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Alternative Nitrate reduction pathways in sandy sediments hosting Submarine Groundwater Discharge (SGD)

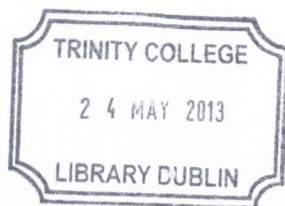
A Thesis Presented to the Academic Faculty
in Partial Fulfillment
of the Requirements for the Degree

Doctor of Philosophy

2012

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Trinity College Dublin



Thesis 10056

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Juan Severino Pino Ibánhez

Summary

Submarine Groundwater Discharge (SGD) is now recognized as an important transport vector of nutrients to the coastal ocean. The role of benthic sandy ecosystems in mitigating SGD NO_3^- loads to coastal marine ecosystems is uncertain. I used a combination of in-situ sampling, experiments under controlled conditions and deterministic modeling techniques in order to identify, quantify and parameterize key biogeochemical processes of N in permeable sediments hosting SGD and thus elucidate the mediation role of SGD seepage faces over N fluxes to the coastal ocean.

After appropriate tests performed over the techniques implemented in this study, benthic reactivity at the seepage face was explored. In order to separate the effects of reaction from those of transport on vertical porewater concentration profiles of NO_3^- at an intertidal groundwater seepage site (Ria Formosa, Portugal), a 1-D Advection-Dispersion-Reaction (ADR) model was used to describe the shape of NO_3^- concentration profiles collected in-situ. Model-derived apparent benthic nitrification and NO_3^- reduction rates (NRR) ranged from 0.01 to 5.2 $\text{mmol m}^{-2} \text{h}^{-1}$, sufficient to explain gross observed changes in NO_3^- fluxes arriving at the seepage face (up to 70 % within the surficial 20 cm depth). Results indicated that the upper limit of the seepage face promoted mitigation of NO_3^- fluxes to the lagoon throughout the year. In contrast, the lower limit of the seepage area promoted net amplification of the NO_3^- fluxes into the lagoon during the warmer months.

A combination of Flow-Through Reactor (FTR) experiments were performed with local sediment from Ria Formosa seepage face in order to further explore benthic reactivity over N fluxes. NRR were obtained by kinetic modeling of the results of FTR experiments and ranged from 0.23 to 1.42 $\text{mmol m}^{-2} \text{h}^{-1}$ thus confirming preliminary reactivity exploration. Furthermore, FTR experiments permitted to identify the presence of Dissimilatory Nitrate Reduction to Ammonium (DNRA) in processing SGD N loads. Experiments permitted to constrain the influence of temperature, oxygen, porewater velocity and organic matter content over NRR. The results show that porewater velocity can enhance net NO_3^- reduction but also that the process might ultimately be limited by benthic organic makeup and content.

Despite the low benthic organic matter content, significant aerobic respiration rates were measured on the top 32 cm at the seepage face ($4.92 \pm 0.57 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$). Advective velocity showed to enhance respiration rates, although under high imposed seepage rates benthic respiration is ultimately limited by organic C. Nevertheless, during active vertical seepage, local respiration rates were sufficient to develop an oxygen minimum zone near the sediment-water interface, thus favoring NO_3^- reduction processes. In these conditions, and during NO_3^- processing within the sediment, benthic reactivity showed to increase the degree of lability of the DOM. The presence of high concentrations of DIN and high discharge velocities act to catalyze the microbial breakdown of refractory organic material. The availability of DIN within SGD enhances the ability of extant microbes to process refractory organic matter, promoting its release in more labile forms. Results show the establishment of an internal positive feedback between benthic organic matter processing and NO_3^- reduction within the seepage face by counteracting previously identified organic matter limitation of NO_3^- reduction rates. Nevertheless, benthic enhancement of SGD N fluxes to the receiving waters is possible due to the presence of DNRA concomitant with the enhancement of organic N fluxes to the receiving waters.

*This work is dedicated to my family, and in particular my two brothers, Oscar and Brais,
always apart, forever together.*

*"The law, in its majestic equality, forbids the rich and the poor alike to sleep under bridges, to
beg in the streets, and to steal bread"*

Anatole France

"Eu son o neno pequeno que tirou o reloxo pola ventana para ver voar o tempo"

"Pepe da Jicha" Faro

"Moto i tal"

Anonymous

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Chapter III: Ibánhez, J.S.P., C. Rocha. Porewater sampling for NH_4^+ with Rhizon Soil Moisture Samplers (SMS): potential artifacts induced by NH_4^+ sorption. Submitted to *Limnology and Oceanography: Methods*.

Chapter IV: Ibánhez, J.S.P., C. Leote, C. Rocha, 2011. Porewater nitrate profiles in sandy sediments hosting Submarine Groundwater Discharge described by an Advection-Dispersion-Reaction (ADR) model. *Biogeochemistry*, doi:103(1-3):159-180. 10.1007/s10533-010-9454-1.

Chapter V: Ibánhez, J.S.P., C. Leote, C. Rocha, *in press*. Seasonal enhancement of Submarine Groundwater Discharge (SGD)-derived nitrate loading into the Ria Formosa coastal lagoon assessed by 1-D modeling of benthic NO_3^- profiles. *Estuarine, Coastal and Shelf Science*. doi: 10.1016/j.ecss.2012.04.015.

Chapter VI: Ibánhez, J.S.P., C. Rocha. Anthropogenic N pollution of a coastal aquifer drives carbon loss from Ria Formosa subterranean estuary. *Submitted to Limnology and Oceanography*.

Chapter VII: Ibánhez, J.S.P., C. Rocha. Processing of Inorganic Nitrogen at a NO_3^- -rich subterranean estuary seepage face: Processes and kinetics. *Submitted to Geochimica et Cosmochimica Acta*.

Chapter VIII. Ibánhez, J.S.P., C. Rocha. Organic matter reactivity at a NO_3^- -rich subterranean estuary seepage face: Implications for benthic modulation of Dissolved Inorganic N loads. *In preparation*.

