer to heterotrophic pathway. Throughout this thesis, I refer terms nitrification and denitrification as the autotrophic and heterotrophic pathway, respectively, unless the alternative mechanism is specified.

Nitrification is the microbial oxidation of reduced NH_4^+ to less reduced forms, NO_2^- and NO_3^- (Fig. 2). Nitrifiers oxide NH_4^+ for generate energy for cell growth and metabolisms. This process is two-step pathway, carried out by separate groups of obligate aerobes bacteria: ammonia and nitrite oxidizers, respectively. They derived their C from CO_2 or carbonates. Denitrification converts NO_3^- to gaseous N forms under anaerobic conditions. Denitrifiers are aerobic facultative, they are capable use NO_3^-, rather than O_2, as a terminal electron acceptor during oxidation of organic matter under low- O_2 conditions (Fig. 1). In this context, denitrification can be an important source of atmospheric N_2O, an recognizable greenhouse that also influences on ozone layer. Despite not being confirmed, ammonia oxidizers also appear able to produce NO arise from NO_2^- reduction, which can also result in the N_2O production (Robertson and Groffman 2007).

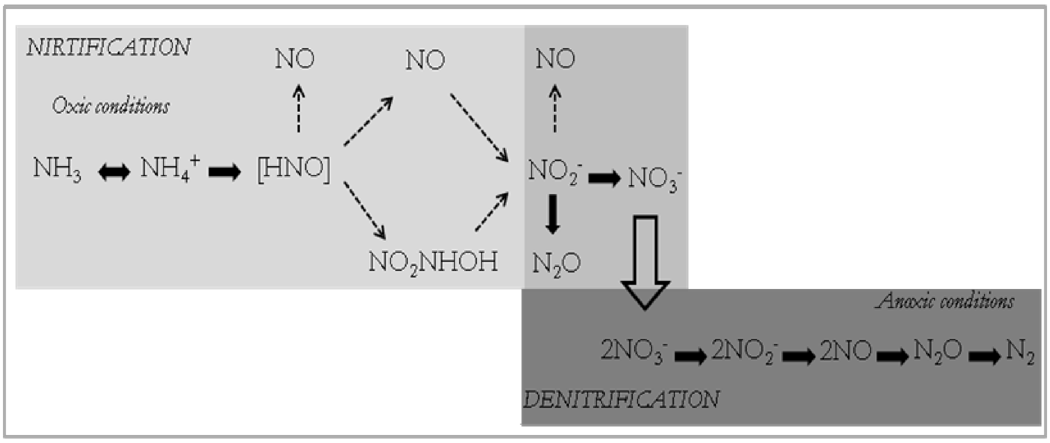

Figure 2. Nitrification and denitrification processes. Dashed arrows indicate unconfirmed pathways. Different grey colors refer different microbial groups. Modified from Robertson and Groffman (2007).

Nitrification and denitrification have been shown to be coupled, moreover, in pristine or low N streams (Kemp and Dodds 2002b), whereby nitrification transforms remineralized NH_4^+ to NO_3^-, which can be denitrified (Seitzinger et al. 2006). Thus, nitrification is central to the accumulation and loss of N (DeLaune et al. 1991) and the importance of nitrifying activity to ecosystem function is highly recognized (Peterson et al. 2001; Bernhard et al. 2002). Although some NO_3^- enters ecosystems as fertilizer, in pristine systems, NO_3^- is formed in situ via nitrification (Kemp and Dodds 2002b). Denitrification closes the N cycle and is the only process that permanently removes N from both terrestrial or aquatic ecosystems back to the atmosphere (Seitzinger et al. 2006; Mulholland et al. 2008). The amount of N that is denitrified and thereby, removed from the ecosystem, will be essentially determined by the activity of the nitrifying bacteria populations when N is low (as in pristine streams), and the total external inputs of NO_3^-. Therefore, factors influencing denitrification rates are of utmost importance when considering long-term N retention and removal efficiency in streams (Bernot and Dodds 2005).

Numerous environmental variables regulating nitrification and denitrification have been shown in aquatic ecosystems. Nitrification and denitrification in streams have been found regulated by changes in temperature dissolved oxygen and sediment redox conditions (e.g. García-Ruiz et al. 1998; Kemp and Dodds 2002a), NH_4^+ (e.g. Kemp and Dodds 2002a; Strauss and Lamberti 2000) and NO_3^- availability (e.g. Strauss et al. 2006; Inwood et al. 2007). In the case of nitrification, pH has also been shown to predict rates variation in North America streams, with low values exerting a negative influence on this pathway (Strauss et al. 2002). Stream organic matter in water and sediments can also limit rates of nitrification and denitrification. While denitrification is enhanced in presence of dissolved organic C and benthic organic matter (e.g. Inwood et al. 2005), especially under high water NO_3^- conditions (Arango et al. 2007; Arango and Tank 2008), nitrification can be seen substantially reduced in sites rich in C levels, likely because nitrifiers must compete with heterotrophs for NH_4^+ (Strauss and Lamberti 2000).

Alternatively, another pathway involve in N transformations is the ***dissimilatory nitrate reduction to ammonium (DNRA)*** (Tiedje 1988). Despite thought to be quantitatively irrelevant in natural ecosystems, as for instance soils, its importance on a ecosystem perspective has generated much interest in the last years in rivers streams, (Burgin and Hamilton 2007) and estuaries (Gardner et al. 2006). This microbially mediated mechanism involves the dissimilatory transformation of NO_3^- to NH_4^+ in the absence of O_2. Compared to NO_3^-, the resultant NH_4^+ is a more biologically availably and a less mobile form than NO_3^-. There is much uncertainty about the fate of NO_3^- that is converted to NH_4^+ via DRNA. Under the appropriate conditions, such NH_4^+ could be back transformed to NO_3^- via nitrification or assimilated into plant biomass (Burgin and Hamilton 2007).

Unfortunately, the unclear importance of such pathway lies on the difficulty of measuring DNRA in the presence of other active N cycle-transformations (Robertson and Groffman 2007).

N cycling from a whole-stream perspective

Once N is delivered to rivers and streams, in-stream processes, as those explained above, exert a substantial influence on its fate (Peterson et al. 2001; Mulholland 2004). This so-called self-purification capacity (Elosegui et al. 1995), makes streams as potential sites impacting the export of N to downstream ecosystems and limiting eutrophication effects in coastal areas (Alexander et al. 2000). Within the context of river networks, small and low-order streams, such as headwater streams, can have a disproportionately large impact on the retention and attenuation of N due to the greater contact between water and benthic biota (Alexander et al. 2000). This interaction optimizes processing and retention of water-column nutrients by active benthos, resulting in reduced nutrient export to downstream ecosystems (Peterson et al. 2001; Ensing and Doyle 2006; Mulholland et al. 2008).

When in-stream N processing is examined at a whole-stream scale, researchers base on concept of nutrient spiraling. Because of the unidirectional flow of water, the coupling of any nutrient cycling and downstream transport was described as "spiraling", where a given nutrient that is regenerated moves downstream before being processed (Webster and Patten 1979) (Fig. 3).

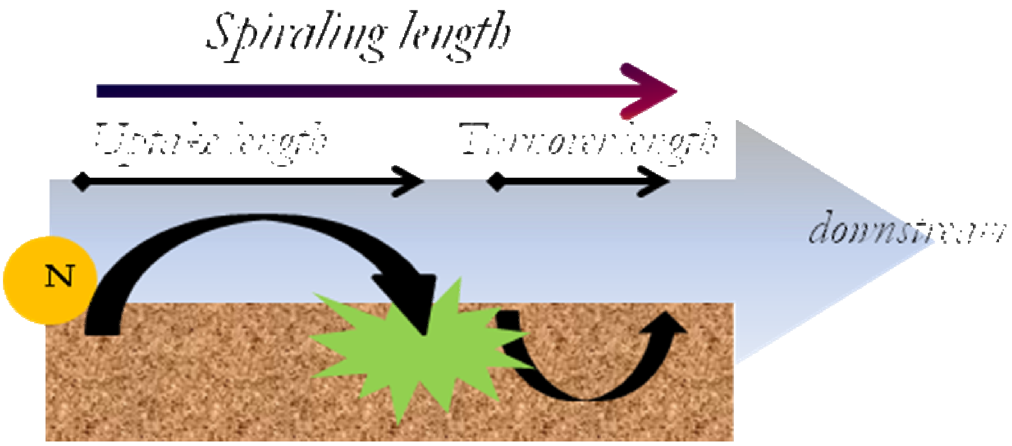

Figure 3. Conceptual scheme of stream nutrient spiraling where the spiraling length is the sum of uptake length (S_w) and turnover length (S_t) Modified from Newbold et al. (1981).

From this theoretical perspective, further Newbold et al. (1981) developed the numerical quantification of spiraling parameters, such as *uptake length* (S_w) and *turnover length* (S_t). S_w for a given nutrient is defined as the distance that this nutrient travels in the water column before being taken into benthos and St was defined as the distance travelled as a part of stream benthos before being released back into the water column (Webster and Valett 2006) (Fig. 3). The total spiral length (S), as the average distance for a nutrient to complete one loop of the spiral, is the sum of individual lengths of each compartment (i.e. $S_w + S_t$). Sw generally constitutes a majority of the spiraling length in stream ecosystems because movement of nutrients while incorporated into biomass is minimal relative to water column movement (Webster and Patten 1979). Noteworthy, S_w integrates all in-stream processes involve on N retention, permanent or temporary (e.g. Martí et al. 1997; Peterson et al. 2001). This parameter reflects in-stream nutrient efficiency relative to the nutrient flux, and shorter S_w values indicate higher efficiencies if compared with larger values. Additionally, from S_w, two other related parameters are often calculated to compare differences in nutrient retention characteristics (Stream Solute Workshop 1990). The amount of a given nutrient that is retained per unit time and area of stream bottom is the *uptake rate* (U) and this metric is often related with stream uptake capacity. The rate of movement of molecules out of the water column is the mass transfer coefficient or *uptake velocity* (V_f), which is indicative of nutrient demand, but also indicated nutrient efficiency relative to nutrient availability (Covino et al. 2010).

Each metric contain useful information and the three parameters use to be employed to quantify changes in N spiraling over time and space in a single stream (e.g. Martí et al. 1997; Simon et al. 2005; von Schiller et al. 2008) and to compare differences among different streams (e.g. Peterson et al. 2001; von Schiller et al. 2008).

Research to date shows several important controls on N uptake metrics (Earl et al. 2006; Hall et al. 2009) and among them, stream discharge and nutrient concentrations have been the most widely observed. S_w has been shown to increase with stream size, because faster, deeper streams carry nutrients farther downstream before removal by benthic processes (Paterson et al. 2001; Hall et al. 2002). Depending on its functional relationship with in-stream biological reactions, stream uptake can respond in a different way N concentration variations (O´Brien et al. 2007). For instance, when biological processes rates are tightly related to available N and are directly proportional to N concentration, uptake capacity or *U* can increase in relation to N, and retention efficiency; as indexed by V_f and S_w, are not expected to change as a function of N. However, when N available supplies exceeds biological demand (Bernot and Dodds 2005), *U* usually increases describing an hyperbolic function (indicative of Michaelis- Menten kinetic) as typically shown by enzimatic processes. As a result of saturation, S_w exhibits a linear increase with the increase in N load, where as V_f would be expected to dramatically decrease along the same gradient, showing in both cases a loss in removal efficiency (O´Brien et al. 2007).

The associated field experiments for reach-scale quantification of in-stream N uptake typically involve isotopic tracer or nutrient additions in a stream reach as the defining spatial unit. Short- term nutrient additions are the most common approach because of their low cost and easy implementation. Besides, the use of isotopic tracers is desirable because the high cost associated with stable isotope (e.g. ^{15}N) and, moreover, the health problems of using radiotracers (e.g. ^{32}P) (Payn et al. 2005). It is important to note, however, that traditional approaches of adding nutrients to measure uptake spiraling metrics assess uptake of the added nutrient (that is, gross uptake, von Schiller et al. 2011), not uptake at nutrient ambient levels. This fact has been considered as one of major shortcomings for nutrient additions experiments, in which ambient uptake rates tend to be unpredicted (Mulholland et al. 2002). As a result, early studies have developed novel methodologies with the aim of evaluating actual uptake spiraling metrics and thus, achieving more accurate results when stream nutrient uptake and its relationship with environmental drivers is evaluated (Payn et al. 2005; Claessens and Tague 2009; Covino et al. 2010).

Streams in Mediterranean semiarid climate regions: potential controls on in-stream N processing

As small stream ecosystems, Mediterranean semiarid streams may play an important role on in-stream N cycling. As early stood out by Sponseller et al. (2013), the distribution and movement of water across landscapes influence a broad suit of ecological, geomorphological and biogeochemical processes. From this perspective, stream draining semiarid catchments may be critical locations for N retention, or "hot spots" (McClain et al. 2003), because biological processing of N elsewhere within the landscape, is limited by water availability, as same as occur in desert lands. The influence of intrinsic stream characteristics associated semiarid climates as well as human pressures, shape the structure of these ecosystems and may also determine their functioning. See Annex 1 for pictures.

Three key factors can interact either directly or indirectly the way of which N is processed in semiarid streams: *hydrological intermittency*, *salinity* and *land use.*

Stream flow intermittency

Seasonality and variability in rainfall is the principal attribute of the Mediterranean type-climate. As a result, most streams draining Mediterranean basin are temporary. They are physically, chemically and biologically shaped by predictable natural events on drying and flooding over an annual cycle (Gasith and Resh 1999). Although seasonally predictable, these disturbances can vary in intensity and duration within and among Mediterranean-climate regions. In arid and semiarid areas, including Southern Spain, temporary streams are the dominant stream-type (Boulton and Suter 1986). This hydrological intermittency is especially severe here and many stream channels only flow after strong rainfall episodes (Uys and O´Keefee 1997). For example, in the province of Murcia, where the study streams of the present thesis are located, ~98% of mapped water courses (at 1:200,000 scale) are temporary and ephemeral (Gómez et al. 2005) many of them so-called *rambla*s (Vida-Abarca 1990; Pulido 1993). Despite temporary systems are typically considered from arid and semi-arid zones they represent a global phenomenon (Larned et al. 2010) and early estimates suggest that the proportion of temporary streams in the global river network is likely to be higher than 50% (Datry et al. 2014).

During droughts, temporary stream are fragmented ecosystems (Stanley et al. 1997); stream groundwater inflow decreases, superficial water gradually disappears (contraction phase), taking form first in isolated pools, and finally streambed completely dries up (Lake 2003; Sabater and Tockner 2010). The drying process can vary longitudinally and depending on catchment features (local gromorphology) streams can exhibit (i) headwaters drying, (ii) mid-reach drying, and (iii) downstream drying (Lake 2003). Consequently, when streams fragment they show a discontinuous surface flow through and streams became a mosaic of wet and dry reaches. After dry phase, rewetting usually begin in autumn and the recovery of entire surface flow reconnects reaches and, physically the stream expands (Stanley et al. 1997). As a result of such hydrological shifts, riparian vegetation in semiarid streams is low dominated by rushes and Mediterranean shrubs thus, inputs of allocthonous organic matter are scarce (Vidal-Abarca et al. 2004). The presence of submerged macrophytes is generally low and biofilm accounts for most primary producer community (Velasco et al. 2003)

Wet-dry cycles shape stream N processing and turnover in Mediterranean streams (Bernal et al. 2012). Flow intermittency can exert an important control on water nutrient concentrations (Gómez et al. 2009; von Schiller et al. 2011) changes in redox status (Baldwin and Mitchell 2000) and organic matter and C quality and availability (Ylla et al. 2010; Dieter et al. 2011), but, moreover, through direct effects on the functional state of microbial communities themselves involve in biogeochemical reactions (Amalfitano et al. 2008; Marxen et al. 2010).

In a recent study, von Schiller et al. (2011) evaluated how N availability and the relative forms NO_3^- and NH_4^+, thoroughly varied in respond to a stream contraction, fragmentation- expansion sequence. Their findings, also supported by others (Gómez et al. 2009), concluded that clearly flow intermittency leads to a high spatial and temporal variability in surface water quality, likely due to changes in underlying N cycling processes. Drought progression represents an important stress for streambed microbial communities in which microorganism must survive to water scarcity by developing physiological and morphological adaptations (e.g. Schimel et al. 2007; Amalfitano et al. 2008). Under dry conditions, microbial activity is very low and in general, comparing with flowing reaches, dry streambeds are biogeochemically quiescent (Larned et al. 2010). Besides the general low activity, the relative importance of N biogeochemical reactions on the base of oxygen and C use change during dry conditions. For instance, whereas streambed mineralization and nitrification are enhanced in response to dry conditions (Steward et al. 2012), denitrification is substantially reduced due to limited anoxic environments (Gómez et al. 2012). In another example, Timoner and co-workers (2012) observed a marked decrease in autotrophic biomass of stream biofilms, whereas heterotrophic communities seemed to be more resistant to desiccation.

As in soils, water pulses associated to rewetting and flow resumes trigger biogeochemical reactions by rehydrating and activating microbes and plants, re-dissolving nutrients and organic matter, moving chemical reactants to reaction sites, and catalysing enzyme-mediated reactions (Larned et al. 2010). As same as during drying, complete saturation can change the predominance of microbial communities, slowing mineralization and nitrification but promoting anaerobic sites for denitrification (Cavanaugh et al. 2006; McIntyre et al. 2009; Austin and Strauss 2011).

In arid and desert soils the response times for biogeochemical and physiological processes range for seconds to weeks (Austin et al. 2004; Schwinning and Sala 2004). However, despite aridland soils and temporary streams appearing comparable (Larned et al. 2010), there is still few information about the response and magnitude of N biogeochemical reactions to rewetting in temporary streams.

From a water quality perspective, initial floods following dry periods may be a fundamental factor determinant of natural nutrient availability and subsequent downstream export in Mediterranean streams. These events, when taking form as flush floods, can release important amounts of N, P and C from sediments to water column (e.g. Tzoraki et al. 2007) and occasionally surpass quality standards (e.g. Skoulikidis and Amaxidis 2009).

Rivers that periodically cease to flow constitute one of the challenges for water-management- related Directives. The *Water Framework Directive* (WFD, EU/2000/60/EC-WFD 2000) binds all member states to the assessment of all water bodies and to design river basin management plans to achieve or maintain a good ecological and chemical status by 2015, and within this context, stream nutrient concentrations might be used. In this frame, *ecological status* is defined as an expression of the quality of the structure and functioning of aquatic ecosystems by integrating biological, physico-chemical and hydromorphological components of water bodies (e.g. Sánchez-Montoya et al. 2012).

The WFD emphasizes that the natural variability of ecological resources and abiotic conditions needs to be quantified (Irvine 2004; Hawkins et al. 2010). It is clear that compared with permanent running waters, streams subjected to intermittent flow, including semiarid streams, may exhibit a natural spatial and temporal variability of water nutrient availability and conductivity (Gómez et al. 2009; von Schiller et al. 2011; Sánchez-Montoya et al. 2012).

The WFD, however, was developed from the perspective of large permanent running waters and mostly ignore temporary streams (Logan and Furse 2002), which to date constitute one of the challenges to be implemented within WFD (Nikolaidis et al. 2013).

Temporary river and streams constitute important links in the water cycle (Nikoliadis et al. 2013) and provide many goods and services for human society both under wet and dry conditions (Steward et al. 2012). The implementation of properly methodologies and tools for the definition of the ecological and chemical status of these ecosystems and their management, is nowadays a major prerequisite for the sustainable development of relevant river basins through the protection of river eco-systems and the preservation of the services that they provide (Nikoliadis et al. 2013; Datry et al. 2014).

Salinity

Besides to their natural hydrological intermittency, many Mediterranean semiarid streams are naturally saline. Saline streams characterize by presenting a natural water salinity or water electrical conductivity ≥ 3 g L^{-1} or 5 mS cm^{-1}, respectively (Velasco et al. 2006). According to the last classification made by Arribas et al. (2009), saline aquatic ecosystems are classified as hypo (3-20 g L^{-1}), meso- hyper (> 20- 100 g L^{-1}) and extremely hyper-saline (> 100 g L^{-1}).

Salt can enter to aquatic ecosystems from a variety of sources, from groundwater, terrestrial material or from atmosphere, transported by wind or rain (William 1987; Baldwin 1996). In the SE Spain, in particular, salt has a geologic origin. As result of marine intrusions incidents to continent over geological time and later evaporation in Triassic period (Millán et al. 2011), gypsum (SO_4Ca) and halite-rich (NaCl) evaporite rocks are abundant in these areas (Muller and Hsü 1987). Associated to natural low flows, water salinity in many streams can reach values of > 50 g L^{-1} (Gómez et al. 2005).

Together with intermittent hydrological regime, salinity also shapes semiarid landscapes. Elevated salinity in soils of stream riparian zones can diminish the presence of riparian vegetation, limited to halotolerant and halophytic species; *Arthrocnemum macrostachyum*, *Sarcocornia fruticosa* and *Suaeda vera*, which are replaced by reed beds *(Pragmites australis)* as salinity decreases (Gómez et al. 2005; Millán et al. 2011). Likewise, biofilm dominates the primary producers community in saline streams (Millán et al. 2011).

Compared with the effects of flow intermittency, the current state of knowledge of the influence of water salinity on in-stream N retention is, if any, more unclear.

In recent review, Santoro (2010) showed how elevated salinity can alter N transformations, affecting to all microbial groups involve in N cycling. However, as far as we know, almost all of our knowledge concerning the effect of salt on N dynamics is derived from short-term studies conducted salt gradient in estuaries, freshwater diversion sites, or laboratory experiments, while hardly any information exist with respect to naturally saline streams.

Furthermore, studies have investigated the effects of salinity on N biogeochemical processes have produced a number of mixed results (Magalhâes et al. 2005; Wu et al. 2008; Yu et al. 2008). For example, across a estuarine gradient, Magalhâes et al. (2005) observed decreased nitrification rates at increasing salinities, but no effect found in relation to denitrification rates. In mangrove microcosms inundated with wastewater, salinity treatments between 0-30 g L^{-1} resulted in potential denitrification being reduced at higher salinities (Wu et al. 2008). Likewise, Yu et al. (2008) found that an increase in salinity caused decreased denitrification activity in freshwater due to saline intrusion (Yu et al. 2008). Furthermore, by using separate salinity treatments of K_2SO_4, NaCl and seawater it was determined that the sulfate content of seawater had no significant effect on denitrification (Yu et al. 2008). However, environments rich in SO_4 can stimulate microbial sulphate reduction leading to elevated HS^-, which has been reported to inhibit nitrification (Ardón et al. 2013).

Salinity can also have direct implications for in-stream N availability through ionic exchange. For example, increasing NaCl concentration led to the immediate release of NH_4^+ and Fe from sediments due to cations competition (Gardner et al. 1991; 2006; Baldwin et al. 2006) and it has been attributed to explain increased N flux in freshwater streams subjected to drought-induced-seawater intrusion (Ardón et al. 2013).

Land use

As other river ecosystems of the world, Mediterranean streams are facing tremendous anthropogenic pressures such agriculture development (Lassaletta et al. 2009). Water transfer between catchments has allowed that the irrigated crops take up large areas and put a strong pressure on freshwater ecosystems, among other environmental problems (Martinez-Fernandez et al. 2000). In fact, the irrigation surpluses and the leached of nutrients and other pollutants from crops generate serious problems of water quality (Gomez et al. 2005). Thus, the presence of truly "pristine" streams in these landscapes is now almost rare (Vidal-Abarca and Suárez 2013).

As introduced above, agricultural activities have lead to increased anthropogenic inorganic nutrient concentrations in receiving waters worldwide, especially NO_3^- (Kemp and Dodds 2001; Rabalais 2002; Royer et al. 2004). This anthropogenic N can saturate the ability of biological communities to attenuate NO_3^- excess under certain conditions (Bernot and Dodds 2005; O´Brien and Dodds 2010). In Mediterranean semiarid catchments in particular, the natural water stress due to the high hydrological fluctuations, and the annual water losses by evapotranspiration relative to inputs from precipitation, constraint these streams to exhibit a limited dilution capacity of anthropogenic inputs (Álvarez-Cobelas et al. 2005), which might exacerbate stream nutrient demand saturation. As a result, water NH_4^+, and especially NO_3^- concentration in surface water of many stream reaches receiving agricultural inputs became chronically high (2-100 mg NO_3^- L^{-1}, Ballester et al. 2003). This has strong implications for quantitative aspects of nutrient export because catchments may serve as nutrient sinks, especially under situation of pronounced endorheism (Álvarez-Cobelas et al. 2010). The strong pressure that some streams are subjected has resulted in a progressive loss of the self-purification capacity, an important ecosystem service (Vidal-Abarca and Suárez 2013).

Compared with flow intermittency and water salinity, the effects of land use on N biogeochemical processes are relatively well described. For example, agriculturally affected sites, usually exhibit higher potential denitrification and nitrification rates compared with undisturbed sites, clearly, due to higher NO_3^- inputs (e.g. Kemp and Dodds 2001; Inwood et al. 2005; Findlay et al. 2011). Besides to changes in inorganic N, stream water dissolved organic C concentrations can substantially increase associated to agricultural practices. For example, Royer and

David (2005) found a peak in DOC concentrations in agricultural streams linked with summer blooms of primary producers indicating a significant source of autotrophic C, that can have a strong interaction on denitrification (Inwood et al. 2005; 2007) and nitrification rates (Strauss and Lamberti 2000; Strauss et al. 2002).

Furthermore, many agricultural streams have also been channelized and cleared of riparian vegetation, enhancing erosion and accumulations of fine sediments (Allan 2004), which ultimately promote denitrification rates through of anoxic environments (e.g. Solomon et al. 2009).

N assimilation, denitrification, nitrification and primary production are capable to respond to excess N by increasing processing rates. However, once N is no longer a limiting factor, the effectiveness of these processes on regulating water N availability is limited, and N removal at whole-reach level is reduced (Bernot et al. 2006; O´Brien et al. 2007; Mulholland et al. 2008).

The positive stimulation of denitrification in agricultural sites can have, however, serious implications for stratospheric ozone when considering denitrification as substantial source of N_2O (Beaulieu et al. 2011). Using a global river network model, these authors observed that microbial N transformations (e.g. denitrification and nitrification) convert at least 0.68 Tg y^{-1} of anthropogenic N inputs to N_2O in river networks, equivalent to 10% of the global anthropogenic N_2O emission rate.

Streams ecosystems and global change

Global change in rivers and streams will result in a decrease in water availability due to anthropogenic pressures, changes in the natural hydrological regime and extended drought as a consequence of climatic change (lower rainfall and higher temperatures and evaporation) (*Intergovernmental Panel on Climate Change,* IPCC 2007). In Mediterranean region, where an important proportion of stream network is already temporary, global change will bring about an increase in the duration, frequency and severity of drought periods (Giorgi and Lionello 2008; Larned et al. 2010; Döll and Schmied 2012). Such effects will be also extend to permanent waters in the temperate zone (IPCC 2007) and stream flow intermittency will became a global phenomenon (Tockner et al. 2009).

Under natural conditions, salinization; defined as the process whereby of dissolved salts increases (Williams 1987; 1999), usually occurs associated to low flow periods. However, climate change in likely to impact this natural balance in some regions of the word (Cañedo- Argüelles et al. 2013). Reduced rainfall and stream discharged could not be an isolated effect of climate changes since an accumulation in salt content could occur in many saline ecosystems due to coupled effect of evaporation but also in freshwater as possible intrusions of saline groundwater and seawater (Sereda et al. 2011). Thus, in the context of the global warming, while some aquatic ecosystems could remain fresh during periods of low flow, others can become sufficiently saline to shift the composition of communities to poor diverse salinity tolerant biota (Nielsen and Brock 2009), especially in Mediterranean regions.

Secondary salinization is a long problem in Australian aquatic ecosystems (Nielsen et al. 2003) in highly-humanized catchments (Cooper et al. 2013) and, recently it is considered as a global threat for many rivers and streams (Cañedo-Argüelles et al. 2013).

In light of such expectations, there is urgent need to deepen in our understanding about possible consequences of climate and land use change on stream ecosystems functioning and services. As pointed out by von Schiller et al. (2011), Mediterranean streams that naturally cease to flow could serve as a template to understand better the biogeochemical and ecological implications of drought in more temperate regions.

References

Alexander RB, Boyer EW, Smith RA, Schwarz GE, Moore RB (2007) The role of headwater streams in downstream water quality. J Am Water Resour As 43:41-59

Alexander RB, Smith RA, Schwarz GE (2000) Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403:758-761

Allan JD (2004) Landscapes and riverscapes: The influence of land use on stream ecosystems. Annu Rev Ecol Evol S 35:257-284

Álvarez-Cobelas M, Rojo C, Angeler DG (2005) Mediterranean Limnology: current status, gaps and the future. J Limnol 64:13-29

Álvarez-Cobelas M, Sánchez-Andrés R, Sánchez-Carrillo S, Angeler DG (2010) Nutrient contents and export from streams in semiarid catchments of central Spain. J Arid Environ 74:933-945

Amalfitano S, Fazi S, Zoppini A, Caracciolo AB, Grenni P, Puddu A (2008) Responses of benthic bacteria to experimental drying in sediments from Mediterranean temporary rivers. Microbial Ecol 55: 270-279

Arango CP, Tank JL, Johnson LT, Hamilton SK (2008) Assimilatory uptake rather than nitrification and denitrification determines nitrogen removal patterns in streams land use. Limnol Oceanogr 53:2558-2572

Arango CP, Tank JL, Schaller JL, Royer TV, Bernot MJ, David MB (2007) Benthic organic carbon influences denitrification in streams with high nitrate concentration. Freshwater Biol 52:1210-1222

Arango CP, Tank JL (2008) Land use influences the spatiotemporal controls on nitrification and denitrification in headwater streams. J N Am Benthol Soc 27:90-107

Ardón M, Morse JL, Comlan BP, Bernhardt ES (2013) Drought-induced saltwater incursion leads to increased wetland nitrogen export. Glob Change Biol 19:2976-2985

Arribas P, Gutiérrez-Cánovas C, Abellán P, Sánchez-Fernandez D, Picazo F, Velasco J, Millán A (2009) Tipificacion de los rios salinos ibericos. Ecosistemas 18:1-13

Austin AT, Yahdjian L, Stark JM, Belnap J, Porporato A, Norton U, Ravetta DA, Schaeffer SM (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141:221-235

Austin BJ, Strauss EA (2011) Nitrification and denitrification response to varying periods of desiccation and inundation in a western Kansas stream. Hydrobiologia 658:183-195

Baldwin DS (1996) Salinity in inland rivers. Australasian Sci 17:15-17

Baldwin DS, Mitchell AM (2000) The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river-floodplain systems: a synthesis. Regul River 16:457-467

Baldwin DS, Rees GN, Mitchell AM, Watson G, Williams J (2006) The short-term effects of salinization on anaerobic nutrient cycling and microbial community structure in sediment from a freshwater wetland. Wetlands 2:455-464

Ballester R, Vidal-Abarca MR, Esteve MA, Suárez ML (2003) Humedales y Ramblas de la Región de Murcia. Dirección general del medio natural, Murcia, Spain, 137 pp

Beaulieu JJ, Tank JL, Hamilton SK, Wollheim WM, Hall RO, Mulholland PJ, Peterson BJ, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Grimm, NB, Johnson SL, McDowell WH, Poole GC, Valett HM, Arango CP, Bernot MJ, Burgin AJ, Crenshaw CL, Helton AM, Johnson LT, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM (2011) Nitrous oxide emission from denitrification in stream and river networks. P Natl Acad Sci USA 108:214-219

Bencala KE (1993) A perspective on Stream-Catchment Connections. J N Am Benthol Soc. 12:44-47

Bernal S, von Schiller D, Sabater F, Martí E (2013) Hydrological extremes modulate nutrient dynamics in mediterranean climate streams across different spatial scales. Hydrobiologia 719:21-42

Bernhardt ES, Hall RO, Likens GE (2002) Whole-system estimates of nitrification and nitrate uptake in streams of the Hubbard Brook experimental forest. Ecosystems 5:419-430

Bernot MJ, Dodds WK (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8:442-453

Bernot M J, Tank JL, Royer TV, David MB (2006) Nutrient uptake in streams draining agricultural catchments of the midwestern United States. Freshwater Biol 51:499-509

Boulton AJ, Suter PJ (1986) Ecology of temporary streams - an Australian perspective. In: De Deckker P, Williams WD (eds) Limnology in Australia. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 313-327

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front Ecol Environ 5:89-96

Burns DA (1998) Retention of NO3 in an upland stream environment: a mass balance approach. Biogeochemistry 40:73:96

Camargo JA, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ. Int 32:831:849

Cañedo-Argüelles M, Kefford BJ, Piscart C, Prat N, Schäfer RB, Schulz C (2013) Salinization of rivers: An urgent ecological issue. Envinon Pollut 173:157-167

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol Appl 8:559-568

Cavanaugh JC, Richardson WB, Strauss EA, Bartsch LA (2006) Nitrogen dynamics in sediment during water level manipulation on the Upper Mississippi River. River Res Applic 22:651-666

Claessens L, Tague CL (2009) Transport-based method for estimating in-stream nitrogen uptake at ambient concentration from nutrient addition experiments. Limnol Oceanogr 7:811-822

Cooper SD, Lake PS, Sabater S, Melack JM, Sabo JL (2013) The effects of land use changes on streams and rivers in Mediterranean climates. Hydrobiologia 719:383-425

Covino TP, McGlynn BL, McNamara RA (2010) Tracer Additions for Spiraling Curve Characterization (TASCC): quantifying stream nutrient uptake kinetics from ambient to saturation. Limnol Oceanogr 8:484-498

Datry T, Larned ST, Tockner K (2014) Intermittent rivers: A challenge for freshwater ecology. BioScience DOI:10.1093/biosci/bit027

DeLaune RD, Salinas LM, Knox RS, Sarafyan MN, Smith CJ (1991) Water quality of a coastal river receiving nutrient inputs: ammonium nitrogen transformations. J Environ Sci Heal A 26:1287-1302

Dieter D, von Schiller D, Garcia-Roger EM, Sánchez-Montoya MM, Gómez R, Mora-Gómez J, Sangiorgio F, Gelbrecht J, Tockner K (2011) Preconditioning effects of intermittent stream flow on leaf litter decomposition. Aquat Sci 73:599-609

Döll P, Schmied HM (2012) How is the impact of climate change on river flow regimes related to the impact on mean annual runoff? A global-scale analysis. Environ Res Lett 7:014037

Donner SD, Kucharik CJ, Foley JA (2004) Impact of changing land use practices on nitrate export by the Mississippi River. Global Biogeochem Cy 18:GB1028

Earl SR, Valett HM, Webster JR (2006) Nitrogen saturation in stream ecosystems. Ecology 87:3140-3151

Elosegui A, Arana X, Basaguren A, Pozo J (1995) Self-purification along a medium size stream. Environ Manage 19:931-939

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135-1142

Ensign SH, Doyle MW (2006) Nutrient spiraling in streams and river networks. J Geophys Res 111:G04009

Fan AM, Steinberg VE (1996) Health implications of nitrate and nitrite in drinking water: An update on methemoglobinemia occurrence and reproductive and developmental toxicity. Regul Toxicol Pharm 23:35-43

Findlay SEG, Mulholland PJ, Hamilton SK, Tank JL, Bernot MJ, Burgin AJ, Crenshaw CL, Dodds WK, Grimm NB, McDowell WH, Potter JD, Sobota DJ (2011) Cross-stream comparison of substrate-specific denitrification potential. Biogeochemistry 104:381-392

Galloway JN, Cowling EB (2002) Reactive nitrogen and the world: 200 years of change. Ambio 31:64-71

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ (2003) The nitrogen cascade. BioScience 53:341-356

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153-226

García-Ruiz R, Pattinson SN, Whitton BA (1998) Denitrification in river sediments: relationship between process rate and properties of water and sediment. Freshwater Biol 39:467-476

Gardner WS, Seitzinger SP, Malczyk JM (1991) The effects of sea salts on the forms of nitrogen released from estuarine and freshwater sediments: Does ion pairing affect ammonium flux? Estuaries 14:157-166

Gardner WS, McCarthy MJ, An S, Sobolev D, Sell KS, Brock D (2006) Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support dynamics in Texas estuaries. Limnol Oceanogr 51:558-568

Gasith A, Resh VH (1999) Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Sys 30:51-81

Giorgi F, Lionello P (2008) Climate change projections for Mediterranean region. Global Planet Change 63:90-104

Gómez R, Hurtado I, Suárez ML, Vidal-Abarca MR (2005) Ramblas in south-east Spain: threatened and valuable ecosystems. Aquat Conserv 15:387-402

Gómez R, García V, Vidal-Abarca R, Suárez L (2009) Effect of intermittency on N spatial variability in an arid Mediterranean streams. J N Am Benthol Soc 28:572-583

Gómez R, Arce MI, Sánchez JJ, Sánchez-Montoya MM (2012) The effects of drying on sediment nitrogen content in an arid Mediterranean stream: a microcosms study. Hydrobiologia 679:43-59

Hall Jr RO, Bernhardt ES, Likens GE (2002) Relating nutrient uptake with transient storage in forested mountain streams. Limnol Oceanogr 47:255-265

Hall Jr RO, Tank JL (2003) Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. Limnol Oceanogr 48:1220-1128

Hall Jr RO, Tank JL, Sobota DJ et al (2009) Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: total uptake. Limnol Oceanogr 54:653-665

Hawkins PC, Olson JR, Hill RA (2010) The reference condition: predicting benchmarks for ecological and water-quality assessments. J N Am Benthol Soc 29:312-343

Inwood SE, Tank JL, Bernot MJ (2005) The influence of land use on sediment denitrification in 9 midwestern streams. J N Am Benthol Soc 24:227-245

Inwood SE, Tank JL, Bernot MJ (2007) Factors controlling sediment denitrification in Midwestern streams of varying land use. Microb Ecol 53:247-258

IPCC (Intergovernmental Panel on Climatic Change) (2007) Fourth assessment report. Climatic change 2007: climatic change impacts, adaptation and vulnerability. http://www.ipcc.ch/

Irvine K (2004) Classifying ecological status under the European Water Framework Directive: the need for monitoring to account for natural variability. Aquat Conserv 14:107e112

Kemp MJ, Dodds WK (2001) Spatial and temporal patterns of nitrogen concentrations in pristine and agriculturally-influenced prairie streams. Biogeochemistry 53:125-141

Kemp MJ, Dodds WK (2002a) The influence of ammonium, nitrate, and dissolved oxygen concentration, and denitrification rates associated with prairie stream substrata. Limnol Oceanogr 47:1380-1393

Kemp MJ, Dodds WK (2002b) Comparisons of nitrification and denitrification in pristine and agriculturally influenced streams. Ecol Appl 12:998-1009

Lake PS (2003) Ecological effects of perturbation by drought in flowing waters. Freshwater Biol 48:1161-1172

Larned ST, Datry T, Arscott D, Tockner K (2010) Emerging concepts in temporary river-ecology. Freshwater Biol 55:717-738

Lassaletta L, García-Gómez H, Gimeno BS, Rovira JV (2009) Agriculture-induced increase in nitrate concentrations in stream waters of a large Mediterranean catchment over 25years (1981–2005). Sci Total Environ 407:6034-6043

Logan P, Furse M (2002) Preparing for the European Water Framework Directive: Making the links between habitat and aquatic biota. Aquat Conserv 12:425-437

Magalhâes CM, Joye SB, Moreira RM, Wiebe WJ, Bordalo AA (2005) Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. Water Res 39:1783-1794

Martì E, Grimm NB, Fisher SG (1997) Pre-and post-flood retention efficiency of nitrogen in a Sonoran Desert stream. J N Am Benthol Soc 16:805-819

Martinez-Fernandez J, Esteve-Selma MA, Calvo-Sendin JF (2000) Environmental andsocioeconomic interactions in the evolution of traditional irrigated lands: a dynamicsystem model. Hum Ecol 28:279-299

Marxsen J, Zoopini A, Wilczek S (2010) Microbial communities in streambed sediments recovering from desiccation. FEMS Microbiol Ecol 71:374-386

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW, Johnston CA, Mayorga E, McDowell WH, Pinay G (2003) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301-312

McIntyre RE, Adams MA, Ford DJ, Grierson PF (2009) Rewetting and litter addition influence mineralisation and microbial communities in soils from a semi-arid intermittent stream. Soil Biol Biochem 41:92-101

McIsaac GF, David MB, Gertner GZ, Goolsby DA (2001) Eutrophication: Nitrate flux in the Mississippi River. Nature 414:166-167

Meyer JL (1990) A blackwater perspective on riverine ecosystems. BioScience 40:643-651

Millán A, Velasco J, Gutiérrez-Cánovas C, Arribas P, Picazo F, Sánchez-Fernandez D, Abellán P (2011) Mediterranean saline streams in southeast Spain: What do we know? J Arid Environ 75:1352-1359

Mulholland PJ, Tank JL, Webster JR et al. (2002) Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. J N Am Benthol Soc 21:544-560

Mulholland PJ (2004) The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch Watershed. Biogeochemistry 70:403-426

Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SE, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Niederlehner BR, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202-205

Muller DW, Hsü KJ (1987) Event stratigraphy and paleoceanography in the Fortuna basin (Southeast Spain): a scenario for the Messinian salinity crisis. Paleoceanography 2:679-696

Newbold JD, Elwood JW, O'Neill RV, VanWinkle W (1981) Measuring nutrient spiralling in streams. Can J Fish Aquat Sci 38:860-863

Nielsen DL, Brock MA, Rees GN, Baldwin DS (2003) Effects of increasing salinity on freshwater ecosystems in Australia. Aust J Bot 51:655-665

Nielsen DL, Brock MA (2009) Modified water regime and salinity as consequence of climate change: prospect for wetlands of Southern Australia. Climatic Change 95:523-533

Nikolaidis NP, Demetropoulou L, Froebrich J, Jacobs C, Gallart F, Prat N, Lo Porto A, Campana C, Papadoulakis V, Skoulikidis N, Davy T, Bidoglio G, Bouraoui F, Kirkby M, Tournoud MG, Polesello S, Barberá GG, Cooper D, Gomez R, Sánchez-Montoya MM, Latron J, De Girolamo AM, Perrin JL (2013) Towards sustainable management of Mediterranean river basins: policy recommendations on management aspects of temporary streams. Water Policy 15:830-849

Niyogi DK, Simon KS, Townsend CR (2004) Land use and stream ecosystem functioning: nutrient uptake in streams that contrast in agricultural development. Arch Hydrobiol 180:471-486

O'Brien JM, Dodds WK (2010) Saturation of NO3− uptake in prairie streams as a function of acute and chronic N exposure. J N Am Benthol Soc 29:627-635

O'Brien JM, Dodds WK, Wilson KC, Murdock JN, Eichmiller J (2007) The saturation of N cycling in Central Plains streams: 15N experiments across a broad gradient of nitrate concentrations. Biogeochemistry 84:31-49

Payn RA, Webster JR, Mulholland PJ, Valett HM, Dodds WK (2005) Estimation of stream nutrient uptake from nutrient addition experiments. Limnol Oceanogr 3:174-182

Peterson BJ, Wollheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Marti E, Bowden WB, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK, Gregory S, Morrall DD (2001) Control of nitrogen export from watersheds by headwater streams. Science 292:86-90

Pingali PL (2012) Green revolution: impacts, limits, and the path ahead. P Natl Acad Sci USA 109:12302-12308

Pulido A (1993) Las ramblas mediterráneas: condicionantes geomorfológicos e hidrológicos. In Actas de la VI Aula de Ecología, Las ramblas mediterráneas. Institutos de Estudios Almerienses, Diputación de Almería, Almería, Spain, pp 135–144

Rabalais NN (2002) Nitrogen in aquatic ecosystems. Ambio 31:102-112

Robertson GP, Groffman PM (2007) Nitrogen transformations. In Paul EA (ed) Soil Microbiology, Biogeochemistry, and Ecology. Springer, New York, NY, USA, pp 341-364

Royer TV, Tank JL, David MB (2004) Transport and fate of nitrate in headwater agricultural streams in Illinois. J Environ Qual 33:1296-1304

Royer TV, David MB (2005) Export of dissolved organic carbon from agricultural streams in Illinois, USA. Aquat Sci 67:465-471

Sabater S, Tockner K (2010) Effects of hydrologic alterations on the ecological quality of river ecosystems. In Sabater S, Barceló D (eds) Water Scarcity in the Mediterranean regions. Springer, Berlin Heidelberg, pp 15-39

Sánchez-Montoya MM, Arce MI, Vidal-Abarca MR, Suárez ML, Prat N, Gómez R (2012) Establishing physico-chemical reference conditions in Mediterranean streams according to the European Water Framework Directive. Water Res 46:2257-2269

Santoro AE (2010) Microbial nitrogen cycling at the saltwater-freshwater interface. Hydrogeol J 18:187-202

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394

Schwinning S, Sala OE (2004) Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. Oecologia 141:211-220.

Seitzinger S, Harrison J A, Böhlke J K, Bouwman A F, Lowrance R, Peterson B, Tobias C, Drecht G V (2006) Denitrification across landscapes and waterscapes: a synthesis. Ecol Appl 16:2064-2090

Sereda J, Bogard M, Hudson J, Helps D, Dessouki T (2011) Climate warming and the onset of salinization: rapid changes in the limnology of two northern plains lakes. Limnologica 41:1-9

Simon Ks, Townsend CR, Biggs BJF, Bowden WB (2005) Temporal variation of N and P uptake in 2 New Zealand streams. J N Am Benthol Soc 24:1-18

Skoulikidis N, Amaxidis Y (2009) Origin and dynamics of dissolved and particulate nutrients in a minimally disturbed Mediterranean river with intermittent flow. J Hydrol 373:218-229.

Smil V (2002) Nitrogen and food production: Proteins for human diets. Ambio 31:126-131

Solomon CT, Hotchkiss ER, Moslemi JM, Ulseth AJ, Stanley EH, Hall RO, Flecker AS (2009) Sediments size and nutrients regulate denitrification in a tropical stream. J N Am Benthol Soc 28:480-490

Sponseller RA, Heffernan JB, Fisher SG (2013) On the multiple ecological roles of water in river networks. Ecosphere 4:art17

Stream Solute Workshop (1990) Concepts and methods for assessing solute dynamics in stream ecosystems. J N Am Benthol Soc 9:95-119

Stanley EH, Fisher SG, Grimm NB (1997) Ecosystem expansion and contraction in streams. BioScience 47:427-435

Steward AL, von Schiller D, Tockner K, Marshall JC, Bunn SE (2012) When the river runs dry: human and ecological values of dry riverbeds. Front Ecol Environ 10:202-209

Strauss EA, Lamberti GA (2000) Regulation of nitrification in aquatic sediments by organic carbon. Limnol Oceanogr 45:1854-1859

Strauss EA, Mitchell NL, Lamberti GA (2002) Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Can J Fish Aquat Sci 59:554-563

Strauss EA, Richardson WB, Cavanaugh JC, Bartsch LA, Kreiling RM, Standorf AJ (2006) Variability and regulation of denitrification in an Upper Mississippi River Backwater. J N Am Benthol Soc 25:596-606

Tank JL, Dodds WK (2003) Responses of heterotrophic and autotrophic biofilms to nutrients in ten streams. Freshwater Biol 48:1031-1049

Tank JL, Webster JR (1998) Interaction of substrate and nutrient availability on wood biofilm processes in streams. Ecology 79:2168–2179

Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In Zehnder AJB (ed) Biology of anaerobic microorganisms. John Wiley & Sons, New York, pp 179-244

Timoner X, Acuna V, von Schiller D, Sabater S (2012) Functional responses of stream biofilms to flow cessation, desiccation and rewetting. Freshwater Biol 57:1565-1578

Tockner K, Uehlinger U, Robinson CT, Siber R, Tonolla D, Peter FD (2009) European Rivers. In Likens GE (ed) Encyclopedia of Inland Waters, 1st edn. Elsevier Academic Press, Waltham, USA, pp 366-377

Tzoraki O, Nikolaidis NP, Amaxidis Y, Skoulikidis NT (2007) In-stream biogeochemical processes of a temporary river. Environ Sci Technol 41:1225-1231.