Chapter I

General introduction

I.1 N in the environment

Nitrogen (N) is present in the biosphere in a wide range of compounds and oxidation states. N is involved in many reaction-transformation-transport patterns in nature that explain its relative high mobility within the ecosystems when compared with other major chemical elements like O, C or S (Vitousek et al. 2002). N is an essential element for life since all organisms need N for growth and it is incorporated into biomass in a relative consistent stoichiometric ratio with C and other essential elements (Redfield 1958). Since early 20th century, the discovery and application of the so-called Haber-Bosch process for NH_4^+ synthesis from atmospheric N_2 and subsequent use mainly as fertilizer in the form of NO_3^- has changed human influence over the global N cycle (Fig. I.1). Because N_2 is an inert gas for most organisms, the application of the Haber-Bosch process is an anthropogenic source of bioavailable N to natural ecosystems. Human activities now introduce more bioavailable N into the biosphere than the remaining Earth's ecosystem, thus enhancing both its availability and mobility within the ecosystems (Vitousek et al. 1997; Vitousek et al. 2002; Galloway et al. 2004). Application of the Haber-Bosch process at an industrial scale is the main anthropogenic activity in introducing bioavailable N in the ecosystems. However other sources such as the cultivation of N-fixing crops or biomass and fossil fuel combustion and subsequent deposition also contribute largely to the actual human-derived reactive N budget (Fig. I.1; Dalton and Brand-Hardy 2003; Fields 2004; Galloway et al. 2004). N excess can promote several environmental problems with direct and indirect impacts over human activities and health. NO_3^- and NO_2^- excess in drinking water has been related with human health problems, like the infant methemoglobinemia or digestive system cancer (Powlson et al. 2008). In addition, toxicity of N compounds in aquatic animals, acidification of freshwater reservoirs and coastal waters, increase in the atmospheric concentration of N greenhouse gases or disruption of the trophic status and degradation of coastal waters are also major effects of N enrichment of natural ecosystems (Vitousek et al. 1997; Galloway et al. 2003; Camargo et al. 2005; Camargo and Alonso 2006).

Anthropogenic N enrichment of natural ecosystems has promoted an exponential increase of N fluxes to the coastal zone (Mackenzie et al. 2002; Smith et al. 2003). Furthermore, projections based on actual trends suggested to double N fluxes to the coastal sea

by 2050 (Seitzinger et al. 2002). N and P availability controls atmospheric C sequestration by marine phytoplankton and determines its exportation and related elements from the surficial waters (Falkowski 1997; Mackenzie et al. 2002). Governmental policies implemented during the 70's and 80's have centred in controlling P fluxes to the coast. As a consequence, recent reviews showed that, at least in most temperate coastal waters, N seems to be now the main nutrient limiting primary producers growth (Howarth and Marino 2006). Increasing N fluxes to the coastal waters can enhance their elevated primary production, promoting the likelihood of trophic crisis events, harmful algal blooms occurrence, accumulation of organic matter, enhancement of oxygen consumption, reduction in water quality and lose of biodiversity (Falkowski 1997; Rabouille et al. 2001). Nutrient enrichment of coastal waters has been shown as the main cause of increasing eutrophication events reported worldwide (Smith et al. 2003; Rabalais et al. 2009). Eutrophication has been shown as responsible for the exacerbation of some of the hypoxia/anoxia events (Dead zones) registered in many parts of the world oceans like the Gulf of Mexico, Arabian Sea and many other coastal and estuarine ecosystems (Diaz and Rosenberg 2008). As a result, mass mortality of vertebrates and changes in ecological structure of these ecosystems has been verified (e.g. Joyce 2000; Diaz and Rosenberg 2008; Rabalais et al. 2009). In this sense, projected environmental changes can enhance the impact of human-derived reactive N in the coastal zone. Acceleration of the hydrological cycle can enhance N fluxes to the coastal zone and thus eutrophication events. This together with increasing seawater temperatures and stratification, will have direct impact in dissolved oxygen concentration in the oceans (Rabalais et al. 2009; Keeling et al. 2010). Thus, higher N fluxes in combination with increasing temperatures can increase the likelihood and persistence of the so-called "Dead zones" (Diaz and Rosenberg 2008).

In order to prevent further deterioration of coastal ecosystems and in anticipation of the negative enhancing effects of global change, control over reactive N introduction in ecosystems has been requested by the scientific community (e.g. Galloway et al. 1995; Camargo and Alonso 2006; Howarth and Paerl 2008; Rabalais et al. 2009). In this sequence, European Union has introduced specific regulations concerning the reactive N levels in inland and coastal waters through the Nitrate Directive (1991) and the European Union Water Framework Directive (2000) (Council of the European Communities 1991; 2000).

Understanding and being able to predict the distribution, reactive patterns and fluxes of reactive N compounds in the ecosystems is of scientific, social and strategic importance.

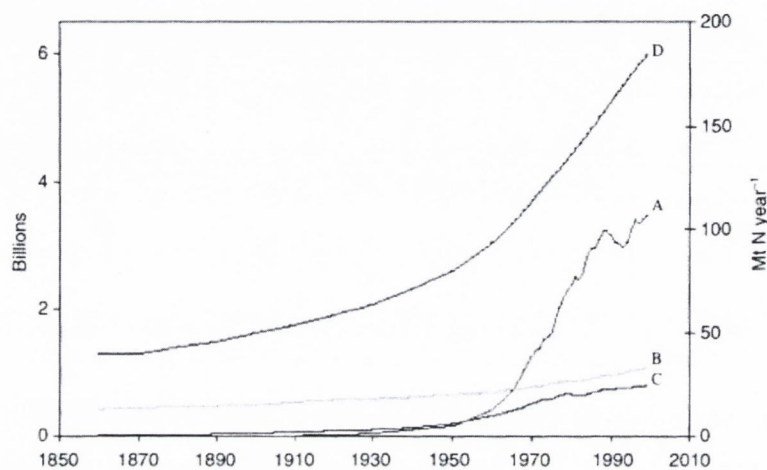


Figure I.1 Evolution of anthropogenic N production and human population since the industrial revolution. A. N synthesis through the Haber-Bosch process. B. N fixation through cultivation of N-fixing crops. C. N production through fossil fuel combustion. D. Human population. Adapted from Dalton and Brand-Hardy (2003).

I.2 N sources to the coastal zone

The main sources of anthropogenic N to the oceans are atmospheric deposition and those directly linked to human activities in the near-shore, like aquaculture, waste water treatment or agricultural run-off which can enter into the coastal zone by direct disposal or riverine and subsurface transport (Smith and Atkinson 1994). Atmospheric deposition has been estimated to account for 30% of the external N input to the oceans and is an important source of reactive N mainly to the open ocean (Duce et al. 2008). The remaining 70% of the external N inputs enters the oceans through coastal areas (Mackenzie et al. 2002). Since point sources of N to the coastal zone such as riverine transport have received considerable attention, the role of other diffuse sources such as subsurface transport has only been explored recently (Moore 1999; Taniguchi et al. 2002; Gallardo and Marui 2006). The difficulty in

identifying and quantifying groundwater discharges and their spatial and temporal variability explain the lack of knowledge of this fresh water source to the sea (Taniguchi et al. 2002; Burnett et al. 2003; Burnett et al. 2006). Nevertheless, global estimations now suggest that groundwater represent in between 5 and 10% of the total freshwater discharge to the coast (Burnett et al. 2003; Slomp and Van Cappellen 2004). Thus, groundwater can be locally an important source of freshwater and associated solutes to the coastal zone. Furthermore, nutrient concentrations in groundwater can be higher than those found in surface waters due to limited denitrification in aquifer sediments (Rivett et al. 2008), groundwater contribution to nutrient fluxes to the coastal zone can rival riverine inputs at a local level (Slomp and Van Cappellen 2004; Moore 2006a; Swarzenski et al. 2006). Thus, groundwater discharge to the coastal sea establishes an important, previously not recognized, linkage between land use and coastal waters.

N content of groundwater arriving to the coastal area is highly variable. High N concentrations in coastal aquifers are reported worldwide (Rivett et al. 2008), mainly in the form of NO_3^- due to agriculture fertilization and subsequent leakage into aquifers (Foster 2000). Other N sources such as sewage and mains leakage, septic tanks, industrial spillages or the use of manure and sewage sludge inland can also contribute to the N budget in coastal aquifers (Wakida and Lerner 2005). Thus, land use and infiltration rate strongly determines the NO_3^- content of groundwater. Subsurface transport, redox conditions and biogeochemical transformation into the aquifer can also modulate the final NO_3^- concentration arriving to the areas in contact with the ocean (Slater and Capone 1987; Bowen et al. 2007; Rivett et al. 2008). Heterotrophic denitrification is assumed to be the main biogeochemical pathway in mitigating NO_3^- content in aquifers (Rivett et al. 2008). Nevertheless, limited NO_3^- removal is likely to occur prior to arrival at the coastal zone mainly due to the lack of labile organic matter (Slater and Capone 1987; DeSimone and Howes 1996). Therefore, groundwater discharge can be an important source of bioavailable N to the coastal zone, and indeed the dominant source in some ecosystems (Cable et al. 1997; Ullman et al. 2003; Leote et al. 2008). Because of the potential impact on the trophic status of the receiving coastal waters, quantification of groundwater-derived nutrient loads to the coastal budget becomes therefore a

relevant scientific and social concern (Verhoeven et al. 2006; Bowen et al. 2007; Moore 2010).

I.3 Submarine Groundwater Discharge. Seepage faces

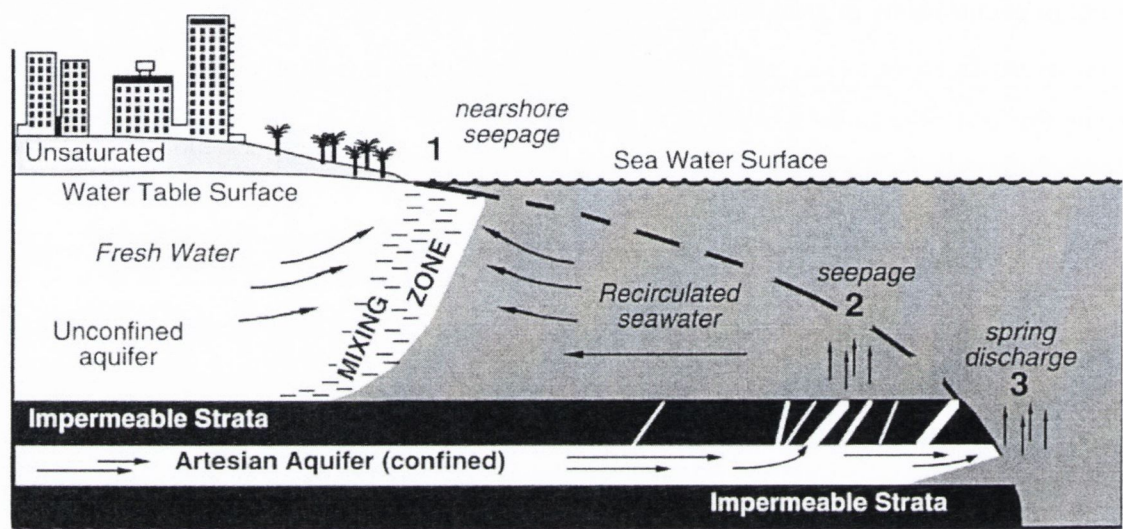


Figure I.2. Schematic view of mixing processes and discharge at the subterranean estuary, a mixing zone between coastal aquifers and sea water. Adapted from Burnett (2001).