UN (United Nations) (2013) World population prospects: the 2012 revision, key findings and advance tables. Working paper No. ESA/P/WP.227. United Nations, New York, 54 pp

USEPA (2009) Draft 2009 Update Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater. EPA-822-D-09-001. United States Environmental Protection Agency, Washington, DC, 192 pp

Uys MC, O'Keeffe JH (1997) Simple words and fuzzy zones: early directions for temporary river research in South Africa. Environ Manage 21:517-531

Velasco J, Millán A, Vidal-Abarca MR, Suárez ML, Guerrero C, Ortega M (2003) Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. Freshwater Biol 48:1408-1420

Velasco J, Millán A, Hernández J, Gutiérrez C, Abellán P, Sánchez D, Ruiz M (2006) Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. Saline Systems 2:1-15

Vidal-Abarca MR (1990) Los ríos de las cuencas áridas y semiáridas: una perspectiva ecológica comparativa y de síntesis. Scientia Gerundensis 16:219–228

Vidal-Abarca MR, Suárez ML, Gómez R, Guerrero C, Sánchez-Montoya MM, Velasco J (2004) Inter-annual variation in benthic organic matter in a saline, semiarid stream of southeast Spain (Chicamo stream). Hydrobiologia 523:199-215

Vidal-Abarca MR, Suárez ML (2013) Which are, what is their status and what can we expect from ecosystems services provided by Spanish rivers and riparian areas?. Biodivers Conserv 22:2464-2503

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: Sources and consequences. Ecol Appl 7:737-750

von Schiller D, Martí E, Riera JL, Ribot M, Argerich A, Fonollà P, Sabater F (2008) Inter-annual, annual, and seasonal variation of P and N retention in a perennial and an intermittent stream. Ecosystems 11:670-687

von Schiller D, Bernal S, Martí E (2011) Technical Note: A comparison of two empirical approaches to estimate in-stream nutrient net uptake. Biogeosciences 8:875-882

Ward MH, Mark SD, Cantor KP, Weisenburger DD, CorreaVillasenor A, Zahm SH (1996) Drinking water nitrate and the risk of non-Hodgkin's lymphoma. Epidemiology 7:465-471

Webster JR, Patten BC (1979) Effects of watershed perturbation on streams potassium and calcium dynamics. Ecolol Monogr 49:51-72

Webster JR, Valett HM (2006) Solute dynamics. In Hauer FR, Lamberti GA (eds). Methods in stream ecology. San Diego, Academic Press, pp:169-185

WFD (Water Framework Directive) (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for the Community action in the field of water policy

Williams WD (1987) Salinization of rivers and streams: an important environmental hazard. Ambio 16:180-185

Williams WD (1999) Salinization: A major threat to water resources in the arid and semi-arid regions of the world. Lake and Reservoirs: Research and Management 4:85-91

Wu Y, Tam NFY, Wong MH (2008) Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms. Mar Pollut Bull 57:727-734

Ylla I, Sanpera-Calbet I, Vázquez E, Romaní AM, Muñoz I, Butturini A, Sabater S (2010) Organic matter availability during pre- and post-drought periods in a Mediterranean stream. Hydrobiologia 657:217-231

Yu K, DeLaune RD, Seo DC (2008) Influence of salinity level on sediment denitrification in a Louisiana estuary receiving diverted Mississippi River water. Arch Agron Soil Sci 54:249-257

Annex to Introduction: pictures of study sites

***Alhárabe**; perennial and freshwater stream*

***Corneros**; perennial and freshwater stream*

***Rambla de la Parra**; temporary and saline stream (up-reach)*

***Rambla de la Parra**; temporary and saline stream (down-reach)*

***Chícamo**; temporary and saline stream (up-reach)*

***Chícamo**; temporary and saline stream (down-reach)*

Turrilla; *temporary and saline stream*

Luchena; *perennial and saline stream*

Rambla de Benito; *temporary and saline stream*

Arroyo Blanco; *perennial and freshwater stream*

Drought in Rambla de la Parra

Rogativa; *temporary and freshwater stream*

Drought in Rogativa

Chícamo stream after a flood

Objectives

Objectives

Most of the knowledge concerning stream biogeochemistry and N retention arises from temperate or xeric climate streams. There is, however, very few research about how N fluxes are modulated in Mediterranean semiarid streams and the potential drivers controlling this role. The outcomes of the present dissertation contribute to the current state of knowledge of the biogeochemistry of stream ecosystems, in general, and of temporary and saline streams, in particular. In this respect, the results of this research also help to understand projected consequences for stream ecosystems associated to global change.

The overarching goal of this dissertation is to examine in-stream N processing in Mediterranean semiarid streams and environmental drivers potentially involved in its spatial and temporal variation.

As introduced in the previous section, agriculture, hydrological intermittence and salinity are, both directly and indirectly, potential drivers on stream N processing variation. Among all explained biogeochemical processes involved in N cycling, denitrification is the only mechanism that removes N permanently from the ecosystem. In this regard, quantifying stream denitrification rates and studying patterns in their variation are essential to understand the fundamental functioning of temporary streams as a basis for sustainable management, especially in agriculturally affected catchments. Therefore, an important part of this thesis sought to deepen into the understanding of how the aforementioned factors impact on denitrification rates. This research employed a combination of experiments, field studies and modeling approaches to accomplish these goals.

This dissertation is prepared in four individual studies, which follow a chronological structure and have been written as independent publications.

Chapter 1: Denitrification rates and controlling factors in two agriculturally influenced temporary Mediterranean saline streams

**Published in Hydrobiologia (2013) 700:169-185

In this first study, sediment denitrification rates were measured in two temporary streams from head to mouth, following a gradient of increasing natural water salinity and agricultural influence, and during pre- and post- drought conditions, with the aim of studying how denitrification varied in response to these factors.

Chapter 2: Implications of flow intermittency on sediment nitrogen availability and processing rates in a Mediterranean headwater stream

**Published in Aquatic Sciences (2013) DOI:10.1007/s00027-013-0327-2

This work aimed to assess how streambed drying influenced the response of in-stream N processing rates to sediment rewetting. This study was conducted in a headwater stream subjected to natural dry-wet cycles. This study focused not only in denitrification, but also in nitrification rates as this process is substantial to provide NO3 for denitrification in pristine headwater streams. Besides, to investigate nitrification and denitrification rates, this work evaluated how sediment nutrient and organic matter availability varied in response to extreme hydrological shifts.

This work was conducted within the frame of the EU 7th framework project *Mediterranean Intermittent River Management* (MIRAGE, FP7-ENV-2007-1). MIRAGE project aimed to set up with the purpose of studying the hydrology and ecology of temporary rivers, defining reference conditions, and creating a tool box for the implementation of the EU-WFD.

Chapter 3: Nitrogen availability and denitrification rates following short-term inundation of dry sediments in a high-nitrogen Mediterranean temporary stream.

**Manuscript in preparation.

The aims of this experimental study were to address changes in water and sediment N concentration following inundation of dry sediments of a N-rich temporary stream, and to evaluate how denitrification responded to such water pulse.

Chapter 4: Variation in nitrate uptake and denitrification rates across a salinity gradient in Mediterranean semiarid streams

**Published in Aquatic Sciences (2014) DOI:10.1007/s00027-014-0336-9

This research evaluated the influence of water salinity on variation in NO_3^- processing. In this study, sediment denitrification rates and NO_3^- uptake at whole-reach level were examined in nine streams of variably water salinity. Furthermore, other stream variables, besides to salinity, were measured as potential controlling factors of NO_3^- processing variation. The contribution of denitrification to NO_3^-

uptake at whole- reach level was also calculated to address the importance of this process within stream NO_3^- retention.

Chapter 1

Denitrification rates and controlling factors in two agriculturally influenced temporary Mediterranean saline streams

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Abstract

In the present study, we tested the hypothesis that agriculture, through its influence on water NO_3^- availability, would control denitrification in agriculturally-influenced temporary saline streams, and that water salinity would not affect this process. We also tested the effect of summer drought on the denitrification process. We approached these objectives by estimating sediment denitrification (using the acetylene inhibition technique) in two temporary Mediterranean streams following an increased natural water salinity and agricultural gradient under pre- and post-drought conditions. During the pre-drought conditions, the water NO_3^- concentration was the main predictor of denitrification rates. Together with the water NO_3^- concentration, sediment redox conditions and water salinity appeared to be significant predictors, the latter showing a negative effect. During the post-drought, denitrification rates dropped significantly in both streams and no abiotic factors seemed to significantly influence this process. Our results suggest that high water salinity and drought affected negatively the stream denitrifying capacity. This study highlights that stressors such as water salinity and hydrological intermittence should be considered in future stream management plans in order to preserve the role of streams on controlling the NO_3^- export, especially in the context of warmer and drier climate.

Introduction

Denitrification plays a significant role in controlling the fate of nitrogen (N) in the stream channel network, particularly in small-order streams (Alexander et al. 2000).

Whereas quantifying denitrification and investigating its variation, and associated controlling variables, have been studied extensively in temperate streams (e.g. Martin et al. 2001; Strauss et al. 2006; Arango et al. 2007), very little attention has been paid to this process in temporary streams in semiarid regions. Studies in arid and semiarid regions are required to not only gain a global understanding of this process, but to develop conceptual models for temporary-stream ecology (Larned et al. 2010).

In the Mediterranean basin, temporary streams are the dominant lotic ecosystem (Gasith and Resh 1999), which undergoes a recurrent dry phase in summer. Among them, streams from arid and semiarid regions are naturally saline (3 g L^{-1} or $\geq$ 5 mS cm^{-1}). Despite these environments being rare in the European context, saline systems are globally distributed in arid lands (Williams 1996). The natural water salinity of Mediterranean saline streams is due to the presence of gypsum and halite-rich evaporite rocks in their watershed (Gómez et al. 2005). Hydrological intermittence and high water salinity values constrain these streams to being biota-restricted ecosystems with limited allocthonous organic matter inputs (Vidal-Abarca et al. 2004), and low algal and streambed biofilm coverage (Velasco et al. 2003).

Similarly to other Mediterranean streams, these systems also drain agricultural watersheds. Consequently, water NO_3^- concentrations are chronically high (2-100 mg L^{-1} Ballester et al. 2003), mostly downstream where irrigated agriculture is denser with extended inorganic N use as a fertilizer. Due to low surface flow (and, thus, limited water pollution "dilution") and potentially low biological uptake, semiarid streams are widely recognised as being especially sensitive to N saturation (Dahm et al. 2003). In this context, denitrification might prove to be an important mechanism for N removal. Despite its potential importance, there is no information on either denitrification or the factors governing this process for temporary saline streams. Studies analysing the effect of water salinity on denitrification in aquatic ecosystems have produced contradictory results. A drop in denitrification with increasing salinity has been observed in ecosystems affected by sporadic changes, as in suburban streams, due to the use of salt as a de-icer (Hale and Groffman 2006), or as in salt water intrusion river sites (Seo et al. 2008). Conversely, no influence of salinity on denitrification has been found in ecosystems naturally affected by salinity fluctuations, such as estuaries, suggesting that halotolerant bacteria dominate denitrifier communities (Magalhâes et al. 2005). Opposite results were reported by Rysgaard et al. (1999), who observed a negative effect of salinity on denitrification in estuaries.

It is wise that heterotrophic denitrification is directly controlled by NO_3^-, organic C and oxygen availability (e.g. Garcia-Ruiz et al. 1998). Unlike the effects of salinity, the influence of these variables on denitrification in streams has been widely documented. Compared with reference streams draining native vegetation, streams draining agricultural land uses through an excessive use of fertilizers show high NO_3^- availability (Carpenter et al. 1998) and usually exhibit higher potential denitrification rates (e.g. Kemp and Dodds 2002; Inwood et al. 2005; Findlay et al., 2011). Both allochthonous and autochthonous DOC sources may contribute high DOC concentrations in agricultural streams through drainage of agricultural soils and exhudates of primary productors, respectively (Royer and David 2005). As nitrate is usually plentiful in agricultural streams, organic C availability may mediate denitrification (Inwood et al. 2007; Arango et al. 2007). However, both DOC concentration and DOC quality in water and sediment compartments have been shown to control denitrification in streams (e.g. Pfenning and McMahon 1997; Baker and Vervier 2004). In fact, when most water DOC is recalcitrant, as in some agricultural streams, sediment organic C has been shown to be a key factor for denitrification by providing labile DOC and by expanding an anoxic habitat via heterothrophic decomposition (Arango et al. 2007; Arango and Tank 2008).

What remains unclear, despite an increasing number of studies, is the effect of droughts on denitrification. During droughts, hydrological connectivity along the stream network is broken as some stream reaches undergo total desiccation. Desiccation of sediments during a drought has been shown to lead to microbiota habitat-changes, lysis of microbial cells, and alterations in the microbial community structure (Fierer et al. 2003; Rees et al. 2006; Amalfitano et al. 2008), thereby potentially affecting sediment denitrification. Previous studies have found decreased denitrification as a result of the increased sediment aerobic conditions associated with the desiccation and oxidation of stream sediments during a drought (Cavanaugh et al. 2006; Austin and Strauss 2011; Gómez et al. 2012). However, our knowledge of the effects of sediment desiccation on denitrification is still limited and, with some exceptions (Cavanaugh et al. 2006), most of it relates to the effects of sediment drying under experimental conditions.

Investigating denitrification in temporary saline streams is relevant within the context of networked river ecosystems. In recent years, researchers have developed N dynamic models along stream networks in the U.S. with particular attention being paid to denitrification and nitrate removal (e.g. Alexander et al. 2000). Potential factors that could influence denitrification are incorporated into models and are commonly used in management techniques. As stated by Helton et al. (2011), one of the major uncertainties of the existing models derives from the difficulty in forecasting denitrification changes in future climate change scenarios. Flow intermittency and salinisation of inland waters would be expected to rise worldwide given the water scarcity scenario forecast as a result of climate and land use changes (IPCC 2007). Thus, there is an urgent need to quantify denitrification under specific hydrological intermittency and salinity conditions in order to integrate these potential controls into networked river models.

The aims of this study were to i) quantify denitrification rates in two agriculturally- affected temporary saline streams and to analyse the potential variables explaining their variation, ii) examine the influence of summer drought on denitrification rates, and iii) analyse the nutrient limitation pattern in stream denitrification and whether it was influenced by agricultural development and drought.

To address these objectives, we estimated sediment denitrification using the acetylene inhibition technique in two streams following a water salinity and agricultural gradient under both pre- and post-drought conditions. By assuming that adapted bacteria would dominate the denitrifier communities in these saline streams, we hypothesised that salinity would not affect denitrification. We also hypothesised that agriculture would control denitrification variation through its influence on NO_3^- water availability. Finally, based on the premise that sediment desiccation-oxiation would negatively influence microbial communities, we hypothesised that drought would negatively affect the denitrification process. Thus, we predicted lower denitrification rates under post-drought conditions if compared to pre-drought conditions.

Methods

Site description

This study was carried out in the province of Murcia (SE Spain, Fig. 1.1), an area with a semiarid Mediterranean climate and an annual mean precipitation of between 250-350 mm, with high inter-annual variability. Annual evapotranspiration is higher than 900 mm with average annual temperatures ranging between 16ºC and 19ºC. Rainfall patterns are highly irregular, although the highest flows usually occur in spring and autumn, when storms can lead to flash flooding. Two temporary saline streams were selected for study: Chícamo and Rambla de la Parra (hereafter CH and RbP, respectively) (Fig. 1.1).

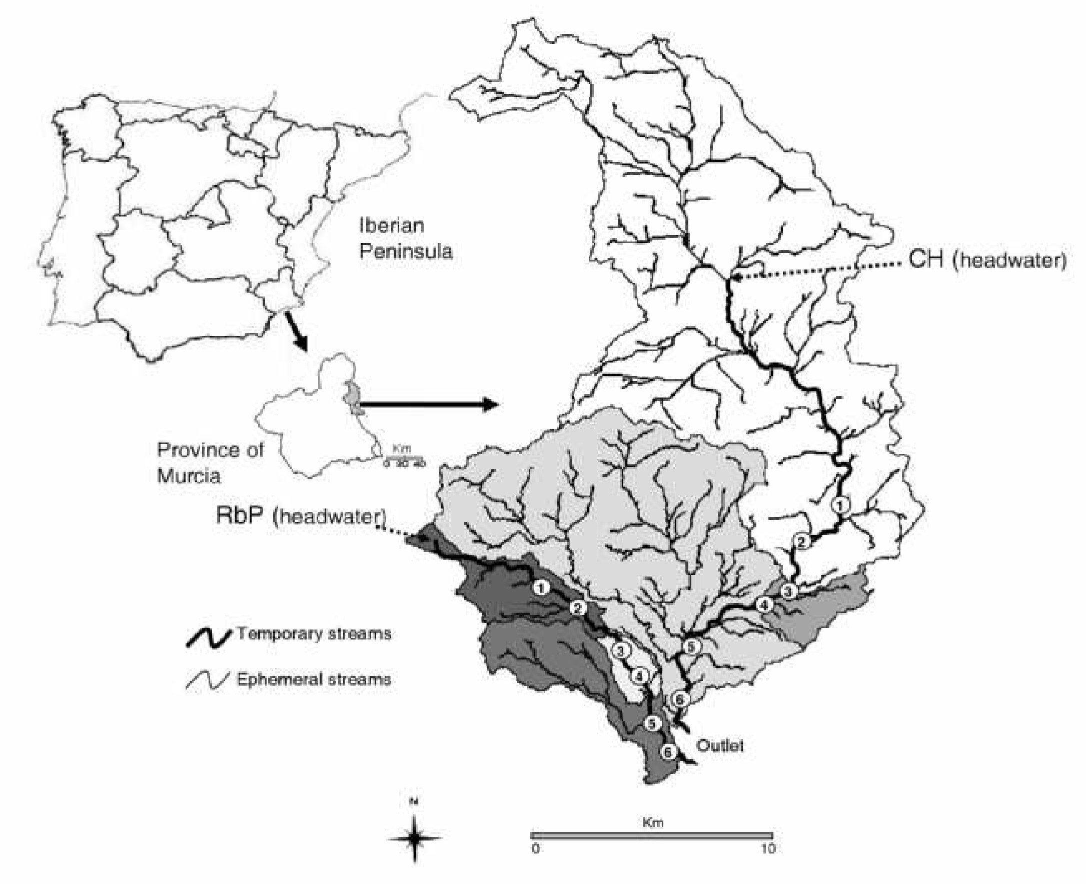

Figure 1.1. Study area and location of sampling reaches. CH= Chícamo stream; RbP= Rambla de la Parra. Codes 1 to 6 correspond to sampling reaches; R1 to R6.

Watersheds (296 Km^2 and 36 Km^2 for CH and RbP, respectively, Table 1.1) are characterised by the presence of halite-rich sedimentary marls (Miocene and Triassic origin). The ionic composition of stream water is dominated by Na$^+$ and Cl$^-$ in the case of CH, and by Ca^{2+} and SO$_4^{2-}$ in the case of RbP (Table 1.2). Streambed sediments consist mainly in gravel and sand upstream, whereas a mixture of silt and gravel dominates downstream. The presence of macrophytes and periphyton covering the channel is, in general, scarce, and is even absent in the upper reaches. However, small patches of periphyton dominated by *Calotrix* sp. (Velasco et al. 2003) are more common downstream. In general, riparian vegetation is also scarce in both streams and is formed by small patches of *Phragmites australis* and sporadic scrubs such as *Tamarix canariensis.*

Table 1.1. The subwatershed characteristics in Chícamo stream (CH) and Rambla de la Parra (RbP).

Drainage subwatersheds	Drainage Area (Km^2)	Forested use (%)	Agricultural use (%)
CH			
Up	189.13	82.8	10.9
Mid	196.71	66.2	27.8
Down	296.43	51.8	43.0
RbP			
Up	13.09	85.6	0.2
Mid	17.56	81.8	7.4
Down	36.39	73.3	20.6

In order to estimate the agricultural influence gradient, three drainage subwatersheds (up-, mid- and down-stream, Table 1.1) were delineated in both streams using a digital elevation model of 10 m resolution (Fig. 1.1). Land uses within the subwatershed were extracted from a remote sensing land cover map (Martínez-López et al. 2011). Data layers were processed using GRASS GIS (version 6.4.0. Open Source Geospatial Foundation, http://grass.osgeo.org). Land was grouped per use into two zones: forested, which included wood, grasslands and Mediterranean scrubs, and agricultural, which included irrigated farms, irrigation ponds and dry-land crops. Only the agricultural land use percentages (hereafter %AG) were used for the data analyses in this study (Table 1.1).

Table 1.2. Mean (SD) concentration (in mg L^{-1}) of anions and cations of the surface water (n=3) in Chícamo stream (CH) and Rambla de la Parra (RbP).

Stream	Cl^-	Br^-	SO_4^{2-}	Ca^{2+}	K^+	Na^+
CH	557 (26)	0.8 (0.03)	310 (41)	117 (10)	8 (1)	473 (15)
RbP	809 (24)	1.0 (0.01)	2220 (67)	591 (10)	18 (0.3)	555 (73)

Field sampling

Six stream reaches were selected in both streams and were coded from R1 to R6 according to their position from up- to downstream (Fig. 1.1). Along the agricultural gradient, three groups of reaches, R1-R2, R3-R4, and R5-R6, were located in the up, mid, and down drainage subwatersheds, respectively.

Streams were first sampled mid-June 2009, one month before cessation of the surface water flow (pre-drought conditions). The summer drought continued until the beginning of October. The post-drought sampling was performed 2- weeks after surface flow recovery. Field observations and an online daily rainfall database from SIAM (Agrometereological Information Service) from the province of Murcia allowed us to track rainfall in the study area.

Ten sediment cores were collected at each stream reach at five sampling points along a 50-m transect. A 28-cm^2 core was used to sample up to a depth of 5 cm. At each reach, cores were pooled into one sediment composite sample to be used for denitrification assays and sediment characterisation. Surface water samples were taken in previously acid-washed polyethylene bottles (125 mL), filtered (Whatman GF/F, 0.7 μm nominal pore size, Whatman International Ltd., Maidstone, England) and kept on ice until the nitrogen (NO_3^- and NH_4^+) and dissolved organic carbon (DOC) analyses were performed in the laboratory (within 24 h of collection). We also collected 1.5 L of unfiltered stream water for the denitrification assays. Surface discharge was estimated as a product of the average water velocity and the cross-sectional area for the fixed recording sites located along the reaches. Water velocity was measured using a current meter (MiniAir2, Schilktnecht Co, Zürich, Switzerland). Water salinity was measured with a conductivity meter (Tretacon 235, WTW, Munich, Germany). Sediment redox potential (Eh) was measured with a portable Eh/pH meter at a depth of 5 cm. Redox measures were corrected by adding 200 mV to the field voltage (value of the standard Ag/AgCl reference electrode) (Vepraskas and Faulkner 2001).

Sediment and water chemical characterisation

Sediment subsamples taken from each composite sample collected at each reach were dried at 100ºC for 24 h to quantify dry mass (DM). Ash-free dry mass (AFDM) was determined as loss on ignition at 550ºC after 4 h to quantify sediment organic matter (%OM). Using the additional sediment samples from each sampling reach, the fine sediments fraction (hereafter %FS) was determined by sieving (2-mm mesh) dry sediments.

Water NO_3^- and NH_4^+ concentrations were measured following a standard colorimetric method (APHA 2002). All these analyses were performed in an automated ion analyser (EasyChem Plus, Systea Analytical Technologies, Italy). DOC samples were acidified and analysed using a Total Organic Carbon Analyser for total non- purgeable organic content (TOC-Vcsh Shimazu).

Laboratory denitrification assays

Sediment denitrification rates were determined with the chloramphenicol-amended acetylene block method (Smith and Tiedje 1979; Royer et al. 2004). In the laboratory, five replicates were run for the denitrification assay from each sediment composite sample. Approximately 125 mL of sediment, excluding particles of > 6 mm, were placed into 250 mL media bottles with a septum lid. Each bottle was filled with 75 mL of unfiltered stream water, and chloramphenicol was added at a final concentration of 8 mM to suppress *de novo* enzyme synthesis. Based on previous assays with the studied sediments, 8 mM was the lowest concentration of chloramphenicol at which N_2O production was linear over a 5-hour incubation period. Bottles were purged with ultra-high purity He for 10 min to create a reducing environment. Approximately 10% of the volume in each bottle was replaced by adding 15 mL of pure C_2H_2 using gas-tight syringes. Bottles were then shaken, vented and incubated at room temperature (20-25°C). The headspace gas in each bottle was sampled at 0.5, 2.5 and 5.0 h using gas-tight syringes and 5 ml vacutainers® (Becton-Dickinson, NJ, USA). Head-space replacement gas (10% C_2H_2, 90% He) was than added to each bottle to maintain constant pressure throughout the assays. Gas samples were analysed in an Agilent C890N gas chromatograph (Agilent Technologies, Silicon Valley, California, USA) equipped with a Porapack Q column and an ^{63}Ni electron-capture detector. Denitrification rates were determined by regressing out the N_2O-N amount in the bottles against time. Because the lab temperature varied between experiments, N_2O solubility was corrected using the appropriate Bunsen Coefficient for temperature.

The acetylene inhibition technique can underestimate denitrification rates in streams with low NO_3^- availability or coupled nitrification-denitrification (Rudolph et al. 1991). Nevertheless, Rudolph et al. (1991) found that if the NO_3^- concentration was $\geq$ 10 μM (0.140 mg L^{-1}), then this technique is acceptable for analysing *in situ* denitrification when using sediment slurries. Thus, given the NO_3^- concentrations of the study streams (0.1 - 23 mg L^{-1}), we believe that this technique is appropriate for the streams examined. Denitrification rates were expressed as N_2O-N produced per g of sediment organic matter (μg N g^{-1}AFDM h^{-1}) and per g of dry sediment mass (μg N g $^{-1}$DM h^{-1}); the latter was selected to perform the statistical analyses. When denitrification rates were below the detection levels, one half of the reporting laboratory value was assigned to carry out the statistical analyses (Helsel 2005).

Nutrient-amended denitrification assays

To determine whether there was a nutrient limitation in the denitrification rates and whether the limitation pattern varied with agricultural development and period (pre- and post-drought), nutrient-amended experiments were conducted in each stream. Given the large number of incubations to be analysed simultaneously, the nutrient-amended experiment performed in each stream was conducted with only the sediments from R1 and R6, which represented the extremes of the agricultural gradient. We conducted four types of treatments in each reach with five replicates per treatment: i) unamended (only unfiltered stream water); ii) with a spike solution of 200 mg NO_3^- L^{-1} such as KNO_3; iii) with a spike solution of 1 g labile organic C L^{-1} as glucose; iv) with both nutrients in order to study a possible co-limitation.

Statistical analyses

To visualise the direction and correlation of %AG and stream variables (Table 1.3) that might contribute to variation in the denitrification rates, we performed principal component analyses (PCA) during each period using the data from both streams. The results of each PCA are hereafter referred to as pre-drought PCA and post-drought PCA. As the variables were measured in very different units, they were standarised to a zero mean and unit variance before being included in the PCA analyses. Having discovered which variables correlated with %AG, this was not used in the remaining analyses.

Pearson correlation coefficients (reported as r) were used to explore the relationship between stream variables (Table 1.3) and denitrification rates using data from both streams. Stepwise multiple regressions were further computed to select the predictors that explained most of the variation in the denitrification rates. To run the step-wise models, we selected independent variables appeared uncorrelated: water salinity, NO_3^- and sediment redox during pre-drought, and NO_3^- and %OM during post-drought.

To improve the fit of the regression model and to reduce the influence of leverage values, a log(x) transformation was applied whenever appropriate. The inclusion criterion for all variables included in the model was $p< 0.05$. Each model was examined using partial regression leverage plots, plots of residuals vs. predicted values, and the Durbin-Watson test to assess the autocorrelation among residuals. A collinearity analysis was also performed for all the variables included in the model. An analysis of covariance (ANCOVA) was performed to examine the interaction between stream and each stream variable on denitrification. Because the interaction term (data not shown) was not significant, we concluded that the influence of each variable on denitrification did not differ between streams. Consequently, the data from both streams were pooled to run the multiple linear regression models.

In each stream, a two-way factorial ANOVA, with NO_3^- and DOC as fixed factors, was used for the nutrient limitation experiment (Tank et al. 2006) for each reach (R1 and R6) and period (pre- and post-drought). We assumed that nutrient limitation by NO_3^- or DOC occurred when amending the denitrification assay bottle with NO_3^- and/or glucose caused a significant increase in the denitrification rate in comparison to the rate of the unamended control. NO_3^- or DOC limitation was indicated when just one factor substantially increased the denitrification rate with no significant interaction term. Co-limitation was elicited when NO_3^- and DOC independently, or NO_3^- + DOC together, increased denitrification.

Table 1.3. Mean (SD) values of discharge (n=2), water chemistry (n=3) and sediment variables (n=5) from up- to down-reaches (from R1 to R6) in Chícamo stream (CH) and Rambla de la Parra (RbP) during the pre- and post-drought periods. *P*< 0.05 shows pre- and post-drought significant differences (paired t-test). n.s. = no significant differences; Q= Discharge; DOC =Dissolved organic carbon; OM= Organic matter; FS= Fine sediments. N/A= data not available due to the absence of surface water.

		Q ($L\ s^{-1}$)		Salinity ($mS\ cm^{-1}$)		NO_3^--N ($mg\ L^{-1}$)		NH_4^+-N ($mg\ L^{-1}$)	
Stream	Reach	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
CH	R1	21 (1.3)	24 (2.3)	2.7 (0.2)	2.8 (0.1)	4.6 (0.2)	4.9 (0.2)	0.03 (0.01)	0.08 (0.02)
	R2	26 (2.0)	17 (1.7)	2.8 (0.1)	2.8 (0.2)	4.7 (0.1)	4.7 (0.1)	0.04 (0.01)	0.07 (0.02)
	R3	22 (1.7)	22 (2.5)	3.1 (0.3)	2.8 (0.2)	5.4 (0.6)	5.0 (0.3)	0.01 (0.01)	0.08 (0.02)
	R4	25 (3.2)	19 (4.3)	3.3 (0.2)	2.7 (0.1)	5.2 (0.3)	5.0 (0.2)	0.01 (0.01)	0.05 (0.01)
	R5	1.3 (0.8)	0.8 (0.2)	19 (0.5)	17 (0.2)	19 (1.1)	18 (0.3)	0.3 (0.04)	0.8 (0.03)
	R6	0.6 (1.0)	0.5 (0.1)	19 (0.1)	19 (0.1)	21 (0.7)	19 (0.4)	0.2 (0.03)	0.7 (0.05)
		n.s.		n.s.		n.s.		$p<0.05$	
RbP	R1	0.2 (0.1)	0.1 (0.1)	11 (0.1)	7.8 (0.3)	0.2 (0.1)	0.1 (0.1)	0.2 (0.05)	0.3 (0.2)
	R2	1.5 (0.2)	0.3 (0.1)	8 (0.3)	8.2 (0.2)	0.1 (0.1)	0.1 (0.3)	0.1 (0.04)	0.3 (0.1)
	R3	0.6 (0.4)	N/A	22 (0.6)	N/A	0.7 (0.2)	N/A	0.3 (0.06)	N/A
	R4	0.5 (1.0)	N/A	39 (0.8)	N/A	0.2 (0.1)	N/A	0.2 (0.05)	N/A
	R5	4.8 (2.0)	1.4 (0.2)	25 (1.2)	23 (0.2)	9.8 (2.2)	22 (3.0)	0.3 (0.01)	0.8 (0.1)
	R6	3.2 (0.3)	0.5 (0.3)	27 (2.6)	26 (0.1)	13 (1.3)	23 (1.0)	0.3 (0.05)	0.8 (0.07)
		$p<0.05$		n.s.		n.s.		$p<0.05$	

		DOC ($mg\ L^{-1}$)		OM (%)		Redox (mv)		FS (%)	
Stream	Reach	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
CH	R1	5.6 (0.3)	13 (2.2)	0.5 (0.1)	0.5 (0.1)	336 (17)	337 (28)	22 (6)	28 (25)
	R2	5.5 (0.4)	13 (2.8)	0.4 (0.2)	0.3 (0.1)	398 (11)	357 (23)	20 (9)	24 (12)
	R3	6.1 (0.5)	12 (1.1)	0.6 (0.1)	0.6 (0.1)	296 (6)	298 (14)	24 (10)	25 (5)
	R4	9.0 (1.5)	13 (1.8)	1.3 (0.1)	0.5 (0.1)	168 (4)	214 (27)	28 (7)	37 (8)
	R5	7.2 (1.8)	18 (1.2)	0.9 (0.2)	0.5 (0.1)	268 (7)	205 (13)	42 (8)	45 (13)
	R6	12 (0.2)	27 (0.5)	1.7 (0.2)	0.6 (0.1)	240 (10)	200 (10)	49 (3)	54 (9)
		$p<0.05$		n.s.		n.s.		n.s.	
RbP	R1	8.2 (0.6)	16 (0.3)	0.5 (0.1)	1.2 (0.2)	335 (32)	308 (18)	19 (4)	25 (18)
	R2	7.1 (0.7)	17 (0.7)	0.6 (0.1)	1.0 (0.2)	310 (27)	310 (44)	23 (11)	19 (4)
	R3	9.5 (5.0)	N/A	0.8 (0.1)	N/A	319 (5)	N/A	36 (8)	N/A
	R4	12 (0.7)	N/A	0.8 (0.3)	N/A	296 (5)	N/A	35 (4)	N/A
	R5	15 (1.4)	25 (1.2)	1.2 (0.7)	2.8 (1.8)	150 (26)	293 (32)	44 (3)	55 (12)
	R6	18 (0.6)	26 (1.2)	1.5 (0.2)	3.0 (1.1)	86 (33)	273 (46)	52 (12)	60 (6)
		$p<0.05$		$p<0.05$		n.s.		n.s.	

The pre- and post-drought differences for the denitrification rates and environmental variables were assessed using paired t-tests for each stream. Streams were compared for denitrification rates by means of independent t-tests.

All the variables followed a normal distribution and were only log(x)- or log (x+1)- transformed to perform the two-way ANOVA and the t-test analyses in order to improve the homogeneity of variances. The results were significant if $p< 0.05$ and were marginally significant if $0.05 < p < 0.1$. Ordination analyses were done using PRIMER 6.0 (PRIMER, Ivybridge, UK), while SPSS 15.0 (SPSS, Chicago, USA) was used for all the other statistical analyses.

Results

Agricultural land use and stream variables

%AG increased in the downstream direction in both streams (Table 1.1). Table 1.3 reflects the average values that the environmental variables displayed from R1 to R6 (i.e., in the downstream direction) in both streams during the pre- and post-drought.

When comparing inorganic N solutes, NO_3^- concentrations were > 1 order of magnitude higher than the NH_4^+ concentrations in both streams and periods, except for R1 to R4 in RbP, which had similar concentrations of both N forms. The NO_3^- and DOC concentrations were of the same order of magnitude in CH, except for R1 to R4 in the post-drought period, when DOC was one order of magnitude higher than NO_3^-. In RbP, the NO_3^- and DOC concentrations, of the same order of magnitude, were found only in R5 and R6 under both the pre- and post-drought conditions, whereas lower NO_3^- concentrations than DOC were present between R1 and R4.

The two principal components of the pre-drought PCA explained 82.3% of the variance observed from the environmental variables in both streams (Fig. 1.2a). The first axis (55.3%) separated down-reaches (R5 and R6) from the rest of the reaches. These reaches showed the highest %AG, nutrient concentrations, salinity, sediment %OM, %FS and the lowest sediment redox values and discharge. The second axis (27.0%) separated the two streams, with CH presenting higher %AG, water NO_3^- and discharge, and lower salinity if compared with RbP (Fig. 1.2a). This separation was more evident in the case of reaches R1 to R4 since R5 and R6 were similar between streams (Fig. 1.2a).

The pre-drought PCA revealed that %AG strongly correlated with the NO_3^--N concentrations (Fig. 1.2a). Some stream variables were also correlated. Water DOC, sediment %FS and %OM all positively correlated with each other, and they all correlated negatively with redox. Water salinity and NH_4^+ also correlated positively. Finally, a negative correlation was observed between sediment %OM and redox, and between NH_4^+ and discharge (Fig. 1.2a).

The two first components of the post-drought PCA condensed 88.8% of variance (Fig. 1.2b) and the axis showed similar trends to the pre-drought PCA. When compared with the pre-drought results, the first axis (67.9%) led to a stronger separation between the down-reaches (R5 and R6) and the rest of the reaches in both streams (Fig. 1.2b). When analysing the second axis (20.9%), we observed that the down-reaches differed more between the streams if compared to the pre-drought PCA, with RbP showing higher %OM than CH (Fig. 1.2b). The post-drought PCA also displayed that %AG correlated moderately with NO_3^-, but strongly and negatively with redox. Stream variables such as water salinity, NH_4^+, NO_3^-, DOC and sediment %FS were positively correlated (Fig. 1.2b).

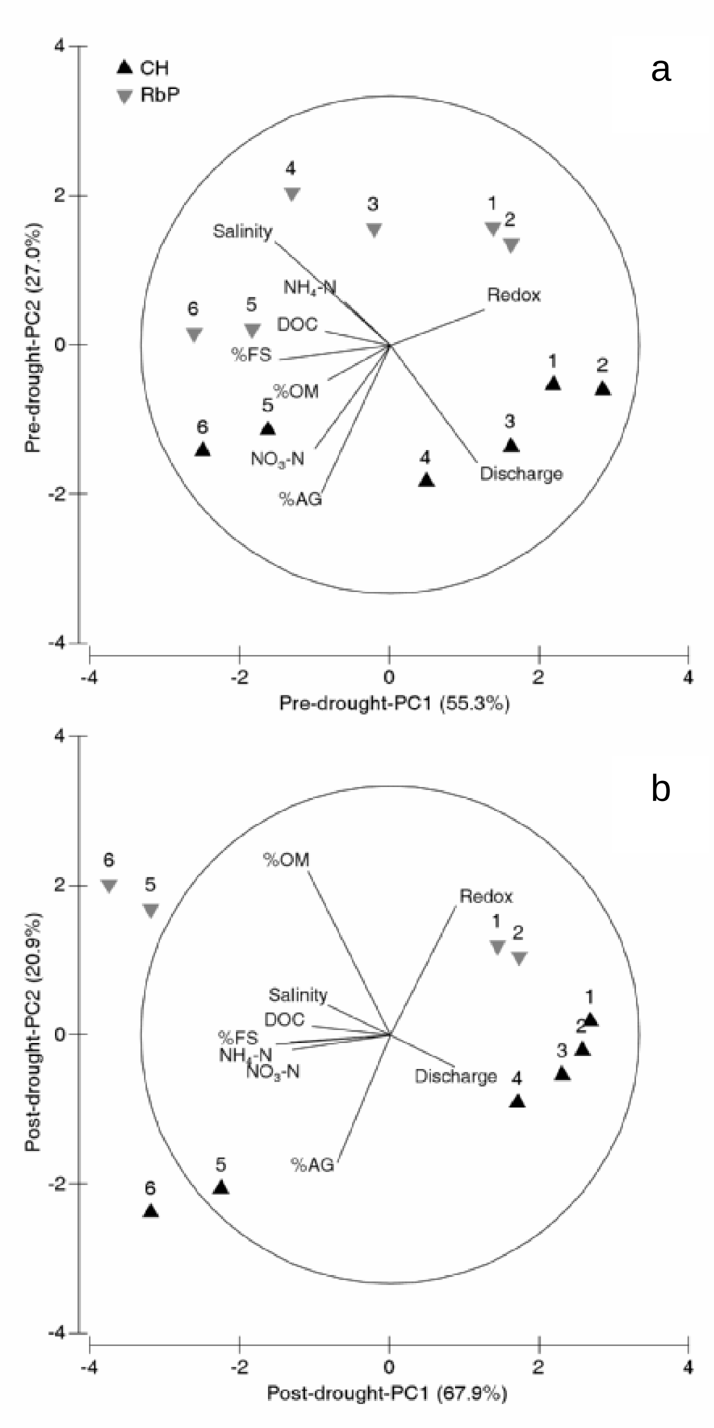

Figure 1.2. Principal component analysis (PCA) of the environmental variables of pre- (a) and post-drought (b). CH= Chícamo stream; RbP= Rambla de la Parra. Codes 1 to 6 correspond to sampling reaches R1 to R6 located from up- to down-stream.

When comparing the pre- and post-drought periods, significant differences were found in some environmental variables (Table 1.3). In both streams, the average water DOC concentrations and NH_4^+ concentrations increased significantly by ~ 2-times if compared to the pre-drought (t-test, $p< 0.05$; Table 1.3). The average discharge value and %OM dropped and increased, respectively, during the post-drought in RbP (t-test, $p< 0.05$; Table 1.3) while no significant differences were detected for these variables in CH.

Denitrification rates and stream predictors

In general, denitrification rates increased from up- to down-stream under pre-drought conditions (Table 1.4). This trend was more gradual in RbP compared to CH (Table 1.4). During the pre-drought, the average denitrification rates expressed per DM and per AFDM in CH were approximately 2 times higher than RbP, although these differences were marginally significant (t-test, $p=0.092$ and $p=0.056$, respectively).

Table 1.4. Mean (SD; analytical replicates) denitrification rates (n=6) in the study stream from up- to down- reaches (from R1 to R6) in Chícamo stream (CH) and Rambla de la Parra (RbP) during the pre- and post-drought periods. $P< 0.05$ shows the pre- and post-drought significant differences (paired t-test). n.s.= no significant differences; DM=dry mass; AFDM= ash-free dry mass; ND= no detected denitrification; N/A= data not available due to the absence of surface water.

		Denitrification (μg N g^{-1} DM h^{-1})		Denitrification (μg N g^{-1} AFDM h^{-1})	
Stream	Reach	Pre-	Post-	Pre-	Post-
CH	R1	0.05 (0.02)	0.04 (0.01)	8.1 (3.2)	7.3 (1.2)
	R2	0.01 (0.07)	0.01 (0.01)	2.7 (1.5)	1.1 (0.2)
	R3	0.10 (0.03)	0.05 (0.02)	16.0 (5.3)	14.2 (1.2)
	R4	0.25 (0.01)	0.10 (0.01)	19.5 (2.6)	23.2 (0.2)
	R5	0.06 (0.01)	0.07 (0.01)	7.2 (0.8)	13.2 (0.5)
	R6	0.28 (0.09)	0.17 (0.06)	19.0 (2.7)	26.0 (1.9)
		p<0.05		n.s.	
RbP	R1	0.001 (0.001)	ND	0.2 (0.01)	ND
	R2	0.002 (0.001)	ND	0.4 (0.02)	ND
	R3	0.001 (0.001)	N/A	0.2 (0.08)	N/A
	R4	0.002 (0.001)	N/A	0.3 (0.05)	N/A
	R5	0.11 (0.01)	0.02 (0.01)	5.6 (1.5)	2.8 (0.2)
	R6	0.40 (0.06)	0.05 (0.01)	30.0 (8.8)	1.3 (0.7)
		p<0.05		p<0.05	

Denitrification rates correlated positively with stream water NO_3^- ($r=0.74$, $p<0.05$) and DOC ($r=0.70$, $p<0.05$). Sediment variables also correlated with denitrification, such as %OM ($r=0.86$, $p<0.05$), %FS ($r=0.64$, $p<0.05$) and sediment redox ($r=0.73$, $p<0.05$).

Table 1.5. Multiple regression model relating the denitrification rate (μg N_2O-N g^{-1}DM h^{-1}) to stream predictors during the pre- (n=12) and post-drought periods (n=10).

Step	Variable included	Model	Partial adjusted r^2	Model adjusted r^2	p- value
	Pre-				
1	Log_{10} (NO_3^-)	Log_{10} Denitrification = 1.10 ($\log_{10}NO_3^-$) - 2.06	0.80	0.80	0.000
2	Redox	Log_{10} Denitrification = 0.92 ($\log_{10}NO_3^-$) - 0.004 (redox) - 1.03	0.09	0.89	0.014
3	Log_{10} (Salinity)	Log_{10} Denitrification = 0.80 ($\log_{10}NO_3^-$) - 0.005 (redox) - 0.60 ($\log_{10}$salinity)	0.05	0.94	0.013
	Post-				
1	Log_{10} (NO_3^-)	Log_{10} Denitrification = 0.97 ($\log_{10}NO_3^-$) - 2.30	0.78	0.78	0.000

The regression model that accounted for most of the variation of denitrification during the pre-drought included the log-tranformed water NO_3^- and salinity, and sediment redox (Table 1.5). NO_3^- was the best predictor of denitrification, explaining 80% of variance. Inclusion of the sediment redox variable in the regression model significantly increased the explained variance (89%). Having taken into account the NO_3^- and sediment redox effects, there was a weak negative effect of water conductivity on denitrification since the inclusion of water conductivity in the model yielded modest improvements in the amount of variance explained by the pre-drought model (94%). Figure 1.3a shows strong agreement between the denitrification rates observed and predicted for this model.

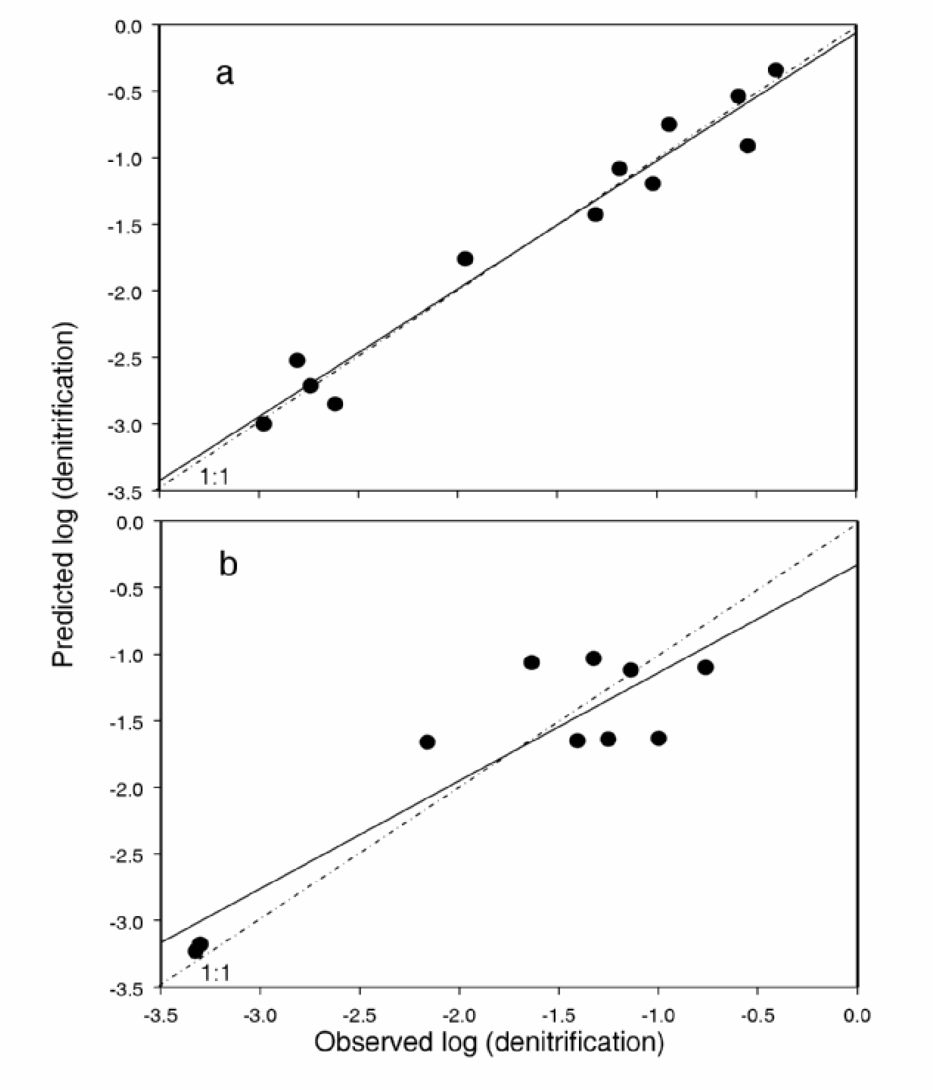

Figure 1.3. Measured denitrification rates (μg N g $^{-1}$DM h^{-1}) vs. predicted rates according to the multiple regression model of the pre- (a) and post-drought periods (b).

Drought effect

Under post-drought conditions, the average denitrification rates expressed per g of DM dropped significantly; 41% and 79%, in CH and RbP, respectively (t-test, $p < 0.05$; Table 1.4). Denitrification rates expressed per g of AFDM were significantly lower in RbP (t-test, $p < 0.05$; Table 1.4), dropping 83%, whereas non-significant differences were detected in CH.

During the post-drought, denitrification showed a marginally significant correlation with NO_3^- ($r=0.60$, $p=0.057$). Besides, the regression model that explained most of the variation of denitrification included only log-transformed NO_3^- (78% of explained variance; Table 1.5). Despite the high r^2 value, this result was spurious since the lowest rates strongly influenced on model fit. Thus, we cannot consider that NO_3^- was a relevant predictor of denitrification during post-drought. In fact, a weaker agreement was found between the observed and predicted denitrification rates (Fig. 1.3b) if compared with the pre-drought model.

Nutrient limitation

When we performed the two-way ANOVA analyses, we found limited denitrification, but only under pre-drought conditions (Fig. 1.4). Besides, when denitrification appeared to be limited, this limitation was due to NO_3^- since the pure NO_3^- treatment had a significant positive effect on denitrification. In CH, denitrification was NO_3^--limited in the least agriculturally affected reach (R1), (two-way ANOVA, NO_3^- factor $p < 0.05$; Fig. 1.4), whereas no limitation was detected in R6. Unlike CH, denitrification in RbP was NO_3^--limited in both reaches (R1 and R6), irrespectively of varying agricultural influence (two-way ANOVA in both reaches, NO_3^- factor $p < 0.05$; Fig. 1.4). It is worth noting that no denitrifying activity was detected in R1 in RbP for any treatment under the post-drought conditions (Fig. 1.4).