Groundwater discharge to the coast occurs where ever a coastal aquifer is hydraulically connected to the sea with a positive head related with sea level (Fig. I.2; Johannes 1980). At the permeable exit point, seawater can infiltrate the aquifer, and the resulting mixed water seeping out at discharge sites usually contains a large proportion of recirculated sea water. The mixed water seeping out in the coast line is termed Submarine Groundwater Discharge by convention (SGD; Burnett et al. 2003). The magnitude of the discharge in terms of fresh water fluxes depends on factors like the hydraulic gradient between the aquifer and the sea or the transmissivity of the aquifer sediment (Johannes 1980). Other factors like the slope of the exit points and the permeability of the seepage face can also control the magnitude of the discharge (Robinson et al. 2007).

In sediment biogeochemistry, high quantities of organic matter and solutes were assumed as crucial for the establishment of significant reactions and fluxes and a dynamic ecology (Boudreau et al. 2001; Rocha 2008). Thus, the biogeochemical role of permeable sediments was ignored until recently. In cohesive sediments, the main solute transport processes are molecular diffusion, bioturbation and bioirrigation (Berner 1980). Other processes such as compaction, compression or dehydration can also be important solute transport processes in cohesive sediments. By contrast, in permeable sediments, the intrinsic permeability allows water movement through the pores induced by pressure gradients (Darcy's law; Darcy 1856). Permeabilities as low as $2 \times 10^{-12} \text{ m}^2$ were suggested as a threshold from which advection can take place (Huettel and Gust 1992; Forster et al. 1996; Glud et al. 1996). When advection is present, solute transport can be orders of magnitude higher than transport caused by diffusion alone (Huettel et al. 1998; Meile and Van Cappellen 2003; Rocha 2008). Surface water movement over permeable sediments can promote advective water interchange between overlying water and porewater. Surface waves (Webb and Theodor 1968; Shum and Sundby 1996; Precht and Huettel 2003) or unidirectional currents interacting with benthic topography (e.g. ripples, obstacles) increase the water flux across the sediment-water boundary (Riedl et al. 1972; Huettel et al. 1998; Røy et al. 2005).

Three main types of SGD are identified: nearshore seepage, offshore seepage and submarine springs (Fig. 1.2; Burnett 2001). In the nearshore, SGD often happens through the lower intertidal and subtidal area of a permeable beach, which is termed the seepage face. At SGD seepage faces, the volumetric discharge promoted by the hydraulic gradient between the aquifer and the sea can be enhanced by surface water movement like waves and tides through a pumping effect (Cartwright et al. 2004; Robinson et al. 2007). Density gradients can also promote water and solute exchange across the sediment-water interface in submerged SGD places. Differences in temperature due to aquifer thermal inertia and in salinity compared with sea water can promote convective transport orders of magnitude higher than diffusive transport (Rocha 2000; Simmons et al. 2001). Thus, convective and advective transport processes can promote high water movement across the sediment-water interface. In mesotidal zones, SGD usually happens, at least partially, through a narrow seepage face in the intertidal zone (e.g. Taniguchi et al. 2006; Leote et al. 2008). Therefore, cyclic sea water infiltration and

SGD seepage modulated by tide are the main mechanical processes controlling solute fluxes (Horn 2002; Rocha et al. 2009). At low tide, understauration of the higher intertidal levels together with the time lag between the aquifer freatic level and the tide determine the circulation pattern inside the sediment. Sea water infiltrates in the higher levels at flood tide and the mixed water seeps out in the lower intertidal area at ebb tide (Horn 2002; Robinson et al. 2007).

Advective motion in porous media is a crucial constrain in sediment reactivity. The heterogeneity of the porous media determines non-uniform water movement into permeable sediments. The macroscopic effect of this heterogeneous movement, mechanical dispersion, induces high mixing rates into the sediment, thus strongly affecting benthic solute distribution (Boudreau 1997; Roychoudhury 2001; Bijeljic and Blunt 2006). Furthermore, recently recognized permeable sediments reactivity can be enhanced by the advective movement of the water (Shum and Sundby 1996; Huettel and Rusch 2000; Rasheed et al. 2003; Rocha 2008). In intertidal permeable sediments affected by SGD, sea water infiltration can transport oxygen and labile organic matter in depth (Slomp and Van Cappellen 2004; Bowen et al. 2007; Santos et al. 2009). In addition, cyclic alternance of infiltration and seepage induced by the tide can promote oscillation of redox and oxygen conditions in the sediment which, in turn, can enhance microbial remineralization processes (Aller 1994; Sun et al. 2002). The high dynamism of permeable intertidal sediments determines the enhancement of reactive processes, thus explaining the lack of organic matter accumulation (Boudreau et al. 2001). As a consequence, organic matter content in sandy sediments is characterized by higher lability and shorter resident times when compared with cohesive sediments, thus promoting positive feedback over microbial heterotrophic processes (Boudreau et al. 2001; Rocha 2008).

I.4 Subterranean Estuary reactive zone

In 1999, Moore established the concept of Subterranean Estuary applied to the subsurface mixing zone between groundwater and sea water due to the similarities of this mixing area with surface estuaries. In subterranean estuaries, chemical reactions between the

mixed waters and the aquifer solids modify water composition (e.g. Postma et al. 1991; Moore 1999; Charette and Sholkovitz 2006). Furthermore, the salinity gradient established into the aquifer determines the distribution of important physical and chemical properties like redox potential or pH, biogeochemical solutes like oxygen or organic matter, and strongly determines the activity coefficients of solutes driven by ionic strength gradients (Slomp and Van Cappellen 2004; Charette et al. 2005; Charette and Sholkovitz 2006). Thus, biotic and non-biotic transformations in subterranean estuaries are likely to occur and can modulate the solute loads transported by groundwater to the coastal zone (Moore 1999).

Recent research has identified subterranean estuaries seepage faces as zones of important NO_3^- processing within the sediment prior to discharge (Nowicki et al. 1999; Ueda et al. 2003; Ullman et al. 2003; Bowen et al. 2007; Kroeger and Charette 2008; Spiteri et al. 2008; Rocha et al. 2009). Both non biotic and biotic processes modulating NO_3^- loads through SGD have been reported in the cited works. Nevertheless, recent work has shown that the expected mitigation role of seepage faces over NO_3^- loads to the receiving waters mainly through heterotrophic denitrification can be uncertain, since other pathways reducing NO_3^- can take place (Bowen et al. 2007; Kroeger and Charette 2008; Rocha et al. 2009). Indeed, enhancement of SGD NO_3^- fluxes at the seepage faces can be possible through alternative NO_3^- reduction pathways and marine organic matter remineralization (Rocha et al. 2009).

Heterotrophic denitrification is considered as the main pathway removing bioavailable N in the oceans (Seitzinger 1988). This pathway, the heterotrophic respiration process using NO_3^- as electron acceptor, has N_2 and N_2O as main end-products, thus effectively acts as a sink of bioavailable N. Heterotrophic denitrification has been already identified and quantified in SGD seepage faces (e.g. Slater and Capone 1987; Nowicki et al. 1999; Ueda et al. 2003). Nevertheless, the discovery of new NO_3^- reduction pathways during the last 15 years raised doubts about previous measurements assuming only heterotrophic denitrification presence and led to the actual global N cycle reformulation (Fig. I.3; Devol 2003; Hulth et al. 2005; Burgin and Hamilton 2007; Brandes et al. 2007; Galloway et al. 2008; Schlesinger 2009; Hanke and Strous 2010). One of these processes is the Anaerobic Ammonium Oxidation (ANAMMOX) process, first identified in fluidized bed reactors in The Netherlands in 1995 (Mulder et al. 1995). ANAMMOX evolves the reaction of both NH_4^+ and NO_2^- , producing N_2 (Dalsgaard

and Thamdrup 2002). Since NO_3^- is the most common oxidized form of N in sediments, NO_2^- can be produced as intermediary product of heterotrophic denitrification, or even through the dissimilatory oxidation of certain organic compounds by the ANAMMOX bacteria (Güven et al. 2005). This process has been identified in marine sediments (Dalsgaard and Thamdrup 2002; Trimmer et al. 2003), anoxic waters (Dalsgaard et al. 2003; Kuypers et al. 2005), arctic ice (Rysgaard and Glud 2004) and recently in SGD faces (Kroeger and Charette 2008). Recent estimations indicated that ANAMMOX could be responsible for up to 50% of the bioavailable N removal in the oceans (Dalsgaard et al. 2005). Thus, ANAMMOX is an important process removing bioavailable N from the ecosystems, but differs from denitrification in many relevant characteristics: ANAMMOX does not produce N_2O , a potent greenhouse gas, and it is an autotrophic process (Van De Graaf et al. 1996). Thus, organic matter presence and composition can be one of the key factors in controlling the relative rates of denitrification and ANAMMOX. Dependence of the ANAMMOX process on temperature and N availability has been also been reported (Dalsgaard and Thamdrup 2002).

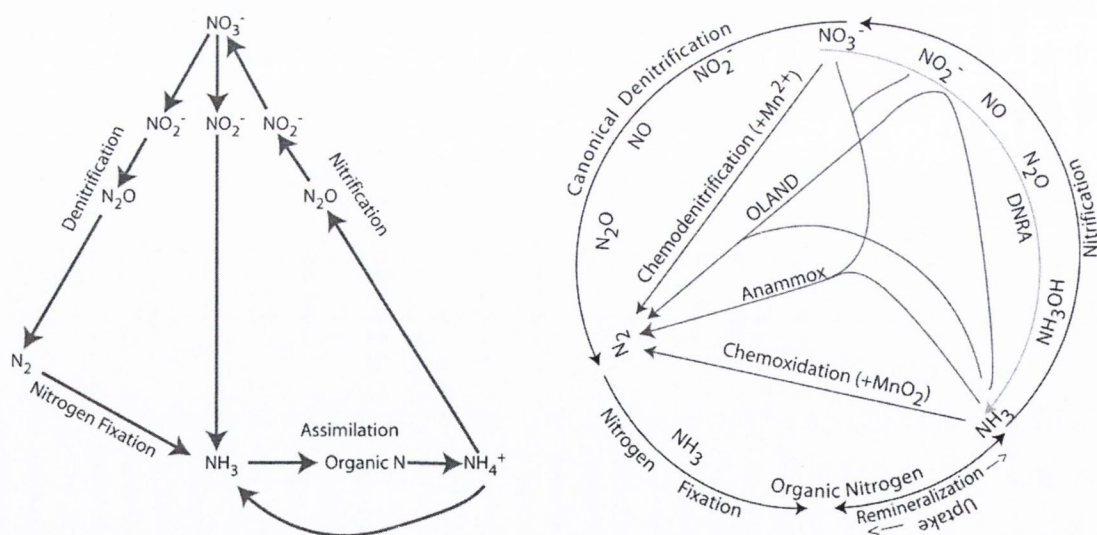


Figure I.3 The marine N cycle. Left. Classic view of main reactive pathways in marine environments. Right. Current diagram model of the revised marine N cycle. Adapted from Banded et al. (2007).

Another globally important NO_3^- reduction pathway suggested as potentially present at SGD seepage faces is the Dissimilatory Nitrate Reduction to Ammonium (DNRA; Rocha et al.

2009). This process is substantially different to the heterotrophic denitrification and ANAMMOX because the main end-product is NH_4^+ , thus recirculating N into available forms for the food chain (Bonin 1996). This fermentative process is being identified as an important process in estuaries (An and Gardner 2002; Dong et al. 2009; Dong et al. 2011), soils (Yin et al. 2002; Huygens et al. 2007), fresh water sediments (Kamp et al. 2006; Burgin and Hamilton 2008) and marine sediments (Christensen et al. 2000; Childs et al. 2002). Moreover, recent research suggested that DNRA could be a dominant process in NO_3^- reduction in tropical estuary sediments (Dong et al. 2011). Its presence was also suggested in NO_3^- -rich SGD places (Rocha et al. 2009). Despite the fact that DNRA is still poorly understood, previous results suggested that DNRA can be a dominant process over heterotrophic denitrification at high NO_3^- concentrations and/or high C/N ratios in the organic matter (Fazzolari et al. 1998; Tomaszek and Rokosz 2007).