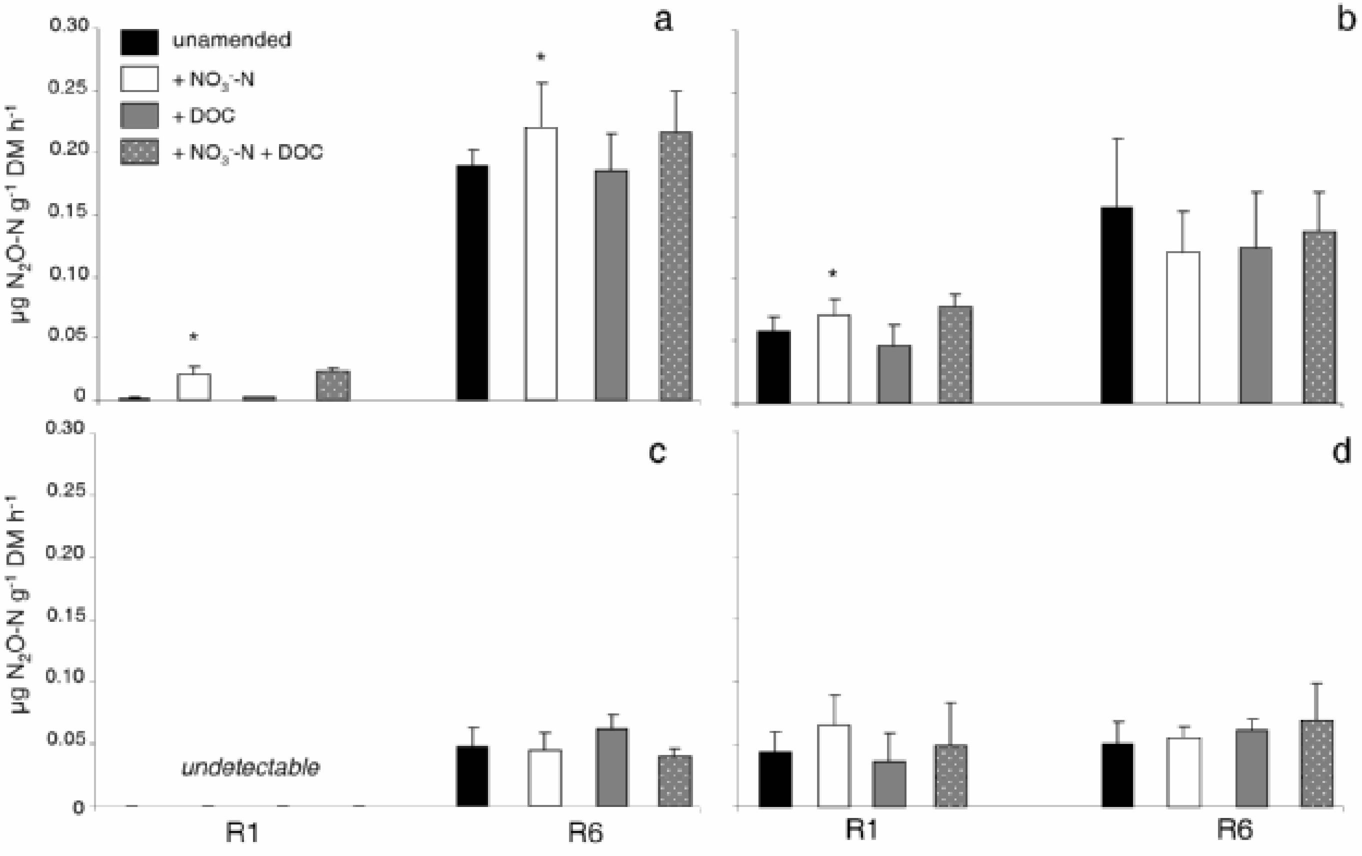

Figure 1.4. Mean (+1 SD) denitrification rates under different treatments in Rambla de la Parra (RbP) under pre- (a) and post-drought (c) conditions, and in Chícamo stream (CH) under pre- (b) and post-drought (d) conditions. Asterisks (*) show significant a NO_3^- or DOC effect in the two-way ANOVA ($P < 0.05$).

Discussion

Denitrification rates and controlling factors in temporary saline streams

Given the numerous methods available to measure denitrification rates in aquatic ecosystems, it is often challenging to compare results among different sites. We compared our results with those obtained from previously published literature which used the C_2H_2 inhibition method with unamended incubations. In our study, the maximum denitrification rates (μg N_2O-N g^{-1}DM h^{-1}) obtained in both streams were lower than those found in other agricultural streams, in spite of our streams accounted for a higher water NO_3^- (Fig. 1.5a) and also large DOC concentrations. However, when we calculated denitrification rates as μg N_2O-N g^{-1}AFDM h^{-1}, our results were within the same order of magnitude, or even higher, as the denitrification rates reported for other agricultural streams (Fig. 1.5b).

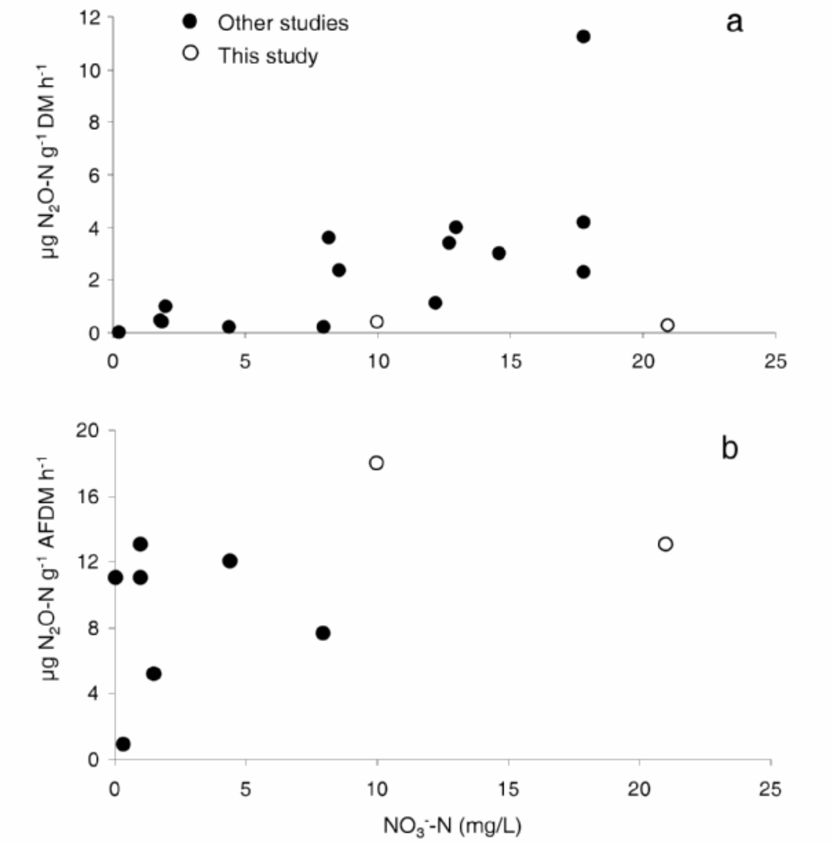

Figure 1.5. Comparison of denitrification rates with the literature. Data show that from the study streams the maximum rates found in this study. Other data presented are from Arango and Tank (2008) (a), Arango et al. (2007) (a), García-Ruiz et al. (1998) (a), Opdyke et al. (2006) (a), Powel and Bouchard (2010) (a), Schaller et al. (2004) (a), Wall et al. (2005) (a,b), Inwood et al. (2005) (a,b), Martin et al. (2001) (b), Herrman et al. (2008) (b), Duff et al. (1984) (b), O´Brien et al. (2006) (b).

Compared to temperate streams, riparian vegetation in semiarid saline streams (mainly woody shrubs) is typically scarce with limited leaf inputs (Vidal-Abarca et al. 2004), which leads to low streambed organic matter stocks. Consequently, a low biomass of heterotrophic organisms may be expected (Marmonier et al. 2010). This situation could explain the low denitrification rates expressed by DM in these streams in relation to other agricultural streams. Despite its potential importance for denitrification, %OM was not a significant predictor, but was negatively correlated with redox, which was an important predictor of denitrification. Presence of sediment %OM could favour denitrification through its coupling with low sediment redox. Anoxic habitats are often associated with high heterotrophic decomposition with greater O_2 demand (Royer et al. 2004), which ultimately results in denitrification hotspots (McClain et al. 2003; Groffman et al. 2009). Alternatively, organic matter stocks may also facilitate denitrification by contributing labile organic compounds to water. Abiotic leaching and microbial degradation with exoenzymes can extract DOC from particulate organic C, providing denitrifiers with a C source (Seitzinger 1988). The strong relationship of sediment organic matter with denitrification, by expanding anaerobic habitats and providing DOC, has been highlighted in other agricultural streams (Arango et al. 2007; Arango and Tank, 2008). In agreement with these studies, we also observed a high correlation of denitrification with %OM and DOC, and also between %OM and DOC, during the pre-drought. This last finding suggests that most water DOC derived from in-stream, which is consistent with results previously obtained in Chícamo stream, where authocthonous organic matter (mainly algal and peryphiton production) has been suggested to be the most important source of water DOC (Vida-Abarca et al. 2001), as in other arid streams (Grimm 1987). The overall higher abundance of DOC relative to NO_3^- could explain the fact that denitrifiers were not limited by DOC when we ran the nutrient-amended denitrification assays during pre-drought, as also described in other agricultural streams (Herrman et al. 2008). The high DOC concentrations found in these streams fell within the range of those reported in the N-limited arid stream Sycamore Creek, Arizona (6.22-7.37 mg L^{-1}), which even showed increased values in low discharge periods (Jones et al., 1996).

Anoxic conditions within streams may also be potentiated at fine sediments sites. The results of the pre-drought-PCA reflected that redox was coupled to the percentage of fine sediments, which in turn, correlated significantly with denitrification. Such findings are consistent with previous studies that have identified a high positive association between denitrification rates and fine sediments of low porosity in agricultural streams (Opdyke et al. 2006; Solomon et al. 2009).

Strean water NO_3^- concentration, which was highly correlated with the percentage of agricultural land use, predicted 80% of the variation in the denitrification rates during the pre-drought. As we predicted, denitrification rates were higher in the most agriculturally-influenced sites, as previously documented in other agricultural watersheds (Kemp and Dodds 2002; Inwood et al. 2005; Arango and Tank 2008; Findlay et al. 2011). Unlike the results obtained by Inwood et al. (2007) in agricultural streams, we observed a positive response of denitrification to NO_3^- addition during pre-drought, although our study streams were rich in NO_3^-, mainly in the down-reaches. The main difference between both studies was that, in our case, the streamwater DOC was higher than NO_3^-. One exception was the reach R6 in CH where, even with a lower DOC than NO_3^-, denitrification did not respond to DOC additions. The fact that denitrification could approach NO_3^- saturation in this down-reach, thus without using the surplus DOC added, could be a plausible explanation for this result. Nevertheless, more research is required to identify when NO_3^- exceeds the half-saturation constant in these streams.

On the other hand, under long-term high salinity conditions, we expected salinity to not be a predictor of denitrification. However, with low explained variance (5%), water salinity negatively affected denitrification under pre-drought conditions once the influence of NO_3^- availability and sediment redox had been corrected. Although we acknowledge that explained variance was low, this finding indicated that stream sites displaying favourable denitrification conditions might show lowered denitrification rates under high water salinity conditions. Contrary to this result, Magalhâes et al. (2005) observed that denitrification was not controlled by salinity in estuaries, and argued that halo-tolerant denitrifier communities would evolve in ecosystems affected by natural salinity fluctuations. However, in agreement with our results, Rysgaard et al. (1999) also reported the negative effect of increased salinity on denitrification in estuarine sediments and argued, be it poorly understood, that salinity causes physiological stress on biological mechanisms.

Bacteria adapt osmotic stress by increasing both intracellular salt content and concentrations of organic compounds, or by producing enzymes that are not influenced by salt. Therefore, they are able to survive and grow. However, the use of these approaches diverts energy from the normal cellular metabolism and result in organisms that may compete less efficiently with those evolving at low salinities (as revised in Hart et al. 1991).

On the other hand, since both autotrophic and heterophic denitrification can take place in saline ecosystems, we also have to bear in mind that large sulphate concentrations may inhibit autothrophic denitrification. However, some studies have reported that inhibition by sulphates began at concentrations above 5 g L^{-1} (Claus and Kutzner 1985) whereas lower concentrations were detected in RbP and CH. Thus, we consider that inhibition by sulphate is not an important factor in controlling nitrate removal in our streams. Nevertheless, although ambient sulphate concentrations could inhibit autothrophic denitrification, heterotrophic denitrification could take place as organic carbon was present, thus ensuring the removal of nitrate from the water column.

If high water salinity affects denitrification, the effect of raised salinity due to increased water evaporation and potential evapotranspiration (Chaouche et al. 2010) might have implications for stream functioning and services. Although the temperature-change scenarios proposed in Europe vary regionally, they all show a clear trend towards warming, with the Mediterranean region appearing to be the most vulnerable (Schröter et al. 2005). By bearing in mind such a trend, climatic changes would lead to rising water nutrient concentrations and salinity values, especially in the Mediterranean region. Therefore, it is possible to consider that both stream-denitrifying capacity and, consequently, water quality might be affected. The effect of salinity on denitrification should be specifically tested in controlled experiments to improve our limited understanding of this issue.

Drought effect on denitrification

We predicted low denitrification rates under post-drought conditions. Apart from the denitrification rates per AFDM, which did not change in CH, after 2 weeks of streambed rewetting and flow recovery, the studied streams showed a denitrifying capacity, but with lower rates than during the pre-drought period. Our results support the hypothesis that drought events negatively affect microbial processes, such as denitrification. Previous studies have documented that drought leads to changes in microbial biomass, and that it alters community composition, metabolic rates and functioning, which could have an impact on biogeochemical processes (Fierer et al. 2003; Rees et al. 2006; Amalfitano et al. 2008; Zoppini et al. 2010). Regarding denitrificaion, Austin and Strauss (2011) performed a desiccation-inundation experiment in a small headwater stream and the found that after a 28-day sediment desiccation period, denitrification rates had still not recovered. Similarly, studies examining the effect of desiccation on denitrification in stream sediments obtained decreased denitrification due to increased aerobic conditions (Cavanaugh et al. 2006; Gómez et al. 2012). On the other hand, Marxsen et al. (2010) observed the persistence of extracellular enzymes in sediments during drought and their reactivation after 4 days of rewetting in a Mediterranean stream; however, the abundance and activity of microbial communities had not completely restored. These authors suggested that microbial-resilient communities are developed in recurrent dry-wet cycles occurring in semiarid regions. The diminished denitrifying activity we observed after 15 days of rewetting in our streams is in the line with this suggestion.

During post-drought, the influence of stream-water NO_3^- on denitrification was spurious. Furthermore, no other stream variable proved to be a significant predictor of denitrification under post-drought conditions. Both findings indicate that after a stressful condition such as sediment drying, denitrification rates might be influenced by processes that directly affect the denitrifying community. This hypothesis was further reinforced by the fact that, unlike the pre-drought conditions, denitrification rates during a post-drought period did not increase with surplus NO_3^- during the nutrient addition experiment, even when the stream-water DOC remained relatively higher.

The clearest drought effect on denitrification was witnessed in RbP (R1 and R2) where, even with nutrient addition in R1, no denitrifying activity was observed during the post-drought period. This highlights the fact that the microbial community itself (i.e. structure and function) was more important in terms of denitrification than nutrient availability after drought.

When combined with the effect of drought on the microbial community, we cannot underestimate the potential effect of drought on denitrification through its effect on DOC quality and its use by microbial biota (Ylla et al. 2010; Vazquez et al.

2011). However, if a change in DOC quality contributes to lower denitrification rates after drought, then a positive response to a highly labile carbon addition (glucose) may be expected. Finally on the basis of our results, we must highlight the importance of both drought frequency and its spatial and temporal extent in annual budgets of NO_3^- exports from watersheds.

Conclusions

The variation in the denitrification rates along the gradient of increased agricultural land use was strongly influenced by the water NO_3^- concentrations during the pre-drought (summarized in Fig. 1.6). In addition to stream-water NO_3^-, low sediment redox (streambed anaerobic conditions) also favoured denitrification under pre-drought conditions. Unexpectedly, and after taking into account the effects of water NO_3^- and sediment redox, denitrification was lower in high water salinity stream reaches, although it only explained 5% of variance. During the pre-drought period, sediment %OM was coupled with denitrification by providing DOC and creating anoxic conditions. In these streams, anoxia was also associated with a high percentage of fine sediments. Our findings also suggested that drought negatively influenced denitrification by affecting denitrifying communities. Lack of

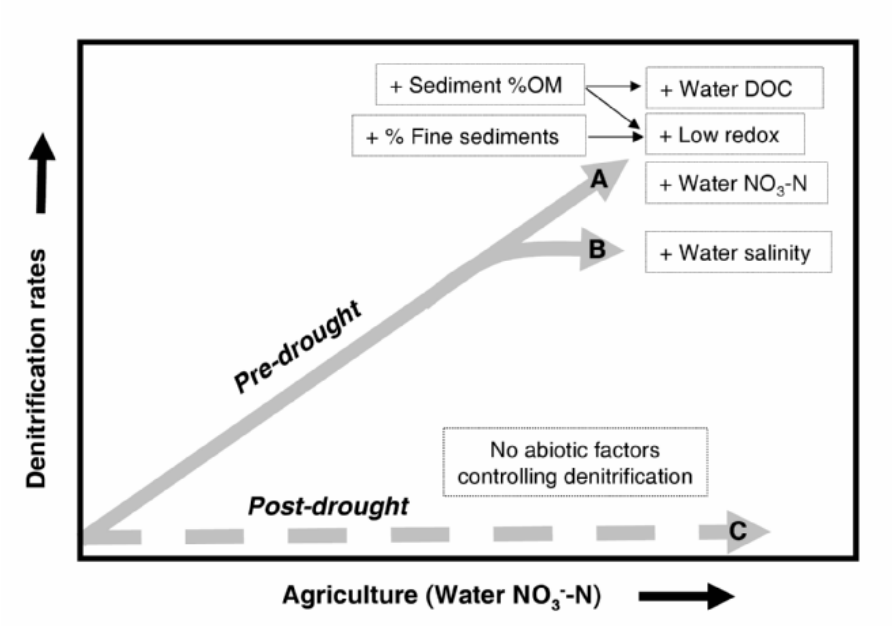

Figure 1.6. Conceptual denitrification model under pre- (a-b) and post-drought (c) conditions in agriculturally-influenced temporary saline streams. Denitrification rates increased with agriculture development because of its influence on water NO_3^- availability, and also with the sediment anaerobic conditions (a). Under these conditions, salinity seemed to modulate the denitrification rates, which dropped with increased water salinity (b). Stream-water DOC, sediment %OM and fine sediments also favoured denitrification stream sites during the pre-drought period. After drought, denitrification dropped and its variation was independent of agriculture (as shown by the dashed line in c) and of any abiotic factor.

abiotic driving factors, combined with the absence of a response to nutrient additions, highlighted the fact that the microbial community was more important in terms of denitrification than abiotic factors after a drought.

Finally, our results have important implications when considering streams as ecosystems which may attenuate NO_3^- export downstream (e.g. Alexander et al. 2000). In this context, stream management plans should focus on providing suitable conditions for denitrification and on guaranteeing stream services relating to N export control, especially given the future climate change scenario expected. First, these plans should focus on developing agricultural practices that ensure less N inputs. Although the agriculturally affected sites "adjust" their response to excess N by enhancing denitrification, chronic NO_3^- inputs to streams minimise the effect of this mechanism in controlling the NO_3^- load.

Second, riparian vegetation clearcutting and woody debris removal must be avoided, especially in agriculturally affected reaches. Thus, avoiding these practices would help maintain scarce riparian vegetation, which contributes organic matter to streams (including woody debris). Rather than providing a DOC source, allowing organic matter to accumulate in these streams would promote benthic substrata heterogeneity, slow water velocities, thus prolonging the contact time of water with denitrifiers, and anoxic sites. This would result in “hotspots” for denitrification within streams, thus optimising stream NO_3^- removal capacity.

Third, management plans should ensure the maintenance of minimum surface discharges in streams to avoid increased water salinity and drought period duration. All these measures are applicable to arid and semiarid regions predisposed to extensive drought periods (Giorgi and Lionello 2008), but also if arid conditions will predominate in the future (IPCC 2007) in a wide range of areas.

Finally, a more thorough assessment of N biogeochemical processes in water salinity fluctuations and under dry conditions (frequency, and the spatial and temporal extent of droughts) needs to be incorporated into N dynamic models along stream networks.

Acknowledgments

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References

Alexander RB, Smith RA, Schwartz GE (2000) Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403:758-761

Amalfitano S, Fazi S, Zoppini A, Caracciolo AB, Grenni P, Puddu A (2008) Responses of benthic bacteria to experimental drying in sediments from Mediterranean temporary rivers. Microb Ecol 55:270-279

Arango CP, Tank JL, Schaller JL, Royer TV, Bernot MJ, David MB (2007) Benthic organic carbon influences denitrification in streams with high nitrate concentration. Freshwater Biol 52:1210-1222

Arango CP, Tank JL (2008) Land use influences the spatiotemporal controls on nitrification and denitrification in headwater streams. J N Am Benthol Soc 27:90-107

APHA (2002) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC

Austin BJ, Strauss EA (2011) Nitrification and denitrification response to varying periods of desiccation and inundation in a western Kansas stream. Hydrobiologia 658:183-195

Ballester R, Vidal-Abarca MR, Esteve MA, Suárez ML (2003) Humedales y Ramblas de la Región de Murcia. Dirección general del medio natural, Murcia, Spain

Baker MA, Vervier P (2004) Hydrological variability, organic matter supply and denitrification in the Garone River ecosystem. Freshwater Biol 49:181-190

Carpenter SR, Caraco NF, Correl DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol Appl 8:559-568

Cavanaugh JC, Richardson WB, Strauss EA, Bartsch LA (2006) Nitrogen dynamics in sediment during water level manipulation on the Upper Mississippi River. River Res Appl 22:651-666

Chaouche K, Neppel L, Dieulin C, Pujol N, Ladouche B, Martin E, Salas D, Caballero Y (2010) Analyses of precipitation, temperature and evapotranspiration in a French Mediterranean region in the context of climatic change. C R Geosci 342:234-243

Claus G, Kutzner HJ (1985) Physiology and kinetics of autotrophic denitrification by *Thiobacillus denitrificans*. Appl Microbiol Biot 22:283-288

Dahm CN, Baker MA, Moore DI, Thibault TR (2003) Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshwater Biol 48:1219-1231

Duff JH, Triska FJ, Oremland RS (1984) Denitrification associated with stream periphyton:chamber estimates from undisrupted communities. J Environ Qual 13:514-518

Fierer N, Schimel JP, Holden PA (2003) Influence of drying-rewetting frequency on soil bacterial community structure. Microb Ecol 45:63-71

Findlay SEG, Mulholland PJ, Hamilton SK, Tank JL, Bernot MJ, Burgin AJ, Crenshaw CL, Dodds WK, Grimm NB, McDowell WH, Potter JD, Sobota DJ (2011) Cross-stream comparison of substrate-specific denitrification potential. Biogeochemistry 104:381-392

García-Ruiz R, Pattinson SN, Whitton BA (1998) Denitrification in river sediments: relationship between process rate and properties of water and sediment. Freshwater Biol 39:467-476

Gasith A, Resh VH (1999) Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Syst 30:51-81

Giorgi F, Lionello P (2008) Climate change projections for Mediterranean region. Global Planet Change 63:90-104

Gómez R, Hurtado I, Suárez ML, Vidal-Abarca MR (2005) Ramblas in south-east Spain: threatened and valuable ecosystems. Aquat Conserv 15:387-402

Gómez R, Arce MI, Sánchez JJ, Sánchez-Montoya MM (2012) The effects of drying on sediment nitrogen content in an arid Mediterranean stream: a microcosms study. Hydrobiologia 679:43-59

Grimm NB (1997) Nitrogen dynamics during succession in a desert stream. Ecology 68:1157-1170

Groffman PM, Butterbach-Bahl K, Fulweiler RW, Gold AJ, Morse JL, Stander EK, Tague C, Tonitto C, Vidon P (2009) Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. Biogeochemistry 93:49-77

Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C, Swadling K (1991) A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia 210:105-144

Hale R, Groffman PM (2006) Chloride effects on nitrogen dynamics in forested and suburban streams debris dams. J Environ Qual 35:2425-2432

Helsel DR (2005) More than Obvious: Better Methods for Interpreting Nondetect data. Environ Sci Technol 39:419A-423A

Helton AM, Poole GC, Meyer JL, Wollheim WM, Peterson BJ, Mullholand PJ, Bernhardt ES, Stanford JA, Arango C, Ashkenas LR, Cooper LW, Dodds WK, Gregory SV, Hall HO, Hamilton SK, Johnson SL, McDowell WH, Potter JD, Tank JL, Thomas SM, Valett HM, Webster JR, Zeglin L (2011) Thinking outside the channel: modeling nitrogen cycle in networked river ecosystems. Front Ecol Environ 9:229-238

Herrman K, Borchard V, Moore RH (2008) Factors affecting denitrification in agricultural headwater streams in Northeast Ohio, USA. Hydrobiologia 598:305-314

Inwood SE, Tank JL, Bernot MJ (2005) The influence of land use on sediment denitrification in 9 midwestern streams. J N Am Benthol Soc 24:227-245

Inwood SE, Tank JL, Bernot MJ (2007) Factors controlling sediment denitrification in Midwestern streams of varying land use. Microb Ecol 53:247-258

IPCC (Intergovernmental Panel on Climatic Change) (2007) Fourth assessment report. Climatic change 2007: climatic change impacts, adaptation and vulnerability. http://www.ipcc.ch/

Jones JB, Fisher SG, Grimm NB (1996) A long-term perspective of dissolved organic carbon transport in Sycamore Creek, Arizona, USA. Hydrobiologia 317:183-188

Kemp MJ, Dodds WK (2002) Comparisons of nitrification and denitrification in praire and agricultural influenced streams. Ecol Appl 12:998-1009

Larned ST, Datry T, Arscott D, Tockner K (2010) Emerging concepts in temporary river-ecology. Freshwater Biol 55:717-738

Magalhâes CM, Joye SB, Moreira RM, Wiebe WJ, Bordalo AA (2005) Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. Water Res 39:1783-1794

Marmonier P, Piscart C, Sarriquet PE, Azam D, Chauvet E (2010) Relevance of large litter bag burial for the study of leaf breakdown in the hyporheic zone. Hydrobiologia 641:203-214

Martin LA, Mulholland PJ, Webster JR, Valett HM (2001) Denitrification potential in sediments of headwater streams in the southern Appalachian Mountains, USA. J N Am Benthol Soc 20:505-519

Martínez-López J, Carreño MF, Palazón JA, Esteve MA, Martínez-Fernández J (2011) Wetland-watershed modelling and assessment: GIS methods for establishing multiscale indicators. In Baranyai A, Benkô D (eds) Wetlands: Ecology, Management and Conservation. Nova Science Publishers, New York., pp 231-250

Marxsen J, Zoopini A, Wilczek S (2010) Microbial communities in streambed sediments recovering from desiccation. FEMS Microbiol Ecol 71:374-386

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW, Johnston CA, Mayorga E, McDowell WH, Pinay G (2003) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301-312

O'Brien JM, Williard KWJ (2006) Potential denitrification rates in an agricultural stream in Southern Illinois. J Freshwater Ecol 21:157-162

Opdyke MR, David MB, Rhoads BL (2006) Influence of geomorphological variability in channel characteristics on sediment denitrification in agricultural streams. J Environ Qual 35:2103-2112

Pfenning KS, McMahon PB (1997) Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. J Hydrol 187:283-295

Powell KL, Bouchard V (2010) Is denitrification enhanced by the development of natural fluvial morphology in agricultural headwater ditches? J N Am Benthol Soc 29:761-772

Rees GN, Watson GO, Baldwin DS, Mitchell AM (2006) Variability in sediment microbial communities in a semipermanent stream: impact of drought. J N Am Benthol Soc 25:370-378

Royer TV, Tank JL, David MB (2004) Transport and fate of nitrate in headwater agricultural streams in Illinois. J Environ Qual 33:1296-1304

Royer TV, David MB (2005) Export of dissolved organic carbon from agricultural streams in Illinois, USA. Aquat Sci 67:465-471

Rudolph J, Frenzel P, Pfennig N (1991) Acetylene inhibition technique underestimates in situ denitrification rates in intact cores of freshwater sediments. FEMS Microbiol Ecol 85:101-106

Rysgaard S, Thastum P, Dalsgaard T, Christensen PB, Sloth NP (1999) Effects of salinity on NH_4^+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. Estuaries 2:21-30

Schaller JL, Royer TV, David MB, Tank JL (2004) Denitrification associated with plants and sediments in an agricultural stream. J N Am Benthol Soc 23:667-676

Seo DC, Yu K, Delaune RD (2008) Influencia of salinity on denitrification in a Louisiana estuary receiving divertid Mississippi River water. Arch Agron Soil Sci 54:249-257

Smith MS, Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. Soil Biol Biochem 11:261-267

Solomon CT, Hotchkiss ER, Moslemi JM, Ulseth AJ, Stanley EH, Hall RO, Flecker AS (2009) Sediments size and nutrients regulate denitrification in a tropical stream. J N Am Benthol Soc 28:480-490

Strauss EA, Richardson WB, Cavanaugh JC, Bartsch LA, Kreiling RM, Standorf AJ (2006) Variability and regulation of denitrification in an Upper Mississippi River Backwater. J N Am Benthol Soc 25:596-606.

Schröter D, Cramer W, Leemans R, Prentice IC, Araújo MB, Arnell NW, Bondeau A, Bugmann H, Carter TR, Gracia CA, de la Vega-Leinert AC, Erhard M, Ewert F, Glendining M, House JI, Kankaanpää S, Klein RJT, Lavorel S, Lindner M, Metzger MJ, Meyer J, Mitchell TD, Reginster I, Rounsevell M, Sabaté S, Sitch S, Smith B, Smith J, Smith P, Sykes MT, Thonicke K, Thuiller

W, Tuck G, Zaehle S, Zierl B (2005) Ecosystems service supply and vulnerability to global change in Europe. Science 310:1333-137

Tank JL, Bernot MJ, Rosi-Marshal EJ (2006) Nitrogen limitation and Uptake. In Hauer FR, Lamberti GA (eds) Methods in Stream Ecology. Academic Press, San Diego, pp 213-238

Vazquez E, Amalfitano S, Fazi S, Butturini A (2011) Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions. Biogeochemistry 102:59-72

Velasco J, Millán A, Vidal-Abarca MR, Suárez ML, Guerrero C, Ortega M (2003) Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. Freshwater Biol 48:1408-1420

Vepraskas MJ, Faulkner SP (2001) Redox Chemistry of Hydric Soils. In Richardson JL, Vepraskas GJ (eds) Wetland Soils. Lewis Publishers, New York, pp 85-107

Vidal-Abarca MR, Suárez ML, Gómez R, Guerrero C, Sánchez-Montoya MM, Velasco J (2004) Inter-annual variation in benthic organic matter in a saline, semiarid stream of southeast Spain (Chicamo stream). Hydrobiologia 523:199-215

Vidal-Abarca MR, Suárez ML, Guerrero C, Velasco J, Moreno JL, Millán A, Perán A (2001) Dynamics of dissolved and particulate organic carbon in a saline and semiarid stream of southeast Spain (Chicamo stream). Hydrobiologia 455:71-78

Wall LG, Tank JL, Royer TV, Bernot MJ (2005) Spatial and temporal variability in sediment denitrification within an agricultural influenced reservoir. Biogeochemistry 76:85-111

Williams WD (1996) The largest, highest and lowest lakes of the world: saline lakes. Verhandlungen Internationale Vereinigung für Theoretische and Angewandte Limnologie 26:61-79

Ylla I, Sanpera-Calbet I, Vázquez E, Romaní AM, Muñoz I, Butturini A, Sabater S (2010) Organic matter availability during pre- and post-drought periods in a Mediterranean stream. Hydrobiologia 657:217-231

Zoppini A, Amalfitano S, Fazi S, Puddu A (2010) Dynamics of a benthic microbiol community in a riverine environment subject to hydrological fluctuations (Mulgaria river, Italy). Hydrobiologia 657:37-51

Chapter 2

Implications of flow intermittency on sediment nitrogen availability and processing rates in a Mediterranean headwater stream

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Abstract

Most streams draining to the Mediterranean basin are temporary. As a result of their hydrological regime, temporary streams are affected by drying and rewetting periods. Drying can alter in-stream nitrogen (N) availability and reduce N processing rates and subsequent retention after re-wetting.

We sought to determine if hydrologic drying modifies reach-scale sediment chemical properties and constrains the response of N processing to rewetting. We compared different abiotic characteristics of sediments and nitrification and denitrification rates between a perennial and intermittent reach in the same stream over a wet period, when surface water flowed in both reaches, and a dry period, when the intermittent reach dried up.

We analyzed N processing rates by incubating sediments with stream water, thereby simulating a rewetting when sediments from the intermittent reach were dry. We found that drying increased the sediment nitrate (NO_3^-) content. Conversely, drying did not reduce the recovery of N processing rates to pre-dry levels after simulated flooding conditions.

Our results suggest that dry reaches may act as a potential NO_3^- source by releasing downstream NO_3^- pulses after stream flow recovery. Given the European Water Framework Directive requirements to assess stream ecological status, these N pulses following rewetting should be considered when designing management plans in temporary streams. Our study highlights the rapid response of in-stream N processing to rewetting period following a drought. This high resilience to process N should be seen as a vital ecosystem service provided by temporary streams despite annual dry periods.

Introduction

In the Mediterranean basin, temporary streams are the dominant lotic ecosystems characterized by hydrological intermittency (Gasith and Resh 1999). In semiarid Mediterranean regions, such as Southeast Spain, the flow of streams is usually interrupted during late spring to early summer, with some stream reaches (i.e., intermittent reaches) becoming completely dry. Consequently, streams become fragmented during the dry period, exhibiting discontinuous surface flow through a series of perennial and intermittent reaches (i.e., stream fragmentation). The entire stream flow recovery usually occurs in late autumn or early winter after intense rainfalls, when reaches are re-connected by continuous surface flow (i.e., stream expansion), which constitutes the wet period. Although the number of studies on temporary streams has greatly increased in recent years (Datry et al. 2011), scientific knowledge on their fundamental functioning as a basis for sustainable management is still lacking (Larned et al. 2010).

Since nitrogen (N) inputs to rivers and streams have increased as a result of anthropogenic activities (Galloway et al. 2003), many studies have investigated the specific environmental factors that control N processing rates such as nitrification and denitrification, especially in temperate climatic regions (Peterson et al. 2001). In these studies, headwater streams have been shown to be highly efficient at processing N by virtue of their large sediment-surface- area- to- water-volume ratio, which maximizes the effect of biogeochemical transformations (Alexander et al. 2000). Despite the importance of these processes, this issue has not received much attention in headwater streams of Mediterranean climates, especially under hydrological shifts (Bernal et al. 2013).

Stream nitrification and denitrification rates are microbial processes directly modulated by N concentration, organic carbon availability and sediment redox conditions (Strauss et al. 2002; Arango et al. 2007), factors that may be influenced by stream water fluctuations (Gómez et al. 2009; von Schiller et al. 2011; Fazi et al. 2013). Changes in water level also exert direct effects on sediment microbial communities. Studies suggest that drying may change microbial biomass and community structure, constraining their functional reactivation after rewetting (Rees et al. 2006; Amalfitano et al. 2008; Zoppini et al. 2010). Therefore, transitions from dry to wet periods may exert a strong influence on in-stream N processing rates and, ultimately, determine stream N availability.

How N transformation rates vary in response to flow intermittency is still an unanswered question. For example, Mitchell and Baldwin (1999) documented that nitrification and denitrification were not notably affected by sediment desiccation, whereas Austin and Strauss (2011) observed a significant reduction in both processes. Drying leads to oxygenated conditions that favour aerobic microbial processes (Baldwin and Mitchel 2000), which may stimulate nitrification and depress denitrification, despite potentially low microbial activity during the dry period (Gómez et al. 2012). Other research has demonstrated that the rewetting of dry soils and sediments stimulates N mineralization (Fierer and Schimel 2002; McIntyre et al.

2009) and denitrification when favourable conditions recover (Roley et al. 2012). Nonetheless, a key issue is whether the microorganisms involved in N processing recover after rewetting and, if so, the time lag to re-gain their previous efficiency. In this respect, some authors found affected recovery rates, when analysing stream N transformation rates to occur either immediately following rewetting (Austin and Strauss 2011) or after 2 weeks of flow resumption (Arce et al. 2013).

As flow intermittency is expected to be amplified worldwide (Sabater and Tockner 2010), there is an urgent need to better understand the role of temporary streams in controlling N transformations and fluxes.

Our aim was to examine i) how hydrologic drying affects the chemical characteristics of streambed sediments, ii) how this drying influences the response of N processing rates to sediment rewetting, and iii) which abiotic characteristics controlled the variation in N processing rates. To approach our objective, we analyzed changes in sediment N (extractable NH_4^+ and NO_3^-), extractable organic carbon (EOC) and organic matter availability (OM) in an intermittent reach of a headwater temporary stream along a sequence of dry-wet periods, and then we compared these changes with those registered in an upstream perennial reach with similar environmental features, but with permanent flow. We also analyzed nitrification and denitrification rates in laboratory assays by incubating sediments with stream water, thereby simulating the rewetting in dry sediment from the intermittent reach during the dry period. We assessed the response of dry sediments to rewetting by comparing N-processing rates of the intermittent reach during the dry and wet periods and, in turn, with those analyzed in the perennial reach.

Based on the assumption that sediment drying would create changes in sediment redox conditions and could stimulate organic matter mineralization and nitrification, we hypothesized that sediment NH_4^+, EOC and OM would decrease while sediment NO_3^- content would increase under dry conditions. We also hypothesized that drying would reduce nitrification and denitrification rates after rewetting compared with pre-dry levels. Based on both hypotheses, we predicted: i) a lower sediment NH_4^+, EOC and OM and a higher sediment NO_3^- content during the dry period than during the wet period in the intermittent reach, and lower rates of N processing rates after rewetting of dry sediments in the dry period than during the wet period; ii) when comparing both reaches, we predicted larger differences in sediment chemical properties during the dry period than during the wet period, and lower nitrification and denitrification rates in the intermittent reach after dry sediment rewetting when compared with the perennial reach and iii) the temporal variability of streambed sediment chemical properties and N processing rates would be higher in the intermittent reach than in the perennial reach.

Materials and Methods

Site description

This study was carried out in Rambla de la Rogativa, a headwater temporary stream in the Taibilla stream catchment (SE Spain, 38°8' N, 2°13' W, 1100 m.a.s.l; Fig. 2.1). The Rambla de la Rogativa catchment covers 47.2 km^2 and has a subhumid Mediterranean climate, 583 mm of average annual rainfall and an average annual temperature of 13.3°C. The dominant lithology consists of marls, limestone, marly limestones and sandstones, and most widespread land cover is natural (91%). In Rambla de la Rogativa, the dry period usually starts in July when streamflow is usually fragmented through a series of perennial reaches that maintain a surface flow, and intermittent reaches that are completely dry. This fragmentation or dry period usually ceases in November-December when intermittent reaches are re-flooded and reconnected with perennial reaches.

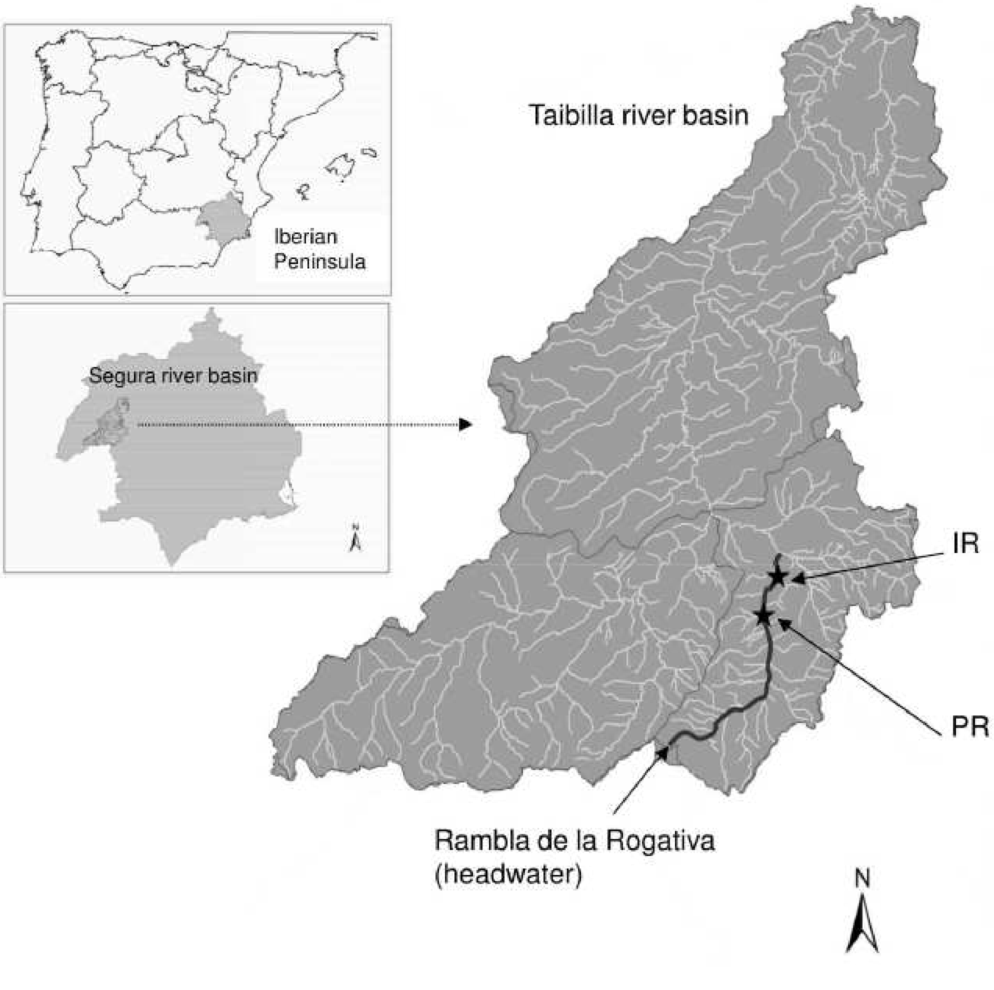

Figure 2.1. The Taibilla catchment, location of Rambla de la Rogativa and sampling reaches. PR: perennial reach; IR: intermittent reach

In our study, we selected two similar 50-m reaches with different hydrological regimes in the upper part of Rambla de la Rogativa: a perennial reach (hereafter PR) and an intermittent reach (hereafter IR), which was located approximately 5 km downstream.

In our study, the advantage of comparing reaches within the same stream is that it minimizes the potential interference of other environmental variables other than the hydrologic effect. Both reaches were similar in geomorphic and biotic variables. Reaches were 2.5 m wide and 6 cm deep, on average, with very shallow sand and gravel sediments. They were characterized by a sequence of small pools and riffles. Water velocity ranged from 0.7 to 1.5 m s^{-1} in PR and from 0.5 to 1.2 m s^{-1} in IR when surface flow existed. Aquatic vegetation was generally scarce and limited to sporadic patches of *Chara* sp. Riparian vegetation was also scarce and was dominated by rushes and Mediterranean shrubs.

Field sampling

Sampling was carried out from October 2009 to November 2010. During the study, the rainfall pattern in Taibilla catchment was highly variable (Fig. 2.2a). As a result of the summer drought of 2009, stream flow in Rambla de la Rogativa was discontinuous from October 2009 to the second half of January 2010. During these months, discharge was low in PR, while IR was completely dry (dry period-09) (Fig. 2.2b). Continuous flow recovered in the second half of January 2010 when rainfalls reached 305 mm, and then IR was re-flooded and re-connected to PR by a continuous surface flow along the entire stream until July 2010 (wet period). From this month, discharge gradually decreased in PR and IR dried up again from August 2010 to November 2010 (dry period-10) (Fig. 2.2b).

Both reaches were sampled monthly with 11 sampling dates per reach. On each date we collected 3 sediment samples along a 50-m reach, one each from the 3 dominant geomorphic structures: pools, riffles and parafluvial zones (water saturated sediments on the lateral margins of the channel). Each sediment sample consisted of a composite sample that was mixed, homogenized and sieved in the field at 4-mm mesh size to separate large rocks, sticks and other debris from sediments. Sediments were collected with a hand shovel from the top 0-5 cm because most biological activity in arid and semiarid sediments is expected to be concentrated in surface sediments (Bennett and Adams 1999), but also because of the shallow sediment depth. Sediment samples were transported in zip-lock bags under ice and dark conditions to the laboratory for analysis (within 24 h).

Surface water samples were collected in 250-mL acid-washed polyethylene bottles at 3 locations along each study reach. *In situ* water temperature, conductivity and dissolved oxygen (DO) also were measured at the same location of water samples using Hach handheld sondes (Loveland, CO, USA). Water-column depth and width, and water velocity (current meter MiniAir2; Schiltknecht Co., Zurich, Switzerland) were also recorded. Surface-water discharge was estimated as the product of the average water velocity and cross-sectional area for the fixed recording sites located along the reaches.

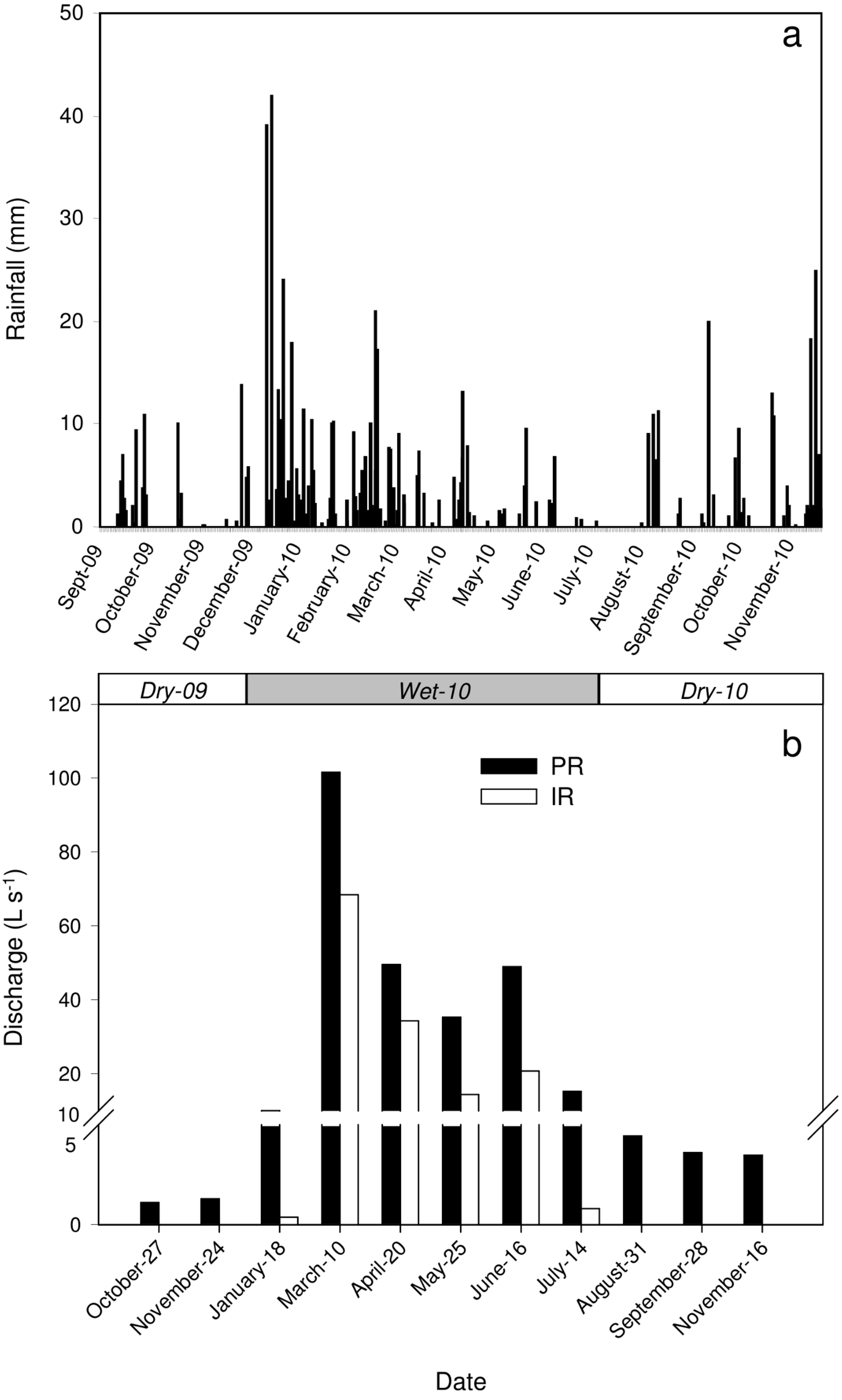

Figure 2.2. Daily mean precipitation in the study catchment (a) and Rambla de la Rogativa discharge throughout the study period (b). PR: perennial reach; IR: intermittent reach

Water and sediment analysis

In the laboratory, surface water samples for chemical analyses were filtered through pre-combusted Whatman GF/F filters (Maidstone, England, UK). The filtered water samples were analyzed for NO_3^- and NH_4^+ following standard colorimetrical methods (APHA 2002) on a Systea EasyChem autoanalyzer (Oak Brook, IL, USA). Water samples taken for dissolved organic carbon (DOC) analyses were acidified before being frozen and were analyzed for non-purgeable organic C using a Shimadzu TOC-5000A Total Organic C analyzer (Columbia, MD, USA). From each sediment sample, 3 subsamples were analyzed for chemical characterization purposes. Subsamples were dried at 60ºC (24 h) to determine the percentage of gravimetric water (GW%). Then, subsamples were further burned at 550ºC for 4 h to estimate the percentage of sediment organic matter (OM%). Cold potassium chloride (KCl) extraction (Mulvaney 1996) was used to estimate the extractable NO_3^- and NH_4^+ sediment concentrations by shaking the sediments (5 g) in 100 mL of 2 M KCl for 1 h on a rotary shaker. Extractable organic carbon (EOC) (i.e., water-soluble sediment organic C) was obtained from sediments after shaking for 1 h in deionized water on a rotary shaker (Starry et al. 2005). All extracts were filtered through pre-combusted Whatman GF/F filters (Maidstone, England, UK) and frozen until analyzed. Extractable NO_3^-, NH_4^+ and EOC were analyzed by the same methods described previously for water. A sorbed C:N molar ratio was used to describe the relative sediment availability of C (EOC) and N (extractable NO_3^- + NH_4^+, Starry et al. 2005).

Nitrification and denitrification rates assays

Biogeochemical processes were measured by incubating sediments in the laboratory with stream water. Therefore, nitrification and denitrification assays were conducted at ambient stream water NH_4^+, NO_3^- or organic C levels at the time of sediment collection. In those cases in which IR became dry (i.e., dry periods), lab assays were run using stream water from PR to simulate rewetting conditions.

Sediment nitrification assays were carried out following the nitrapyrin method (Strauss and Lamberti 2000). Four subsamples were run per sediment sample. Each subsample consisted of two flasks containing 25 mL of sediment and 100 mL of unfiltered stream water. Nitrification was inhibited in one flask by adding 10 mg L^{-1} (final concentration) of nitrapyrin previously dissolved in dimethyl sulphoxide (DMSO). The other flask received only DMSO as a control. Flasks were loosely covered with aluminum foil and were incubated at room temperature in the dark for 12 h on an orbital shaker. After completing the incubations, 100 mL of 2M KCl were added to the flasks for NH_4^+ extraction by shaking sediments for 1 h on a rotary shaker. NH_4^+ concentrations were determined as described in the water analyses. Standards were made by using a matrix of DMSO, nitrapyrin, KCl and deionized water in appropriate proportions to address the matrix effects on NH_4^+ concentrations. The gross nitrification rates (μg N g^{-1} DM h^{-1}) were calculated by subtracting the NH_4^+ concentration (µg L^{-1}) in the control flask from the NH_4^+ concentration in the nitrapyrin-inhibited flask, and by adjusting for sediment mass and incubation time.

Sediment denitrification rates were determined by the chloramphenicol-amended acetylene block method (Royer et al. 2004). Three subsamples per sediment sample were run in the laboratory for the denitrification assay. Approximately 125 mL of sediment were placed into 250 mL media bottles with a septum lid. Each bottle was filled with 75 mL of unfiltered stream water, and chloramphenicol at a final concentration of 5 mM was added to suppress the *de novo* enzyme synthesis. Bottles were purged with argon for 10 min to create anoxic conditions. Approximately 10% of the headspace volume in each bottle was replaced by adding 15 mL of pure C_2H_2 using gas-tight syringes. Bottles were then shaken, vented and incubated at lab temperature. The headspace gas in each bottle was sampled at 0.5, 2.5 and 5.0 h using gas-tight syringes and 5 mL Vacutainers® (Becton-Dickinson, Franklin Lakes, NJ, USA). The N_2O concentration was measured with a gas chromatograph (Agilent C890N; Agilent Technologies, Silicon Valley, CA, USA) equipped with a Porapack Q column and an electron-capture detector. Denitrification rates were determined by regressing the N_2O concentration in the bottles against time after correcting for N_2O diffusion in water with the appropriate Bunsen coefficient. Denitrification rates expressed as N_2O-N produced per g of dry sediment mass and incubation time (µg N_2O-N g^{-1}DM h^{-1}) were used. Previous assays determined that 5 mM was the lowest chloramphenicol concentration at which N_2O production was linear over a 5-hour incubation period. Denitrification rates were calculated using data from the linear phase, thus they were appropriate to establish comparisons.

Data analysis

In our study, sediment chemical characteristics and processes were repeatedly measured over time from the same experimental unit or reach with 3 replicate samples per reach and date. Prior to statistical analysis, sampling dates were grouped into a dry period (dry-09 and dry-10) and wet period (wet-10) (Fig. 2.2b). To examine the effects of period (dry vs. wet) and reach (PR vs. IR) on response measures, we used a mixed-model repeated-measures analysis. In our models, reach and period were included as fixed-effect factors, and sediment sample and sampling date as random-effect factors to control their intrinsic source of variation on fixed-effects factors. Given our initial predictions, we focused on period x reach interactions effects in the mixed model results, rather than examining single factor effects. When the period x reach interaction was significant, each factor was analyzed independently. For that, a set of mixed model analyses for each response measure were run fixing each factor to test i) if differences between PR and IR appeared only in the dry period and ii) if differences between the wet and dry periods occurred only in IR. In both cases, the error rate at p=0.05 was controlled for each set of comparisons by Bonferroni correction (p= 0.025 for individual comparisons). The differences between reaches in water characteristics during the wet period were examined by t-tests.

To investigate the temporal variation of sediment chemical characteristics and N transformation rates throughout the study period, we used the coefficient of variation calculated among the sampling dates in each reach (CV_t, in %). A variance-ratio test (Zar 2010) was done to determine if variation in sediment characteristics and N processing rates statistically differed between reaches.

We used Generalized Linear Models to analyze which environmental characteristics (water and sediment characteristics) contributed to explain the variation in nitrification and denitrification rates during the lab assays. The interaction between environmental drivers and reach and period was also assessed. We used the Akaike Information Criteria corrected for small sample size (AICc; Burnham and Anderson 1998) to select from among numerous models those few that best fit the data. We then calculated the AICc difference (ΔAICc) for each model, which is the difference in the AICc between a candidate model and the model with the lowest AICc (i.e., the best model). We considered that models with a ΔAICc less than 2 were equally likely in their abilities to describe the data (Burnham and Anderson 1998). For each candidate model, r^2 was also calculated. .

The normality of the data was tested by a Kolmogorov-Smirnov test and were log- (x) or log- (x+1)-transformed whenever necessary. Except when we used the Bonferroni correction, results were significant if $p < 0.05$ and marginally significant if $0.05 < p < 0.1$. SPSS software package (vs. 15.0, Chicago, IL, USA) was used for the statistical analyses.

Results

Stream water characteristics variation

During the wet period, when reaches were connected, most of the water physico-chemical characteristics were similar between PR and IR. Reaches had similar average water temperature and conductivity values (t-test, $p > 0.05$) (Fig. 2.3), whereas the average water DO concentrations were significantly higher in PR than in IR (t-test, t= 3.16, df= 10, $p = 0.01$) (Fig. 2.3). Water DOC and NH_4^+

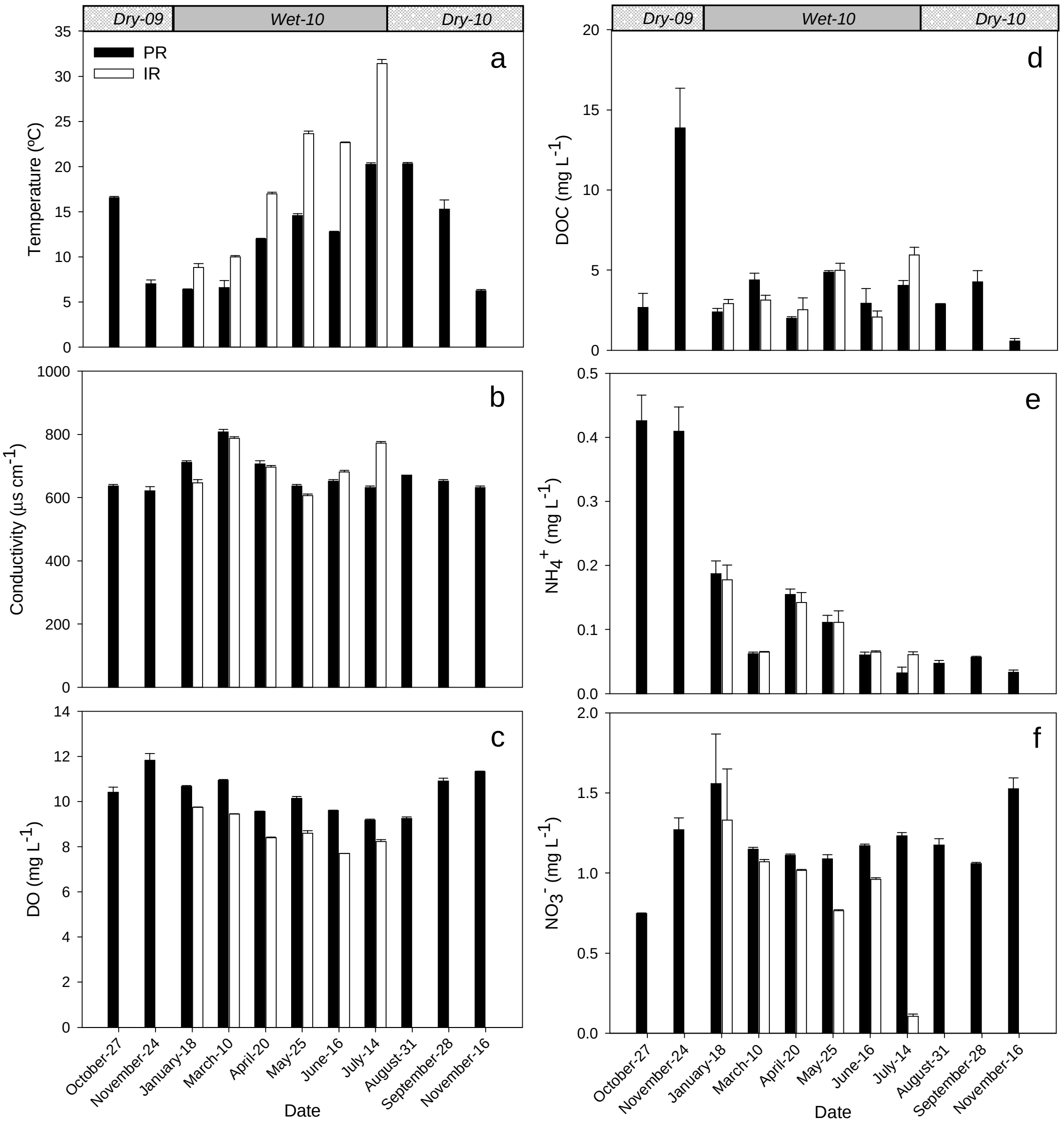

Figure 2.3. Mean (+SE, n=3) values per date of surface water characteristics in the perennial (PR) and intermittent reach (IR) during the study period. During the dry periods, IR was dry and no surface water was present. DO: dissolved oxygen; DOC: dissolved organic carbon.

concentrations in PR averaged 3.43 mg L^{-1} and 0.10 mg L^{-1}, respectively, and were similar to IR (3.60 and 0.10 mg L^{-1}, respectively) (t-test, $p > 0.05$). Marginally significant differences were found for NO_3^-, which was lower in IR (0.87 mg L^{-1}) relative to PR (1.22 mg L^{-1}) (t-test, t= 1.85, df= 10, p= 0.09), especially in August when the concentration in IR was 10-fold lower than in PR (0.11 and 1.23 mg L^{-1}, respectively, Fig. 2.3c).

The CV_t of water temperature and DO in both reaches was similar (Table 2.1). Regarding water DOC and NH_4^+ concentrations, the CV_t in PR doubled that in IR (2.0 and 2.2-fold, respectively), but the differences were not statistically significant. For water NO_3^- concentrations, IR had a significantly higher CV_t (2.6-fold) than PR (Table 2.1).

Table 2.1. Coefficients of temporal variation (CV_t) (x 100) and variance ratio s^2 of the water and sediment characteristics in the perennial (PR) and intermittent reach (IR). DOC: dissolved organic carbon; DO: dissolved oxygen; GW: gravimetric water; OM: organic matter; EOC: extractable organic carbon; F: statistic test used for comparison.
* = $P< 0.1$, ** = $P<0.05$. n= number of dates

Characteristics	CV_t (PR)	CV_t (IR)	Comparison: PR vs.IR
Water	n=11	n=6	s^2 (F)
NO_3^- (mg L^{-1})	18	47	22.47**
NH_4^+ (mg L^{-1})	101	45	4.52*
DOC (mg L^{-1})	85	42	3.61
Temperature (ºC)	42	46	1.16
Conductivity (µs cm^{-1})	7	9	1.67
DO (mg L^{-1})	8	8	1.07
Sediment	n=11	n=11	
GW (%)	19	57	22.61**
OM (%)	32	29	1.00
NO_3^- (µg N g^{-1} DM)	67	127	5.10**
NH_4^+ (µg N g^{-1} DM)	74	72	1.41
EOC (µg C g^{-1} DM)	67	53	1.26
C:N molar ratio	52	125	3.44**
Nitrification (µg N g^{-1} DM h^{-1})	113	164	1.81
Denitrification (µg N_2O-N g^{-1} DM h^{-1})	53	97	1.10

Sediment chemical characteristics variation

When performing the mixed models, the period x reach interaction was significant only for sediment GW%, sediment NO_3^- concentration and sediment C:N molar ratio (Table 2.2). Between periods, sediment GW% only differed significantly in IR (F=41.66, df (n,d)=1, 29, $p< 0.001$), where GW% decreased on average to 9% during the dry period as compared to the wet period (27%, Fig. 2.4a). Conversely, GW% did not vary in PR during the study period (F=0.001, df (n,d)=1, 29, p=0.977). IR had significantly lower GW% than PR, but only during the dry period (F=66.63, df (n,d)=1, 4, p=0.001) ,whereas reaches had similar GW% during the wet period (F=4.72, df (n,d)=1, 4, p=0.259) (Fig. 2.4a). Sediment NO_3^-

concentration in IR was significantly higher during the dry period than during the wet period (F=36.35, df (n,d)=1, 29, *p*< 0.0001), whereas sediment NO_3^- did not change between periods in PR (F=2.89, df (n,d)=1, 43.18, *p*=0.096) (Fig. 2.4d). The differences noted in sediment NO_3^- concentration between reaches occurred only during the dry period (F=247.65, df (n,d)= 1, 4, *p*<0.0001), with no significant changes detected during the wet period (F=0.13, df (n,d)=1, 3.95, *p*=0.738). The sediment C:N molar ratio only varied significantly between periods in IR (F=6.77, df (n,d)=1, 29, *p*=0.014) compared with PR (F=0.00, df (n,d)=1, 28.13, *p*=0.974) (Fig. 2.4f). The C:N molar ratio differed between reaches during the dry period (F=20.42, df (n,d)= 1, 4, *p*=0.011), when IR had on average a lower ratio. Conversely, reaches had a similar C:N molar ratio during the wet period (F=3.94, df (n,d)= 1, 5.87, *p*=0.095) (Fig. 2.4f).

Table 2.2. Mixed model results for each dependent variable. Reach (IR and PR) and period (dry and wet) are the fixed-effect factors. NS: not significant (*p*>0.05), df: degree freedom, n: numerator, d: denominator. Subscript *s*: sediment. See Table 1 for the variables codes and units.

Dependent variable	Factor	df (n,d)	F	*p*
GW	Period	1, 58	13.89	<0.001
	Reach	1, 4.02	15.47	0.017
	Period x Reach	1, 58	13.54	<0.001
$NO_3^-{}_s$	Period	1, 11.96	48.86	<0.001
	Reach	1, 10.94	14.94	0.003
	Period x Reach	1, 11.96	24.48	<0.001
$NH_4^+{}_s$	Period	1, 26.61	1.42	NS
	Reach	1, 12.27	6.65	0.024
	Period x Reach	1, 26.61	0.34	NS
OM	Period	1, 12.04	0.19	NS
	Reach	1, 13.01	0.20	NS
	Period x Reach	1, 12.04	1.58	NS
EOC	Period	1, 7.98	57.78	<0.001
	Reach	1, 8.05	0.70	NS
	Period x Reach	1, 7.98	0.71	NS
C:N molar ratio	Period	1, 9.31	8.30	<0.001
	Reach	1, 4.32	4.59	NS
	Period x Reach	1, 9.31	19.13	0.002
Denitrification rate	Period	1, 22.51	0.12	NS
	Reach	1, 14.1	0.52	NS
	Period x Reach	1, 22.51	0.19	NS
Nitrification rate	Period	1, 58	17.55	<0.001
	Reach	1, 4.20	10.80	0.028
	Period x Reach	1, 58	1.56	NS

The mixed model results also indicated significant differences between periods and reaches for sediment EOC and NH_4^+ concentrations (Table 2.2 and Fig. 2.4b,e, respectively). No differences in sediment OM% were observed between periods and reaches (Table 2.2 and Fig. 2.4c). CV_t was significantly greater in IR than PR for GW%, sediment NO_3^- concentration and the C:N molar ratio (Table 2.1). However, CV_t of sediment OM%, NH_4^+ and EOC did not differ between reaches (Table 2.1).

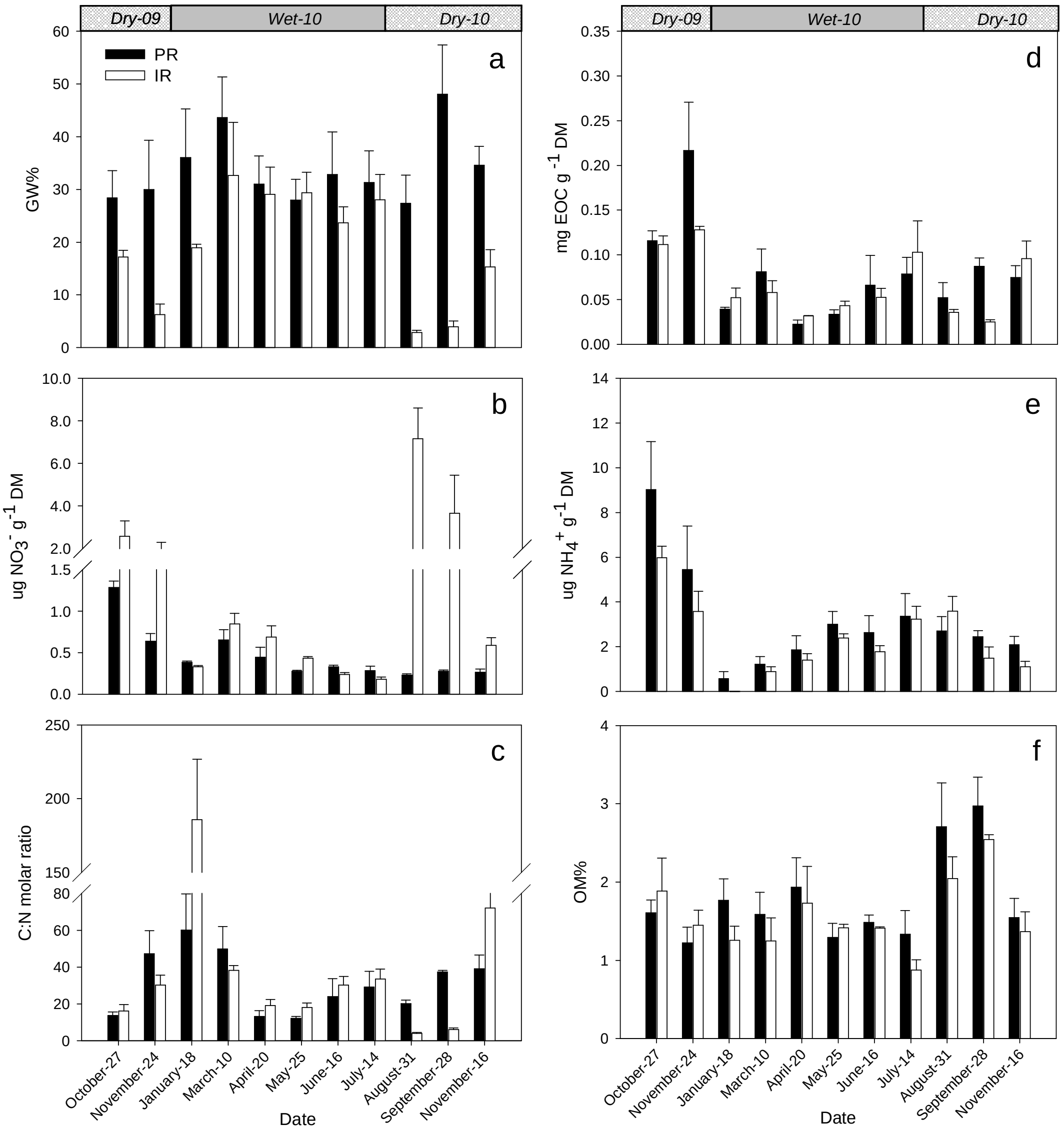

Figure 2.4. Mean (+SE, n=3) values per date of sediment characteristics in the perennial (PR) and intermittent reach (IR) during the study period. GW: gravimetric water, OM: organic matter, EOC: extractable organic carbon.

N-processing rates and controlling factors

In both reaches and time periods, nitrification rates were higher than denitrification rates, showing 6- and 3-fold higher average values in PR and IR, respectively (Fig. 2.5a). For nitrification, the mixed model detected significant effects of reach and period but no interactive effects were found (Table 2.2). IR showed generally lower average nitrification rates than PR in both the wet period and after sediment rewetting. During the dry period, sediments from PR and rewet sediments from IR showed higher nitrification rates than during the wet period (Fig 2.5a). There were no significant differences between periods and reaches in denitrification rates (Table 2.2). However, a peak value was noted in IR during the dry-10 period in September, when the denitrification rate in IR sediments after rewetting was 3.3-fold higher than in PR (Fig. 2.5b). In IR, N-processing rates after rewetting of dry sediments in 2010 did not exhibit any pattern regarding the duration of the sediment desiccation period (Fig. 2.5a and 2.5b). That is, rates analyzed after 4 months of sediment desiccation did not seem to differ from those analyzed during the first month of sediment desiccation.

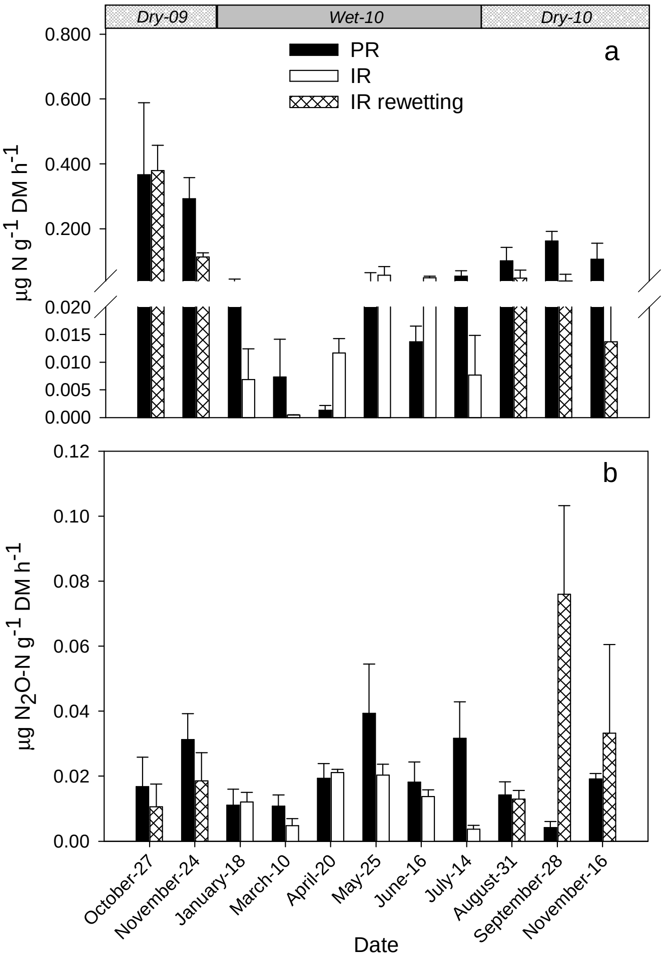

Figure 2.5. Mean (+SE, n=3) values per date of nitrification (a) and denitrification rates (b) in the perennial (PR) and intermittent reach (IR) during the study period. DM: dry mass. During the dry period, dry sediments from IR were incubated using stream water from PR, thereby simulating rewetting conditions (indicated by striped bars).

The CV$_t$ for both nitrification and denitrification rates were slightly higher in IR than in PR (1.8- and 1.4-fold, respectively); however, the differences between reaches were not statistically significant (Table 2.1).

Table 2.3. Candidate regression models relating the variability of N processing rates to water and sediment characteristics. Each significant model represents a best fit based on the smallest ΔAICc. The single models are ranked from best to least good. Models with a ΔAICc value < 2 were considered equally likely. Best single models were selected to build the interaction with factors. "+" indicates positive effect, "-" indicates negative effect, "x" indicates interaction. The *p*-value and the coefficient of explained variation (r^2) by each model are also shown. Subscript *s*: sediment; subscript *w*: water; P: period factor; R: reach factor. Cond.: conductivity. Except for water characteristics, OM, GW and EOC, all the variables were log-transformed. n= 66 for sediment variables, n=22 for water variables. See Table 2.1 for the variables codes and units.

Response variable	Candidate model	AICc	ΔAICc	r^2	*p*-value
Nitrification rate	+ NH_4^+*s*	161.66	0.00	0.25	<0.0001
	+ NH_4^+*w*	177.19	15.53	0.18	<0.0001
	+ EOC	183.19	21.53	0.11	<0.001
	+ DOC	185.81	24.15	0.07	0.02
	+ OM	187.15	25.49	0.05	0.05
	- C:N ratio	188.07	26.41	0.01	0.33
	- Cond.	189.16	27.50	0.02	0.20
	+ GW	190.78	29.12	0.00	0.98
P effect	+ NH_4^+*s* + (NH_4^+*s* x P)	156.99		0.34	<0.0001
R x P effect	+ NH_4^+*s* + (NH_4^+*s* x R x P)	161.80		0.32	<0.0001
R effect	+ NH_4^+*s* + (NH_4^+*s* x R)	163.94		0.25	<0.0001
Denitrification rate	- C:N ratio	92.48	0.00	0.06	0.04
	+ NO_3^+*w*	93.18	0.70	0.05	0.03
	- Cond.	94.28	1.80	0.04	0.07
	+ DOC	96.67	4.19	0.01	0.41
	+ NO_3^+*s*	96.71	4.23	0.01	0.43
	- GW	96.90	4.42	0.01	0.51
	- OM	97.33	4.85	0.00	0.95
	- EOC	97.34	4.86	0.00	0.97
P effect	- C:N ratio + (C:N x P)	94.61		0.06	0.14
R x P effect	- C:N ratio + (C:N x R x P)	97.75		0.08	0.23
R effect	- C:N ratio + (C:N x R)	94.57		0.06	0.13
P effect	+ NO_3^+*w* + (NO_3^+*w* x P)	95.40		0.06	0.12
R x P effect	+ NO_3^+*w* + (NO_3^+*w* x R x P)	98.83		0.08	0.24
R effect	+ NO_3^+*w* + (NO_3^+*w* x R)	95.35		0.06	0.12
P effect	- Cond. + (Cond. x P)	96.20		0.05	0.18
R x P effect	- Cond. + (Cond. x R x P)	97.98		0.09	0.17
R effect	- Cond. + (Cond. x R)	96.53		0.04	0.21

According to AICc analysis, the top ranked-model to explain variation in nitrification included extractable NH_4^+ concentration (25% of the variance; Table 2.3), which had a positive effect on this transformation. After selecting this single model, the joint inclusion of the factors reach and period significantly improved fit, as shown by the AICc value, which lowered from 161.66 to 156.99 (Table 2.3). In this case, the influence of sediment NH_4^+ on nitrification was seen in both reaches, but only during the dry period (log nitrification = +1.56 (log NH_4^+s) - 1.95, r^2= 0.53, n=30) with no significant influence found during the wet period.

In the case of denitrification, the best AICc model included C:N molar ratio (Table 2.3). Amongst the others candidates, single models that included water NO_3^- concentration and conductivity had a ΔAICc value less than 2. However, the importance of these three variables to explain denitrification rates variation was negligible, as shown by the low explained variance (r^2 only up to 6%, Table 2.3). In any case, the inclusion of the factors period and reach did not improve neither the AICc nor r^2 values compared with the respective single models (Table 2.3).

Discussion

Effect of drying on sediment characteristics

As hypothesized, stream sediment drying led to an increase in sediment NO_3^- concentrations. However, no significant effect on the other sediment chemical characteristics was detected. As expected, differences between reaches in sediment NO_3^- concentrations appeared only during the dry period, resulting in a lower C:N ratio in IR than PR. As a result of drying effects, temporal variability in NO_3^- content and C:N ratio was higher in IR than in PR.

Such an increase in sediment NO_3^- concentrations during stream fragmentation was not surprising given that oxygenated dry riverbeds favor N-mineralization from organic matter, and subsequently nitrification (Gómez et al. 2012), even under low gravimetric water sediment conditions (Giambiagi et al. 1993). Conversely, denitrification is absent under dry-oxic conditions (Baldwin and Mitchell 2000; Gómez et al. 2012), leading to an accumulation of NO_3^- within dry stream beds (Steward et al. 2012), as observed in our study. Increased nitrification and an N-enriched upper soil layer flush after a low biological demand period (flushing hypothesis; Poor and McDonnell 2007) have been conventionally linked to increased surface water NO_3^- concentrations after flood events (Obermann et al. 2007; Skoulikidis and Amaxidis 2009). Although N processing rates were not directly estimated in dry sediments, our results suggest that the elevated NO_3^- observed in IR sediments might result from continuous nitrification throughout the dry period when the processes that remove NO_3^- from sediments are presumably depressed.

The fact that no differences were found in sediment NH_4^+, OM% and EOC between reaches and periods might result from the final balance between mechanisms acting as sinks and sources, both of which can co-exist in dry beds. Several factors may counteract the potential loss of sediment NH_4^+ via nitrification when no water is present, such as atmospheric deposition (Belnap et al. 2005) or organic matter mineralization (Mitchell and Baldwin 1999). Likewise, the input of organic matter and dissolved organic carbon due to slight rainfalls (Vázquez et al. 2007) and plant litter into dry stream channels (Acuña et al. 2007) might balance organic C loss by mineralization. Shifts in sediment water levels may cause microbes to undergo osmotic shock, possibly inducing cell lysis and a release of intracellular solutes (Halverson et al. 2000), which could contribute to both EOC and NH_4^+ sediment stocks (Xiang et al. 2008; Austin and Strauss 2011).

Recovery and controlling factors of N processing rates after rewetting

It has been reported that sediment drying and rewetting represent common physiological stresses for microorganisms (Halverson et al. 2000; Fierer et al. 2003).

The widespread opinion is that water scarcity in sediments impacts microbial-processing rates and reduces their recovery after rewetting (Rees et al. 2006; Amalfitano et al. 2008; Austin and Strauss 2011). However, our results showed a quick recovery of N processing rates to pre-dry levels, or even higher, when dry sediments were rewetted. In our study, the rapid recovery was observed within 5 h to 12 h (the incubation time for denitrification and nitrification, respectively), which fall within the response ranges reported in desert soils necessary to trigger biogeochemical reactions (Austin et al. 2004; Schwinning and Sala 2004). However, our results contrast with most studies examining the recovery of nitrification and denitrification rates after rewetting, which reported a variable lag (~30 days or more) between rewetting and rates returning to pre-dry levels (Zaman and Chang 2004; Austin and Strauss 2011). In addition, the negative effect of duration of the sediment desiccation period on N processing rates recovery after rewetting was not observed in our study because rewet sediments responded similarly anywhere after 1 to 4 months of desiccation. In this sense, we must keep in mind the potential effect that small water pulses (summer rainfalls) or even dew formations can exert on the microbial community in the study stream during dry periods. Such effects are likely non-existent when sediment drying is simulated in experimental studies under controlled conditions (Austin and Strauss 2011).

Our findings support the notion that microorganisms exhibit physiological and morphological adjustments, which allow them to rapidly react to changes in water availability (Mamilov and Dilly 2002; Schimel et al. 2007) and suggests a rapid acclimation to new nutrient status conditions (Kemp and Dodds 2002; Roley et al. 2012). The presence of microbial extracellular enzymes preserved during drought has been considered of great importance for the recovery of microbial communities and for re-establishing enzyme functions after sediment rewetting (Marxsen et al. 2010; Pohlon et al. 2013). Both mechanisms (microbial osmo-regulation and persistence of extracellular enzymes) could contribute to rapid N processing activation observed in IR dry sediments following rewetting, and could explain the lack of drought influence. Under dry conditions, the hyporheic zone may be the most favorable compartment for biogeochemical processes within streams (Dahm et al. 2003). Nonetheless, given the limited hyporheic zone in our stream, it is suspected that the implication of this compartment for N processing is negligible.

This study agrees with the idea that microbes frequently exposed to water stress, such as in arid, semiarid and Mediterranean regions, are better adapted to drought conditions than those residing in soils and sediments that rarely undergo moisture content fluctuations such as in temperate ecosystems (Fierer and Schimel 2002; Fierer et al. 2003). This suggestion contrasts with earlier research done in temporary, but saline streams, which detected impaired post-drought denitrification rates (Arce et al. 2013). These conflictive results suggest that other stressors, like water salinity, could influence biogeochemical responses to flow intermittency.

During our study, the environmental characteristics controlling N processing rates throughout the study period were similar in PR and IR, despite their different hydrological regimes. As previous studies have reported (e.g., Triska et al. 1990), sediment NH_4^+ concentration was the best predictor of nitrification, and it had a positive effect on this process in both reaches. The effect of sediment NH_4^+

availability on nitrification was considerable in both reaches, but only during the dry period when discharge was low in PR and dry sediments from IR were rewetted. This result is likely due to a stable sediment NH_4^+ concentration during the wet period compared with the dry period. In aquatic sediments, pH and organic C have been reported to strongly control nitrification rates (Butturini et al. 2000; Strauss et al. 2002). Throughout our study, variation in pH was minimal (pH 8.5-8.9; data not shown) and no influence on nitrification rates was expected. No effect of water DOC and sediment EOC concentration on this process was observed. Nonetheless, this possible effect cannot be ruled out as we have no information on organic C quality, which has been suggested to modulate nitrification variability (Butturini et al. 2000).

Unlike nitrification, no environmental feature between the potential candidates was considered relevant to explain denitrification variation. It is known that denitrification rates can be strongly modulated by NO_3^- availability (Herrman et al. 2008) and organic C quantity and quality (Arango et al. 2007). However, the lack of influence of both factors detected in our study possibly arises from the relatively high NO_3^- and organic C background in the surface water and sediments of the study stream. The qualitative characteristics of dissolved organic matter in Mediterranean streams are highly sensitive to hydrological changes (Fazi et al. 2013). As inferred with nitrification, variables related with water or sediment organic C quality features could be substantial predictors of the variability in denitrification rates in streams subjected to dry and wet periods.

Stream management implications

Although spatially restricted to a Mediterranean temporary stream, our results suggest that dry stream reaches may act as potential NO_3^- sources in intermittent fluvial networks after brief rewetting and flow recovery pulses. This finding can have serious repercussions for stream water quality as a main proportion of the stream channel in a temporary watershed can occasionally dry (e.g., 43.2% of the Rambla de la Rogativa total length dried out in September 2009; González G., personal communication). Although estimates of the amount of NO_3^- storage in sediments are merely potential and are subjected to uncertainty (spatial heterogeneity, sediment bulk density variability), it was calculated from the average sediment NO_3^- concentration during the dry period that 1 km of dry reach can store 137 g (± 14, SD) of NO_3^- as compared with 24 g (± 6, SD) of a wet reach. From this estimation, it can be interpreted that in Rambla de la Rogativa, a dry reach could potentially release ~ 6 times more NO_3^- into the water column after rewetting than a perennial reach of the same size. Given the European Water Framework Directive (WFD) requirements (EU 2000) to assess ecological status of freshwaters, after flow recovery, these N pulses should be considered when designing management plans in temporary watersheds. Indeed, initial flood events following dry periods have proven to be more important for dissolved and particulate nutrient loads than individual months with high runoffs and considerable sediment transport in Mediterranean temporary streams (Skoulikidis and Amaxidis 2009).

When the reference threshold equivalents to a high-good class boundary for NO_3^- are established for temporary streams, this “natural” increase in NO_3^- concentrations might be confused with anthropogenic perturbation and may lead to underestimates of ecological quality of a particular temporary stream. Defining the exact location of the sampling site is essential when analyzing the physico-chemical conditions to assess ecological status (Sánchez-Montoya et al. 2012).

Water legislation that considers all streams similarly does not seem appropriate for temporary streams (Dallas 2013). A review of WFD criteria to define good ecological status (EU 2000) for temporary streams is necessary, especially if we consider the future scenario of water scarcity conditions, in which the number of temporary streams is expected to increase worldwide. The results from this study and others (e.g., Gómez et al. 2009; von Schiller et al. 2011) also help explain the spatial and temporal variability observed in nutrient availability in streams subjected to intermittent flows.

This study highlights the rapid reactivation of biogeochemical processes, especially denitrification, following rewetting of dry sediments during the early expansion phase, which could potentially dampen the high pulse of NO_3^-. This subject is particularly relevant when drying affects headwater streams, as these ecosystems play an essential role on dampening N excess from terrestrial to downstream ecosystems. The high resilience of these ecosystem processes can be considered as a vital ecosystem service that must be maintained from anthropogenic impacts. In Mediterranean semiarid regions, stream drybeds are usually impacted by rubbish dumping, sand and gravel extraction activities and channel bed impermeabilization since they are considered as unsuitable sites (Gómez et al. 2005). The protection and conservation of conventionally, poorly recognized drybeds should be incorporated into the European water legislation (European Union WFD), since they may constitute valuable elements for maintaining stream surface water quality after flow resumption (Steward et al. 2012).

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References

Acuña V, Muñoz I, Giorgi A, Omella M, Sabater F, Sabater S (2005) Drought and post drought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. J N Am Benthol Soc 24:919-933

Alexander RB, Smith RA, Schwartz GE (2000) Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403:758-761

Amalfitano S, Fazi S, Zoppini A, Caracciolo AB, Grenni P, Puddu A (2008) Responses of benthic bacteria to experimental drying in sediments from Mediterranean temporary rivers. Microbial Ecol 55:270-279

APHA (2002) Standard Methods for the Examination of Water and Wastewater. American Public Health Association: Washington DC

Arango CP, Tank JL, Schaller JL, Royer TV, Bernot MJ, David MB (2007) Benthic organic carbon influences denitrification in streams with high nitrate concentration. Freshw Biol 52:1210-1222

Arce MI, Gómez R, Suárez ML, Vidal-Abarca MR (2013) Denitrification rates and controlling factors in two agriculturally influenced temporary Mediterranean saline streams. Hydrobiologia 700:169-185

Austin BJ, Strauss EA (2011) Nitrification and denitrification response to varying periods of desiccation and inundation in a western Kansas stream. Hydrobiologia 658:183-195

Austin AT, Yahdjian L, Stark JM, Belnap J, Porporato A, Norton U, Ravetta DA, Schaeffer SM (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141:221-235

Baldwin DS, Mitchell AM (2000) The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river-floodplain systems: a synthesis. Regul River 16:457-467

Belnap J, Welter JR, Grimm NB, Barger N, Ludwig JA (2005) Linkages between microbial and hydrologic processes in arid and semiarid watersheds. Ecology 86:298-307

Bennett LT, Adams MA (1999) Indices for characterising spatial variability of soils nitrogen semi-arid grasslands of northwestern Australia. Soil Biol Biochem 31:735–746

Bernal S, von Schiller D, Sabater F, Martí E (2013). Hydrological extremes modulate nutrient dynamics in mediterranean climate streams across different spatial scales. Hydrobiologia 719:21-42

Burnham KP, Anderson DR (1998) Model selection and multimodel inference. Springer: New York

Butturini A, Battin TJ, Sabater F (2000) Nitrification in stream sediments biofilms: the role of ammonium concentration and DOC quality. Water Res 34:629-639

Dahm CN, Baker MA, Moore DI, Thibault JR (2003) Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshw Biol 48:1219-1231

Dallas HF (2013) Ecological status assessment in mediterranean rivers: complexities and challenges in developing tools for assessing ecological status and defining reference conditions. Hydrobiologia 719:483-507

Datry T, Arscott D, Sabater S (2011) Recent perspectives on temporary river ecology. Aquat Sci 73:453-457

European Commission (2000) Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Working Group REFCOND. Guidance document no 10. Rivers and lakes-Typology, reference conditions and classification systems

Fazi S, Vázquez E, Casamayor EO, Amalfitano S, Butturini A (2013) Stream hydrological fragmentation drives bacterioplankton community composition. PLoS One 8(5):e64109

Fierer N, Schimel JP (2002) Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biol Biochem 34:777-787

Fierer N, Schimel JP, Holden PA (2003) Influence of drying-rewetting frequency on soil bacterial community structure. Microb Ecol 45:63-71

Galloway JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, Cosby BJ (2003) The nitrogen cascade. BioScience 53:341-356.

Gasith A, Resh VH (1999) Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Sys 30:51-81

Giambiagi N, Rimolo M, Pirolo T (1993) Influence of drought on the production of mineral nitrogen in a typical argiudol of the Pampas. Soil Biol Biochem 25:101-108

Gómez R, Arce MI, Sánchez JJ, Sánchez-Montoya MM (2012) The effects of drying on sediment nitrogen content in an arid Mediterranean stream: a microcosms study. Hydrobiologia 679:43-59

Gómez R, García V, Vidal-Abarca R, Suárez L (2009) Effect of intermittency on N spatial variability in an arid Mediterranean streams. J N Am Benthol Soc 28:572-583

Gómez R, Hurtado I, Suárez ML, Vidal-Abarca MR (2005) Ramblas in south-east Spain: threatened and valuable ecosystems. Aquatic Conserv Mar Freshw Ecosyst 15:387-402

Halverson LJ, Jones TM, Firestone MK (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci Soc Am J 64:1630-1637

Herrman K, Borchard V, Moore RH (2008) Factors affecting denitrification in agricultural headwater streams in Northeast Ohio, USA. Hydrobiologia 598:305-314

Kemp M J, Dodds WK (2002) Comparisons of nitrification and denitrification in prairie and agriculturally influenced streams. Ecol Appl 12:998-1009

Larned ST, Datry T, Arscott BD, Tockner K (2010) Emerging concepts in temporary river-ecology. Freshw Biol 55:717-738

Marxsen J, Zoppini A, Wilczek S (2010) Microbial communities in streambed sediments recovering from desiccation. FEMS Microbiol Ecol 71:374-386

Mamilov AS, Dilly OM (2002) Soil microbial eco-physiology as affected by short-term variations in environmental conditions. Soil Biol Biochem 34:1283-1290

McIntyre R, Adams MA, Grierson PF (2009) Nitrogen mineralization potential in rewetted soils from a semi-arid stream landscape, north-west Australia. J Arid Environ 73:48-54

Mitchell AM, Baldwin DS (1999) The effects of sediment desiccation in the potential for nitrification, denitrification, and methanogenesis in an Australian reservoir. Hydrobiologia 392:3-11

Mulvaney RL (1996) Methods of soil analysis: chemical methods. Soil Science Society of America: Madison

Obermann M, Froebrich J, Perrin J, Tournoud M (2007) Impact of significant floods on the annual load in an agricultural catchment in the Mediterranean. J Hydrol 334:99-108

Peterson BJ, Wollheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Marti E, Bowden WB, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK, Gregory S, Morrall DD (2001) Control of nitrogen export from watersheds by headwater streams. Science 292:86-90

Pohlon E, Mätzig C, Marxsen J (2013) Desiccation affects bacterial community structure and function in temperate stream sediments. Fundam Appl Limnol 182:123-134

Poor CJ, McDonnell JJ (2007) The effect of land use on stream nitrate dynamics. J Hydrol 332:54-68

Rees GN, Watson GO, Baldwin DS, Mitchell AM (2006) Variability in sediment microbial communities in a semipermanent stream: impact of drought. J N Am Benthol Soc 25:370-378

Roley SS, Tank JL, Williams MA (2012) Hydrologic connectivity increases denitrification in the hyporheic zone and restored floodplains of an agricultural stream. J Geophys Res 117:G00N04

Royer TV, Tank JL, David MB (2004) Transport and fate of nitrate in headwater agricultural streams in Illinois. J Environ Qual 33:1296-1304

Sabater S, Tockner K (2010) Effects of hydrologic alterations on the ecological quality of river ecosystems. In Sabater S, Barceló D (eds) Water Scarcity in the Mediterranean regions. Springer, Berlin Heidelberg, pp 15-39

Sánchez-Montoya MM, Arce MI, Vidal-Abarca MR, Suárez ML, Prat N, Gómez R (2012) Establishing physico-chemical reference conditions in Mediterranean streams according to the European Water Framework Directive. Water Res 46:2257-2269

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394

Schwinning S, Sala OE (2004) Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. Oecologia 141:211-220

Skoulikidis N, Amaxidis Y (2009) Origin and dynamics of dissolved and particulate nutrients in a minimally disturbed Mediterranean river with intermittent flow. J Hydrol 373:218-229

Starry OS, Valett HM, Schreiber ME (2005) Nitrificaction rates in a headwater stream: influences of seasonal variation in C and N supply. J N Am Benthol Soc 24:753-768

Steward AL, von Schiller D, Tockner K, Marshall JC, Bunn SE (2012) When the river runs dry: human and ecological values of dry riverbeds. Front Ecol Environ 10:202-209

Strauss EA, Lamberti GA (2000) Regulation of nitrification in aquatic sediments by organic carbon. Limnol Oceanogr 45:1854-1859

Strauss EA, Mitchell NL, Lamberti GA (2002) Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Can J Fish Aquat Sci 59:554-563

Triska FJ, Duff JH, Avanzino RJ (1990) Influence of exchange of flow between the channel and hyporheic zone on nitrate production in a small mountain stream. Can J Fish Aquat Sci 47:2099-2111

Vázquez E, Romaní AM, Sabater F, Butturini A (2007) Effects of the dry-wet hydrological shift on dissolved organic carbon dynamics and fate across stream-riparian interface in a Mediterranean catchment. Ecosystems 10:239-251

von Schiller D, Acuña V, Graeber D, Martí E, Ribot M, Sabater S, Timoner X, Tockner K (2011) Contraction, fragmentation and expansion dynamics determine nutrient availability in a Mediterranean forest stream. Aquat Sci 73:485-497

Xiang S, Doyle A, Holden PA, Schimel JP (2008) Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biol Biochem 40:2281-2289

Zaman M, Chang S X (2004) Substrate type, temperature, and moisture content affect gross and net N mineralization and nitrification rates in agroforestry systems. Biol Fertil Soils 39:269-279

Zar JH (2010) Biostatistical Analyses. Prentice Hall: Upper Saddle River, New Jersey

Zoppini A, Amalfitano S, Fazi S, Puddu A (2010) Dynamics of a benthic microbial community in a riverine environment subject to hydrological fluctuations (Mulgaria river, Italy). Hydrobiologia 657:37-51

Chapter 3

Nitrogen availability and denitrification rates following short-term inundation of dry sediments in a high-nitrogen Mediterranean temporary stream

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Abstract

Mediterranean climate regions predispose streams to experience extreme drought and stream-dry beds to become completely dry during long periods. Changes in hydrological conditions affect nitrogen (N) biogeochemical processes, which can have important consequences for nitrate (NO_3^-) retention, especially concerning when streams drain agricultural landscapes. During drying, continued microbial nitrogen mineralization and reduced denitrification can lead to NO_3^- accumulation the in stream bed. These dry periods are usually punctuated by water pulses that take form as large pools than can re-inundate the stream-bed for hours to days.

Here, a microcosm study was conducted to evaluate the stream N availability and denitrification rates after a water pulse to 3 months dry sediments and over the course of a subsequent inundation for 14 days.

Flooding of dry sediments induced a rapid stimulation of denitrification rates. Whereas a slight increase of water NO_3^- was found the first 24h of inundation, sediment NO_3^- decreased substantially since the beginning of the experiment, indicating that an important part of NO3 stored in sediments starts to be processed, including via denitrification, rather than being totally release to water column. After 24 h, a considerable reduction of NO_3^- in both compartments was observed until the end of the inundation period. However, a noticeable increase in the ammonium (NH_4^+) concentration was detected in water and sediments, suggesting that another fraction of NO_3^- pool is being reduced to NH_4^+.