The reduction of NO_3^- can be driven by other processes, like the reduction catalyzed by Fe or Mn-minerals (van der Zee and van Raaphorst 2004; Weber et al. 2006). These NO_3^- reduction processes can be biotic and abiotic mediated. Little information is available about biotic-mediated mechanisms of NO_3^- catalysis with Fe and Mn (Hulth et al. 2005). The abiotic NO_3^- catalysis has been identified as likely to occur in organic-poor, neutral to basic pH anoxic or suboxic waters, conditions close to those often representative of coastal aquifers (Ottley et al. 1997). Postma and colleagues identified the presence of this NO_3^- reduction pathway in a subterranean estuary based in electron balances across the redoxcline between the groundwater and the seawater (Postma et al. 1991). N_2 has been proposed as final reduced form of N in these processes but also NH_4^+ and intermediary N forms (Weber et al. 2006). Fe and Mn-driven NO_3^- reduction is strongly dependant on the local geology and thus could be a relevant pathway in a local perspective (Hulth et al. 2005).

The possible coexistence of different reactive pathways with different end-products involving land-derived NO_3^- processing in SGD places raises uncertainty about the mediation role of the seepage face. Thus, in order to clarify the modulation capacity of seepage faces over NO_3^- loads associated with groundwater it is necessary to identify and quantify the processes involved and to elucidate the main parameters controlling the final mediation role of the seepage face.

I.5 Elucidating N processing in SGD seepage faces

The main working hypothesis of this research is that SGD seepage faces can process NO_3^- loads through different NO_3^- reduction pathways. Moreover, I hypothesized that main environmental and biogeochemical parameters (benthic organic matter quantity and composition, advective porewater velocity, oxygen concentration, temperature) control the presence and relative contribution of the different pathways, thus determining NO_3^- loads to the receiving waters. Thus, the general objectives of this research are to identify, quantify and parameterize key biogeochemical processes of N in permeable sediments hosting SGD and correlate these processes with in-situ flux measurements, while using selected representative N molecules as proxies. The main objective of this research is therefore to explore the main biogeochemical and environmental parameters modulating the presence and magnitude of NO_3^- reduction processes in order to elucidate the mediation role of SGD seepage faces over N fluxes to the coastal ocean.

Preliminary field and modeling work aimed to describe NO_3^- behavior in the seepage face of SGD sites and to identify main parameters constraining benthic reactivity. The study site was located at the Ria Formosa coastal lagoon (SW Iberian peninsula; Chapter II). The sampling site was located in the inner part of the Ancão peninsula (77°00'04''N, 7°88'57''W), on one of the two peninsulas of the Ria Formosa coastal lagoon. Large amounts of NO_3^- concomitant with low salinity water seep out of the intertidal beach face during low tide (Leote et al. 2008). At the seepage face, porewater distribution of relevant N compounds and interfacial fluxes were analyzed in order to obtain preliminary explanations for the benthic reactivity and to identify main reactive horizons for subsequent isolation in laboratory experiments (Chapter IV and V).

Different methods have been used to quantify the discharge of groundwater to the sea (Burnett et al. 2006). Geochemical tracers such as Ra, Rn and He isotopes or CH_4 have been successfully used in quantifying groundwater contribution to the coastal sea (Cable et al. 1996; Moore 2006b; Povinac et al. 2008). Other methods such as aquifer mass balances and transport modeling have been also successfully implemented in quantifying the volumetric contribution of groundwater discharge to the sea (e.g. Sutula et al. 2001; Oberdorfer 2003;

Ataie-Ashtiani 2007). Nevertheless, in intertidal SGD seepage faces, direct measurements of the seepage are also possible (Taniguchi et al. 2006; Leote et al. 2008). In permeable sediments, seepage rates are proportional to sediment permeability and the local pressure gradient (Darcy 1856). Thus, pressure measurements using sets of piezometers permit mass quantification of SGD in the intertidal area (Lewis 1987; Rocha et al. 2009). Other technique commonly implemented for SGD measurement is the use of Lee-type seepage meters (Lee 1977). Lee-type seepage meters are 10-15 cm deep cylinders with one end closed and inserted with the open end down into the sediment surface. Water seepage forces the displacement of the internal volume of the seepage meters which is collected in bags attached to a sampling port of the cylinders. The operation with seepage meters requires some precautions in order to minimize the resistance of the connecting tube and bag, and to avoid interferences of the surface water movement with the volume collected in the bags (Shaw and Prepas 1989; Cable et al. 1997; Burnett et al. 2006). Other disadvantages are the detection limit of the seepage meters which make them suitable only in places with significant seepage rates (a few $\text{cm}^3 \text{ cm}^{-2} \text{ day}^{-1}$) and the necessity of deployment of many devices in order to minimize the natural spatial and temporal variability of SGD (Cable et al. 1997). Despite seepage meters being difficult to operate and time consuming, this is the only technique that permits simultaneous measurements of seepage rates together with associated solute fluxes by analyzing the seeped water (Lee 1977; Cable et al. 1997; Taniguchi et al. 2006). Therefore seepage meters are the only technique available accounting for the biogeochemical mediation of the seepage faces over SGD-derived NO_3^- loads. This technique was already successfully used in Ria Formosa sediments (Leote et al. 2008), and results were used to explore in-situ vertical NO_3^- distribution and to constraint porewater flow in experiments under controlled conditions (Chapters IV-VIII).