We demonstrated that after three months of desiccation, stream functionality, in terms of N processing, can be significantly activated after a short-term inundation period. From a water quality perspective, however, these natural floods are probably not effective at removing inorganic N before stream flow is recovered. We highlight that catchment management plans at local scale are needed to improve the quality of the water before it reaches dry stream-beds.

Introduction

Because natural fluctuations in hydrological regime, most streams draining the Mediterranean region are temporary as they periodically cease to flow (Gasith and Resh 1999). Yet temporary streams are not only restricted to arid and semiarid regions since these ecosystems currently account for likely around 50% in the global river network and they are expanding in response to global change (Datry et al. 2014). Climate change and water abstraction for irrigated agriculture and other human uses are expected to cause more intense and longer periods of no water while at the same time heavy precipitations events are likely to raise (Larned et al. 2010). Thus, the state of knowledge arisen from Mediterranean temporary streams could provide valuable information as a first step to understand future consequences in other temperate and xeric areas (von Schiller et al. 2011; Bernal et al. 2013; Timoner et al. 2012). In fact, several studies, including those carried out in Mediterranean catchments, have long increased our understanding of temporary streams ecology (Datry et al. 2011). However, information about functional aspects, such as, nitrogen (N) processing in streams and its link with hydrology, is still limited (Larned et al. 2010).

In terms of landscape-level N cycling, rivers and streams are considered as potential reactors. Streams can store, transform, and retain N through different in-stream processes (Alexander et al. 2009), dampening the export of exceeded N to downstream ecosystems (Hall et al. 2009). N transformation integrates a set of processes that represent sources (N fixation, organic matter mineralization and nitrification) and sinks (microbial assimilation, plant uptake and denitrification). Among the later processes, denitrification is comparably the most significant since this mechanism represents the only permanent sink of N through the conversion of NO_3^- to gaseous forms of N (i.e. N_2O and N_2). The contribution of denitrification to mitigate the excess of NO_3^- is especially relevant when streams experience high N inputs, as typically occur in agricultural landscapes (Lassaletta et al. 2009). Mediterranean streams are highly susceptible to N inputs effects derived from human pressure because of their natural low discharge and scarce stream biomass (Alvarez-Cobelas et al. 2005; Arce et al. 2013a). This is particularly worrying in the driest areas, such as Southern Spain, where stream water NO_3^- concentrations can reach over 20 mg L^{-1} in down reaches due to the agricultural development (Arce et al. 2013a).

As described for arid and semiarid systems (Schwinning and Sala 2004; Collins et al. 2008; Austin 2011), N transformation and loss rates in Mediterranean streams may be spatially and temporarily discontinuous due to intense drying-wetting cycles and time frame over which biogeochemical processes respond to moisture pulses (Bernal et al. 2013). When a stream reach dries up, sediments are biogeochemically quiescent compared with wet sediments as water loss slows down the microbial activity (Larned et al. 2007; Tzoraki et al. 2007; McIntyre et al. 2009a). Despite the reduced microbial activity, the increasing oxic conditions in the sediments during desiccation process may stimulate mineralization and nitrification, even under modest soil moisture (e.g. Giambiagi et al. 1993), whereas denitrification is substantially reduced (e.g. Gómez et al. 2012). Thus, a continued microbial nitrification during a period of low biological N demand can provide NO_3^- accumulates in stream sediments during extended dry phases (Stewards et al. 2012; Arce et al. 2013b). In addition, microbial mortality during desiccation progress release significant amounts of N (Amalfitano et al. 2008), which can be further stored in sediments as precipitated solutes via evaporation (McLaughlin 2008).

In Mediterranean streams, periods of no flow are usually punctuated by short and sporadic water pulses. As seen in other arid and semiarid streams such pulses result of the arrival of water to stream driven by rainfall-runoff (Stanley et al. 1997) or by slight advancing wetted front traveling down towards to dry stream bed (Corti and Datry 2012). These events rarely generate running waters but water usually remains into scattered pools or other wetted areas distributed along the stream channel for hours or days. In streams subjected to natural drying-wetting cycles, microbial activity tends to recover rapidly after sediment rewetting (Amalfitano et al. 2008; Marxsen et al. 2010; Timoner et al. 2012). Along these lines, denitrification and nitrification processes have been reported to be rapidly re-activated in stream sediments (Arce et al. 2013b) as same as in arid soils, where the recovery can occur in a range of minutes (Austin et al. 2004). The time frame in which denitrification rates return to pre-dry levels, however, seems to be variable (from hours to weeks) and to depend on several factors associated with local characteristics such as length, intensity and natural recurrence of the desiccation events (Cavanaugh et al. 2006; Austin and Strauss 2011). Unlike running water, water inundation leads to saturated soils, which then provide anaerobic conditions and devoid of NO_3^- because of reduced nitrification but can activate ammonification and denitrification (Robertson and Groffman 2007; Cavanaugh et al. 2006).

Besides to trigger biogeochemical activity, rewetting of desiccated stream-bed sediments has been reported to result in a flush of N, phosphorus (P) and carbon (C) (Fierer and Schimel 2002; Baldwin et al. 2005; Butterly et al. 2009). The osmotic shock of microorganisms upon rewetting (Halverson et al. 2000; Williams and Xia 2009), the rapid mineralization of accumulated organic matter in the dry channel (Appel 1998), the desorption and redissolution of salts (Baldwin and Mitchell 2000) or the release of NO_3^- enhanced in streambed sediments through desiccation (Gómez et al. 2012; Arce et al. 2013b), are all processes that could be implicated in the flush of N after a drying-rewetting event. The implications of such N pulse for the surface water quality can be noticeable, especially in agricultural watersheds where the N stored in sediments can be very high. In fact, initial flood events (following dry periods) have been occasionally described to surpass good quality standards in disturbed Mediterranean temporary streams (e.g. Skoulikidis and Amaxidis 2009).

Thus, a fundamental concern is whether such brief inundation periods that take place in intermittent reaches during the dry period, trigger denitrification and thereby, dampen the effect of the potential N flush from sediments and reduce the high water NO_3^- concentration in the water column.

In this study, we conducted a microcosm experiment to simulate sediment inundation in pools by waterfront advance, using sediments from an agriculturally affected and intermittent down reach. Our aims were to evaluate i) the changes in sediment and water column N availability following sediment inundation and ii) the response and time over which sediment denitrification responds to rewetting. Since N processing is substantially linked to organic C availability (e.g. Arango et al. 2007), we also explored how the organic carbon availability varied following sediment inundation.

Based on previous evidences, we expect a substantial NO_3^- release from sediments to the water column after the inundation of dry sediments. We predicted that denitrification would be rapidly activated in the inundated sediments, thus modulating the NO_3^- availability in the water column.

Materials and Methods

Site description

The sediments used in this study were collected from an intermittent reach of Chícamo stream (1º03'08" W; 38º12'44" and 172 m s.a.l). Chícamo stream drains a watershed of 501.8 km^2 and it is located in one of the most arid areas of Southern Spain (the Murcia Region). This area is characterized by a semi-arid Mediterranean climate and presents an average annual temperature and precipitation of ~18ºC and of < 300 mm, respectively. Watershed lithology is dominated by sedimentary marls rich in NaCl and SO_4Ca that provide high conductivity to stream water (~ 20 mS cm^{-1}). Riparian vegetation at the study reach is very scarce and dominated by sporadic halophytic plants. The land use of the draining watershed is a mix of agricultural (43%; irrigated farms and ponds) and forest (52%; grasslands and Mediterranean scrubs) (Arce et al. 2013a). As a result of the dense irrigated agriculture in the watershed and low discharge (2.3 L s^{-1}), the water dissolved organic C (DOC) and nutrient concentrations in the study reach are high during baseline flow conditions (DOC= 1.6 mg L^{-1}; NO_3^-=4.2 mg L^{-1}; ammonium (NH_4^+)= 0.02 mg L^{-1}) (Gómez et al. 2012). Chícamo stream is hyposaline as water conductivity ranges 5 - 30 mS cm^{-1} (Arribas et al. 2009). Main anions and cations contributing to salinity are SO_4^{-2} (73%), Cl^- (14%), Ca^{2+} (3%), K^+ (0.2%) and Na^+ (10%) (Arce et al. 2013a). As other semiarid streams, the presence of submerged macrophytes is generally scarce, and the biofilm covering the streambed accounts for most primary production (Velasco et al. 2003).

The study reach is characterized by hydrologic extremes-droughts. Reach drying usually begins in late June to early July, and normally continues until late October to early November when rainstorms occur and the stream flow is restored. Until the flow is restored, the dry period is usually punctuated by sporadic and short flooding events originating wetted areas and small pools, which can remain along the stream channel from hours to several days.

Field sampling

In July 2009, wet sediments were collected under pre-drought conditions when no surface water flow was present. Sediments were collected along a 50 m-length in 10 sampling points from the top 5 cm as most biological activity is expected to be concentrated in the upper surface sediments in semi-arid systems (Bennet and Adams 1999). Then, sediments were placed randomly in plastic tanks for running the microcosms experiment (explained below). The collected stream sediments were characterized by 64.3 ± 1.7% of gravel (<6 mm) and by a fraction of <2 mm, 72.1 ± 1.3% of sand, 22.8 ± 1.5% of silt and 5.2 ± 0.6% of clay (mean ± SE, n=10) (Gómez et al. 2012).

In early October 2009, rainfalls re-flooded the dry stream-bed of the study reach and ~50 L of stream surface water was collected in a carboy to simulate the re-flooding in the microcosms experiment. Surface water conductivity and pH were measured using Hach handheld probes (Loveland, CO, USA). Three water replicates were filtered through GF/F Whatman (Maidstone, England, UK) glass fiber filters (0.7 µm) for DOC and nutrient determination and they were refrigerated (≤ 24h) until further analyses.

Microcosms set-up

After collecting sediments in field, they were transported to the University of Murcia outdoor premises and they were distributed in 24 tanks (28 cm × 18 cm × 15.5 cm deep). Room temperature and humidity were recorded throughout the whole experimental period. Once in the tanks, sediments had a depth of approximately 5 cm. We kept sediments air drying until total desiccation for 3 months before they were subjected to re-flooding. For that, sediments were slowly inundated by direct stream water application until achieving whole sediment saturation and a water column of 2 cm-high over the sediment surface in all experimental tanks. The water column was maintained constant throughout the experimental period. We established eight sampling events: one sampling to describe pre-inundation conditions in water and sediments (t=0) (field stream water and dry sediments, respectively), and seven samplings after sediment re-flooding at 1h, 2h, 8h, 24h, 48h, 168h (i.e.7d) and 336h (i.e.14d), using three tanks as replicates per sampling event. On each sampling date, tanks were removed from the outdoor premises and were carried to the laboratory for collecting and processing water and sediment samples.

Sampling and laboratory analyses

On each sampling date, overlaying water conductivity, pH and temperature in each tank were measured using Hach handheld probes (Loveland, CO, USA). Variations of water conductivity through the study period let us to control changes in N and C concentrations due to non-biological processes. Water column samples were collected by triplicate per tank and then filtered through GF/F Whatman (Maidstone, England, UK) glass fiber filters (0.7 µm) for DOC and N determinations and refrigerated (≤ 24h) until further analyses.

Denitrification assays

Initially, we measured denitrification rates using a modification of the acetylene block technique (Knowles 1990) as previously done by Gómez et al. (2012). On each sampling date, after finalizing the water column sampling, two septum hermetic bottles (250 ml), cut at the bottom, were introduced to the total depth of the sediments (5 cm) in each tank. To ensure a good acetylene distribution through the sediment column inside the bottles, this was injected into the sediment with a syringe and was distributed at four points. We took samples from the air space of the bottles at 15 min, 2 h and 4 h, and we stored them in evacuated glass tubes. The N_2O concentration was determined in an Agilent C890N gas chromatograph (Silicon Valley, CA, USA), equipped with a Porapack Q column and an electron-capture detector (ECD). The N_2O concentration dissolved in the aqueous phase was corrected with the appropriate Bunsen´s coefficient. We determined denitrification rates by regressing out the linear relationship between the N_2O concentration in the bottles and the incubation time. After removing gas samples for denitrification estimates, sediments from hermetic bottles were removed and dried at 60ºC (24h) to determine sediment bulk density (BD) (g ml^{-1} or g cm^{-3}). BD was further used to calculate denitrification rates and sediment nutrient content (see below) on an areal basis. Denitrification was reported as N_2O-N produced per m^2 h^{-1}. For literature comparison, we reported rates per g of sediment dry mass (DM).

As denitrification rates were not redox-optimized and there was not enrichment of NO3 and DOC, these rates were acceptable to approach *in situ* rates. We calculated the rates for a given date as the average denitrification rates calculated from the three tanks, where the rate of each tank was the average denitrification rates calculated from the two assay bottles per tank.

Sediment analyses

Redox potential (Eh) of saturated sediments was measured at six sampling points in each tank using a Eutech- Eh/pH-meter (Eutech Instruments, Landsmeer, The Netherlands). Redox measures were corrected by adding 200 mV to the field voltage (value of the standard Ag/AgCl reference electrode) (Vepraskas and Faulkner 2001). After collecting the water column and gas samples, the remainder surface water was removed from experimental tanks for allowing sediment sampling. Then, sediments from each tank were homogenized and subsamples were obtained by triplicate for subsequent analyses. Samples were dried at 60ºC (24 h) to determine the percentage gravimetric water content (GW).

The percentage of sediment organic matter (OM) was determined by loss on ignition by burning dry samples at 550ºC for 4h. To estimate the extractable NO_3^- and NH_4^+ sediment concentrations, cold KCl extraction (Mulvaney 1996) was used. Five g of fresh weight of each sample were placed in 50 ml of 1 M KCl and shaken for 1 h on a rotary shaker. Interstitial organic carbon (IDOC) (i.e. water-soluble sediment organic C) was similarly obtained, but using MilliQ deionized water as the extracting agent (sensu Baker et al. 2000). All the N and C extracts were filtered through pre-combusted Whatman GF/F filters (and acidified in the case of IDOC) and stored at 4ºC until analysis within 48 h using the methods described below for the water samples. For the KCl-extracted samples, KCl standards were prepared to address the possible effects of KCl on determining the sediment NO_3^- and NH_4^+ concentrations. Both, N and C content in sediments were standardized per g of dry mass after correcting water percentage. BD values were employed to express sediment N and C concentrations per sediment DM (mg N g DM^{-1}) on an areal basis (mg N m^{-2}). The dissolved inorganic N (DIN) in sediments was calculated as the sum of sediment NO_3^- and NH_4^+.

PH and conductivity were also measured in the sediment extracts. Five g of fresh sediments were placed into 50 ml of MilliQ deionized water and shaken for 1 h on a rotary shaker. Then, pH and conductivity measurements were taken from the supernatant. We calculated all the sediments chemical parameters for a given date as the average from the three tanks, where chemical parameters of each tank were the average calculated from the three sediment subsamples.

Water analyses

Both stream water and the microcosms water column, were analyzed colorimetrically for the NO_3^- and ammonium (NH_4^+) concentrations following the reduction-diazotization method (Wood et al. 1967) and the phenol-hypochlorite method (Solorzano 1969), respectively, in a Systea EasyChem autoanalyzer (Oak Brook, IL, USA). They were also analysed for dissolved organic carbon (DOC) using a Shimadzu TOC-5000A Total Organic C (TOC) analyzer (Columbia, MD, USA). Before the analyses, DOC samples were preserved with acid in the dark at 4ºC (Hunter and Faulkner 2001). As for sediments, water DIN was calculated as the sum of NO_3^- and NH_4^+ concentrations.

Data analyses

Before statistical analyses, water conductivity values were converted to Cl^- concentrations. For that, we employed a relationship previously done between water conductivity and Cl- using surface water from Chícamo to which we added known concentrations of NaCl. Afterwards, Cl^-concentrations were used to calculate NO_3^-: Cl^-, NH_4^+: Cl^- and DOC: Cl^- - ratios. Examining the temporal dynamics of the ratio between a non-conservative- and conservative- solute allowed us to discern between biological and non-biological processes involve in N or C variation. No significant change of ratio over the inundation period is indicative that variation of either nutrients or DOC are long coupled to Cl^- changes, and therefore mediated by non-biological processes. To examine temporal changes in the dominance of each fraction over the inundation period, the ratio of NO_3^-: NH_4^+ in the water column and sediments was calculated.

One-way ANOVA analyses were used to examine general differences over the experimental time. Because the interval in the factor time (i.e. between sampling events) is unequal, we performed regression models between the study variables and time as independent and continue variable to identify whether there was a particular temporal trend in the variation of the study variables over the experimental period. In addition to linear model, we tested quadratic and cubic trends. While a linear pattern represents a straight-line relationship through the group means, a quadratic represents a U-shaped relationship with a single "change of direction", and the cubic represents a more complex pattern with two "changes of direction" (Quinn and Keough 2002).

To explore the relationship between the study variables we used Pearson correlations. Then, we conducted simple regression analyses to examine relationship between variables. In case of finding a different model of linear, we reported the best fit model that described the data distribution.

All the row variables were normally distributed (Kolmogorov-Smirnov test). They were log (x)- or arcsine (√x) (for percentage data)- transformed whenever necessary to improve the heterocedasticity (Levene test) in the variance analyses. Statistical analyses were carried out using SPSS vs. 19.0 (Chicago, IL, USA). The significance of results was considered at $p \leq 0.05$ and results reported as non significant had p values > 0.05. When denitrification rates were below the detection levels, one half of the reporting laboratory value was assigned to carry out the statistical analyses (Helsel 2005). Average values reported in the result section are accompanied by standard error (±SE).

Table 3.1. *P*- values of one-way analyses results for differences throughout the experimental period on sediment characteristics and deitrification rates. Regression coefficients (r^2) and *p*-values for the examined temporal trends. n.s.: non- significant; GW: gravimetric water content; DIN: dissolved inorganic nitrogen; IDOC: interstitial dissolved organic carbon; OM: organic matter. *subscript s*: sediment.

	GW	NO_3^- *s*	NH_4^+ *s*	DIN *s*	IDOC *s*	Conductivity *s*	pH *s*	OM	Redox	Denitrification rate
Time	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.019$	$P < 0.001$	n.s.	$P < 0.001$	n.s.	$P = 0.002$	$P < 0.001$
Temporal trend										
Linear	n.s.	$r^2 = 0.34$ $P = 0.003$	n.s.	n.s.	n.s	n.s.	$r^2 = 0.59$ $P < 0.001$	n.s.	$r^2 = 0.48$ $P = 0.001$	$r^2 = 0.16$ $P = 0.046$
Quadratic	n.s.	$r^2 = 0.71$ $P < 0.001$	$r^2 = 0.76$ $P < 0.001$	$r^2 = 0.46$ $P = 0.001$	n.s	n.s.	$r^2 = 0.79$ $P < 0.001$	n.s.	$r^2 = 0.68$ $P < 0.001$	$r^2 = 0.42$ $P = 0.003$
Cubic	n.s.	$r^2 = 0.78$ $P < 0.001$	$r^2 = 0.77$ $P < 0.001$	$r^2 = 0.46$ $P = 0.005$	n.s	n.s.	$r^2 = 0.85$ $P < 0.001$	$r^2 = 0.41$ $P = 0.012$	$r^2 = 0.72$ $P < 0.001$	$r^2 = 0.70$ $P < 0.001$

Results

Water and sediment chemical characteristics

One-way ANOVA analyses detected significant differences over the experimental period for all study variables except in the case of sediment OM and conductivity and water $NH_4^+:Cl^-$ ratio (Table 3.1 and 3.2). Besides, saved sediment GW and IDOC, significant temporal trends over the experimental period were found for the rest of study variables (Table 3.1 and 3.2).

After three months of drying, sediments had an average GW of 0.42±0.03 % (t=0h) (Fig. 3.1a). Immediately after being inundated (t=1h), the average sediment GW increased significantly up to 12±1 % (Fig. 3.1a). After that, values remained practically constant over the inundation period (18% on average) with no significant temporal changes (Table 3.1).

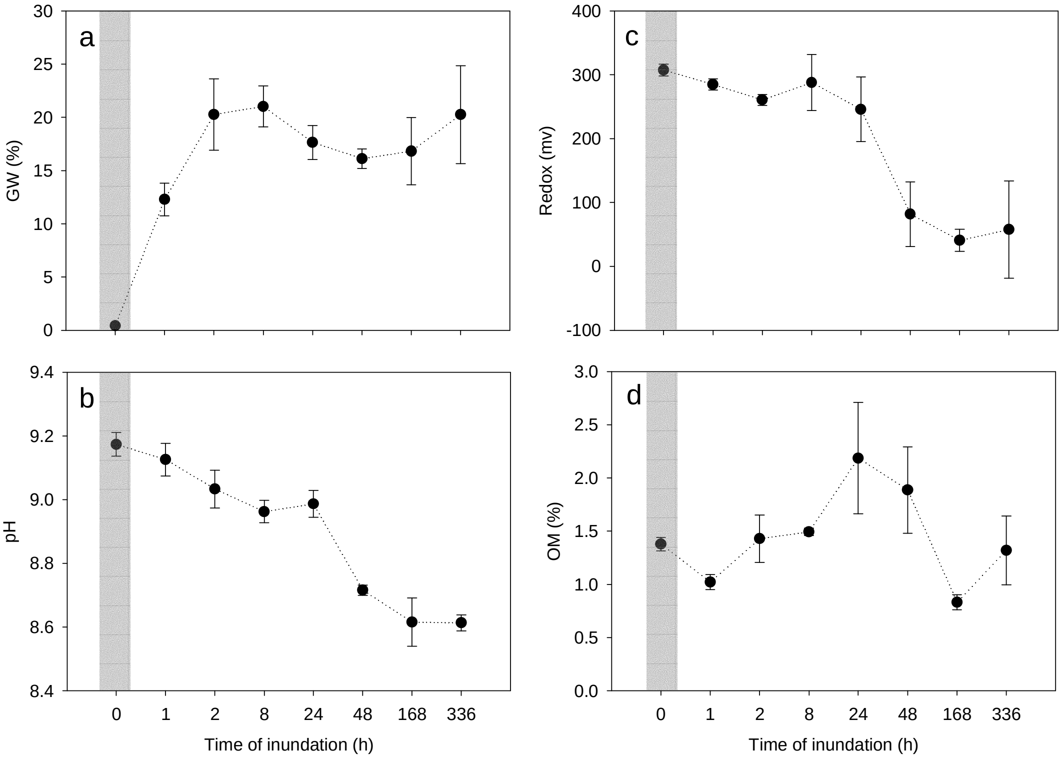

Figure 3.1. Mean (±1SE, n=3) percentage of sediment gravimetric water content (GW) (a), sediment pH (b), sediment redox (c) and percentage of sediment organic matter (OM) (d) for each sampling event over the inundation period Grey bar is shown to indicate initial conditions (t0; pre-inundation). The discontinuous line connecting sampling events has been used for helping in the visualization of temporal variation of data but does not implicate actual trends between events.

Table 3.2. *P*- values of one-way analyses used for differences throughout the experimental period on water characteristics. Regression coefficients (r^2) and *p*-values for the examined temporal trends. n.s. non-significant; Cl: Chloride; DOC: dissolved organic carbon. *subscript w*: water.

	NO$_3^-$ *w*	NH$_4^+$ *w*	DIN *w*	DOC *w*	Cl$^-$ *w*	NO$_3^-$:Cl$^-$ *w*	NH$_4^+$:Cl$^-$ *w*	DOC:Cl$^-$ *w*
Time	$P= 0.002$	$P= 0.001$	$P= 0.002$	$P < 0.001$	$P< 0.001$	$P < 0.001$	n.s	$P =0.015$
Temporal trend								
Linear	$r^2= 0.63$ $P < 0.001$	$r^2= 0.64$ $P < 0.001$	n.s	$r^2= 0.74$ $P < 0.001$	$r^2= 0.88$ $P < 0.001$	$r^2= 0.71$ $P < 0.001$	$r^2= 0.22$ $P =0.030$	n.s.
Quadratic	$r^2= 0.75$ $P < 0.001$	$r^2= 0.70$ $P < 0.001$	n.s	$r^2= 0.75$ $P < 0.001$	$r^2= 0.92$ $P < 0.001$	$r^2= 0.79$ $P < 0.001$	$r^2= 0.34$ $P =0.023$	$r^2= 0.47$ $P = 0.003$
Cubic	$r^2= 0.82$ $P < 0.001$	$r^2= 0.71$ $P < 0.001$	$r^2= 0.62$ $P < 0.001$	$r^2= 0.78$ $P < 0.001$	$r^2= 0.92$ $P < 0.001$	$r^2= 0.88$ $P < 0.001$	$r^2= 0.41$ $P = 0.022$	$r^2= 0.47$ $P = 0.010$

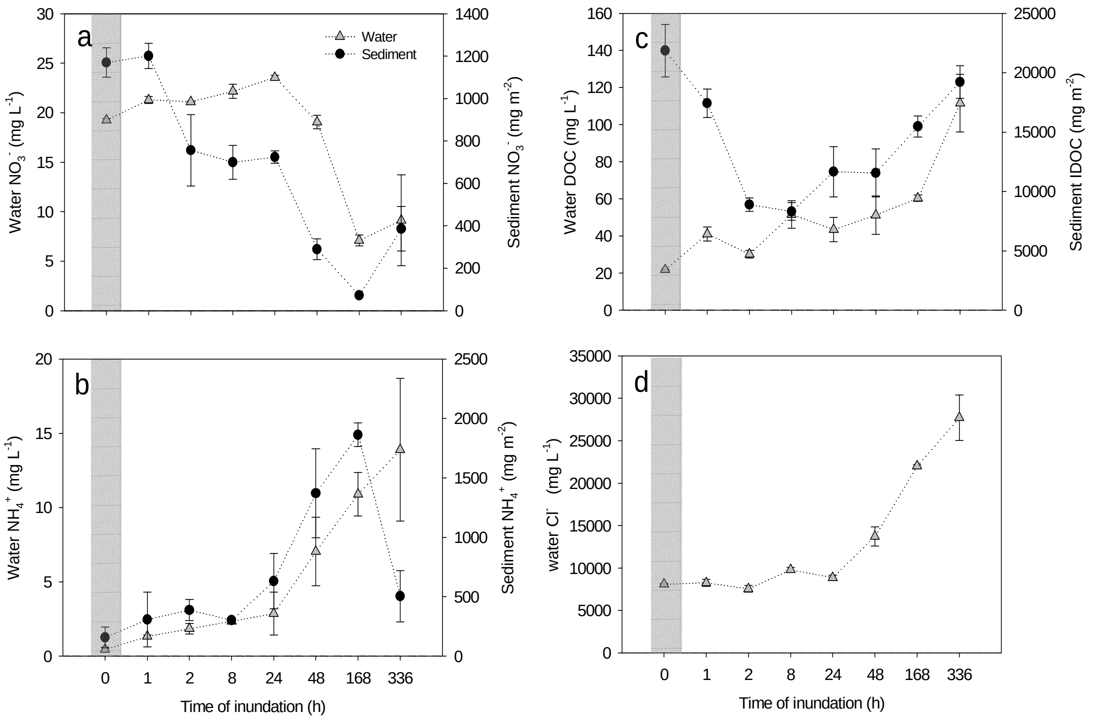

Figure 3.2. Mean (±1SE, n=3) NO_3^- (a), NH_4^+ (b), organic C (c) concentration in water column and sediments, and Cl^- levels in water (d) for each sampling event over the inundation period. Grey bar is shown to indicate initial conditions (t0; pre-inundation). The discontinuous line connecting sampling events has been used for helping in the visualization of temporal variation of data but does not implicate actual trends between events. DOC: dissolved organic carbon; IDOC: interstitial dissolved

The surface water of Chícamo stream used to inundate sediments had high NO_3^- concentration, 19.25±0.17 mg L^{-1} (Fig. 3.2a). When sediments were inundated in the microcosms after 3 months drying, we observed a slight increase in NO_3^- concentrations in the water column, with a maximum average value of 23.6±0.21 mg L^{-1} after 24h inundation (Fig. 3.2a). However, afterwards, a sharply drop was found at 168h, when average water concentration fell down to 7.0±0.5, almost 3 times lower with respect to pre-inundation t=0 (Fig. 3.2a). Unlike water, NO_3^- content in dry sediments experienced a rapid and marked drop after the first 2h of inundation (from 1,170±69 to 756±168 mg NO_3^- m^{-2}) (Fig. 3.2a) and tended to decrease until 168h (72±5 mg NO_3^- m^{-2}). At the end of the inundation period, a slight increase in NO_3^- was observed in both water column and sediment, showing average values of 9.1±4.6 mg L^{-1} and 386±105 mg m^{-2}, 2- and 3- times lower, respectively, with respect to pre-inundation conditions (Fig. 3.2a). In the case of NH_4^+, initial concentrations in water and sediment (t=0; 0.45±0.16 mg L^{-1} and 157±86 mg m^{-2}, respectively) increased immediately after inundating the sediment, with a sharply rise at 48h, 15.6- and 8.7- times higher than the initial conditions (Fig. 3.2b). At the end of the inundation period, NH_4^+ concentration in the water column remained high, reaching a maximum average concentration of 13.8±4.8 mg L^{-1}, 30 times higher than the initial concentration. Conversely, a drop in sediment NH_4^+

content was detected, with an average value of 504±90 mg m^{-2}, still higher than that found at the pre-inundation moment (i.e. t=0) (Fig. 3.2b).

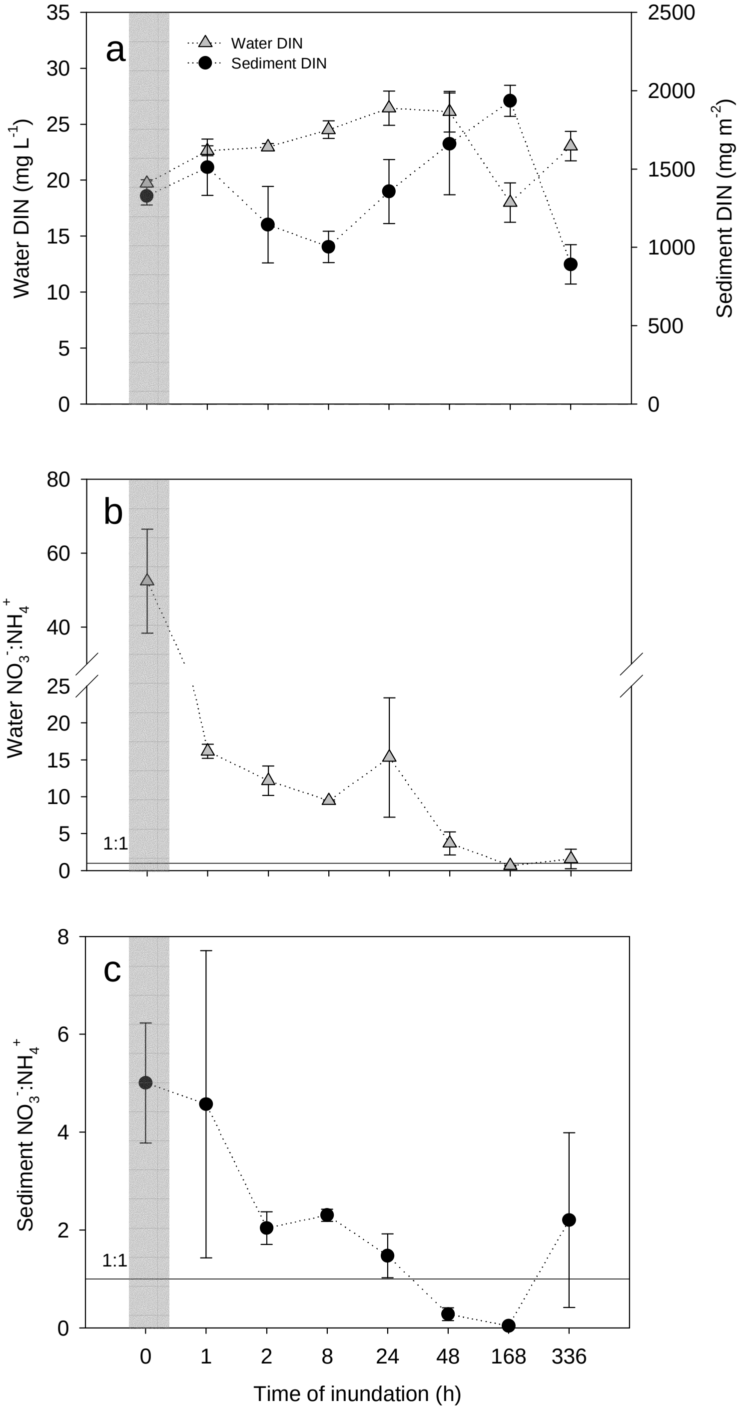

Figure 3.3. Mean (±1SE, n=3) dissolved inorganic N (DIN) in water column and sediments (a) and ratio of NO_3^-: NH_4^+ in water (b) and sediments (c) for each sampling event over the inundation period. Grey bar is shown to indicate initial conditions (t0; pre-inundation).
The discontinuous line connecting sampling events has been used for helping in the visualization of temporal variation of data but does not implicate actual trends between events.
The continuous line is shown for helping in the interpretation of the ratio variation, where ratios > 1:1 reflect dominance of NO_3^- over NH_4^+; ratios < 1:1 reflect dominance of NH_4^+ over NO_3^- and ratios = 1:1 reflect equal dominance.

As a result of the opposite pattern showed by NO_3^- and NH_4^+, DIN levels in water and sediment remained constant at the end of the experimental period (Fig. 3.3a). Before inundation, the dominant fraction of the initial DIN in water and dry sediments was clearly NO_3^- (97% and 88%, respectively) (Fig. 3.3b,c). Such dominance continued until 48h, moment when water NO_3^- and NH_4^+ concentration tended to match until the conclusion of the experiment (40 and 60 %, respectively)

(Fig. 3.3b). In the case of sediment, NH_4^+ was 82% of sediment DIN after 48h of inundation, however, both fractions tended to match at the end of the experiment (NH_4^+=56% and NO_3^-= 44% of sediment DIN) (Fig. 3.3c).

Water DOC concentration in the surface stream water of Chícamo was also high (21.8±0.3 mg L^{-1}). After sediment inundation, DOC concentration in the water column of tanks tended to increase steadily over the inundation period (Fig. 3.2c) achieving finally a concentration of 111.7±15.5 mg L^{-1}. On the contrary, sediment IDOC showed a significant decrease during the first 8h of inundation with respect to dry conditions (from 21,861±2,197 to 8,897±577 mg m^{-2}) (Fig. 3.2c). After that, IDOC increased sharply in sediments until reaching an average value close to that obtained during dry conditions (19,914±1380 mg m^{-2}) (Fig. 3.2c).

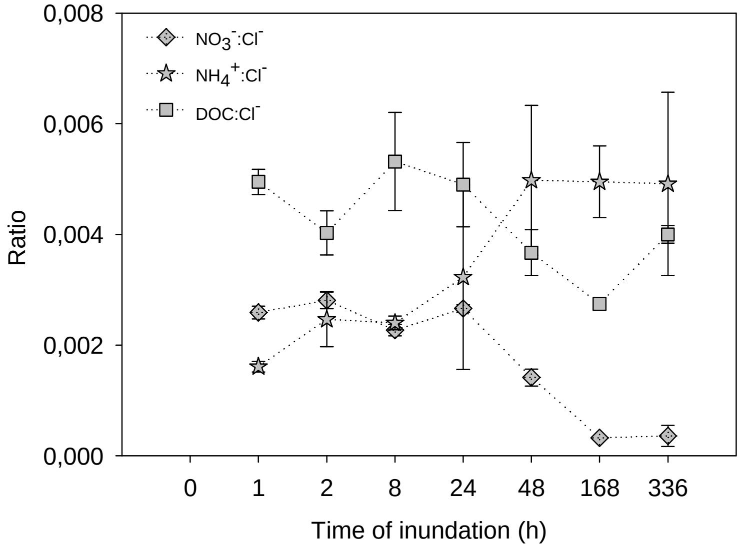

Figure 3.4. Mean (±1SE, n=3) ratio of NO_3^-:Cl^-, NH_4^+:Cl^- and DOC:Cl^- in water column for each sampling event over the inundation period. The discontinuous line connecting sampling events has been used for helping in the visualization of temporal variation of data but does not implicate actual trends between events.

Despite the fact that water volume in tanks was stable over the inundation period, water Cl^- concentration changed substantially (Fig. 3.2d) whereas no significant changes were detected in sediment conductivity (Table 3.1). Consequently, we examined how N:Cl^- and DOC:Cl^- ratios varied over the inundation period (Fig. 3.4). The three ratios; NO_3^-: Cl^-, NH_4^+: Cl^- and DOC:Cl^- followed significant temporal trends, mainly in the case of NO3:Cl^- where a cubic trend explained an important data variability (Table 3.2). However, in the case of NH_4^+: Cl^- ratio, the one-way ANOVA did not detect important changes through experiment (Table 3.2), indicating that part of the variation seen for water NH_4^+ could be attributed to mechanisms involved in Cl^- dynamics.

Unlike conductivity, water column pH did not change over the inundation period, with average values of 8.3±0.0 and 8.2±0.0 at the beginning and conclusion of the experiment, respectively. Conversely, pH in sediments tended to decrease over the whole experiment with a change from 9.17 to 8.6 at the end of the inundation period (Fig.3.1b). Sediment redox conditions (307 mV at t=0h) also showed a substantial and steadily decrease, mainly during the last days of inundation (57 mV at t= 336h) (Fig. 3.1c). Throughout the study period, the percentage of sediment OM (1.37% on average, n=8) did not show significant changes through the study period (Fig. 3.1d).

Table 3.3. Pearson coefficients calculated between studied variables during the study period. Only variables showed any significant correlations are shown. n.s.: non- significant result; IDOC: interstitial dissolved organic carbon; DOC: dissolved organic carbon. subscripts "s "and "w" refer sediment and water, respectively.
*: significant coefficient at $p<0.05$; **: significant coefficient at $p<0.01$. n=21 for water variables; n=24 for sediment variables.

	Denitrification	Redox	pH_s	$IDOC_s$	$NH_4^+{}_s$	$NO_3^-{}_s$	DOC_w	$NH_4^+{}_w$
$NO_3^-{}_w$	n.s.	0.775**	0.745**	-0.574**	-0.568**	0.697**	-0.699**	-0.881**
$NH_4^+{}_w$	0.445*	-0.863**	-0.776**	0.623**	0.571**	-0.720**	0.799**	
DOC_w	n.s.	-0.606**	-0.638**	0.640**	n.s.	-0.460*		
$NO_3^-{}_s$	-0.683**	0.808**	0.873**	n.s.	-0.763**			
$NH_4^+{}_s$	0.628**	-0.736**	-0.627**	n.s.				
$IDOC_s$	n.s.	n.s.	n.s.					
pH_s	-0.685**	0.773**						
Redox	-0.636**							

Table 3.3 shows the results of significant Pearson correlations calculated between study variables throughout the incubation period. We observed that ~ 70% variability of water NO_3^- concentration was positively correlated with sediment NO_3^- content (Table 3.3). After examining regression analyses, we found that the positive influence of sediment NO_3^- on water content fit better a logarithmic model ($r^2= 0.63$, $p< 0.001$, n=21). Likewise, a positive correlation (57% of variance) was observed in relation to NH_4^+ in both compartments during the whole experimental period, which followed a robust linear fit ($r^2= 0.84$, $p< 0.001$, n=18) once excluded data from 336h. The same relationship was found between water DOC and sediment IDOC ($r^2= 0.66$, $p< 0.001$, n=18) but excluding data at 1h. Overall, variation of the N forms in the water column and sediments were coupled during the experiment. Over the inundation period, water NO_3^- concentration in the water column decreased lineally as a function of increasing water NH4 concentration ($r^2= -0.77$, $p< 0.001$, n=21) while this negative relationship fit better to an exponential model in the case of sediment content ($r^2= -0.75$, $p< 0.001$, n=24). Significant correlations were also found between N and C availability, in both, water and sediments, as well as with sediment redox and pH (Table 3.3).

Denitrification rates

Before sediment inundation, denitrification rates in dry sediments were below the detection levels (<0.01 mg N m^{-2} h^{-1}, Fig. 3.5). After 1h of sediment inundation, however, denitrification rates rose to 0.031±0.002 mg N m^{-2} h^{-1} and rates persisted significantly higher with respect to dry conditions until the end of the inundation period. Over the first 24h of inundation, denitrification rates tended to increase, with a marked raise at 24h and a peak value of 0.830±0.460 mg N m^{-2} h^{-1}. From this moment, a light decrease was observed, with an average value of 0.287±0.08 mg N m^{-2} h^{-1} at the end of the inundation period (Fig. 3.5).

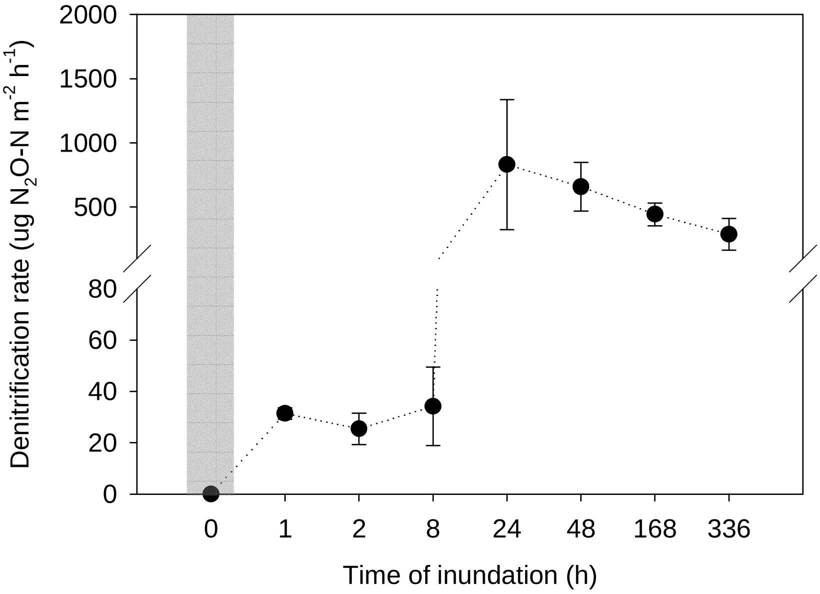

Figure 3.5. Mean (±1SE, n=3) denitrification rate for each sampling event over the inundation period. Grey bar is shown to indicate initial conditions (t0; pre-inundation). The discontinuous line connecting sampling events has been used for helping in the visualization of temporal variation of data but does not implicate actual trends between events.

Over the whole study, denitrification rates correlated negatively with the sediment NO_3^-, redox and pH, but positively with the water and sediment NH_4^+ (Table 3.3). The regression analyses showed that sediment NO3 decreased linearly in response of denitrification (r^2= 0.47, p< 0.001, n=24). Unlike NO_3^- in sediments, denitrification appeared no correlate with water NO_3^- over the inundation period.

Noteworthy, the drop of the NO_3^- concentration in the water column registered since 24 h of sediment inundation coupled with the maximum rates observed in denitrification over the study period.

Discussion

Modifications in the streams physical conditions, including water availability, can result in profound changes in N-cycling processes and thus, nutrient availability (Mitchell and Baldwin 1999; Fierer and Schimel 2002). Since soil microorganisms are key drivers of biogeochemical cycling, the way they respond to changes to sudden changes in moisture could be a fundamental factor for predicting changes in temporary ecosystems functioning. We initially expected that a pulse of NO_3^- to water column would occur after rewetting dry sediments, and that denitrification would be rapidly activated modulating the NO_3^- availability in the water column. According to our expectations, we detected a significant recovery in denitrifying activity after 1h of inundation. This could account for the lack of substantial flush of NO_3^- in water concentration. Despite not finding a great increase, a slight pulse of NO_3^- was observed during the first 24 h of sediment inundation.

Although denitrification was absent in dry sediments, this process exhibited a quick recovery once stream sediments were rewetted. As in soils, saturated sediment conditions favoured that the sediment redox-potential spanned values < +300 mV, thereby, promoting denitrifying bacteria to use NO_3^- over the course of the inundation (Vepraskas and Faulkner 2001; Otero and Macías 2003). Noteworthy, denitrification rate peaked at 24h (0.010 μg N_2O-N g^{-1} DM h^{-1} on average), when sediment redox was on average 254 mV and it seemed to recover pre-dry levels, since they were similar to rates observed by Gómez et al. (2012) during pre-dry conditions in Chícamo stream using the same microcosms method (0.011 μg N_2O-N g^{-1} DM h^{-1} on average). In agreement with our findings, we also observed rapid returning times of denitrification at 4h of rewetting in a headwater stream, after a natural dry period of 4 months, with rates even higher than those observed to pre-dry conditions (Arce et al. 2013b). The differences found between both studies with respect to pre-dry recovery times (4 h *vs.* 24 h), could be attributed to the experimental conditions in the present work. Unlike the microcosms conditions, sporadic rainfalls or dew formation that can occur in headwater streams can provide certain levels of soil humidity. This may help microbial communities to be active during the summer drought, and to rapidly recover upon rewetting, once favourable conditions are established (i.e. NO_3^- and C availability and low redox). Also supported by our findings, many studies have argued that in climatic areas subjected to natural water fluctuations, drying-wetting cycles enable the biota to adapt to extreme hydrological changes (Fierer et al. 2003; Amalfitano et al. 2008) by adjusting their physiological and morphological features (Schimel et al. 2007). Nonetheless, regardless of climatic region, the recovery of microbial functions is highly dependent of grade of sediment desiccation (Marxen et al. 2010). Hence, as occur in temperate regions (Cavanaugh et al. 2006; Austin and Strauss 2011), even in semiarid streams, denitrification can recover with delay after extreme drought periods once stream flow resumes (Arce et al. 2013a). Contemporary research has emphasized the importance of multifunctionality extracellular enzymes in recovering ecosystem function after desiccation, not only in Mediterranean but also in temperate streams

(Marxen et al. 2010; Pohlon et al. 2013). However, their implications on N processing are unclear.

In agreement with previous studies, the sediment NO_3^- content in our microcosms after three months of desiccation (expressed per g DM) was high (13 μg g^{-1} DM on average). This level was similar to the maximum observed by Gómez et al. (2012) in the same stream under the driest conditions (< 2% of GW, ~11 μg NO_3^- g^{-1} DM on average). These authors reported levels ~ 6 times higher than the NO_3^- content in wet sediments (12% of GW, ~2 μg NO3 g^{-1} DM on average) (Gómez et at. 2012). After sediment inundation, we indeed detected a progressive loss of sediment NO_3^-, but the magnitude of NO_3^- lost we found during the first 24h of sediment inundation; 445 mg NO_3^- m^{-2} on average, was not translated into a proportional increase in NO_3^- concentration in the water column. Compared with the NO_3^- content in sediments, the NO_3^- in the water column, expressed in mg NO_3^- m^{-2}, varied from 426 to 471 mg m^{-2} on average, thus, increasing only 45 mg m^{-2}. These results support the idea that the NO_3^- accumulated in sediments during the dry period began to be transformed immediately after sediment rewetting, rather than being completely released to water column, at least a noticeable proportion. Hence, the rapid recovery of the denitrifying activity in sediments that we observed supports this hypothesis. Besides, over the inundation period, sediment NO_3^- variation correlated negatively with denitrification, reinforcing the fact that a proportion of NO_3^- from this compartment decreased in response to denitrification.

Although denitrification could be an important process in reducing the stream NO_3^- concentration, given these results, we must consider alternative mechanisms that could also be involved in the total NO_3^- loss during the inundation period. In contrast to NO_3^-, the NH_4^+ concentration in water and sediment increased sharply over the inundation period. This is an indicative result that nitrification was steadily reduced in saturated sediments (Baldwin and Mitchell 2000; Cavanaugh et al. 2006). The increases in NH_4^+ water and sediment concentration were more acute at 24h coinciding with the lower sediment redox. Unexpectedly, we noticed a drop in water NH_4^+ concentration at the end of the study period. Although we were not aware of evident growth of primary producers in tanks, the development of some biofilm in the surface of sediments at the end of the study could support an uptake of N in form of NH_4^+ and therefore explain such artifact. Saved this finding, to explain the gradual increase in NH_4^+ observed we must consider possible pathways acting as a source of this nutrient such as ammonification (Baldwin and Williams 2007). Ammonification can occur in anoxic soils mediated by anaerobic microbes (Robertson and Groffman 2007) and, hence, N mineralization could have taken place in our microcosms stimulated by rewetting, as described in semiarid soils (McIntyre et al. 2009b). Zaman and Chang (2004), suggested, however, that optimal soil moisture for mineralization is wet yet with good aeration. In our study, variables indicatives of potential source of mineralizing organic N, such as sediment OM, did not change substantially over the 14 days of inundation, and DOC and IDOC, rather than being reduced over time, they were gradually increasing, likely due to full saturation conditions This discards organic matter mineralization and ammonification as possible source of $NH_4^+$and support the stable OM and accumulation of both DOC and IDOC at the end of the experiment.

There is a widespread opinion that drying-rewetting events result in large but temporary pulses of NH_4^+ and organic C from microbial cells (Appel 1998, Halverson et al. 2000). Researchers examining dried soils and sediments that were rewetted detected increased NH_4^+ concentration upon re-flooding, presumably due to the lysis of bacterial cells due to abrupt osmotic changes (Humphries and Baldwin 2003). Although NH_4^+ pulses have been suggested to occur instantaneously, we do not discard that the general increase of sediment NH_4^+, and subsequently in water, could be derived of nutrient cell release, at least partially. However, we must note that water NH_4^+ fluctuated coupled to water conductivity, which varied over the course of the experiment from 19 to 66 mS cm^{-1}. Therefore, physical mechanisms such as cation exchange (Gardner et al. 1991; Baldwin et al. 2006) and redissolution of salts from sediments (Baldwin and Mitchell 2000) could provide NH_4^+ to total water and sediment pool, at least partially. Despite being less known in streams in terms of nitrate removal (Burgin and Hamilton 2007), the dissimilatory reduction of NO_3^- to NH_4^+ under anoxic conditions (DNRA) could take part, since NO_3^- decreased as a function of increasing NH_4^+ in both water and sediments. Interestingly, there are evidences to suggest that under salty environments, dissimilatory NO_3^- reduction is promoted (Gardner et al. 2006) while nitrification is reduced through increased toxicity of HS^- via SO_4^{2-} reduction (Joye and Hollibaugh 1995; Ardón et al. 2013). Although the redox-potential measured in sediments did not achieve values < 100 mV, indicative of complete anoxia (Vepraskas and Faulkner 2001; Otero and Macías 2003), we do not discard complete anoxic conditions in some microsites since some black areas were present during sampling (M.I. Arce, personal observation). These effects, together with the high conductivity values could synergistically increase NH_4^+ availability in pools; by supporting DNRA as a additional pathway of NO_3^- retention (Burgin and Hamilton 2007), and by reducing nitrification. In these lines, it has been suggested that sulphate concentrations could also inhibit autotrophic denitrification in saline environments when concentrations are ≥ 5 g L^{-1} (Claus and Kutzne 1985). We consider, however, that since organic C was present in our microcosms, thereby supporting heterotrophic denitrification, the plausible inhibition by sulphate would not be an important factor in controlling NO_3^- removal over the inundation period in the study site (Arce et al. 2013a).

Recent studies have observed a flush of respiratory C together with an increase in the respiration and enzymatic degradation rates following rewetting (Williams and Xia 2009) and inundation (Wilson et al. 2011) of dry soils. The drop in sediment IDOC we observed the first 2h of rewetting could be attributed to a fast use of C mediated by respiratory activity of sediments. The subsequent gradual increase, not only in IDOC but also in water DOC, could arise from the C flushes, presumably derived of the accumulated intracellular osmolytes (Halverson et al. 2000) and from the limited mineralization-respiration once imposed anaerobic conditions in the saturated sediments (McIntyre et al. 2009a) as previously discussed.

Conclusions and implications

Our microcosms experiment demonstrated that water pulses and the subsequent short-term flooding periods during drought can rapidly stimulate stream-bed biogeochemical processes, including denitrification, and is an important driver of transformations of inorganic N. Rather than promote a noticeable NO_3^- release from sediments to water column, NO_3^- is rapidly processed by active sediments once rewetted. These pools are typically anoxic and, if NO_3^- and DOC are plentiful to support denitrification, as occur in the study site (Arce et al. 2013a), these environments are optimal for long-term N loss. Nonetheless, although it seems to exist a contribution potentially relevant of denitrification to total water inorganic N loss other transformations that could take place under saturated/anoxic conditions appears to simply recycle the inorganic N content in water and sediments. If part of the observed loss of NO_3^- in water and sediments was associated to DNRA, as hypothesized, it would involve that NO_3^- was only temporarily removed over the course of the inundation. Furthermore, if nitrification is reduced under high SO_4^- conditions (Ardón et al. 2013), the subsequent increase of NH_4^+ stocks in the ecosystem will be only transformed back to NO_3^- via nitrification if pool is dried out and sediments are exposed again to air/oxic conditions. Drying and rewetting of soils and sediments have been proposed to effectively promote the sediment removal in wetlands (Qui and McComb 1996), lakes (Scholz et al. 2002) and soils (Fierer and Schimel 2002). In sites receiving water pulses rich in N from agriculture, as Chícamo stream, the high total NO_3^- pool may supply different microbial processes apart from denitrification. Therefore, unless all NO_3^- is completely denitrified, it may be difficult to detect significant net reductions in total DIN (Cavanaugh et al. 2006) as we observed in sediments from Chícamo stream.

Our results have important implications for the management excess of N in temporary and streams draining agricultural landscapes. Isolated pools, as the system simulated in our study, are naturally created not only during dry phase but also during stream hydrological contraction (Stanley et al. 1997). Conditions of no water transport maximize interaction between water and active sediments, thus, promoting the effectiveness of denitrification on dampening part of NO_3^- excess. However, other part can be transformed to NH_4^+ which, unless is nitrified and/or assimilated by a well developed biofilm, may achieve acute values. Although NO_3^- is a N form easily mobilized in water compared with NH_4^+ (Bernot and Dodds 2005), excess of NH_4^+ in water can be toxic for some aquatic organisms if it reaches to downstream ecosystems (Camargo and Alonso 2006). Therefore, in this respect, if these pools are not disconnected of the stream channel or do not dry back after a strong flow resumption (flush flood), a delivery of NH_4^+ could be temporarily transported downstream.

In conclusion, our results suggest that if utilization of nutrient fertilizers does not decrease in the terrestrial lands, the chronic excess of N in draining reaches would be very difficult to regulate by natural stream-bed drying-rewetting cycles. Given that, there is a need for local management strategies in order to improve water quality before the water reaches downstream ecosystem when temporary streams drain agricultural landscapes.

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Dedication

This paper is dedicated to the memory of our colleague José Javier Sánchez, who suddenly passed away at a young age.

References

Alexander RB, Böhlke JK, Boyer EW, David MB, Harvey JW, Mulholland PJ, Seitzinger SP, Tobias CR, Tonitto C, Wollheim WM (2009) Dynamic modeling of nitrogen looses in river networks unravels the coupled effects of hydrological and biogeochemical processes. Biogeochemistry 93:91-116

Álvarez-Cobelas M, Rojo C, Angeler DG (2005) Mediterranean limnology: current status, gaps and the future. J Limnol 64:13-29

Amalfitano S, Fazi S, Zoppini A, Caracciolo AB, Grenni P, Puddu A (2008) Responses of benthic bacteria to experimental drying in sediments from Mediterranean temporary rivers. Microbial Ecol 55: 270-279

Appel T (1998) Non-biomass soil organic N - the substrate for N mineralization flushes following soil drying–rewetting and for organic N rendered CaCl2-extractable upon soil drying. Soil Biol Biochem 30: 1445-1456

Arango CP, Tank JL, Schaller JL, Royer TV, Bernot MJ, David MB (2007) Benthic organic carbon influences denitrification in streams with high nitrate concentration. Freshwater Biol 52:1210-1222

Arce MI, Gómez R, Suárez ML, Vidal-Abarca MR (2013a) Denitrification rates and controlling factors in two agriculturally influenced temporary Mediterranean saline streams. Hydrobiologia 700:169-185

Arce MI, Sánchez-Montoya MM, Vidal-Abarca MR, Suárez ML, Gómez R (2013b) Implications of flow intermittency on sediment nitrogen availability and processing rates in a Mediterranean headwater stream. Aquat Sci (DOI: 10.1007/s00027-013-0327-2)

Ardón M, Morse JL, Comlan BP, Bernhardt ES (2013) Drought-induced saltwater incursion leads to increased wetland nitrogen export. Glob Change Biol 19:2976-2985

Arribas P, Gutiérrez-Cánovas C, Abellán P, Sánchez-Fernandez D, Picazo F, Velasco J, Millán A (2009) Tipificacion de los rios salinos ibericos. Ecosistemas 18:1-13

Austin AT (2011) Has water limited our imagination for aridland biogeochemistry?. Trends Ecol Evol 26:229-235

Austin BJ, Strauss EA (2011) Nitrification and denitrification response to varying periods of desiccation and inundation in a western Kansas stream. Hydrobiologia 658: 183-195

Austin AT, Yahdjian L, Stark JM, Belnap J, Porporato A, Norton U, Ravetta DA, Schaeffer SM (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141: 221-235

Baker MA, Valett HM, Dahm CN (2000) Organic carbon supply and metabolism in a shallow groundwater ecosystem. Ecology 81:3133-3148

Baldwin DS, Mitchell AM (2000) The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river-floodplain systems: a synthesis. Regul River 16:457-467

Baldwin DS, Williams J (2007) Differential release of nitrogen and phosphorus from anoxic sediments. Chem Ecol 23:243-249

Baldwin DS, Rees GN, Mitchell AM, Watson G (2005) Spatial and temporal variability of nitrogen dynamics in an upland stream before and after a drought. Mar Freshwater Res 56:457-464

Baldwin DS, Rees GN, Mitchell AM, Watson G, Williams J (2006) The short-term effects of salinization on anaerobic nutrient cycling and microbial community structure in sediment from a freshwater wetland. Wetlands 2:455-464

Bennett LT, Adams MA (1999) Indices for characterising spatial variability of soils nitrogen semi-arid grasslands of northwestern Australia. Soil Biol Biochem 31:735–746

Bernal S, von Schiller D, Sabater F, Martí E (2013) Hydrological extremes modulate nutrient dynamics in mediterranean climate streams across different spatial scales. Hydrobiologia 719:21-42

Bernot MJ, Dodds WK (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8:442-453

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front Ecol Environ 5:89-96

Butterly CR, Bünemann EK, McNeill AM, Baldock JA, Marschner P (2009) Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biol Biochem 41:1406-1416

Camargo JA, Alonso Á (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int 32:831-849

Cavanaugh JC, Richardson WB, Strauss EA, Bartsch LA (2006) Nitrogen dynamics in sediment during water level manipulation on the Upper Mississippi River. River Res Appl 22:651-666

Claus G, Kutzner HJ (1985) Physiology and kinetics of autotrophic denitrification by *Thiobacillus denitrificans*. Appl Microbiol Biot 22:283-288

Collins SL, Sinsabaugh RL, Crenshaw C, Green L, Porras-Alfaro A, Stursova M, Zeglin LH (2008) Pulse dynamics and microbial processes in aridland ecosystems. J Ecol 96:413-420

Corti R, Datry T (2012) Invertebrates and sestonic matter in an advancing wetted front traveling down a dry river bed (Albarine, France). Freshwater Sci 31:1187-1201

Datry T, Arscott D, Sabater S (2011) Recent perspectives on temporary river ecology. Aquat Sci 73: 453-457

Datry T, Larned ST, Tockner K (2014) Intermittent rivers: A challenge for freshwater ecology. BioScience, bit027

Fierer N, Schimel JP (2002) Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biol Biochem 34:777-787

Fierer N, Schimel JP, Holden PA (2003) Influence of drying-rewetting frequency on soil bacterial community structure. Microbiol Ecol 45:63-71

Gardner WS, McCarthy MJ, An S, Sobolev D, Sell KS, Brock D (2006) Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. Limnol Oceanogr 51:558-568

Gardner WS, Seitzinger SP, Malczyk JM (1991) The effects of sea salts on the forms of nitrogen released from estuarine and freshwater sediments: Does ion pairing affect ammonium flux? Estuaries 14:157-166

Gasith A, Resh VH (1999) Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Sys 30: 51-81

Giambiagi N, Rimolo M, Pirolo T (1993) Influence of drought on the production of mineral nitrogen in a typical argiudol of the Pampas. Soil Biol Biochem 25:101-108

Gómez R, Arce MI, Sánchez JJ, Sánchez-Montoya MM (2012) The effects of drying on sediment nitrogen content in an arid Mediterranean stream: a microcosms study. Hydrobiologia 679:43-59

Hall Jr RO, Tank JL, Sobota DJ et al (2009) Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: total uptake. Limnol Oceanogr 54:653-665

Halverson LJ, Jones TM, Firestone MK (2000) Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci Soc Am J 64:1630-1637

Helsel DR (2005) More than Obvious: Better Methods for Interpreting Nondetect data. Environ Sci Technol 39:419A-423A

Humphries P, Baldwin DS (2003) Drought and aquatic ecosystems: an introduction. Freshwater Biol 48:1141-1146

Hunter RG, Faulkner SP (2001) Denitrification potentials in restored and natural bottomland hardwood wetlands. Soil Sci Soc Ac J 65:1865–1872

Joye SB, Hollibaugh JT (1995) Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. Science 270:623-625

Knowles R (1990) Acetylene inhibition technique: Development, advantages, and potential problems. In: Revsbech NP, Sorensen J (eds) Denitrification in soil and sediment. Plenum Press, New York, pp 151-166

Lassaletta L, García-Gómez H, Gimeno BS, Rovira JV (2009) Agriculture-induced increase in nitrate concentrations in stream waters of a large Mediterranean catchment over 25years (1981–2005). Sci Total Environ 407:6034-6043

Larned ST, Datry T, Arscott BD, Tockner K (2010) Emerging concepts in temporary river-ecology. Freshwater Biol 55:717-738

Larned ST, Datry T, Robinson CT (2007) Invertebrate and microbial responses to inundation in an ephemeral river reach in New Zealand: effects of preceding dry periods. Aquat Sci 69:554-567

Marxsen J, Zoppini A, Wilczek S (2010) Microbial communities in streambed sediments recovering from desiccation. FEMS Microbiol Ecol 71:374-386

McIntyre RE, Adams MA, Ford DJ, Grierson PF (2009a) Rewetting and litter addition influence mineralisation and microbial communities in soils from a semi-arid intermittent stream. Soil Biol Biochem 41:92-101

McIntyre RE, Adams MA, Grierson PF (2009b) Nitrogen mineralization potential in rewetted soils from a semi-arid stream landscape, north-west Australia. J Arid Environ 73:48-54

McLaughlin C (2008) Evaporation as a nutrient retention mechanism at Sycamore Creek, Arizona. Hydrobiologia 603:241-252

Mitchell AM, Baldwin DS (1999) The effects of sediment desiccation in the potential for nitrification, denitrification, and methanogenesis in an Australian reservoir. Hydrobiologia 392:3-11

Otero XL, Macías F (2003) Spatial variation in pyritization of trace metals in salt marsh soils. Biogeochemistry 62:59-86

Pohlon E, Mätzig C, Marxsen J (2013) Desiccation affects bacterial community structure and function in temperate stream sediments. Fundam Appl Limnol 182:123-134

Qiu S, McComb AJ (1996) Drying-induced stimulation of ammonium release and nitrification in reflooded lake sediment. Mar Freshwater Res 47:531–536

Quin GP, Keough MJ (2002) Experimental design and data analysis for Biologists. Cambridge University Press, Cambridge, UK

Robertson GP, Groffman PM (2007) Nitrogen transformations. In Paul EA (ed) Soil Microbiology, Biogeochemistry, and Ecology. Springer, New York, NY, USA, pp 341-364

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394

Scholz O, Gawne BEN, Ebner B, Ellis I (2002) The effects of drying and re-flooding on nutrient availability in ephemeral deflation basin lakes in western New South Wales, Australia. River Res Appl 18:185-196

Schwinning S, Sala OE (2004) Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. Oecologia 141:211-220

Skoulikidis N, Amaxidis Y (2009) Origin and dynamics of dissolved and particulate nutrients in a minimally disturbed Mediterranean river with intermittent flow. J Hydrol 373:218-229

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr 14:799-801

Stanley EH, Fisher SG, Grimm NB (1997) Ecosystem expansion and contraction in streams. BioScience 47:427-435

Steward AL, von Schiller D, Tockner K, Marshall JC, Bunn SE (2012) When the river runs dry: human and ecological values of dry riverbeds. Front Ecol Environ 10:202-209

Timoner X, Acuna V, von Schiller D, Sabater S (2012) Functional responses of stream biofilms to flow cessation, desiccation and rewetting. Freshwater Biol 57:1565-1578

Tzoraki O, Nikolaidis NP, Amaxidis Y, Skoulikidis NT (2007) In-stream biogeochemical processes of a temporary river. Environ Sci Technol 41:1225-1231

Velasco J, Millán A, Vidal-Abarca MR, Suárez ML, Guerrero C, Ortega M (2003) Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. Freshwater Biol 48:1408-1420

Vepraskas MJ, Faulkner SP (2001) Redox Chemistry of Hydric Soils. In: Richardson JL, Vepraskas GJ (eds) Wetland Soils. Lewis Publishers, New York, pp 85-107

von Schiller D, Acuña V, Graeber D, Martí E, Ribot M, Sabater S, Timoner X, Tockner K (2011) Contraction, fragmentation and expansion dynamics determine nutrient availability in a Mediterranean forest stream. Aquat Sci 73:485-497

Williams MA, Xia K (2009) Characterization of the water soluble soil organic pool following the rewetting of dry soil in a drought-prone tallgrass prairie. Soil Biol Biochem 41:21-28

Wilson JS, Baldwin DS, Rees GN, Wilson BP (2011) The effects of short-term inundation on carbon dynamics, microbial community structure and microbial activity in floodplain soil. River Res Appl 27:213-225

Wood ED, Armstrong FA, Richards FA (1967) Determination of nitrate in seawater by cadmium copper reduction to nitrite. J Mar Biol Assoc UK 47:23–31

Zaman M, Chang SX (2004) Substrate type, temperature, and moisture content affect gross and net N mineralization and nitrification rates in agroforestry systems. Biol Fert Soils 39:269-279

Chapter 4

Variation in nitrate uptake and denitrification rates across a salinity gradient in Mediterranean semiarid streams

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Abstract

Streams are significant locations for nitrate (NO_3^-) processing within landscapes. This is especially important in dry climates given the limited water availability for biological processes elsewhere. In arid and semiarid regions, many streams are naturally saline. Elevated salinity can constrain the structure and function of aquatic organisms, which is expected to increase worldwide being associated to global warming. We investigated whole-reach NO_3^- uptake and denitrification in nine semiarid streams of variable water salinity (i.e. from freshwater to hyposaline) to test if NO_3^- processing would decrease with increasing salinity. We used pulse additions and Tracer Addition for Spiraling Curve Characterization (TASCC) to measure whole-reach uptake of added NO_3^-, and the acetylene block technique to measure sediment denitrification. TASCC results showed that only five of nine streams were able to retain added NO_3^-. Of these five retentive streams, four were saline; however, salinity did not control significantly the variation in whole-reach NO_3^- uptake observed across streams. Other measured environmental variables such as streambed NH_4^+ and organic carbon availability were better at explaining this variation. Denitrification was detected in all streams except one and its variation across streams was also independent of salinity. Although denitrification rates tended to be high, their contribution to whole-reach NO_3^- uptake was insignificant ($\leq$ 2.16%). Alternative pathways, heterotrophic assimilation and/or dissimilatory NO_3^- reduction to NH_4^+, were probably responsible for most whole-reach NO_3^- uptake. Together, our results highlight that the function of streams in controlling external NO_3^- inputs is highly variable and salinity does not apparently constrain this role.

Introduction

Streams play a critical role in landscape-level nitrogen (N) cycling since they can modify nitrate (NO_3^-) fluxes via channel routing and in-stream processes, thereby dampening the export of NO_3^- to downstream ecosystems (Alexander et al. 2009; Hall et al. 2009). In Mediterranean semiarid catchments in particular, streams may be hotspots for NO_3^- uptake because the biological reactions involved in nutrient processing in these landscapes are generally limited by water availability, as typically occurs in arid or desert catchments (Belnap et al. 2005; Sponseller et al. 2013). Yet at the same time, scarce precipitation combined with low discharges constrain these streams to exhibit a limited dilution capacity of anthropogenic inputs (Álvarez-Cobelas et al. 2005), which might saturate the ability to attenuate NO_3^- excess under certain conditions (Bernot and Dodds 2005). Despite such evidence, most of our knowledge about stream NO_3^- processing is limited to cold temperate catchments, and very few studies have been conducted in arid or semiarid catchments (see Martí et al. 1997; Grimm et al. 2005). Thus, we know very little about how NO_3^- fluxes are modulated in this type of ecosystems and the environmental factors controlling this role.

In association with aridity and the presence of gypsum and halite-rich rocks in catchments, many Mediterranean semiarid streams are naturally saline (Gómez et al. 2005; Millán et al. 2011). Saline streams are characterized by water salinity or conductivity values of ≥ 3 g L^{-1} or ≥ 5 mS cm^{-1}, respectively (Velasco et al. 2006). Although these ecosystems are widely neglected in the world-wide context, saline streams are geographically widespread, especially in arid and semiarid regions (Williams 1996), such as the southeast Iberian Peninsula. To date, most research on Mediterranean saline semiarid streams has focused on structural aspects (Millán et al. 2011), and although some studies have evaluated stream metabolism (Gutierrez-Cánovas et al. 2009) or organic matter dynamics (Vidal-Abarca et al. 2004), very little is known about functional aspects, such as NO_3^- processing (Arce et al. 2013).

In-stream NO_3^- uptake comprises a set of processes that integrates assimilation into the biomass of stream biota or NO_3^- reduction to gaseous forms of N under anaerobic conditions through the denitrification process, of which the latter results in permanent loss (Mulholland et al. 2008). To our knowledge, no research has evaluated the influence of salinity on NO_3^- uptake by addressing measurements at the whole-reach scale, and any information currently available is based mostly on studies that have examined denitrification rates in estuaries (Rysgaard et al. 1999; Magalhâes et al. 2005), saline lakes (Kulp et al. 2007) or saline streams (Arce et al. 2013). In addition, while some studies have shown low rates at higher salinity, others have found no effect. Theoretically from a physiological basis, when high salinity conditions are imposed, salt-tolerant types of microorganisms with similar functions may be able to replace previous communities progressively (Hart et al. 1991). Nonetheless, it has been suggested that salinization alters the composition and function of microbial communities, particularly for all the microbial groups involved in N cycling (Santoro 2010). Surviving under high salt concentrations is bioenergetically taxing since organisms must maintain an osmotic balance between their cytoplasm and the surrounding medium, while excluding ions from the cell interior (Kulp et al. 2007). Salt-tolerant microorganisms must, therefore, supply sufficient energy to fulfil the cellular requirements for osmoadaptation by diverting less energy in other processes (Oren 1999). If this occurs, it may result in organisms being less efficient for certain processes, such as N uptake, if compared with similar organisms living under less saline or freshwater conditions.

Under natural conditions, usually salinization occurs in association with low flow periods in many wetlands and rivers (Nielsen et al. 2003). However, long-term salinization has become a problem in many highly-humanized catchments (Cooper et al. 2013) and it is also expected to increase in relation to global warming (IPCC 2007). Since salt concentrations have exceeded the critical threshold of biota, salinization has long since been recognized as a major environmental problem in Australian inland waters (Nielsen et al. 2003). Recently it has also been identified as a serious disturbance for many rivers and streams (Cañedo-Argüelles et al. 2013). Likewise, albeit in the opposite direction, decreased salinity due to freshwater inputs from agriculture has also been shown to alter the biological assemblages and metabolism of naturally saline streams (Velasco et al. 2006; Gutiérrez-Cánovas et al. 2009). Whatever the future evolution for both fresh and saline waters in terms of salinity, we still know very little about how salinity interacts with the way nutrients are processed (Baldwin et al. 2006).

In this study, we aimed to investigate in-stream NO_3^- processing in semiarid Mediterranean streams and how it is affected by water salinity. Thus, we measured whole-reach NO_3^- uptake using field solute enrichment experiments and denitrification using laboratory bioassays in streams located across a gradient of salinity that included freshwater and hyposaline streams. We measured denitrification rates at ambient nutrient concentrations and under optimum redox conditions to identify an upper bound on its contribution to whole-reach NO_3^- uptake. Given the proposed linkages between salinity and N processing, we predicted that the rates of whole-reach uptake of added NO_3^- and denitrification would be lower across the gradient of increasing water salinity to reflect a stress-induced reduction in the capacity to process this nutrient.

Materials and Methods

Study sites

The Segura river catchment (18,815 km²) is located in the southeast Iberian Peninsula (Fig. 4.1). The climate ranges from sub-humid Mediterranean (average annual rainfall > 1,000 mm, temperature = 13ºC) to semiarid Mediterranean (< 300 mm and 18ºC) (CHS 2007). The latter dominates most of the catchment and is responsible for the fact that almost 80% of the river network is temporary (Vidal-Abarca 1990). The catchment area is covered mostly by agriculture (52%) and forest/seminatural systems (45%) (Belmar 2013). Geology varies from limestone at the uplands to Miocene salt-rich marl in the mid- and lowlands. As a result of the variable geology and climate patterns, freshwater and saline streams dominate the sub-humid and the semiarid areas, respectively.

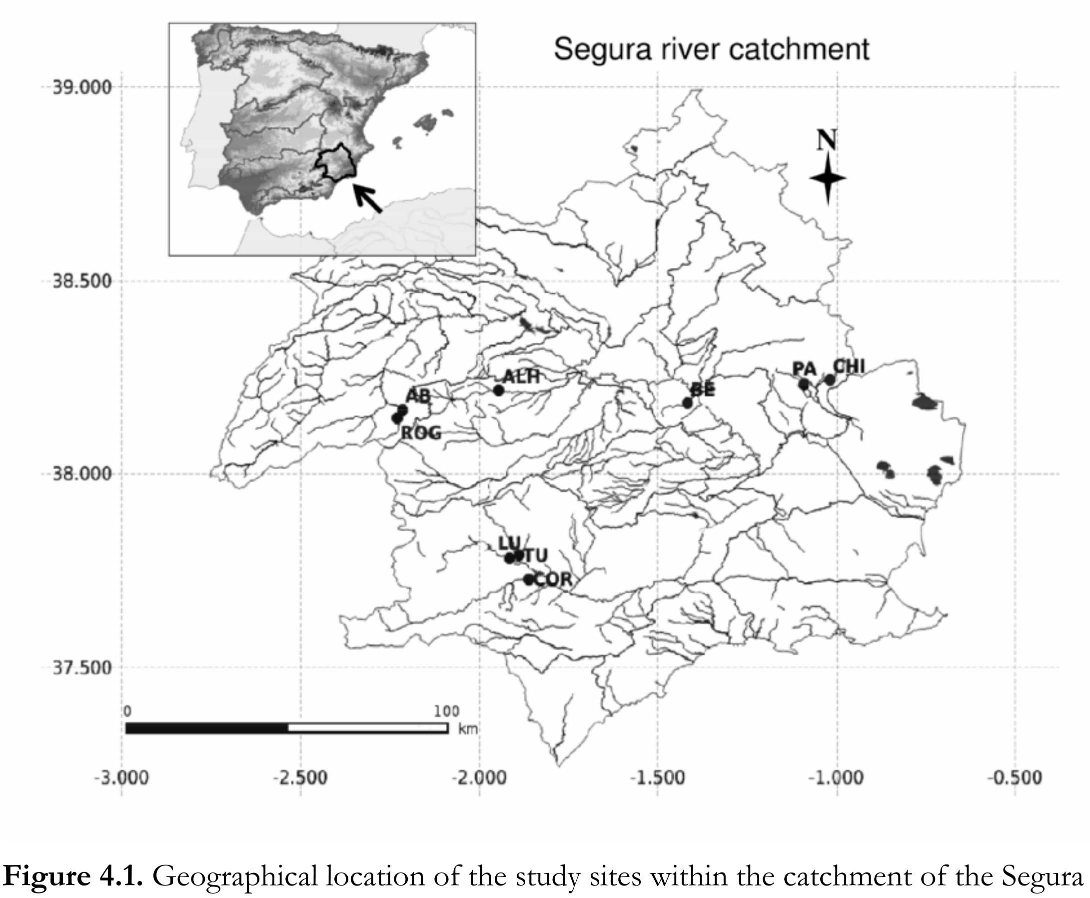

Figure 4.1. Geographical location of the study sites within the catchment of the Segura river (SE Spain). See Table 4.1 for stream codes.

For this study, we selected nine streams (Fig. 4.1, Table 4.1) located along a gradient of water salinity or conductivity (0.3-8 g L^{-1} or 500-12,000 μS cm^{-1}) (Table 4.2). According to the classification by Arribas et al. (2009), the strictly saline streams in the study gradient were hyposaline (3-20 g L^{-1}).

Table 4.1. Geographical location and hydraulic characteristics of the study sites. Width and depth values are reported as mean ± SE (n=5-12). Q= discharge, Vel.= flow velocity.

Stream	Code	Longitude (W)	Latitude (N)	Altitude (m a.s.l)	Q (L s^{-1})	Vel. (m s^{-1})	Width (m)	Depth (m)
Alhárabe	ALH	1º 57' 46"	38º 12' 50"	701	94.6	0.32	2.05±0.23	0.14±0.02
Arroyo Blanco	AB	2º 12' 59"	38º 09' 54"	1,035	32.3	0.34	1.67±0.17	0.07±0.02
Rambla de la Rogativa	ROG	2º 13' 41"	38º 08' 29"	1,105	20.7	0.25	1.59±0.21	0.08±0.01
Corneros	COR	1º 51' 39"	37º 43' 37"	475	41.7	0.35	1.99±0.20	0.07±0.01
Luchena	LU	1º 53' 18"	37º 46' 18"	261	125.2	0.34	2.86±0.15	0.30±0.02
Chícamo	CHI	1º 01' 09"	38º 14' 25"	503	28.9	0.25	1.88±0.25	0.07±0.01
Rambla de la Parra	PA	1º 05' 20"	38º 13' 51"	262	1.5	0.06	0.62±0.11	0.03±0.01
Turrilla	TU	1º 53' 13"	37º 46' 28"	503	14.9	0.20	2.42±0.35	0.05±0.01
Rambla de Benito	BE	1º 24' 58"	38º 10' 51"	247	1.6	0.07	0.90±0.15	0.02±0.01

Table 4.2. Stream water physicochemical characteristics. Values are reported as mean ± SE (n= 3). The streams are ordered as a function of increasing salinity. T= temperature, DOsat= dissolved oxygen saturation, SRP= soluble reactive phosphorus, DOC= dissolved organic carbon. See Table 4.1 for stream codes.

Stream	Salinity (µS cm^{-1})	T (ºC)	DOsat (%)	pH	SO_4^{2-} (mg L^{-1})	Cl^- (mg L^{-1})	Ca^{2+} (mg L^{-1})	K^+ (mg L^{-1})	Na^+ (mg L^{-1})	NO_3^- (mg L^{-1})	NH_4^+ (µg L^{-1})	SRP (µg L^{-1})	DOC (mg L^{-1})
ALH	555±1	20.4±0.0	106.8±0.7	8.5±0.0	106±21	38±12	62±3	1.9±0.2	25±4	0.75±0.02	22.8±4.3	2.9±0.7	30.1±2.0
AB	656±2	16.9±0.0	105.5±0.1	8.5±0.0	87±5	13±1	55±1	1.9±0.0	10±0	0.36±0.01	35.6±4.0	3.0±0.8	21.0±2.1
ROG	777±1	18.3±0.1	109.1±0.3	8.4±0.0	186±13	24±1	74±4	5.7±0.4	23±2	0.63±0.01	48.0±12.9	3.2±1.0	17.9±0.1
COR	1,267±1	22.8±0.1	102.7±0.6	8.4±0.0	204±5	94±2	94±4	3.6±0.3	81±5	2.31±0.03	41.4±12.9	7.1±2.2	27.9±1.9
LU	3,130±0	25.8±0.0	101.7±0.3	8.9±0.0	885±45	212±24	324±12	8.7±1.1	162±17	0.24±0.01	49.4±3.9	1.6±0.2	19.1±1.0
CHI	3,553±3	26.7±0.2	114.0±0.6	8.5±0.0	3,100±27	577±15	117±6	7.9±0.7	437±8	4.52±0.01	6.1±3.0	1.2±0.3	9.5±2.7
PA	6,713±3	23.4±0.1	109.5±1.3	8.1±0.1	2,220±39	810±14	591±5	18.2±0.2	556±42	0.01±0.00	74.9±9.3	4.8±1.3	17.9±0.4
TU	9,270±2	24.7±0.1	117.7±0.8	8.2±0.0	2,546±69	1,803±18	505±23	14.4±0.3	770±2	4.92±0.10	111.5±13.1	2.3±0.3	28.2±5.2
BE	11,740±2	20.9±0.1	122.3±0.8	8.1±0.1	2,076±9	3,279±44	606±3	14.0±0.3	1,603±9	0.16±0.01	69.9±4.0	8.2±1.9	17.8±0.5

Riparian vegetation was variable and ranged from well-developed forests (*Populus* spp. and *Salix* spp.) in the freshwater streams to communities of open Mediterranean scrubs (*Tamarix* spp. and *Nerium oleander*) and halophytic small-sized plants (e.g. *Sarcocornia* spp. and *Arthrocnemum* spp.) in the saline streams. In general, the presence of submerged macrophytes (*Chara* spp., *Cladophora* spp. and *Enteromorpha* spp.) is scarce in semiarid streams, and the biofilm covering the streambed accounts for most primary production, especially in saline streams (Velasco et al. 2003). In Arroyo Blanco (AB), the streambed was also covered by *Nostoc* spp. In general, the streambed substrate consisted of a mix of pebbles and sands in freshwater streams, whereas fine gravels and sands were more common in the saline streams. Allochthonous benthic particulate organic matter was scarce, and coarse particles, mainly leaves, were limited to freshwater streams.

Field sampling

All the study sites were sampled between late May and early July 2011 under base flow conditions.

In-situ NO_3^- *additions*

To measure whole-reach NO_3^- uptake, we conducted pulse additions (i.e. slugs) of NO_3^- (as $NaNO_3^-$) and a conservative tracer (Cl^-, as NaCl). In each stream, sampling was conducted in a representative reach dominated by riffles and pools. The selected experimental reaches were 50-160 m long depending on stream discharge and size. The average wetted width and depth was determined from cross-sectional transects (5-12) separated equidistantly along the reach. Prior to additions (i.e. background conditions), we collected stream water samples and measured conductivity, the saturation percentage of dissolved oxygen (DO), pH and temperature at the upstream, middle and downstream parts of the study reach using Hach handheld probes (Loveland, CO, USA). The slug solution (reagents dissolved in 2-3 L of stream water) was injected as a single pulse to the stream at a point of high turbulence to assure complete mixing. We measured water conductivity in real time (logging at 10-s intervals) and collected grab samples across the breakthrough curve (BTC) (n=20-30 grab samples per release, depending on the slope of the BTC) at the base of the experimental reach. Water samples were filtered immediately through previously combusted Whatman GF/F (Maidstone, England, UK) glass fiber filters (0.7 μm), which were transported to the laboratory on ice and then refrigerated at 4°C for ≤ 24 h (NO_3^- samples) or frozen in the laboratory (remaining samples) until further analysis.

Slug additions were more suitable than constant rate additions for this particular study given the high NO_3^- background and/or salt concentrations of some study sites. Another advantage of using slug additions in our study was its easy short-term replicability and its low-cost since several additions using the conservative tracer were required to determine the amount of salt and the stream length required to detect water conductivity changes. Finally, the use of slug additions allowed us to apply the Tracer Addition for Spiraling Curve Characterization (TASSC) approach (Covino et al. 2010b) to determine whole-reach uptake metrics (see below).

Sediment sampling

To measure different sediment physico-chemical variables and denitrification rates, six sediment samples from the top 0-5 cm (Inwood et al. 2005) were randomly collected along the study reach before the additions using a corer of known inner diameter. Samples were transported to the laboratory in zip-lock bags on ice under dark conditions and were stored until further processing (within 24 h). We also collected 2.5 L of unfiltered stream water.

To estimate the clorophyll *a* (Cl*a*) content, as an indicator of the biomass of primary producers (biofilm and filamentous algae), six samples of streambed substrata were also collected with the same corers prior to additions, but only the top 0-3 cm of sediment were considered. Whenever coarse substrates (pebbles and gravels) were collected, the coarse substrates area was estimated with aluminium foil after Cl*a* extraction. These sediment samples were transported to the laboratory on ice in zip-lock bags and were kept frozen until analyzed (within 1 month).

Laboratory analyses

Water analysis

Background and grab samples were analyzed colorimetrically for the NO_3^- concentration in a Systea EasyChem autoanalyzer (Oak Brook, IL, USA) following the reduction-diazotization method (Wood et al. 1967). Background samples were further analyzed for the ammonium (NH_4^+) concentration in the same autoanalyzer following the phenol-hypochlorite method (Solorzano 1969), for soluble reactive phosphorus (SRP) by hand using a spectrophometer and following the molibdate method (Murphy and Riley 1962), for dissolved organic carbon (DOC) using a Shimadzu TOC-5000A Total Organic C (TOC) analyzer (Columbia, MD, USA), and for the main cations (Na^+, K^+ and Ca^{+2}) and anions (Cl^-, Br^- and SO_4^{-2}) using a Fisher Scientific ICP-OES Iris Intrepid II XDL Thermo (Waltham, MA, USA). Before the analyses, DOC samples were preserved with acid in the dark at 4ºC (Hunter and Faulkner 2001).

Sediment analysis

To determine the percentage of sediment organic matter (OM), samples were oven-dried at 110ºC for 24 h and further combusted in a muffle furnace at 550ºC for 4 h. Sediment bulk density (g cm^{-3}) was estimated as the oven dry mass of sediment divided by core volume. Bulk density was further used to calculate denitrification on an areal basis. To estimate the extractable NO_3^- and NH_4^+ sediment concentrations, cold KCl extraction (Mulvaney 1996) was used.

Approximately 5 g of fresh weight were placed in 50 ml of 1 M KCl and were shaken for 1 h on a rotary shaker. Interstitial organic carbon (IDOC) was similarly obtained, but using deionized water as the extracting agent (sensu Baker et al. 2000). All the extracts were filtered through pre-combusted Whatman GF/F filters and stored at 4ºC until analysis within 48 h using the same methods described previously for stream water. For the KCl-extracted samples, KCl standards were prepared to address the possible effects of KCl on determining the sediment NO_3^- and NH_4^+ concentrations. The fine sediment percentage was determined by sieving (2-mm mesh) dry sediments. The Cl*a* content was established by spectophotometry following extraction in 90% acetone and correcting for phaeopigments by acidification (Steinman et al. 2006). From each sediment sample, three analytical replicates were analyzed, except for the Cl*a* measurements and the fine sediment percentage (one analytical replicate per sample).

Denitrification assays

We measured sediment denitrification following the acetylene block technique (Knowles 1990) and controlling potential microbial growth in response to lab conditions by amending chloramphenicol (Royer et al. 2004). For each sediment sample, three analytical replicates were run. Approximately 100 mL of sediment were transferred to 250-mL bottles equipped with rubber septa to then amended with 75 ml of unfiltered stream water. Bottles were purged with Ar to create anoxic conditions, and were then injected with 10 mL acetylene and incubated in the dark at room temperature. We took head-space gas samples at the beginning and end of incubation (5 h) with intermediate sampling. The N_2O concentration was determined in an Agilent C890N gas chromatograph (Silicon Valley, CA, USA), equipped with a Porapack Q column and an electron-capture detector. The N_2O concentration dissolved in the aqueous phase was corrected with the appropriate Bunsen´s coefficient.

Parameter calculations

Hydraulic parameters

The slug injection method allowed quantifying hydraulic parameters, such as discharge and water velocity. We calculated discharge (Q, L s^{-1}) as the ratio between the conductivity in the injected solution and the value resulting from the integration of the area of the conductivity BTC obtained at the base of the experimental reach (Gordon et al. 1992). The average water velocity (m s^{-1}) was estimated as the ratio between the experimental reach length and the time at which the half of the maximum conductivity was reached (Gordon et al. 1992).

Whole-reach NO_3^- uptake

To quantify the NO_3^- uptake metrics, we used the TASSC approach (Covino et al. 2010b). First, we developed a relationship between conductivity and the Cl^-

concentration in the lab (r^2= 0.999) for each stream to transform the conductivity curve to a Cl^- concentration curve. For that, we used stream water collected at the same date of the slug experiments to which we added different NaCl amounts until we covered the conductivity BTC range.

Spiraling metrics were obtained across a range of NO_3^- concentrations by comparing the ratio of the mass of NO_3^- and Cl^- recovered in each sample throughout the BTC of the grab samples to that of the injectate.

To evaluate if the study sites retained NO_3^- added during the experiments, we plotted the background-corrected NO_3^-:Cl^- ratio vs. time since the injection and we assessed this against the injectate NO_3^-:Cl^- ratio (Covino et al. 2010a). When the background-corrected NO_3^-:Cl^- was lower than the injectate ratio, it demonstrated significant NO_3^- uptake (see the example in Fig. 4.2a). Conversely, when the NO_3^-:Cl^- ratio was near to or higher than the injectate ratio (i.e. near the BTC peak), it indicated that both solutes came through conservatively, thus the stream reach did not retain the added NO_3^- (see the example in Fig. 4.2b).

One benefit of using the TASCC approach is that it allows to develop a distribution of spiraling metrics in accordance with the nutrient concentration and to extrapolate the uptake parameters to the ambient nutrient concentration; (i.e. background NO_3^- uptake without the influence of added NO_3^- (similarly to Payn et al. 2005). Thus, in those streams where the added NO_3^- was retained, we first calculated the added nutrient longitudinal uptake rates ($k_{\text{w-add-dyn}}$) for each grab sample by plotting the natural log of the NO_3^-:Cl^- ratio of the injectate and each background corrected grab sample collected at the end of the experimental reach against the stream reach distance (Covino et al. 2010b). Therefore, $k_{\text{w-add-dyn}}$ was then the respective slope of the line of these data pair for each sample. From $k_{\text{w-add-dyn}}$, we calculated the dynamic uptake length ($S_{\text{w-add-dyn}}$) of the added NO_3^- per grab sample as -1/ $k_{\text{w-add-dyn}}$. The uptake length at the ambient levels ($S_{\text{w-amb}}$) was then inferred from the significant relationship between the $S_{\text{w-add-dyn}}$, and NO_3^- concentration (per grab sample) (SigmaPlot vs. 10, San José, CA, USA) by back-extrapolating to the background NO_3^- concentration (see the example in Fig. 4.2c). From $S_{\text{w-amb}}$, we estimated the ambient areal uptake rate (U_{amb}) and uptake velocity or demand ($V_{\text{f-amb}}$) using the following equations:

$U_{\text{amb}} = (Q\ [NO_3^-{}_{\text{amb}}]\ /\ (S_{\text{w-amb}}\ w)$

$V_{\text{f-amb}} = U_{\text{amb}}\ /\ [NO_3^-{}_{\text{amb}}]$

where $NO_3^-{}_{\text{amb}}$ is the ambient stream water NO_3^- concentration and w is the average wetted width of the study reach.

In this paper, we report the values of $S_{\text{w-amb}}$, U_{amb} and $V_{\text{f-amb}}$ to examine the influence of salinity and other environmental variables on whole-reach NO_3^- uptake. As S_w is a metric that strongly depends on stream size and discharge (Webster and Valett 2006), we focused on U_{amb} and $V_{\text{f-amb}}$ for the statistical analyses and literature comparisons.

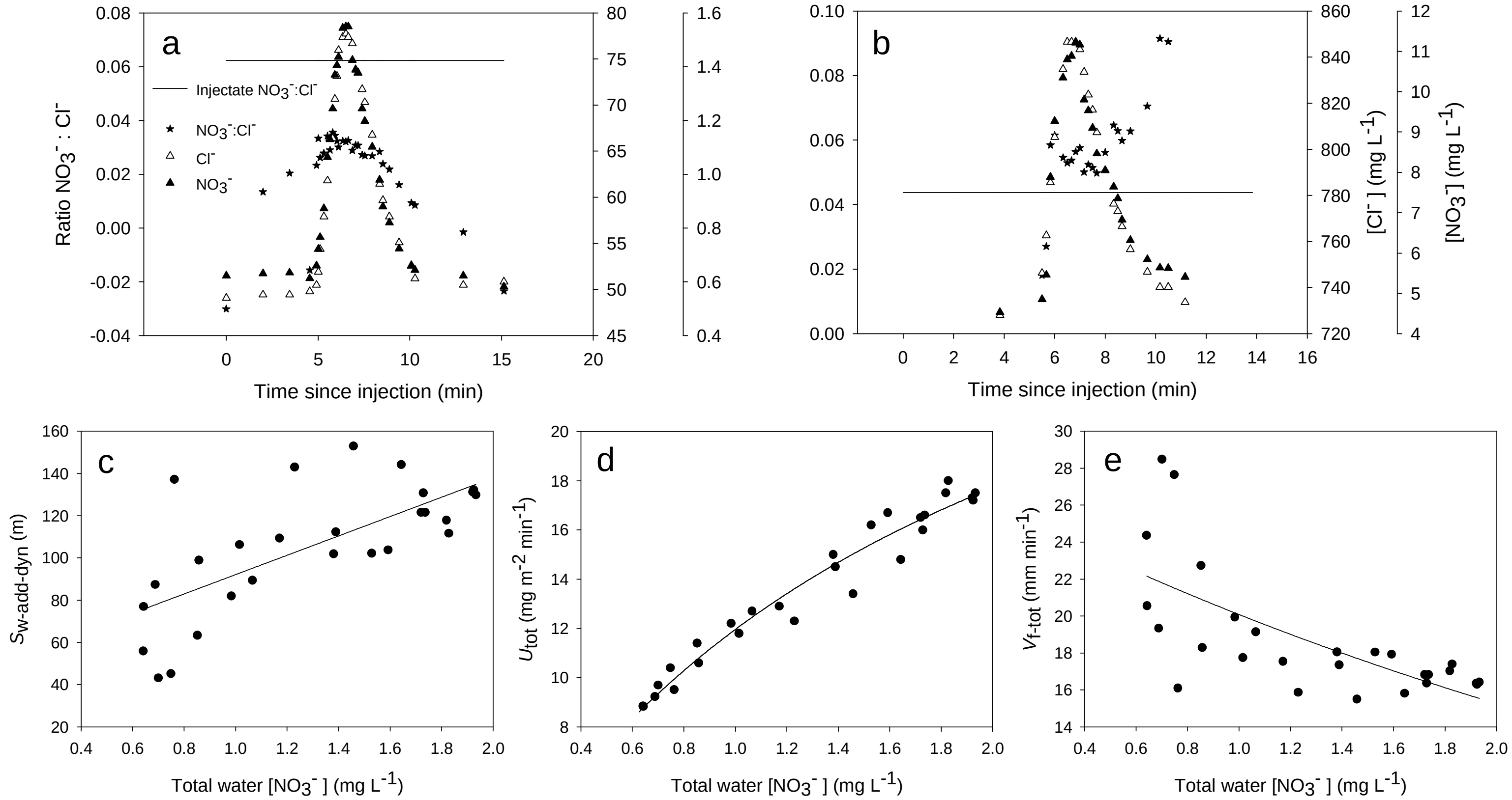

Figure 4.2. (a-b) Examples of two study sites displaying the time series of the Cl$^-$ and NO$_3^-$ concentrations, along with the NO$_3^-$:Cl$^-$ ratio sampled at the base of the experimental reach, showing uptake (a; ROG) and no uptake (b; CHI) of added NO$_3^-$ during the slug additions. The injectate ratio (continuous line) added at the head of the experimental reach at time 0 is shown as a reference for interpreting the changing ratio at the base of the reach. (c) Lineal relationship between $S_{\text{w-add-dyn}}$ and total water [NO$_3^-$] calculated to extrapolate the uptake length at the ambient levels ($S_{\text{w-amb}}$) in the stream example (ROG) showing added NO$_3^-$ uptake. (d-e) Uptake curves of the total NO$_3^-$ areal uptake rate (U_{tot}) and the total NO$_3^-$ uptake velocity ($V_{\text{f-tot}}$) as a function of total water [NO$_3^-$] in the stream example (ROG) showing uptake of added NO$_3^-$. Total [NO$_3^-$] is the geometric mean of the conservative and observed total NO$_3^-$ concentration in each grab sample. See Table 4.1 for stream codes.

Another benefit of the TASCC approach is that it allows the quantification of total nutrient uptake. Similarly to ambient metrics, the added nutrient spiraling metrics were also calculated as:

$U_{\text{add-dyn}} = (Q\ [NO_3^-{}_{\text{add-dyn}}] / (S_{\text{w-add-dyn}}\ w)$

$V_{\text{f-add-dyn}} = U_{\text{add-dyn}} / [NO_3^-{}_{\text{add-dyn}}]$

By combining the added ($U_{\text{add-dyn}}$ and $V_{\text{f-add-dyn}}$) and ambient (U_{amb} and $V_{\text{f-amb}}$) metrics, we also quantified the total spiraling parameters (U_{tot} and $V_{\text{f-tot}}$). Using these total spiraling values, we further examined the relationships between uptake and the water NO_3^- concentrations, which are useful to characterize the stream response to the variable nutrient concentration. We assigned appropriate kinetic models to our U_{tot} and $V_{\text{f-tot}}$ data (SigmaPlot vs. 10) (see the example in Figs. 4.2d, e). For U_{tot}, we determined maximum uptake $U_{\max}$ and the half saturation constant K_m from Michaelis-Menten(M-M) models (SigmaPlot vs.10):

$U = (U_{\max}\ C) / K_m + C$

where C is the total observed water NO_3^- concentration.

More detailed information about the calculations of the uptake metrics used in this paper is provided in Covino et al. (2010b). Given the large number of investigated streams, only ROG and CHI are shown as examples of the stream sites presenting uptake and no uptake of added NO_3^-, respectively (Fig. 4.2). The TASCC results for the streams not shown in figure 4.2 can be found in the Annexes 4.1-4.5 of this chapter.