The advective movement of the porewater in SGD sites promotes interfacial fluxes orders of magnitude higher than diffusion transport and strongly determines the porewater distribution of solutes (e.g. Huettel et al. 1998; Falter and Sansone 2000; Rapaglia and Bokuniewicz 2009). Thus, sampling techniques need to avoid interference with main transport mechanisms (Seeberg-Elverfeldt et al. 2005). Poly-ether sulfone membranes (Rhizon SMS-10 cm; 0.1 μm pore size; Rhizosphere, The Netherlands) were used by being inserted into the

sediment with the aid of acrylic profilers following the design and method developed by Seeberg-Elverfeldt et al. (2005), covering the first 20cm depth into the sediment. The use of Rhizon SMS has been revealed as a suitable technique for sampling permeable sediments since it doesn't interfere with the main transport pathways (Alberto et al. 2000; Song et al. 2004; Sauter et al. 2005; Seeberg-Elverfeldt et al. 2005). Nevertheless, porewater composition obtained with these sampling devices must be representative of in-situ conditions. Tests over Rhizon devices have revealed NH_4^+ adsorption characteristics in the membrane's surface. The adsorption characteristics of rhizon SMS were explored and constrained in chapter III, and better use and improved cleaning procedures to minimize interference over porewater composition were suggested.

Diagenetic modeling of vertical NO_3^- distribution in the porewater obtained in-situ together with flux measurements permitted to identify main apparent vertical reactive horizons in modulating NO_3^- loads to the receiving waters. Vertical biogeochemical zonation thus explored was used in order to identify vertical horizons thus ensuring the isolation of the main net NO_3^- reduction layer at the seepage face for subsequent laboratory work. Also, preliminary reaction rates obtained from this analysis along with seepage rates permitted to design experiments representative of in-situ conditions. Nevertheless, interpretation of solute distribution in advective dominated environments like the Ria Formosa sampling site, requires to take into account the effect of mechanical dispersion and advection over NO_3^- porewater gradients (Berner 1980; Boudreau 1997). Thus, in Chapter IV, an Advection-Dispersion-Reaction model was developed in order to discriminate between the transport and reaction effects over NO_3^- concentration gradients in porewater profiles obtained at Ria Formosa sampling site. The model was successfully applied in describing NO_3^- porewater profiles at the seepage face, and seasonal reactivity at the seepage face was explored (Chapter V).

An important parameter controlling benthic NO_3^- reactivity in sediments is the availability and composition of organic matter (Fitzsimons et al. 2006; Rivett et al. 2008; Santos et al. 2009). The potential organic matter limitation of benthic bacterial processes can increase the likelihood of alternative metabolic pathways being present, including DNRA or ANAMMOX (Hulth et al. 2005; Bowen et al. 2007; Brandes et al. 2007; Kroeger and Charette 2008). In organic matter poor groundwater arriving to the mixing zone, the cyclic infiltration

of sea water strongly controls the distribution and composition of benthic organic matter into the sediment (Bowen et al. 2007; Rocha et al. 2009; Santos et al. 2009). Mobile and non mobile organic matter pools are explored in order to elucidate their dynamics at the seepage face (Chapter VI) and the influence over benthic NO_3^- reduction (Chapter VIII).

Different methods have been used to study the kinetics of natural reactive processes in sediments. For this, static incubations of sediment cores can be used, and benthic reactive processes are inferred from the changes in the composition of the overlying water (e.g. Andersen et al. 2001). Thus, benthic reactivity is measured through resulting diffusive fluxes across the sediment-water interface. Due to the enhancement of reaction rates and fluxes by porewater advection, this technique can underestimate reaction rates in sandy sediments (Boudreau et al. 2001). Another common technique in benthic reactivity studies is the use of sediment slurries (e.g. Han et al. 2008). This technique consists on the incubation of sediment in a water solution with known composition. Reaction rates are determined following changes in target compounds over time. Here, water volume is in excess compared with sediment, thus artificially enhancing sediment specific surface in contact with the solutes and hence overestimating reaction rates (Meile and Tuncay 2006). Thus, although these methods offer valuable information about the presence of reactive processes, extrapolation of results to in-situ conditions is not possible in permeable sediments (Roychoudhury et al. 1998). Another common technique for kinetic studies in marine sediments is the use of Flow-Through Reactors (FTR; e.g. Roychoudhury et al. 2003; Laverman et al. 2006; Laverman et al. 2012). FTR consists of a closed reactor containing a water-saturated sediment slice. The design of the reactors permits the supply of a solution with known characteristics to be forced to flow through the sediment (Roychoudhury et al. 1998; Pallud et al. 2007). Thus, it is possible to replicate field transport pathways, permitting kinetic experiments to be performed in close to in-situ conditions. In Chapters VI-VIII, FTR were used together with a modified HTH corer (Renberg and Hansson 2008), which permits undisturbed sediment slices to be used maintaining the pore structure. FTR experiments were performed in order to explore organic matter dynamics (Chapters VI and VIII) and to elucidate NO_3^- reduction processes and kinetics present at SGD selected sites (Chapter VII).

Chapter II

Ria Formosa coastal lagoon

II.1 Introduction

Ria Formosa is a 110 Km² coastal lagoon extending from 36°58'N 8°20'W to 37°30'N 7°32'W and covering over half of the coast of Algarve (SW Iberian peninsula; Fig. II.1). The lagoon is separated from the open sea by a system of barrier islands that protect a complex system of channels, salt marshes, tidal creeks, sandy deposits and an important human activity. The external sandy barrier is composed of two peninsulas and five islands. Water exchange with the surrounding Atlantic Ocean is limited by the tidal prism of the six inlets (Pacheco et al. 2007; Fig. II.1).