Denitrification rates

We determined denitrification rates by regressing out the linear relationship between the N_2O concentration in the bottles and the incubation time. All the denitrification rates were calculated by averaging only those analytical replicates that exhibited linear N_2O production over time. This ensured that denitrification measurements represented the original enzymatic status of sediment samples, and were comparable among streams. We expressed the rates as N_2O-N produced per g of dry sediment mass (DM) h^{-1}, per gram of ash free dry mass (AFDM) h^{-1} and as the areal rate h^{-1}. Additionally, we estimated the contribution of denitrification to the whole-reach NO_3^- uptake as: denitrification areal rate / U_{amb}.

We acknowledge that the acetylene-block technique can yield underestimated denitrification rates when using ambient nutrient incubations in low N-streams due to nitrification inhibition (Seitzinger et al. 1993). In N-limited systems, nitrification and denitrification are often tightly coupled, and this method has the potential to reduce experimental rates. These potentially confounding effects were taken into account when interpreting our results. Nonetheless, when the water NO_3^- concentrations are > 10 μM or 0.140 mg L^{-1} (as observed for most study sites), these redox-optimized ambient nutrient incubations are acceptable to approach *in situ* rates (Royer et al. 2004; Inwood et al. 2005), representing upper-bound estimates of ambient *in situ* rates (Arango et al. 2008).

Statistical analyses

To examine which variables contributed to variation across streams, we performed two separate principal component analyses (PCA) (Primer vs. 6.0, Plymouth, England, UK) with the water and sediment variables (hereafter water-PCA and sediment-PCA, respectively). All the variables were standardized to mean 0 and variance 1 before being included in the PCA analyses. The significance of the variables on the PCA axes was determined through Pearson correlations (R) (SPSS vs. 16, Chicago, IL, USA) between variables and axis scores.

We conducted simple linear regressions (SPSS vs. 16) to investigate the relationship between water salinity and the NO_3^- uptake metrics and the sediment denitrification rates across stream sites. As the three metrics we used to express the denitrification rates were strongly correlated ($r^2 \geq 0.93$, $p < 0.001$), we selected the areal rates to conduct the statistical analyses. We also used simple regressions to examine which environmental variables constrained the spatial variation of NO_3^- uptake and denitrification using the scores of the water-PCA and sediment-PCA components as independent variables. Finally, when considering only the stream sites that presented NO_3^- uptake and sediment denitrifying activity, we ran simple regressions between the environmental variables and the uptake and denitrification rates. When more than one regression model significantly described the data, we conducted a simple regression with the model that best fitted the data.

Variables were log(x+1)-transformed whenever they had zero values to improve the symmetry of the distribution and to also meet the assumption of normality whenever necessary (Kolmogorov–Smirnov test). Results at $p < 0.05$ were considered significant.

Results

Environmental variables

Q varied > 2 orders of magnitude (1.5-125.2 L s^{-1}) across the study sites (Table 4.1). Study sites also covered a wide range in water conductivity (555-11,740 µS cm^{-1}), while water temperature (16.9-26.7ºC), pH (8.1-8.9) and DO saturation (> 100% in all the streams) were less variable across streams (Table 4.2). Except in BE, the ionic composition of surface water was dominated generally by SO_4^{2-}, while Ca^{2+} and Na^+ were the most abundant cations (Table 4.2). Water DOC (9.5-30.1 mg L^{-1}) was generally higher than the nutrient concentrations; however, NO_3^- (0.01-4.92 mg L^{-1}) and NH_4^+ (6.1-111.5 µg L^{-1}) showed wider ranges (> 1 order of magnitude) than DOC (Table 4.2). Water SRP was less variable (1.2-8.2 µg L^{-1}) and, as a rule, its concentration was below the inorganic N concentration, indicating that most study sites were potentially P-limited (Table 4.2).

The sediment concentration of NH_4^+ (0.03-2.17 µg g^{-1} DM) tended to be higher and more variable across streams than that of NO_3^- (0.03-0.20 µg g^{-1} DM) (Table 4.3). Sediment IDOC content (19.4 - 40.6 µg g^{-1} DM) varied moderately across streams and concentrations were also higher, but less variable, than the sediment nutrient concentrations (Table 4.3). The Cl*a* content per unit of area of substratum was generally high (0.8-4.5 g cm^{-2}), especially in COR and CHI. The sediment OM percentage varied moderately across streams (1.1-3.5%), as did the fine substratum percentage (29–68%) (Table 4.3).

Table 4.3. Stream sediment physicochemical characteristics. Values are reported as the mean ± SE (n= 6). The streams are ordered as a function of increasing salinity. IDOC= interstitial dissolved organic carbon, Cl*a*= chlorophyll *a*, OM= organic matter. See Table 4.1 for stream codes.

Stream	NO_3^- (µg g^{-1}DM)	NH_4^+ (µg g^{-1}DM)	IDOC (µg g^{-1}DM)	Cl*a* (g cm^{-2})	OM (%)	Fine substratum (%)
ALH	0.11±0.03	0.85±0.30	19.7±3.5	2.1±0.5	3.1±0.4	29.0±10.8
AB	0.07±0.03	0.03±0.01	19.4±2.0	1.6±0.4	2.7±0.1	32.2±13.6
ROG	0.08±0.01	1.19±0.38	40.6±4.0	2.5±0.3	2.4±0.3	61.3±18.3
COR	0.06±0.04	0.74±0.22	28.0±1.6	4.5±0.9	1.7±0.2	24.3±4.2
LU	0.12±0.01	1.60±0.66	30.2±2.5	2.4±0.6	2.6±0.2	68.0±11.9
CHI	0.04±0.02	0.49±0.18	30.6±2.6	3.7±0.9	1.3±0.2	36.4±17.7
PA	0.03±0.01	2.17±0.86	38.9±2.7	0.8±0.2	3.5±0.2	50.0±7.4
TU	0.20±0.09	1.96±0.82	25.9±4.6	2.8±0.5	2.3±0.5	41.5±4.9
BE	0.03±0.01	0.10±0.05	26.2±1.6	1.1±0.5	1.1±0.1	40.0±8.5

The water-PCA accounted for 62% of the variance of the water environmental variables we observed across stream sites. The first axis score (38.5% of explained variance) correlated positively with water conductivity ($R= 0.91$, $p< 0.01$), NH_4^+ ($R= 0.70$, $p< 0.05$) and DO saturation ($R= 0.88$, $p< 0.01$), and negatively with Q (R= -0.78, $p< 0.05$) (Fig. 4.3a). The second axis score (23.5% of explained variance) condensed negatively the water NO_3^- (R= -0.72, $p< 0.05$) and temperature (R= -0.80, $p< 0.01$) (Fig. 4.3a).

The sediment-PCA explained 65.9% of the variance observed in the stream sediment characteristics we measured (Fig. 4.3b). The first axis score (42.1% of explained variance) correlated positively with sediment NH_4^+ (R= 0.86, $p< 0.01$), the OM percentage (R= 0.68, $p< 0.05$), IDOC (R= 0.67, $p< 0.05$) and the fine substratum percentage (R= 0.81, $p< 0.01$) (Fig. 4.3b). The second axis score (23.8% of explained variance) correlated only with sediment NO_3^- content (R= 0.92, $p< 0.01$) (Fig. 4.3b).

A certain level of inter-correlation was found between some environmental variables of the water and sediment compartments. Water NO_3^- correlated positively with sediment NO_3^- content (R= 0.83, p= 0.077) and with Cl*a* (R= 0.67, p= 0.046).

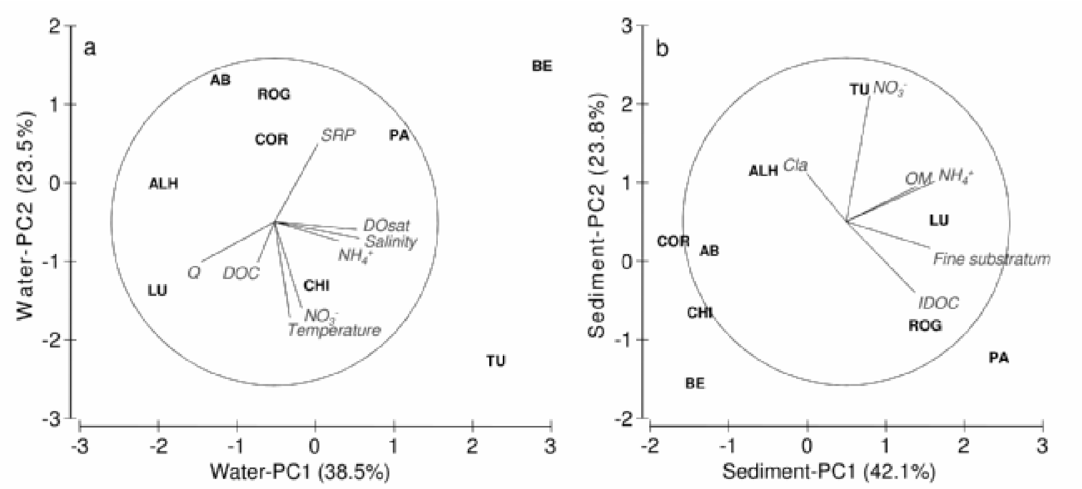

Figure 4.3. Principal component analysis (PCA) of the water (a) and sediment (b) variables. Principal components are weighted linear combinations of the original variables. See Table 4.1 for stream codes and Tables 4.1, 4.2 and 4.3 for the variables included in each PCA.

Whole-reach NO_3^- uptake

According to NO_3^-:Cl^- ratio, we found retention of added NO_3^- in only five of the nine study sites, including the most saline (see the example in Fig. 4.2a, Annex 4.1 and Table 4.4). In most freshwater streams, as well as in the saline stream CHI, there was no retention of added NO_3^-, as indicated by the fact that the NO_3^-:Cl^- ratio did not change over time and was close, or even higher, than the injectate ratio line (see the example in Fig. 4.2b and Annex 4.2). In those streams where added NO_3^- was retained, we observed that $S_{w\text{-add-dyn}}$ increased significantly ($p< 0.05$) following a linear relationship with water NO_3^- concentration (see the example in Fig. 4.2c and Annex 4.3). When we extrapolated $S_{w\text{-amb}}$

Table 4.4. Whole-reach NO_3^- uptake and sediment denitrification parameters. NO_3^- ambient uptake length ($S_{w\text{-}amb}$), ambient areal uptake rate (U_{amb}), ambient uptake velocity ($V_{f\text{-}amb}$), Michaelis-Menten (M-M) model parameters, sediment denitrification rates and contribution of denitrification to the whole-reach uptake rate in the study streams. The streams are ordered as a function of increasing salinity. U_{max} is the maximum rate of uptake and K_m is the half saturation coefficient. Denitrification rates are reported as the mean ± SE (n= 6). DM= dry mass, AFDM= ash free dry mass. (-)= absence of uptake of added NO_3^- , na= data not available. nd= not detected. See Table 4.1 for stream codes.

Stream	$S_{w\text{-}amb}$ (m)	$V_{f\text{-}amb}$ (mm min $^{-1}$)	U_{amb} (mg NO_3^- m^{-2} min^{-1})	U_{max} (mg NO_3^- m $^{-2}$ min^{-1})	K_m (mg NO_3^- L^{-1})	r^2 M-M model	F M-M model	*p*-value M-M model	Denitrification rate (µg N g $^{-1}$DM h^{-1})	Denitrification rate (µg N g $^{-1}$AFDM h^{-1})	Denitrification rate (mg N m $^{-2}$ h^{-1})	% U_{amb} attributed to denitrification
ALH	-	-	-	-	-	-	-	-	0.014±0.004	3.33±1.07	0.88±0.26	na
AB	-	-	-	-	-	-	-	-	0.002±0.001	0.68±0.37	0.12±0.06	na
ROG	74.9	10.4	6.51	34.2	1.85	0.96	551.9	<0.0001	0.006±0.002	4.16±2.04	0.40±0.14	0.10
COR	-	-	-	-	-	-	-	-	0.040±0.005	22.94±3.70	2.77±0.20	na
LU	66.6	39.4	9.10	11.8	0.05	0.38	7.6	0.0150	0.004±0.002	2.16±1.24	0.24±0.13	0.04
CHI	-	-	-	-	-	-	-	-	0.075±0.010	40.97±6.06	4.84±0.28	na
PA	60.7	2.3	0.03	0.3	0.08	0.88	183.0	<0.0001	nd	nd	nd	na
TU	38.3	6.0	28.92	60.5	5.04	0.96	656.5	<0.0001	0.060±0.024	20.38±7.47	2.52±0.07	0.14
BE	44.4	2.4	0.36	3.6	1.56	0.97	1,119.5	<0.0001	0.005±0.001	3.60±0.23	0.46±0.14	2.16

from these relationships, we observed differences across the five streams (Table 4.4). $S_{\text{w-amb}}$ was in general short and varied from 38.3 m in TU to 74.9 m in ROG (Table 4.4). Pronounced differences were found for U_{amb} across streams, which ranged from 0.03 in PA to 28.92 in TU. $V_{\text{f-amb}}$ varied between 2.3 and 39.4 with minimum and maximum values in PA and LU, respectively (Table 4.4).

The use of total nutrient spiraling values allowed us to fit the kinetic models to U_{tot} and $V_{\text{f-tot}}$. In all five retentive streams, the relationship between U_{tot} and the total water NO_3^-concentration was significantly hyperbolic ($p < 0.05$), which is indicative of M-M kinetics (see the example in Fig. 4.2d and Annex 4.4). The $U_{\max}$ values (mg m^{-2} min^{-1}) ranged from 0.3 in PA to 60 in TU, while the K_m values (mg L^{-1}) varied from 0.05 in LU to 5.04 in TU (Table 4.4), and except in LU and TU, NO_3^- concentrations indicated by K_m were well above the ambient levels (Table 4.1). Similarly, we found a negative exponential decay of $V_{\text{f-tot}}$ as a function of the total water NO_3^-concentration in these sites (see the example in Fig. 4.2e and Annex 4.5).

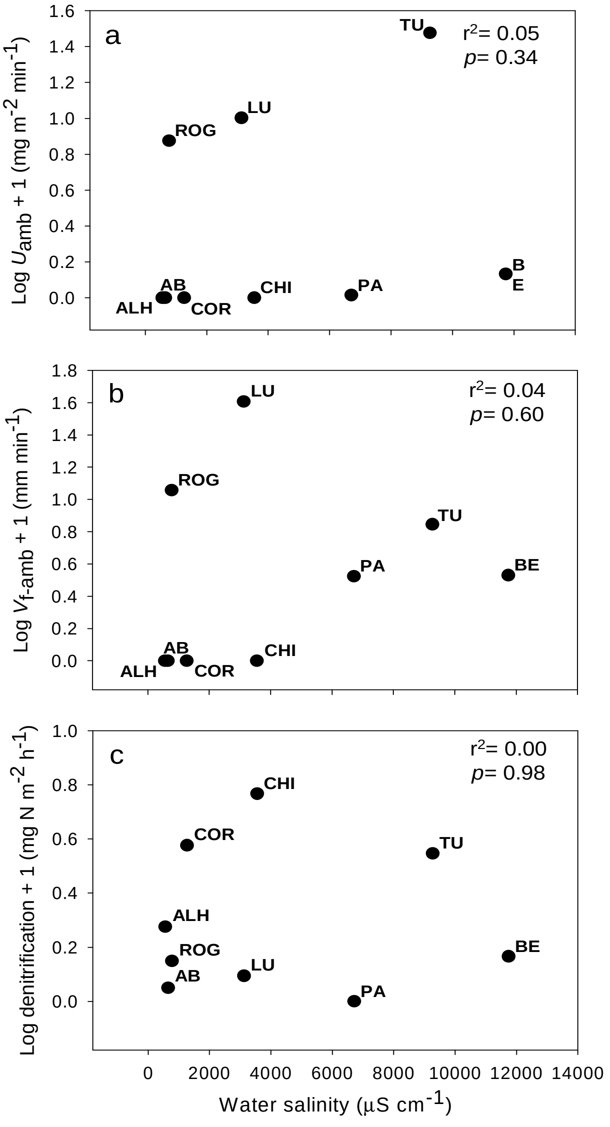

Figure 4.4. Relationship between water salinity (expressed as conductivity) and the whole-reach ambient uptake metrics and sediment denitrification rates. See Table 4.1 for stream codes.

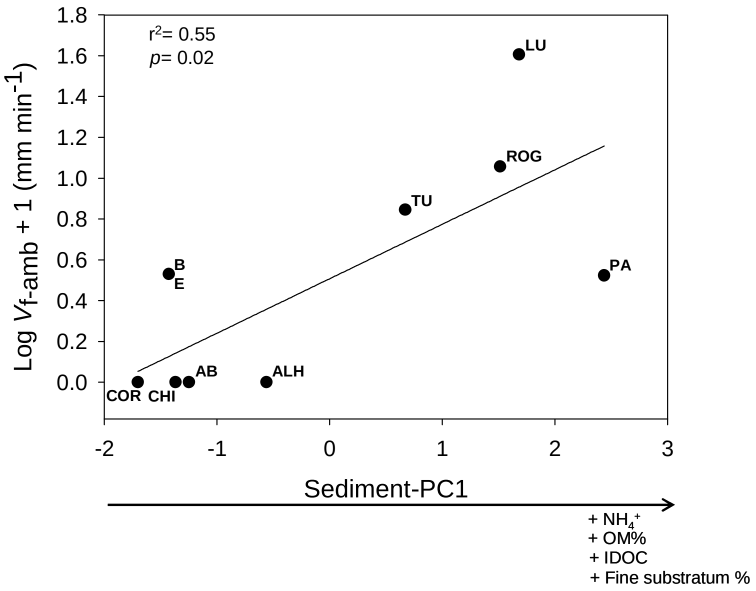

Figure 4.5.

Relationship between whole-reach ambient NO_3^- uptake velocity ($V_{f\text{-amb}}$) and the first axis of sediment-PCA (n=9). The significant variables (p<0.05, Pearson coefficients) associated with the positive PCA axis are shown with their respective positive (+) weight. See Table 4.1 for stream codes.

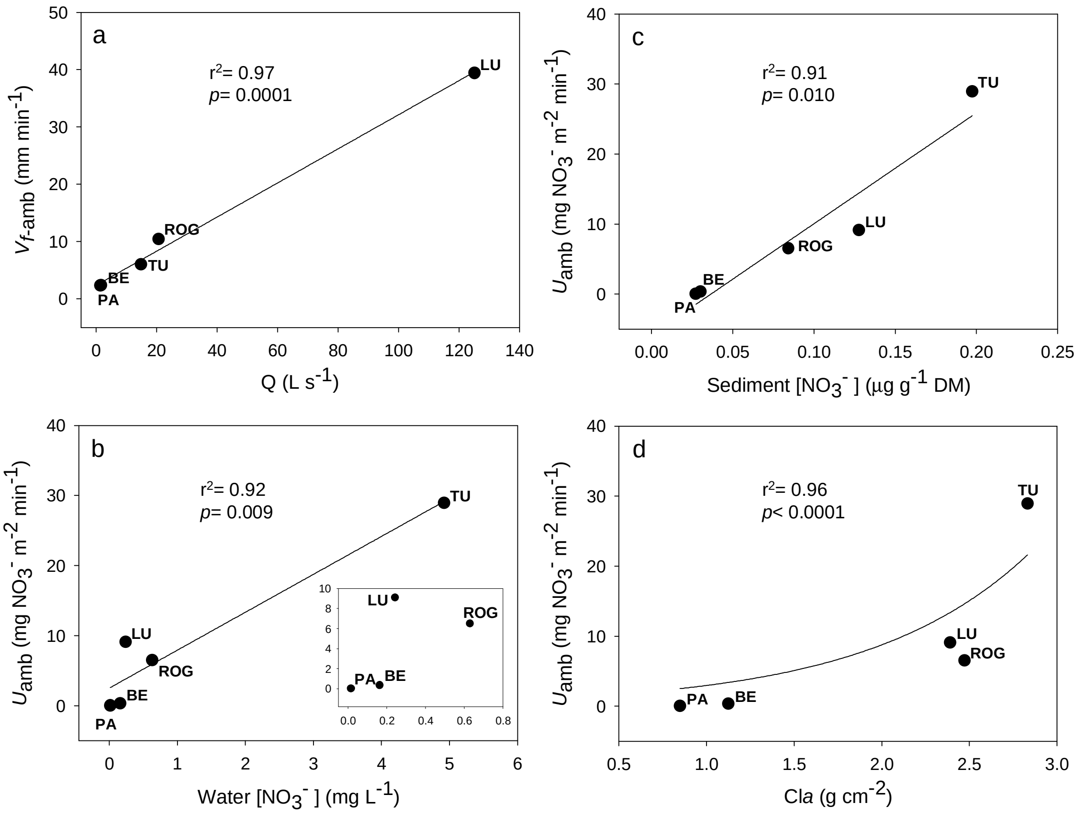

Figure 4.6. Relationship between the whole-reach ambient uptake metrics (ambient NO_3^- uptake velocity; $V_{f\text{-amb}}$ and ambient NO_3^- areal uptake rate; U_{amb}) and the physico-chemical variables of stream water and sediments. Data derive from the streams that showed a significant uptake of added NO_3^- during the instantaneous slug additions (n=5). The non significant relationship found after removing outliers is also shown (n=4). See Table 4.1 for stream codes.

When fixing water salinity alone as an independent variable, we observed no significant relationship with $V_{f\text{-amb}}$ (Fig. 4.4a) and U_{amb} (Fig. 4.4b). However, when using both the water and sediment PCA scores, we found a significant positive relationship between $V_{f\text{-amb}}$ and the first axis of the sediment-PCA, which was related to other environmental variables (Fig. 4.5). Increases in NO_3^- demand were related to increases in the sediment NH_4^+ concentration, OM, IDOC and the fine substratum percentage (Fig. 4.5). We observed no significant relationship between U_{amb} and the sediment-PCA and water-PCA scores ($p > 0.05$). Using data from the five streams where uptake of added NO_3^- was detected and each single environmental variable as an independent predictor, we observed a significant linear relationship between $V_{f\text{-amb}}$ and Q (Fig. 4.6a). We also found that U_{amb} increased linearly with water NO_3^- (Fig. 4.6b), sediment NO_3^- (Fig. 6c), and exponentially with sediment Cl*a* (Fig. 4.6d). However, when considering the presence of outliers, we detected that the relationship between U_{amb} and water NO_3^- was not significant when TU was removed ($p > 0.05$) (Fig. 4.6b).

Sediment denitrification rates

We detected sediment denitrification in all the streams except PA (Table 4.4). As for NO_3^- uptake, sediment denitrification did not vary significantly across streams as a function of water salinity (Fig. 4.4c). In addition, we found no significant relationship between denitrification rates and PCA scores ($p > 0.05$ in all cases). When considering only the study sites that showed denitrifying activity (n=8), we observed that denitrification varied linearly in accordance with water NO_3^- (Fig. 4.7a) and Cl*a* (Fig. 4.7b).

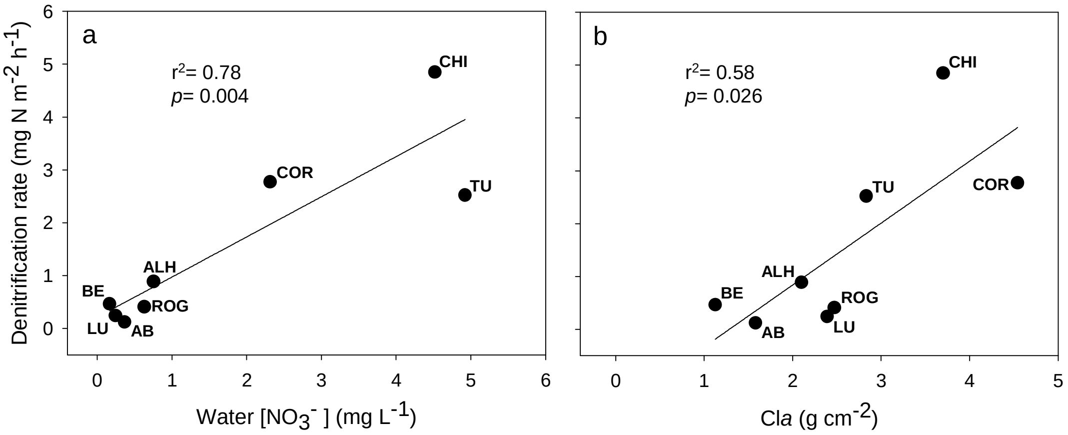

Figure 4.7. Relationship between denitrification rates and the physico-chemical variables of stream water and sediments. Data derive from the streams where denitrifying activity was detected (n=8). See Table 4.1 for stream codes.

Across the nine streams, denitrification rates were not a significant predictor of U_{amb} and $V_{f\text{-amb}}$ ($p > 0.05$). When fixing the data from the study sites where uptake of added NO_3^- was present (n=5), denitrification showed a linear and positive relationship with U_{amb} (Fig. 4.8a), U_{max} (Fig. 4.8b) and K_m (Fig. 4.8c).

However, these relationships were not significant when TU was removed from the regressions (Fig. 4.8).

Overall, the percentage of U_{amb} that was attributed to denitrification was low, with a maximum value of 2.16% in BE (Table 4.4).

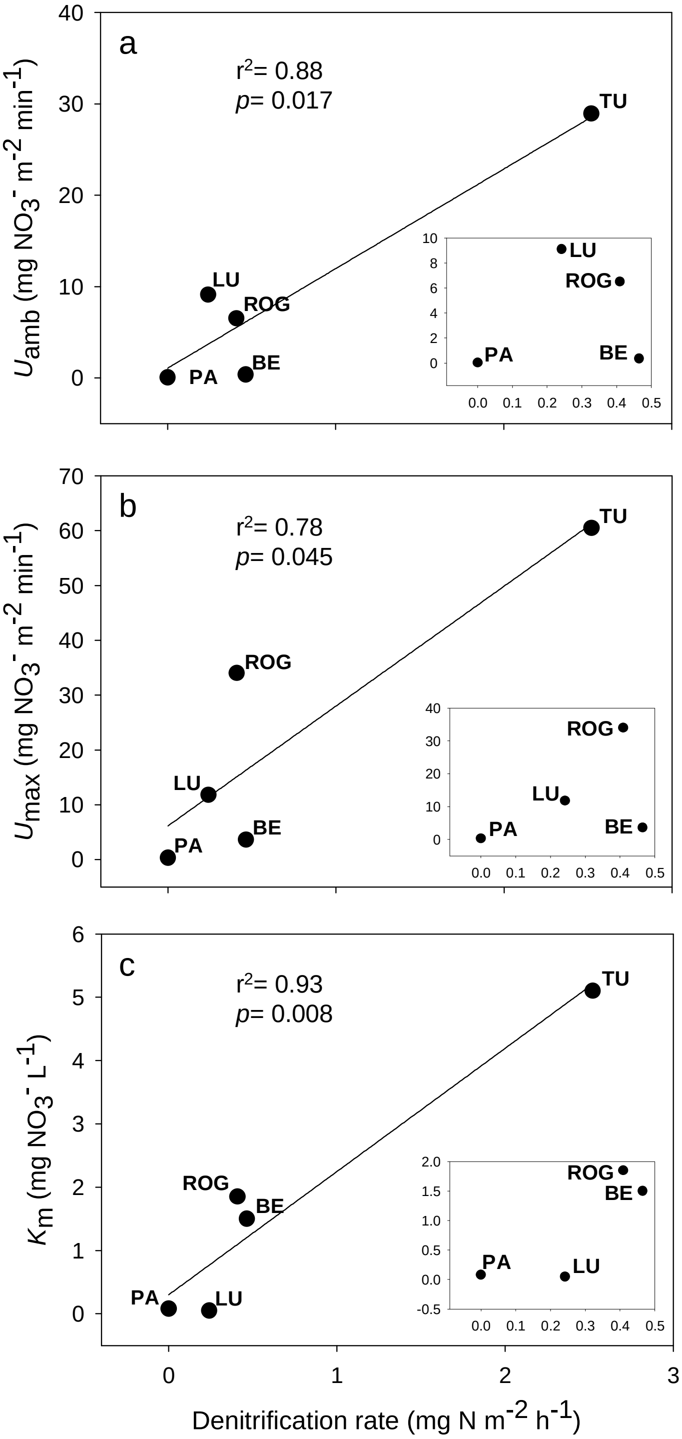

Figure 4.8.

Relationship between the uptake metrics (ambient NO_3^- areal uptake rate; U_{amb}) and the kinetic metrics derived from the M-M models (the maximum uptake capacity; U_{max} and half the saturation constant K_m) and denitrification rates. Data derive from the streams that showed a significant uptake of added NO_3^- during the instantaneous slug additions (n=5). The non significant relationship found after removing outliers is also shown (n=4). See Table 4.1 for stream codes.

Discussion

Whole-reach NO_3^- uptake and sediment denitrification

In this study, we used the recently developed TASCC approach (Covino et al. 2010b) to quantify whole-reach NO_3^- uptake. One of the main advantages of the TASCC approach is that it allows the rapid assessment of nutrient uptake across a range of nutrient concentrations. In addition, the results obtained by TASCC allow the extrapolation of the ambient spiraling metrics from nutrient addition experiments (Payn et al. 2005). We detected whole-reach uptake of added NO_3^- in 56% of the study sites. Comparisons made with the results from previous studies using the TASCC approach or ^{15}N tracer additions suggest that the study streams that showed uptake of added NO_3^- were highly efficient. In a previous research using the TASCC approach, Covino et al (2010b) calculated $V_{f\text{-amb}}$ in two stream reaches (2.1 and 2.6 mm min^{-1}), which are similar to the minimum values we obtained in our study. If compared with the V_f values obtained using $^{15}NO_3^-$ tracer additions across different regions of the USA and Puerto Rico (69 streams, range: 0.024-18 mm min^{-1}, Hall et al. 2009), our $V_{f\text{-amb}}$ were consistently above the median value (0.42 mm min^{-1}1). Regarding other Mediterranean streams, our $V_{f\text{-amb}}$ were one order of magnitude higher than the range reported by von Schiller et al. (2009) from ^{15}N tracer experiments (V_f: 0.044-0.162 mm min^{-1}). Collectively, these results suggest that the semiarid and saline stream type with capacity to retain additional NO_3^- can exhibit a high efficiency in taking up this nutrient.

The efficiency that we observed in some streams contrasts, however, with the fact that uptake of added $NO_3^-$was lacking at other sites. So whereas some streams appeared to be significant locations to control the fate of external NO_3^- within the catchment (Bernhartd et al. 2005; Alexander et al. 2009), others proved inefficient in reducing the downstream additional NO_3^- flux. This finding can be related to the fact that these streams presented high background water NO_3^- concentrations (except for PA) and that the presence of macrophytes and streambed organic matter stocks was poor, thus the associated microbial biomass was reduced. If both factors operate concomitantly, they can diminish the whole-reach NO_3^- assimilation capacity and facilitate N uptake saturation (Bernot and Dodds 2005). According to the saturation concept, NO_3^- concentration could reach a level at which the biological capacity cannot process further NO_3^- (O´Brien et al. 2007). Therefore, as streams approach N saturation, additional N loading to the systems can be transported downstream without being processed (Earl et al. 2006).

Examination of the uptake kinetic responses, which was also done by the TASCC methodology, is important for understanding how these streams can respond to short-term increases in nutrient load since, occasionally, these streams are subjected to NO_3^- inputs from irrigated agriculture. In all the streams where we detected uptake of added NO_3^-, the U_{tot} and $V_{f\text{-tot}}$ data followed M-M kinetics. U_{tot} exhibited a hyperbolic relationship with an increasing NO_3^- concentration, whereas

$V_{f\text{-tot}}$ sharply dropped along the same gradient. The parameters from the M-M models, particularly the half saturation coefficient K_m, provided a relative indication of ecosystem nutrient limitation. Of the five study streams that showed significant added NO_3^- uptake, two streams, LU and TU, gave K_m values that were either below or very close to the background concentrations. Thus, despite their ability to take up added NO_3^- during our study period, additional NO_3^- would no longer stimulate increased cycling (O´Brien et al. 2007). Conversely, in the other three streams, ROG, PA and BE, the K_m values above the ambient NO_3^- levels suggested no sign of saturation, thus additional NO_3^- would stimulate the processing of this nutrient (O´Brien et al. 2007).

Denitrification has been conventionally proposed to exert a substantial effect on the NO_3^- flux downstream because this process removes N permanently from the ecosystem (Seitzinger 1988). We detected sediment denitrification rates in 89% of the study sites. The denitrification sediment rates we found in some streams were high, above the upper value reported in a previous inter-biome study using whole reach ^{15}N-tracer additions (0-0.9 mg N m^{-2} h^{-1}, Mulholland et al. 2008) and, in general, they fell within the range of values that derived from studies using the acetylene block technique (< 0.1-15 mg N m^{-2} h^{-1}, Royer et al. 2004). Across the investigated streams, denitrification did not correlate with the NO_3^- uptake metrics, which suggests that despite these high rates, this process had a limited influence on whole-reach added NO_3^- uptake. The minor contribution of areal denitrification to U_{amb} that we estimated ($\leq$ 2.16%) supports the poor effectiveness of this process in controlling the concentration and load of NO_3^- in the study streams. Despite assuming that our denitrification assays came close to the ambient rates, as done in past studies (Royer et al. 2004; Arango et al. 2008), we acknowledge the difficulties of using incubation experiments to extrapolate denitrification results to the whole-reach scale (Mulholland et al. 2009). Studies using ^{15}N additions with the aim to determine more accurately denitrification rates at the whole-reach scale have shown that the contribution of this process to total U_{amb} can be highly variable, ranging from negligible to 100% (e.g. O´Brien et al. 2007; Mulholland et al. 2008; von Schiller et al. 2009). Although caution should be taken, our results suggest that Mediterranean semiarid streams may fall within the lower part of this range.

Controls on the spatial variation of NO_3^- uptake and denitrification

Our study streams were selected following a natural water salinity gradient for the purpose of examining the influence of this variable on whole-reach NO_3^- uptake and sediment denitrification. Although biotic effects of salinity are relatively well-described (Nielsen et al. 2003), few studies have addressed such effects on the biogeochemical rates of N cycling under both lab and field conditions. We expected that NO_3^- processing rates would decrease along the gradient of increasing water salinity, reflecting a stress-induced reduction in the capacity to process this nutrient. Our findings, however, are consistent with the results from those studies that did not observe a clear effect of salinity (reviewed in Santoro 2010). Indeed, far from observing any negative effect, we found that most sites where added NO_3^- uptake was present were saline. Our results appear to support the alternative idea that salinity selects simply for the new physiological types that are able to tolerate a given salt level, but which maintain similar processing functions (Hart et al. 1991), at least within the covered salinity levels. Another explanation for the lack of salinity effect might be related with the salinity range spanned by the study streams (i.e. from freshwater to hyposaline). Perhaps, the maximum water salinity represented in our gradient (8 g L^{-1} or 12,000 $\mu S\ cm^{-1}$) was not high enough to detect a possible *"in situ"* effect on the NO_3^- processing rates as previously shown in the same study area (Arce et al. 2013). Therefore, studies in streams with higher salinity are needed to examine this possible effect.

The gradient of varying water salinity across the study streams represents an ideal scenario to examine the effects of long-term salinization under natural conditions. However, one drawback is that many environmental conditions also varied along this gradient, and this limitation does not allow us to completely conclude if salinity determines the way N is processed or not. Unless a given salt level strongly modulates the uptake processing rates, working under field conditions alone makes it difficult to elucidate an effect of salinity by itself. Salinity effects can be addressed in future studies by combining mesocosm assays, where salinity alone is modified over a broad range of concentrations, and field experiments to check whether a given salinity level remains as a significant driver once the remaining variables involved in NO_3^- uptake are present.

We found that the stream environmental factors other than salinity better explained the variation in the whole-reach uptake of added NO_3^- and denitrification across the study sites. An increase in $V_{f\text{-amb}}$ along the gradient of increasing sediment NH_4^+, OM, IDOC and the fine sediments percentage was observed across all the stream sites. Fine sediments rich in detritus can provide anaerobic conditions, thus resulting in a sediment rich in NH_4^+ which cannot be oxidized via nitrification (Kemp and Dodds 2001; Baldwin and Williams 2007). The correlation found between $V_{f\text{-amb}}$ and these variables suggests that part of the variation observed in NO_3^- demand during our study seemed to be linked to the activity of heterotrophic organisms that are associated with organic C decomposition places. In fact, the results obtained in a recent study conducted in the same streams indicate greater heterotrophic microbial activity (estimated by the fluorescein diacetate assay, or FDA) in those stream sites with larger OM percentages (Gómez et al. unpublished data). Besides, this result supports the fact that the NO_3^- saturation of the ecosystem in those sites with no significant uptake of added NO_3^- can partially arise from the scarce organic matter standing stocks, as mentioned in the previous section. Nutrient uptake by heterotrophic assimilation has been considered an important component in the control of NO_3^- outputs in temperate streams (Mulholland et al. 2004) and in Mediterranean streams (von Schiller et al. 2009). For example, Arango et al. (2008) suggested that assimilatory demand accounted for most NO_3^- uptake in relation to denitrification across 18 USA streams of variable land use, and Mulholland et al (2008) found the same result across 72 NO_3^- tracer experiments conducted in USA streams. Heterotrophic uptake has been commonly reported during autumn since it has been associated with the decomposition of allochthonous leaf inputs (Goodale et al. 2009). Unlike temperate streams, semiarid streams, and even those freshwater sites showing more developed riparian vegetation, do not present major organic matter inputs or marked seasonality (Vidal-Abarca et al. 2004). Thus, the presence of such heterotrophic activity might be linked to highly decomposed autochthonous organic matter rather than to allochthonous sources (Vidal-Abarca et al. 2004). In general, semiarid streams have been pointed out to be potential autotrophic systems because of the open canopies and unlimited light (Gasith and Resh 1999; Millan et al. 2011). In fact, when considering only the five retentive streams, we found that U_{amb} was higher at those sites with large values of sediment Cl*a*, so NO_3^- supplemented autotrophic production, which also contributed to added NO_3^- uptake (Niyogi et al. 2010). Yet, despite the potential significance of this autotrophic production, differences in the stream heterotrophic activity pattern would ultimately drive part of the variation of NO_3^- demand we observed across the nine streams.

We must note, however, that the correlation we observed between $V_{\text{f-amb}}$ and the environmental gradient may not be solely attributable to assimilatory demand. Mechanisms such as the dissimilatory reduction of NO_3^- to NH_4^+, a process that would lead to increases in NH_4^+, might also be implicated in the variation observed for $V_{\text{f-amb}}$ across streams. Interestingly, the results derived from research conducted in estuaries have suggested that a high sea salt concentration may enhance the rates of dissimilatory NO_3^- reduction to NH_4^+ (Gardner et al. 2006). Although we know comparatively little about the role of this pathway in the stream NO_3^- uptake context, there is evidence for the growing importance of this alternative N processing mechanism in aquatic ecosystems (Burgin and Hamilton 2007).

When considering only the five streams with significant uptake, we also found other relationships between the environmental variables and uptake metrics. As expected, U_{amb} tend to increase with the water NO_3^- concentration across sites (Hall et al. 2009), but this relationship was more robust with the NO_3^- sediment content. Uptake studies have rarely provided sediment NO_3^- measurements since the water NO_3^- concentration directly controls the magnitude of U_{amb} values. Although the NO_3^- in both compartments might be linked in some stream sites, the NO_3^- stored in sediments might also be a useful variable to explain the inter-site variability of nutrient uptake, which occurs mostly in the streambed. Unexpectedly, we also found that $V_{\text{f-amb}}$ increased with increasing Q. This result suggests a strong hydraulic influence on this uptake metric (Doyle et al. 2003), with higher NO_3^- demand in relation to supply, in the streams with a higher flow. This result can at least be partially explained by the low variation in the reach-scale uptake length ($S_{\text{w-amb}}$) among streams, despite the relatively strong differences in hydraulic parameters (i.e. discharge, width).

Water NO_3^- concentration was related to the across-site variability in denitrification rates as widely reported (e.g. Mulholland et al. 2008). The importance of NO_3^- for explaining the variation of this biological process has also been observed within streams through enrichment experiments (Herrman et al. 2008). In a previous study performed in the stream PA, denitrification was found to be NO_3^- limited (Arce et al. 2013), a fact that explained the undetected sediment denitrification rates we observed in this site at ambient concentrations. In addition, as denitrification is probably coupled strongly to nitrification in PA (Seitzinger et al. 2006), the plausible negative side effects of acetylene on denitrification via nitrification inhibition during the lab assays can help to explain the undetected rates in this low NO_3^- stream. Denitrification also increased in accordance with Cl*a*, indicating a possible link between denitrification and stream biofilms (Kemp and Dodds 2002). However, the positive correlation between Cl*a* and water NO_3^- did not allow us to assert the influence of the biofilm itself on the variation of this process.

Conclusions and Implications

As far as we know, this is the first study that has evaluated whole-reach NO_3^- processing across a gradient of semiarid saline streams. One important finding was that water salinity is not a factor that constrains stream denitrification rates and uptake of added NO_3^-. One plausible explanation for this result is that the organisms in these environments are already adapted to salinity, and once they have adapted, other factors can limit nutrient transformation rates. One alternative explanation is that the salinity gradient covered in our study was not wide enough to detect a feasible effect. The results from this study show that there is a wide variability in the potential contribution of semiarid streams in regulating additional NO_3^- fluxes within the catchment ranging from streams that have an influential role to those that have a limited control on the fate of external inputs.

Despite the magnitude of the denitrification rates having increased in proportion to water NO_3^-, our results provide evidence that denitrification plays a minimal role in the whole-reach uptake of this nutrient, and that alternative pathways to this process, such as assimilatory uptake or dissimilatory NO_3^- reduction to NH_4^+, can control the variation we observed in relation to NO_3^- demand. If denitrification is an insignificant sink for NO_3^- in these streams (up to 2.16%), it will mean that the large NO_3^- fraction that is removed from the water column is only temporarily stored and can be occasionally exported downstream in organic and remineralized forms (Arango et al. 2008; von Schiller et al. 2009).

N supply must be seriously managed in semiarid catchments when considering these small-sized streams as potential hotspots for N processing within the landscape. Once N is delivered to streams, the ecosystem itself plays a critical role in modifying N fluxes (Alexander et al. 2009). Thus, the magnitude to which the system responds with permanent NO_3^- loss (denitrification) or temporal storage (assimilatory uptake) in part determines the degree of stream alteration by the excess NO_3^- load (Hall et al. 2009). Even small changes in the N retention rates, albeit temporarily through assimilatory uptake, as suggested, may translate into large changes in the N flux to downstream ecosystems (Mulholland et al. 2008). Therefore, management plans in semiarid catchments should focus on strategies to improve the ability of these low-discharge streams to control NO_3^- outputs. Management efforts should focus on: i) reducing excess NO_3^- inputs from terrestrial upland; ii) avoiding major loss of discharges through water derivation or groundwater extraction since it may result in NO_3^- saturation stages for the stream ecosystem due to impairments in the dilution capacity; and iii) maintaining the scarce riparian vegetation that provides the source of streambed organic matter stocks to support higher nutrient assimilatory stream compartments and to also trigger denitrification by providing anoxic places (Seitzinger et al. 2006). This last aspect would increase the relative contribution of denitrification to whole-stream uptake and would optimize the NO_3^- stream removal capacity in semiarid streams (Arce et al. 2013).

Given the expected increase in semiarid and arid-type streams due to climate change and the gap of knowledge on water salinity evolution for both freshwater and saline streams, more research is needed in these stream types to understand several issues. First, it is important to deal with the key question if these streams can always retain additional NO_3^- or not, and how this relative role varies throughout spatial and temporal scales. Second, more accurate denitrification measurements are needed to ensure the apparent small contribution of this mechanism to N retention. Finally, a more thorough assessment of salinity effects on stream N processing should be made since our current knowledge deriving from either field or lab isolated studies is still incomplete (Santoro 2010). Filling these gaps will provide valuable contributions to our overall understanding of the functioning of these abundant, yet still unknown ecosystems, and to foresee the possible consequences of global change on stream nutrient processing.

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References

Alexander RB, Böhlke JK, Boyer EW, David MB, Harvey JW, Mulholland PJ, Seitzinger SP, Tobias CR, Tonitto C, Wollheim WM (2009) Dynamic modeling of nitrogen looses in river networks unravels the coupled effects of hydrological and biogeochemical processes. Biogeochemistry 93:91-116

Álvarez-Cobelas M, Rojo C, Angeler DG (2005) Mediterranean Limnology: current status, gaps and the future. J Limnol 64:13-29

Arango CP, Tank JL, Johnson LT, Hamilton SK (2008) Assimilatory uptake rather than nitrification and denitrification determines nitrogen removal patterns in streams land use. Limnol Oceanogr 53:2558-2572

Arce MI, Gómez R, Suárez ML, Vidal-Abarca MR (2013) Denitrification rates and controlling factors in two agriculturally influenced temporary Mediterranean saline streams. Hydrobiologia 700:169-185

Arribas P, Gutiérrez-Cánovas C, Abellán P, Sánchez-Fernandez D, Picazo F, Velasco J, Millán A (2009) Tipificacion de los rios salinos ibericos. Ecosistemas 18:1-13

Baldwin DS, Rees G N, Mitchell A M, Watson G, Williams J (2006) The short-term effects of salinization on anaerobic nutrient cycling and microbial community structure in sediment from a freshwater wetland. Wetlands 26:455-464

Baldwin DS, Williams J (2007) Differential release of nitrogen and phosphorus from anoxic sediments. Chem Ecol 23:243-249

Baker MA, Valett HM, Dahm CN (2000) Organic carbon supply and metabolism in a shallow groundwater ecosystem. Ecology 81:3133-3148

Belmar O (2013) Ecological Basis for Assessing Environmental Flow Regimes in the Segura Basin. Dissertation, University of Murcia

Belnap J, Welter JR, Grimm NB, Barger N, Ludwig JA (2005) Linkages between microbial and hydrologic processes in arid and semiarid watersheds. Ecology 86:298-307

Bernhardt ES, Likens GE, Hall Jr RO, Buso DC, Fisher SG, Burton TM, Meyer JL, Mcdowell WH, Mayer MS, Bowden MW, Findlay SEG, Macneale KH, Stelzer RS, Lowe WH (2005) Can´t see the forest for the stream? In-stream processing and terrestrial nitrogen exports. BioScience 55:219-230

Bernot MJ, Dodds WK (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 8:442-453

Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front Ecol Environ 5:89-96

Cañedo-Argüelles M, Kefford BJ, Piscart C, Prat N, Schäfer RB, Schulz C (2013) Salinization of rivers: An urgent ecological issue. Environ Pollut 173:157-167

CHS (2007) Estudio general sobre la Demarcación Hidrográfica del Segura. Confederacion Hidrográfica del Segura. Ministry of Environment. Murcia, Spain

Cooper SD, Lake PS, Sabater S, Melack JM, Sabo JL (2013) The effects of land use changes on streams and rivers in Mediterranean climates. Hydrobiologia 719:383-425

Covino TP, McGlynn B, Baker M (2010a) Separating physical and biological uptake kinetics from ambient to saturation in successive mountain stream reaches. J Geophys Res 115:G04010

Covino TP, McGlynn BL, McNamara RA (2010b) Tracer Additions for Spiraling Curve Characterization (TASCC): quantifying stream nutrient uptake kinetics from ambient to saturation. Limnol Oceanogr 8:484-498

Doyle MW, Stanley EH, Harbor JM (2003) Hydrogeomorphic controls on phosphorus retention in streams. Water Resour Res 39:1147

Earl SR, Valett HM, Webster JR (2006) Nitrogen saturation in stream ecosystem. Ecology 87:3140-3151

Gardner W S, McCarthy M J, An S, Sobolev D, Sell K S, Brock D (2006). Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. Limnol Oceanogr 51:558-568

Gasith A, Resh VH (1999) Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Sys 30:51-81

Gómez R, Hurtado I, Suárez ML, Vidal-Abarca MR (2005) Ramblas in south-east Spain: threatened and valuable ecosystems. Aquatic Conserv Mar Freshw Ecosyst 15:387-402

Goodale CL, Thomas SA, Fredriksen G, Elliott EM, Flinn KM, Butler TJ, Walter MT (2009) Unusual seasonal patterns and inferred processes of nitrogen retention in forested headwaters of the Upper Susquehanna River. Biogeochemistry 93:197-218

Gordon ND, McMahon TA, Finlayson BL (1992) Stream hydrology: an introduction for ecologists. John Wiley and Sons, West Sussex

Grimm NB, Sheibley RW, Crenshaw CL, Dahm CN, Roach J, Zeglin LH (2005) N retention and transformation in urban streams. J N Am Benthol Soc 24:626-642.

Gutiérrez-Cánovas C, Velasco J, Millán A (2009) Effects of dilution stress on the functioning of a saline Mediterranean stream. Hydrobiologia 619:119-132

Hall Jr RO, Tank JL, Sobota DJ et al (2009) Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: total uptake. Limnol Oceanogr 54:653-665

Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C, Swadling K (1991) A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia 210:105-144

Herrman K, Borchard V, Moore RH (2008) Factors affecting denitrification in agricultural headwater streams in Northeast Ohio, USA. Hydrobiologia 598:305-314

Hunter RG, Faulkner SP (2001) Denitrification potentials in restored and natural bottomland hardwood wetlands. Soil Sci Soc Ac J 65:1865–1872

Inwood SE, Tank JL, Bernot MJ (2005) The influence of land use on sediment denitrification in 9 midwestern streams. J N Am Benthol Soc 24:227-245

IPCC (Intergovernmental Panel on Climatic Change) (2007) Fourth assessment report. Climatic change 2007: climatic change impacts, adaptation and vulnerability. http://www.ipcc.ch/

Kemp MJ, Dodds WK (2001) Centimeter-scale patterns in dissolved oxygen and nitrification rates in a prairie stream. J N Am Benthol Soc 20:347-357

Kemp MJ, Dodds WK (2002) The influence of ammonium, nitrate, and dissolved oxygen concentration, and denitrification rates associated with prairie stream substrata. Limnol Oceanogr 47:1380-1393

Knowles R (1990) Acetylene inhibition technique: Development, advantages, and potential problems. In: Revsbech NP, Sorensen J (eds) Denitrification in soil and sediment. Plenum Press, New York, pp 151-166

Kulp TR, Han S, Saltikov CW, Lanoil BD, Zargar K, Oremland RS (2007) Effect of imposed salinity gradients on dissimilatory arsenate reduction, sulphate reduction and other microbial processes in sediments from two California soda lakes. Appl Environ Microb 73:5130-5137

Magalhâes CM, Joye SB, Moreira RM, Wiebe WJ, Bordalo AA (2005) Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. Water Res 39:1783-1794

Martí E, Grimm NB, Fisher SG (1997) Pre-and post-flood retention efficiency of nitrogen in a Sonoran Desert stream. J N Am Benthol Soc 16:805-819

Millán A, Velasco J, Gutiérrez-Cánovas C, Arribas P, Picazo F, Sánchez-Fernandez D, Abellán P (2011) Mediterranean saline streams in southeast Spain: What do we know? J Arid Environ 75:1352-1359

Mulholland PJ, Hall RO, Sobota JD, Dodds WK, Findlay SEG, Grimm NB, Hamilton SK, McDowell WH, O'Brien JM, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Gregory SV, Johnson SL, Meyer JL, Peterson BJ, Poole GC, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Helton AM, Johnson LT, Niederlehner BR, Potter JD, Sheibley RW, Thomas SM (2009) Nitrate removal in stream ecosystems measured by 15N addition experiments: denitrification. Limnol Oceanogr 54:666-680

Mulholland PJ, Helton AM, Poole GC, Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SE, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Niederlehner BR, O'Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202-205

Mulholland PJ, Valett HM, Webster JR, Thomas SA, Cooper LW, Hamilton SK, Peterson BJ (2004) Stream denitrification and total nitrate uptake rates measured using ^{15}N tracer addition approach. Limnol Oceanogr 49:809-820

Mulvaney RL (1996) Nitrogen-Inorganic forms. In: Sparks DL (ed) Methods of soil analysis: chemical methods. SSSA Book Series 5. Soil Science Society of America and American Society of Agronomy, Madison, pp 1123-1184

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Nielsen DL, Brock MA, Rees GN, Baldwin DS (2003) Effects of increasing salinity on freshwater ecosystems in Australia. Aust J Bot 51:655-665

Niyogi DK, Bandeff JM, Selman C, Menke DE (2010) Nutrient flux, uptake and transformation in a spring-fed stream in the Missouri Ozarks, USA. Aquat Sci 72:203-212

O'Brien JM, Dodds WK, Wilson KC, Murdock JN, Eichmiller J (2007) The saturation of N cycling in Central Plains streams: ^{15}N experiments across a broad gradient of nitrate concentrations. Biogeochemistry 84:31-49

Oren A (1999) Bioenergetic aspects of halophilism. Microbiol Mol Biol Rev 63:334-348

Payn RA, Webster JR, Mulholland PJ, Valett HM, Dodds WK (2005) Estimation of stream nutrient uptake from nutrient addition experiments. Limnol Oceanogr 3:174-182

Royer TV, Tank JL, David MB (2004) Transport and fate of nitrate in headwater agricultural streams in Illinois. J Environ Qual 33:1296-1304

Rysgaard S, Thastum P, Dalsgaard T, Christensen PB, Sloth NP (1999) Effects of salinity on NH_4^+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. Estuaries 2:21-30

Santoro AE (2010) Microbial nitrogen cycling at the saltwater-freshwater interface. Hydrogeol J 18:187-202

Seitzinger S, Harrison J A, Böhlke J K, Bouwman A F, Lowrance R, Peterson B, Tobias C, Drecht G V (2006). Denitrification across landscapes and waterscapes: a synthesis. Ecol Appl 16:2064-2090

Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Limnol Oceanogr 33:702-724

Seitzinger SP, Nielsen LP, Caffrey J, Christensen PB (1993) Denitrification measurements in aquatic sediments: a comparison of three methods. Biogeochemistry 23:147-167

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr 14:799–801

Sponseller RA, Heffernan JB, Fisher SG (2013) On the multiple ecological roles of water in river networks. Ecosphere 4:17

Steinman AD, Lamberti GA, Leavitt PR (2006) Biomass and pigments of benthic algae. In: Hauer FR, Lamberti GA (eds) Methods in Stream Ecology. Academic Press, San Diego, pp 357-379

Velasco J, Millán A, Hernández J, Gutiérrez C, Abellán P, Sánchez D, Ruiz M (2006) Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. Saline Systems 2:1-15

Velasco J, Millán A, Vidal-Abarca MR, Suárez ML, Guerrero C, Ortega M (2003) Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. Freshw Biol 48:1408-1420

Vidal-Abarca MR (1990) Los ríos de las cuencas áridas y semiáridas: una perspectiva ecológica comparativa y de síntesis. Scientia Gerundensis 16:219–228

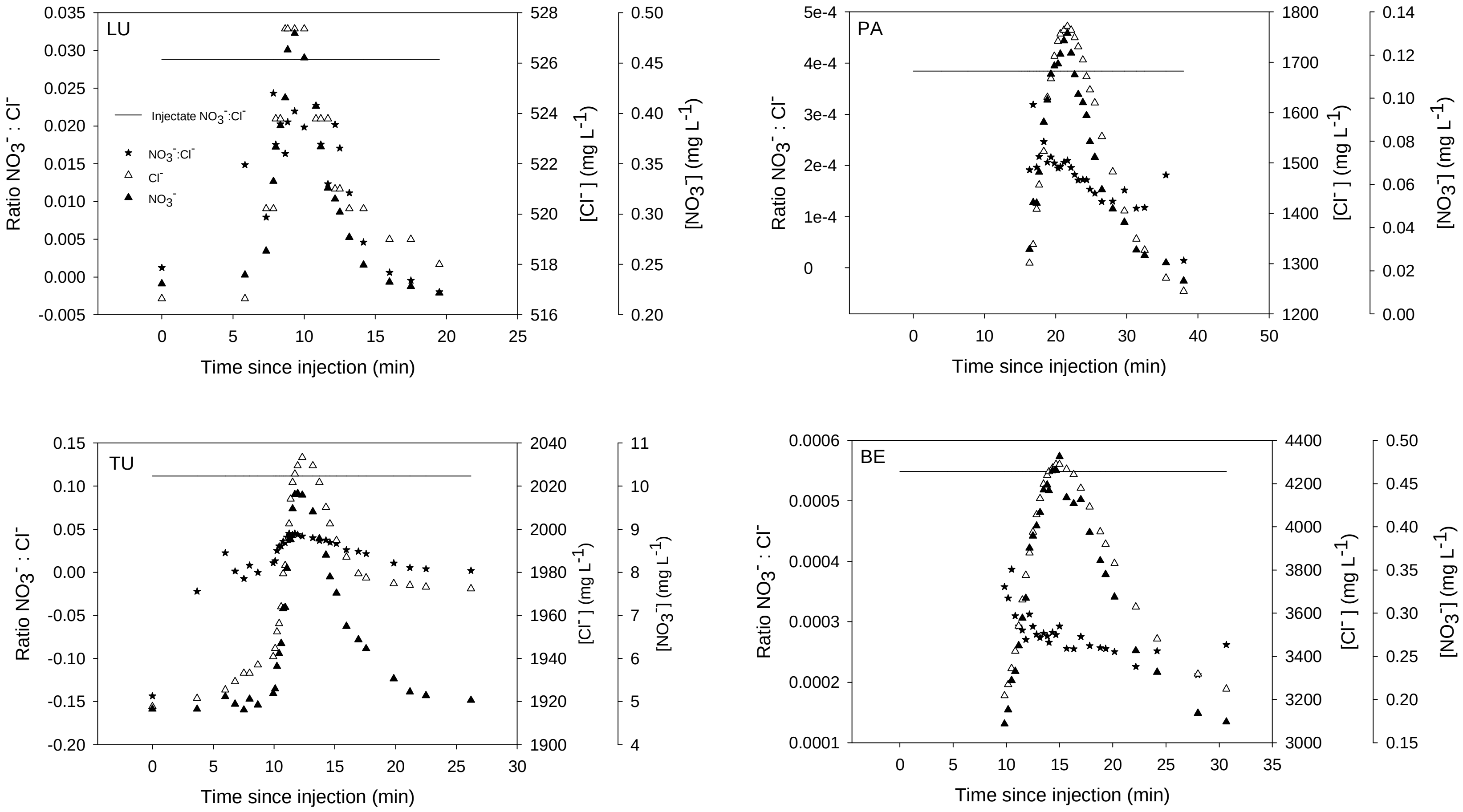
Vidal-Abarca MR, Suárez ML, Gómez R, Guerrero C, Sánchez-Montoya MM, Velasco J (2004) Inter-annual variation in benthic organic matter in a saline, semiarid stream of southeast Spain (Chícamo stream). Hydrobiologia 523:199-215

von Schiller D, Martí E, Riera JL (2009) Nitrate retention and removal in Mediterranean streams bordered by contrasting land uses: a ^{15}N tracer study. Biogeosciences 6:181-196

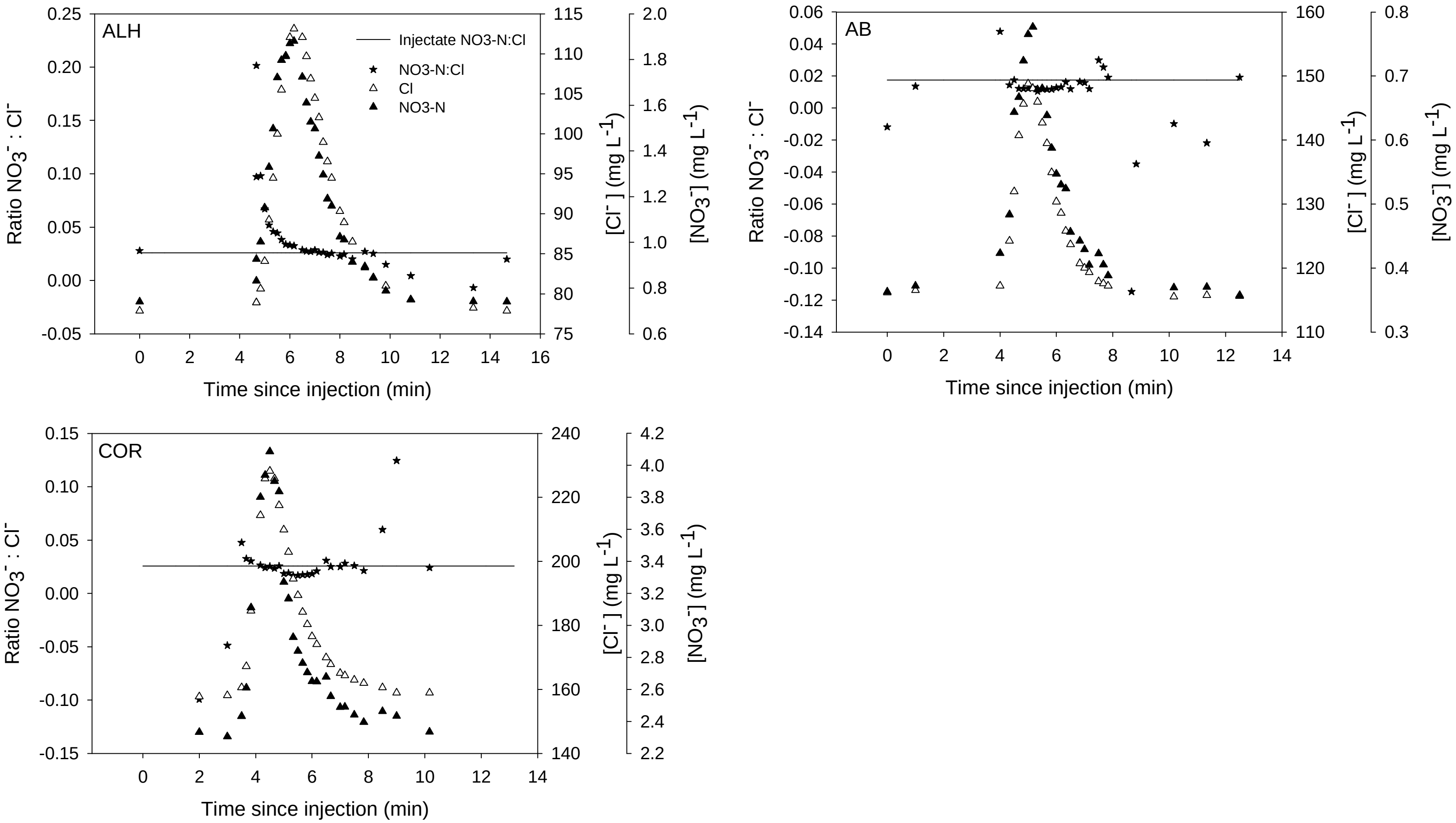
Webster JR, Valett HM (2006) Solute dynamics. In: Hauer FR, Lamberti GA (eds) Methods in Stream Ecology. Academic Press, San Diego, pp 169-185

Williams WD (1996) The largest, highest and lowest lakes of the world: saline lakes. Verhanlungen Internationale Vereiniging für Theoretische und Angewandte Limnologie 26:61-79

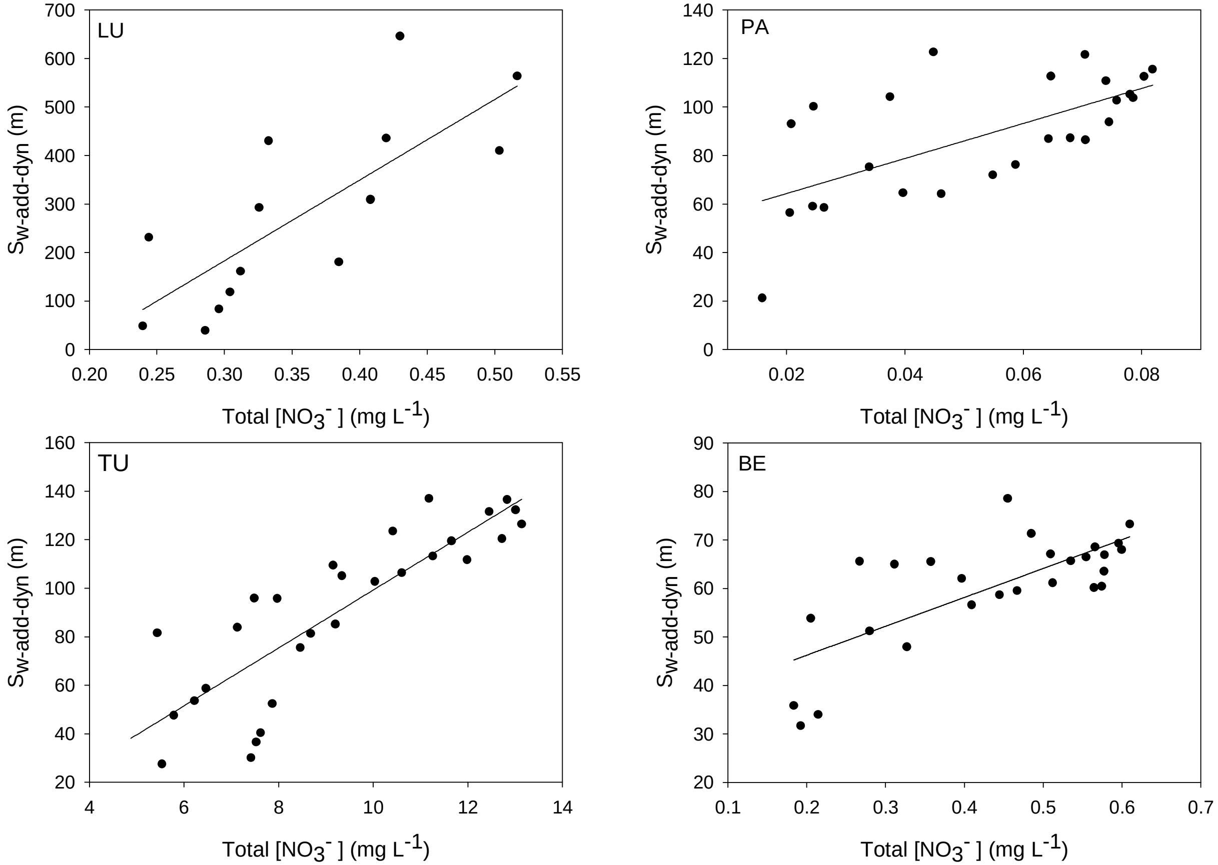
Wood ED, Armstrong FA, Richards FA (1967) Determination of nitrate in seawater by cadmium copper reduction to nitrite. J Mar Biol Assoc UK 47:23–31



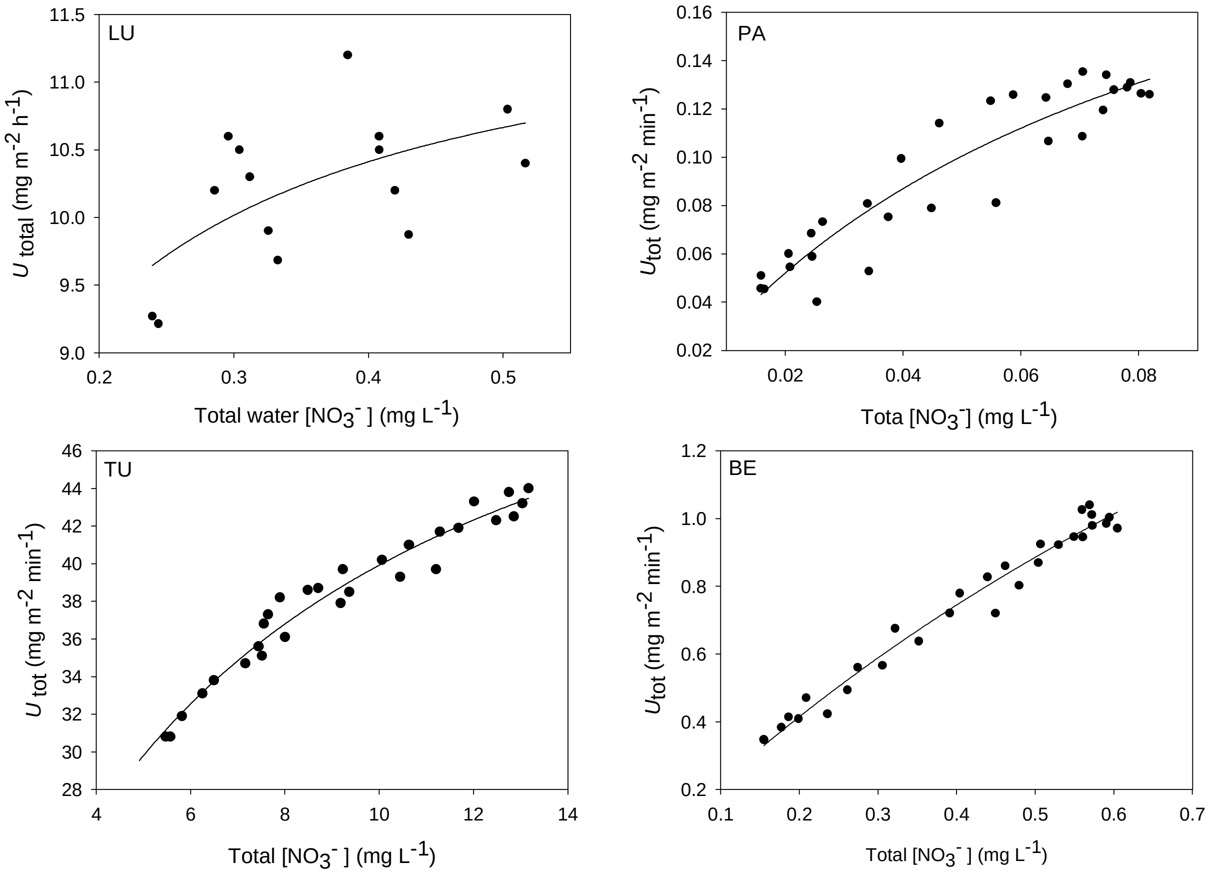
Annex 4.1. Time series of Cl^- and NO_3^- concentrations along with the changing NO_3^- : Cl^- sampled at the base of the study reach of sites showed uptake of added NO_3^- during the instantaneous slug additions. The injectate ratio (continuous line) added at the head of the experimental reach at time 0 is shown as reference for interpreting the changing ratio at the base of the reach.



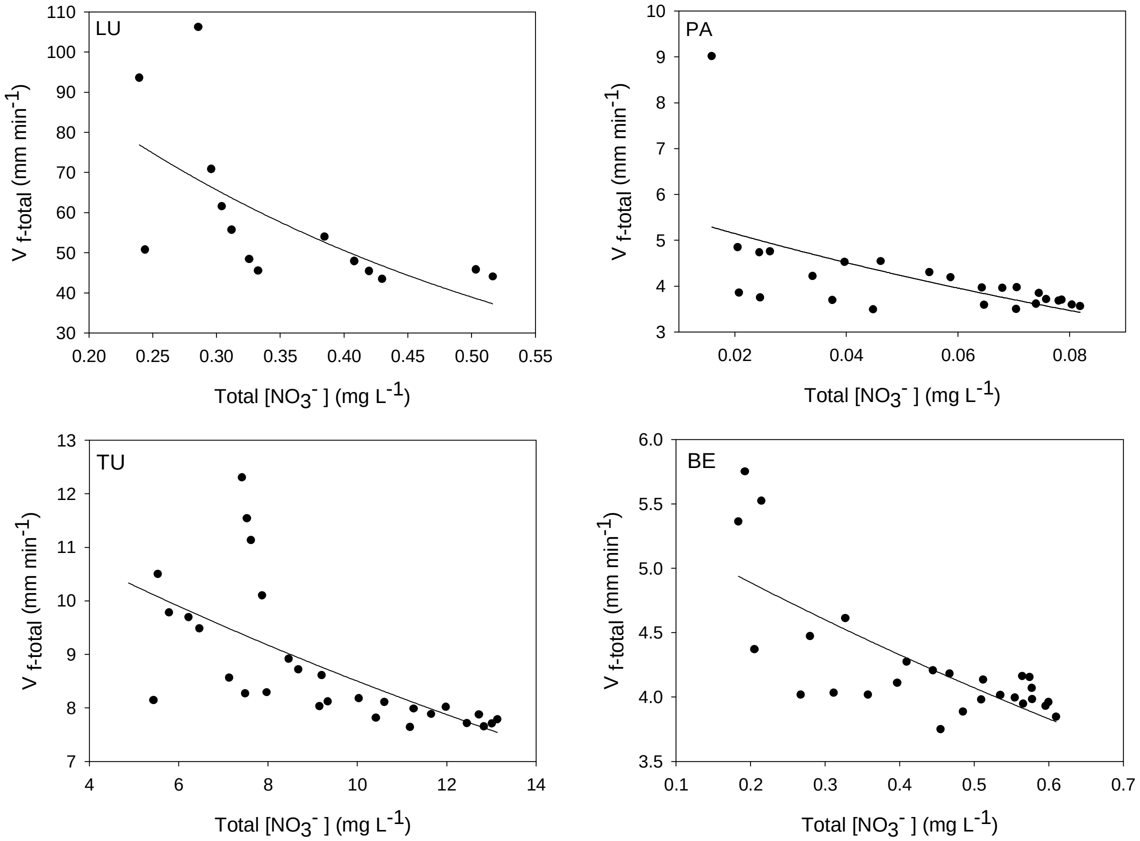
Annex 4.2. Time series of Cl^- and NO_3^- concentrations along with the changing NO_3^- : Cl^- sampled at the base of the study reach of sites showed no uptake of added NO_3^- during the instantaneous slug additions. The injectate ratio (continuous line) added at the head of the experimental reach at time 0 is shown as reference for interpreting the changing ratio at the base of the reach



Annex 4.3. Linear regressions of $S_{\text{w-add-dyn}}$ versus total [NO_3^-] to estimate ambient uptake lengths ($S_{\text{w-amb}}$) in the study sites showing uptake of added NO_3^- . Total [NO_3^-] is the geometric mean of conservative and observed total NO_3^- concentration.



Annex 4.4. Uptake curves of total NO$_3^-$ areal uptake rate (U_{tot}) as a function of total water [NO$_3^-$] in the study sites showing uptake of added NO$_3^-$. Total [NO$_3^-$] is the geometric mean of conservative and observed total NO$_3^-$ concentration in each grab sample.



Annex 4.5. Uptake curves of total NO_3^- uptake velocity($V_{\text{f-tot}}$) as a function of total water [NO_3^-] in the study sites showing uptake of added NO_3^- Total [NO_3^-] is the geometric mean of conservative and observed total NO_3^- concentration in each grab sample.

General Conclusions

General Conclusions

1. As widely described in other climatic regions, denitrifcation rates in semiarid streams increased following the gradient of increased agricultural influence, mostly in response to high water NO_3^- concentrations. Furthermore, stream reaches showing fine sediments rich in organic matter also supported higher denitrification rates by expanding anoxic environments.

2. Despite the fact that in agriculturally affected sites the excess of NO_3^- is "adjusted" by enhancing denitrifcation rates, chronic NO_3^- inputs likely minimize the effect of dentirification at impacting NO_3^- fluxes downstream. Thus, especial efforts should be done to reduce NO_3^- inputs in agriculturally affected watersheds.

3. Benthic organic matter stocks in semiarid streams can play an important role at controlling the magnitude of denitrification rates and thus, the effectiveness of this pathway at removing NO_3^- from water column, especially in NO_3^- rich-stream sites. Rather than provide a organic C source, local management plans that allows inputs of benthic organic matter to stream beds from riparian zones, would support "hot spots" for denitrification rates and nitrate removal by i) expanding anoxic sites; via stimulating aerobic respiration and ii) raising the opportunity of active sediments to process NO_3^- via increasing the stream water residence times.

4. The recovery of biogeochemical processes' capacity following rewetting of dry sediments, and time in which rates return to pre- drought levels likely depend on the local conditions associated to each stream ecosystem such as i) the dry-wet cycles history, ii) the grade of sediment desiccation during drought and iii) another streams factors driving microbial activities, such as nutrient status.

5. The rapid response of N processing rates to rewetting that temporary streams can show, must be considered seen as an essential ecosystem service provided temporary streams despite annual dry periods. Thus, the protection and conservation of conventionally, poorly recognized stream dry beds should be incorporated into the European water legislation (European Union WFD).

6. Annual intermittent flow can modulate water quality in temporary streams. Dry stream reaches can accumulate NO_3^- in desiccated sediments and act as potential sources of NO_3^- to downstream ecosystems if the flush of NO_3^- is not dampened by assimilatory or dissimilatory processes. Therefore, compared with permanent running waters, temporary streams experience natural dry-wet cycles, can exhibit an intrinsic high annual variability on water N concentrations.

7. Given the European Water Framework Directive (WFD) requirements to assess ecological status of freshwaters, these N pulses, which take place when flow resumes, should be considered when designing management plans in temporary watersheds. Water legislation that considers all streams similarly does not seem appropriate for temporary streams. A review of WFD criteria to define good ecological status for temporary streams is necessary, especially if we consider the future scenario of water scarcity conditions, in which the number of temporary streams is expected to increase worldwide.

8. In high-N intermittent reaches, short-term inundation that can take forms as pools in drybeds, can stimulate denitrification rates after a desiccation period. Stimulated denitrification can dampen a high proportion of NO_3^- in both water and sediment. However, a great part of NO_3^- can be transformed to NH_4^+, a less mobile form of DIN, yet it can achieve high and toxic concentrations. Unless NH_4^+ is back transformed to NO_3^- via nitrification, NH_4^+ can be delivered downstream in the subsequent flush flood.

9. At reach level, the role of semiarid streams at retaining additional NO_3^- was highly variable, from sites showing a great NO_3^- uptake efficiency and capacity to sites having an irrelevant function on the fate of this nutrient.

10. Despite denitrification rates tended to high, in the upper part of the reported range in stream literature, the potential contribution of denitrification to whole-reach NO3 uptake was minimal (≤ 2.16%).

11. Alternative pathways to denitrification, heterothrophic assimilation and desasimilatory NO_3^- reduction to NH_4^+ can have a substantial influence on modifying NO_3^- fluxes in semiarid saline streams.

12. Water salinity in a range of 0.5 to 13 mS cm^{-1} appears not to be involved in the spatial variation of denitrification rates and whole-reach NO_3^- uptake. However, salinity values close to 40 mS cm^{-1} can constraint denitrification rates once no one factor, such as NO_3^- and low redox, limits the mechanisms.

Resumen en Español

Resumen

En la actualidad, casi toda la información existente sobre el papel de los arroyos en la retención de nitrógeno (N), y los procesos biogeoquímicos implicados en esta, se ha obtenido en ríos de regiones templadas. Sin embargo, muy poco es sabido sobre la funcionalidad de los ecosistemas fluviales mediterráneos de ambientes semiáridos. Los resultados de la presente tesis doctoral aportan información sobre aspectos generales biogeoquímicos de ecosistemas fluviales, en general, y de ríos intermitentes y salinos, en particular. Las previsiones del cambio ambiental global ponen de manifiesto que uno de los efectos más importantes sobre los ecosistemas de aguas continentales será el aumento del estrés hídrico y, en consecuencia, el aumento de la intermitencia de flujo y la temporalidad hídrica en los ecosistemas acuáticos continentales siendo la región mediterránea la más vulnerable al cambio global en el contexto europeo. Paralelamente, uno de los efectos del estrés hidrico en muchos rios es el incremento de los valures de conductividad o salinidad del agua, como puede ocurrir en rios que ya son salinos de forma natural. En este sentido, los hallazgos de la presente tesis ayudar a entender las posibles consecuencias del cambio global sobre el funcionamiento de estos ecosistemas.

Objetivos

El objetivo general de este trabajo fue examinar la retención de N en ríos mediterráneos semiáridos y los factores que en potencia pueden controlan su variación a escala temporal y espacial.

En las regiones mediterráneas, las intensa actividad agrícola, la intermitencia de caudales y las condiciones naturales de salinidad en los suelos pueden afectar, tanto directa como indirectamente a la variación de la retención de N. De todos los procesos implicados en el ciclo del N, la desnitrificación, es el único proceso que puede eliminar N del ecosistema de forma permanente, gracias a que transforma el nitrato (NO_3^-) en N gas. En este sentido, cuantificar las tasas de desnitrificación en los ríos y examinar patrones espaciales y temporales de su variación es esencial para entender el funcionamiento de los ríos intermitentes como base para una gestión sostenible, especialmente en cuencas hidrológicas impactadas por la agricultura. Por tanto, una importante parte de esta tesis se centra en estudiar como agricultura, sequía y salinidad afectan a la variabilidad en las tasas de desnitrificación en ríos semiáridos.

Esta tesis doctoral ha empleado una combinación de estudios de campo y experimentales, además de aproximaciones conceptuales para llevar a cabo estos objetivos

La presente ha sido preparada en 4 estudios individuales, los cuales siguen una estructura cronológica y han sido escritos como trabajos independientes para ser publicados en revistas de impacto en el campo de la ecología acuática.
A continuación se detallan los objetivos específicos por cada uno de los capítulos de esta tesis:

Capitulo 1: Tasas de desnitrificación y factores implicados en dos ríos temporales, salinos e influenciados por la agricultura.

**Estudio publicado en Hidrobiologia (2013)

En este primer estudio, se midieron las tasas de desnitrificación en sedimentos de dos ríos intermitentes desde su cabecera hasta su desembocadura, siguiendo un gradiente de salinidad natural y de influencia agrícola. Además se midieron antes y después de la sequía. Este trabajo tuvo como objetivo evaluar como la desnitrificación varió en respuesta a estos cambios espaciales y temporales.

Capitulo 2: Implicaciones de la intermitencia del caudal en la disponibilidad de nitrógeno y su retención en un río Mediterráneo de cabecera.

**Estudio publicado en Aquatic Sciences (2013)

Este trabajo tuvo como objetivo evaluar la influencia de la sequía en la recuperación de las tasas del procesado biogeoquímico del N en sedimentos del lecho del río al re-humedecimiento. Para ello, este trabajo fue llevado a cabo en un río temporal sometidos a ciclos naturales de sequía y re-humedecimiento. Este estudio se centró, no solo en las tasas de desnitrificación, sino que también, analizó el proceso de nitrificación. Este proceso suele estar acoplado a la desnitrificación y es muy importante en ríos de cabecera prístinos que no suelen presentar elevadas concentraciones de NO_3^- en el agua. Además de estudiar estos dos procesos biogeoquímicos, en este estudio se evaluó como la intermitencia en el caudal puede afectar a la disponibilidad de materia orgánica y N contenido de los sedimentos del lecho del río.

Esta investigación de enmarcó dentro del proyecto Europeo (MIRAGE; FP7-ENV-2007-1), del 7º programa marco de la Unión Europea. El proyecto MIRAGE se desarrollo con el objetivo de estudiar la hidrología y la ecología de los ríos temporales, definir sus condiciones de referencia y de crear herramientas adecuadas para su correcta implementación en la Directiva Marco del Agua.

Capitulo 3: Disponibilidad de nitrógeno y tasas de desnitrificación tras el re-humedecimiento de sedimentos secos del lecho de un río Mediterráneo temporal con alto contenido de nitrógeno.

**Estudio en preparación para su posterior publicación

Los objetivos de este trabajo experimental se centraron en examinar los cambios en la concentración de N en el agua y en los sedimentos tras un periodo de re-humedecimiento e inundación de sedimentos secos de un tramo de río intermitente rico en N por la influencia de la agricultura. Paralelamente se evaluó la respuesta del proceso de desnitrificación al ese pulso de agua que generó el re-humedecimiento, y a lo largo del subsiguiente periodo de inundación.

Capitulo 4: Variación de la retención de nitrato y tasas de desnitrificación a lo largo de un gradiente de salinidad en ríos Mediterráneos semiáridos

**Publicado en Aquatic Sciences (2014)

En este trabajo se investigó la posible influencia de la salinidad sobre la variación de la retención de NO_3^-. En este sentido, se midieron las tasas de desnitrificación y la asimilación de nitrato a escala de tramo en 9 ríos de salinidad variable, desde ríos de agua dulce a ríos hiposalinos (salinidad $\leq$ 20 g L^{-1}). Adicionalmente, otras variables fueron analizadas en agua y sedimentos para evaluar su posible influencia sobre la retención de NO_3^-. Finalmente, se estimo la contribución del la desnitrificación al total de la retención de NO_3^- medida a nivel de tramo, para así evaluar la relevancia de este proceso desde una perspectiva funcional a una mayor escala.

Resumen del Capítulo 1:

En este trabajo se testo la hipótesis de que la agricultura, a través de su influencia en la disponibilidad de NO_3^- del agua en los ríos controla las tasas de desnitrificación en ríos salinos agrícolas y que, sin embargo, variaciones en la salinidad del agua no afecta a este proceso microbiano. En paralelo, también se testo el efecto de la influencia del estiaje de verano o la sequía sobre las tasas de desnitrificación. Ambos objetivos se evaluaron midiendo las tasas des desnitrificación (usando la técnica de inhibición por acetileno) en sedimentos de ríos siguiendo un gradiente espacial de influencia agrícola y salinidad, además de medirse bajo condiciones de pre-estiaje y post-estiaje.

Durante el pre-estiaje, la concentración de NO_3^- del agua fue el principal factor que explico la variación espacial de las tasas de desnitrificación. Junto con este nutriente, las condiciones redox del sedimento y la salinidad del agua parecieron ser factores importantes, este último, en particular, ejerciendo una influencia negativa. Durante el post-estiaje, una vez que se el caudal creció, las tasas de desnitrificación disminuyeron considerablemente y ningún factor medioambiental estuvo implicado en su variación espacial. Los resultados de este trabajo sugieren que tanto la salinidad del agua como el estiaje pueden limitar las tasas de desnitrificación en ecosistemas fluviales. Este resultado implica que ambas presiones naturales deben ser considerados en futuros planes de gestión de ríos con el fin de preservar el buen funcionamiento de estos ecosistemas en controlar los flujos de NO_3^-, especialmente en el contexto de cambio climático.

Resumen del Capítulo 2:

La mayoría de ríos y arroyos que drenan la cuenca Mediterránea son intermitentes o temporales. Como resultado de su régimen hidrológico, los ríos intermitentes están afectados por periodos de sequía y re-humedecimiento. La pérdida de agua en los sedimentos de los lechos puede alterar la disponibilidad de N y reducir las tasas de transformación de este nutriente tras el re-humedecimiento debido al efecto en las comunidades microbianas. El objetivo de este estudio fue analizar si el estiaje del río puede modificar las propiedades químicas de los sedimentos del lecho y si limita la respuesta de los procesos biogeoquímicos tras su re-humedecimiento. Para la consecución de este objetivo, se compararon diversas características de los sedimentos y las tasas de nitrificación y de desnitrificación entre un tramo permanente y otro intermitente, ambos localizados en el mismo río. Las variables de estudio se compararon entre tramos, durante un periodo húmedo (cuando ambos llevaron agua) y durante un periodo seco (cuando el tramo intermitente se seco).

Las tasas de nitrificación y de desnitrificación se analizaron mediante incubaciones en laboratorio de los sedimentos del lecho usando agua superficial del río de estudio. De esta forma se simulo un re-humedecimiento en los sedimentos secos del tramo intermitente durante el periodo seco.

Los resultados de este estudio indicaron que el estiaje parece incrementar el contenido de NO_3^- acumulado en los sedimentos. Por el contrario, la sequía no parece limitar la recuperación de las tasas de nitrificación y desnitrificación tras el pulso de agua y re-humedeciendo. Estos hallazgos sugieren que los tramos secos de los ríos pueden actuar como "fuentes" de NO_3^- tras la recuperación del flujo hídrico. Consecuentemente, esta variación natural en los ríos intermitentes o temporales en cuando a las concentraciones de NO_3^- del agua, deberían ser considerados para la gestión y la evaluación ecológica de los ecosistemas fluviales temporales en el contexto de la Directiva Europea Marco del Agua.

Por otro lado, este trabajo resalta la rápida respuesta que tienen los tramos secos para procesar y retener N, una vez el caudal es restablecido o incluso tras pequeños pulsos de agua (después de ligeras lluvias). Esta alta resiliencia debería pues, ser considerada como un servicio ecosistémico proporcionado por los ríos temporales, a pesar de los periodos de sequía intrínsecos en su ciclo hidrológico.

Resumen del Capítulo 3:

Las regiones de clima Mediterráneo predisponen a los ríos que discurren bajo su influencia a que presenten fuertes periodos de estrés hídrico. De madera que muchos de estos ríos llegan a secarse completamente durante largos periodos de tiempo. Es sabido que los cambios en las condiciones hidrológicas pueden afectar de manera significativa a los procesos biogeoquímicos que regulan la disponibilidad de N, situación que puede tener fuertes repercusiones para la retención global de NO_3^-, especialmente, cuando ríos temporales drenan paisajes agrícolas.

Durante el proceso se estiaje o sequía, los sedimentos quedan expuestos al aire, estimulando los procesos de mineralización del N, como la nitrificación, mientras que la desnitrificación se ve limitada a pequeñas zonas anóxicas en los sedimentos.

Ocasionalmente, estos periodos de sequía se ven interrumpidos por pulsos de agua que formar pozas inundando los sedimentos durante horas y días. En este estudio, se llevo a cabo un experimento de microcosmos para evaluar como la disponibilidad de N y las tasas de desnitrificación pueden variar en respuesta a dichos pulsos y a lo largo de un periodo de inundación. Usando sedimentos de un tramo de río intermitente y afectado por las actividades agrícolas, se simulo una inundación en pozas durante 14 días tras 3 meses de sequía en condiciones de microcosmos. Las tasas de desnitrificación y la concentración de N se midieron antes y a lo largo de la inundación a diferentes tiempos de muestreo.

Se observó que el re-humedecimiento y la inundación de los sedimentos secos estimulo rápidamente la capacidad desnitrificante. Mientras que un modesto incremento en la concentración de NO_3^- del agua fue observado las primeras 24 h de experimento, el NO_3^- del sedimento, sin embargo callo desde el inicio del re-humedecimiento. Este resultado indicó que una proporción importante del pool de NO_3^- del sedimento comienza a ser procesado tras el pulso de agua (parte de este vía desnitrificación), en lugar de ser liberado por completo a la columna del agua.

A pesar de esta reducción de N en forma de NO_3^- , un importante aumento en el contenido de NH_4^+ tanto en la columna del agua como en los sedimentos fue registrado. Esto indicó, que una fracción de NO_3^- puede transformándose a NH_4^+ bajo condiciones de anaerobiosis.

Este estudio demuestra que tras 3 meses de sequía, la funcionalidad de los ríos, en términos de retención de N, puede verse significativamente activa tras una pequeña inundación. Sin embargo, desde un punto de vista de calidad de agua, estas inundaciones que de forma ocasional tienen lugar, no parecen ser efectivas en términos de eliminación neta de N inorgánico del ecosistema fluvial. Los resultados de este trabajo destacan que planes de gestión de cauces a escala local de cuenca deben llevarse a cabo con el objetivo de reducir las concentraciones de N antes de que el agua de escorrentía llegue a los lechos fluviales secos.

Resumen del Capítulo 4:

Dentro de un paisaje terrestre, los arroyos o ríos de pequeño tamaño están considerados zonas de especial retención de nutrientes, como el NO_3^-. Esto es especialmente importante en zonas de clima seco, como las regiones áridas o semiáridas, dado que la generalizada baja disponibilidad de agua puede limitar las reacciones biológicas. En zonas áridas y semiáridas, muchos ríos son naturalmente salinos. Condiciones de elevada salinidad en el agua puede dar lugar a modificaciones en la estructura y función de los organismos acuáticos, y esta salinidad puede llegar a incrementar asociada al calentamiento global.

En este trabajo se estudio la retención de NO_3^- a escala de tramo de río y las tasas de desnitrificación en los sedimentos de 9 ríos de variable salinidad (desde ríos de agua dulce a hiposalinos) con el objetivo de testar si el procesado de NO_3^- se ve reducido a medida que incrementa la salinidad del agua.

Los resultados indicaron que de los 9 ríos estudiados, solo 5 fueron capaces de retener el NO_3^- añadido. Además 4 de esos 5 ríos fueron salinos. Sin embargo, la salinidad, como factor ambiental, no explico la variación observada en la retención de NO_3^- . Otras variables medioambientales estudiadas, como la disponibilidad de NH_4^+ y materia orgánica de los sedimentos, explicaron de forma significativa esta variación.

A diferencia de la retención a escala de tramo, la actividad desnitrificante fue detectada en todos los ríos, sin embargo, su variación espacial fue también independiente de la salinidad.

A pesar de que las tasas de desnitrificación tendieron a ser elevadas, según una comparación hecha con la literatura, su contribución en la retención de NO_3^- a escala de tramo fue irrelevante ($\leq 2.16\%$). Procesos alternativos a la desnitrificación, como la asimilación heterótrofa y/o la reducción desasimilatoria del NO_3^- a NH_4^+, podrían ser responsables de la variabilidad asociada a la retención de NO_3^- a escala de tramo. Este trabajo destaca que la función de los ríos como sumideros de NO_3^- adicional es altamente variable, y la salinidad no parece estar implicada en este papel.

Conclusiones Generales

1. Como ha sido ampliamente descrito en otras regiones climáticas, las tasas de desnitrificación en ríos semiáridos incrementaron siguiendo un gradiente de influencia agrícola creciente, mayormente, en respuesta al alto contenido de NO_3^- del agua. Además, aquellos tramos de río que mostraron sedimentos finos con elevado contenido en materia orgánica mostraron alta capacidad desnitrificante gracias a que favorecieron condiciones anóxicas, necesarias para este proceso.

2. A pesar de que los tramos agrícolas "ajustan" el exceso de NO_3^- por medio de elevadas tasa de desnitrificación, las entradas crónicas de NO_3^- a los ríos probablemente minimiza el control o la efectividad de este proceso en sentido de regular los flujos de este nutriente aguas abajo.

3. La materia orgánica bentónica en los ríos semiáridos puede desempeñar un papel fundamental en la magnitud de las tasas de desnitrificación y por tanto de la efectividad de este proceso en la retención de NO_3^- del agua, especialmente en sitios con niveles elevados, como los tramos agrícolas. Herramientas de gestión riparia a nivel local que aumenten las entradas de materia orgánica a los tramos, podrían optimizar zonas "hot spots" o de elevada capacidad, para el proceso de desnitrificación y la retención de NO_3^- favoreciendo: i) zonas anóxicas y ii) incrementando los tiempos de retención del agua sobre los sedimentos activos, así favoreciendo la oportunidad de este proceso para eliminar NO_3^-.

4. La recuperación de los procesos biogeoquímicos al re-humedecimiento tras la sequía y el tiempo en el cual las tasas alcanzan los niveles pre-estiaje, pueden depender de condiciones locales intrínsecas al ecosistema fluvial como i) la frecuencia natural de ciclos de intermitencia hídrica ii) del grado de desecación de los sedimentos durante el estiaje y iii) otros factores diferentes al hidrológico que puedan modular las funciones microbianas, como los niveles de nutrientes en el agua.

5. La rápida respuesta al re-humedecimiento que los ríos intermitentes o temporales pueden mostrar en términos de retención de N, debería ser considerado como servicio ecosistémico. De esta manera, es esencial la protección y la conservación de los tramos secos, muchas veces infravalorados por la sociedad y olvidados por las políticas de gestión y conservación de cauces.

6. La intermitencia de flujo hídrico anual puede modular la calidad del agua superficial en ríos temporales. Los tramos secos pueden almacenar NO_3^- en los sedimentos y así actuar como fuentes potenciales de este soluto hacia cuerpos de agua localizados aguas abajo si los procesos implicados en la transformación de NO_3^-. Por tanto, comparado con tramos o ríos de flujo permanente, los ríos intermitentes, afectados por ciclos naturales de sequía, pueden presentar una elevada variabilidad natural en las concentraciones de N que es intrínseca a sus características hidrológicas.

7. Dados los requisitos de la Directiva Europea Marco del Agua para la evaluación ecológica de los cuerpos de agua, estos pulsos de N deben ser considerados a la hora de evaluar el estado químico y ecológico de un río temporal. Una revisión de los criterios de la Directiva Marco es, por lo tanto, urgentemente necesaria, sobretodo, si se consideran que el numero de ríos intermitentes o temporales puede incrementar globalmente asociado al cambio climático.

8. En tramos intermitentes con alto contenido en N, las inundaciones temporales que toman forma de pozas durante el periodo de estiaje pueden estimular notoriamente las tasas de desnitrificación y por tanto de eliminación de NO_3^- del sistema. Sin embargo, hay que tener en cuenta que una parte de este NO_3^- puede ser transformado a NH_4^+, una fracción menos móvil, pero que en elevadas concentraciones pueden llegar a producir condiciones de toxicidad. A menos que este NH_4^+ sea transformado a NO_3^- vía nitrificación, ese NH_4^+ podría ser exportado aguas abajo si una inundación más fuerte tiene lugar a continuación.

9. A escala de tramo, el papel de los ríos semiáridos como sistemas de retención de NO_3^- adicional es altamente variable; desde sitios que muestran una alta eficiencia y capacidad de retención de este soluto, a tramos donde esta función es irrelevante.

10. A pesar de que las tasas de desnitrificación tendieron a ser elevadas, su contribución a escala de tramo en la retención global en ríos semiáridos fue mínima ($\leq 2.16\%$).

11. Mecanismos alternativos a la desnitrificación, como la asimilación por parte de organismos heterótrofos y la reducción desasimilatoria del NO_3^- a NH_4^+ podrían tener un importante papel en la función que tienen los ríos semiáridos, de controlar los flujos de NO_3^-, especialmente los de agua salina.

12. La salinidad del agua en un gradiente de 0.5 a 13 mS cm^{-1} no parece ejercer ningún papel en la variación de las tasas de desnitrificación y de la retención de NO_3^- a escala de tramo. Sin embargo, bajo condiciones favorables para la desnitrificación; buena disponibilidad de NO_3^- y carbono y bajos potenciales redox, valores de salinidad en torno a 40 mS cm^{-1} puede llegar a afectar negativamente a este proceso.