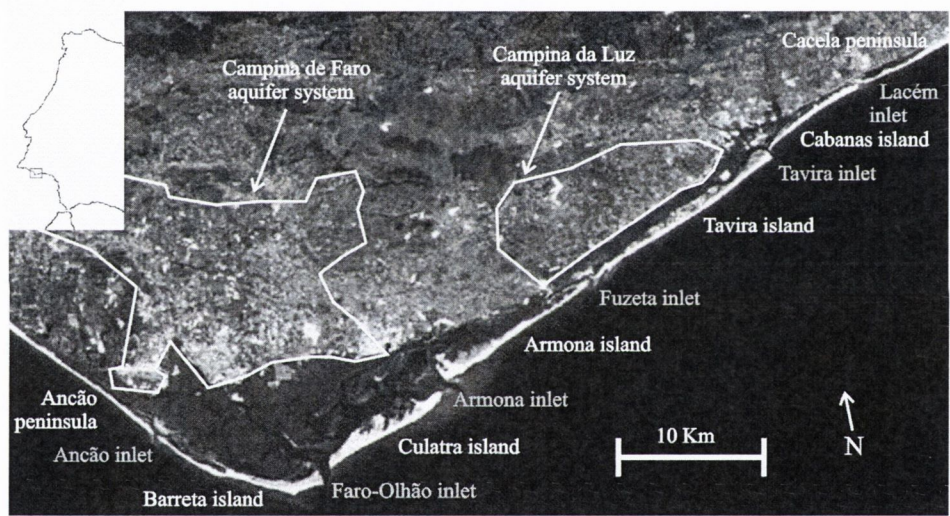


Figure II.1 Ria Formosa coastal lagoon. Location, inlets, islands and peninsulas part of the beach-barrier-lagoon system. Main in-land aquifer systems surrounding Ria Formosa are also shown.

The adjacent coastal area presents a semidiurnal, mesotidal regime. Tidal amplitude varies from 1.3m at neap tides to 2.8m during spring tides, with extreme amplitudes of up to 3.5m (Vila-Concejo et al. 2004). The tidal regime together with the low average depth in the lagoon (2m; (Andrade et al. 2004) develops an extensive intertidal area. The flooded area during spring tides is estimated to be approximately 86Km² (Andrade et al. 2004), inclusive of tidal channels (29Km²), salt marshes (32Km²), mud and muddy sand flats (10Km²), sandy sediments (14Km²) and macrophytes' beds (8Km²) (Gamito 2008).

The climate in the region of the Ria Formosa is Mediterranean. Average temperature ranges between 11°C during the winter and 24°C during the summer. Annual precipitation averages 480mm in the city of Faro (West) and 580 in Tavira (East) (Salles 2001). Surface water streams are seasonal and sporadic. The low freshwater inputs compared to the volume of the lagoon ($210 \times 10^6 \text{ m}^3$; Ferreira et al. 2003) explain the low variation of salinity inside the lagoon (Andrade et al. 2004). The average annual water temperature in the lagoon ranges from 18.1°C in the East sector to 20.6 °C in the West. Only during sporadic torrential precipitation events can surface freshwater influence these parameters.

II.2 Ecological and economic importance

As a coastal lagoon, Ria Formosa represents an important ecological and economic ecosystem due to the goods and services played by these type of sedimentary coastal systems (C sequestration, buffer effect over anthropogenic pressure over the coastal ecosystems, high productivity; Smith and Atkinson 1994; Verhoeven et al. 2006). Furthermore, Ria Formosa is on the main route of several migratory bird species, serves as breeding and nursing grounds for several mollusk and fish species and represents one of the most important populations of sea grass in Portugal (Cunha et al. 2009). As recognition of its ecological value, Ria Formosa is protected by the Portuguese legislation under the National Park designation. It is also catalogued as a Ramsar wetland, a protected area through the Birds Directive (79/409/EEC) and a member of the Natura 2000 Network.

The lagoon is a valuable socio-economic resource for the region mainly due to tourism, fisheries, aquaculture (especially shellfish) and salt extraction. Up to 10% of the lagoon's surface is modified by these activities (ICBN 2004). The largest commercial activity in the lagoon is the harvesting of clam *Ruditapes decussatus*. Indeed approximately 90% of the clams harvested in Portugal are produced in Ria Formosa (~2000-7000 tons per year; Cachola 1996), representing 27% of the total seafood cultured in Portugal (INE 2007). As many as 10000 people rely directly or indirectly on the natural resources of the lagoon (INE 2007).

II.3 Geology and origin

Ria Formosa coastal lagoon presents some atypical characteristics when compared with other beach-barrier-lagoon systems (Pilkey Jr. et al. 1989; Vila-Concejo et al. 2003):

- The surrounding subtidal area presents a high vertical depth gradient,, uncommon for this type of sedimentary system
- Occurrence on the higher tidal range limit for the existence of barrier islands
- Heavy impact of erosive effects of tides and waves on the lagoon's morphology
- Uncommon cape shape of Ria Formosa when compared with other similar sedimentary systems

The lagoon lays on barrier platform sands roughly shaped into a prism by the slowdown of Relative Sea-Level Rise from 6000 BP, the different wave and current conditions on both sides of the lagoon and the effect of storms (Bettencourt 1994; Dias et al. 2000; Salles 2001). Nevertheless, different theories have been proposed to explain the origin and formation of Ria Formosa. Pilkey Jr. et al. (1989) suggested that it originated with the flooding of ancient dune deposits during the last deglaciation. After analyzing a complete set of high resolution, 30m depth sediment cores from the lagoon, Bettencourt (1994) identified the relative uniformity of the sedimentary facies, which he interpreted as tidal paleodeltas (Fig. II.2). After analysis of 5m sediment cores taken from the Ancão inlet (Western sector), (Andrade et al. 2004) confirmed the sedimentary homogeneity of the deeper sandy deposits. They characterized this unit as sandy deposits with marine origin prior to the formation of the Ria Formosa system. The surficial deposits showed a transition in the sedimentary regime from subtidal sands to intertidal muddy plains. The authors also pointed out the absence of carbonate fossils in the upper limit of the sandy deposits. Analysis of the diatoms present indicated tolerance to fresh water inputs and the authors suggested the results were caused by seasonal freshwater infiltration.

Ria Formosa coastal lagoon

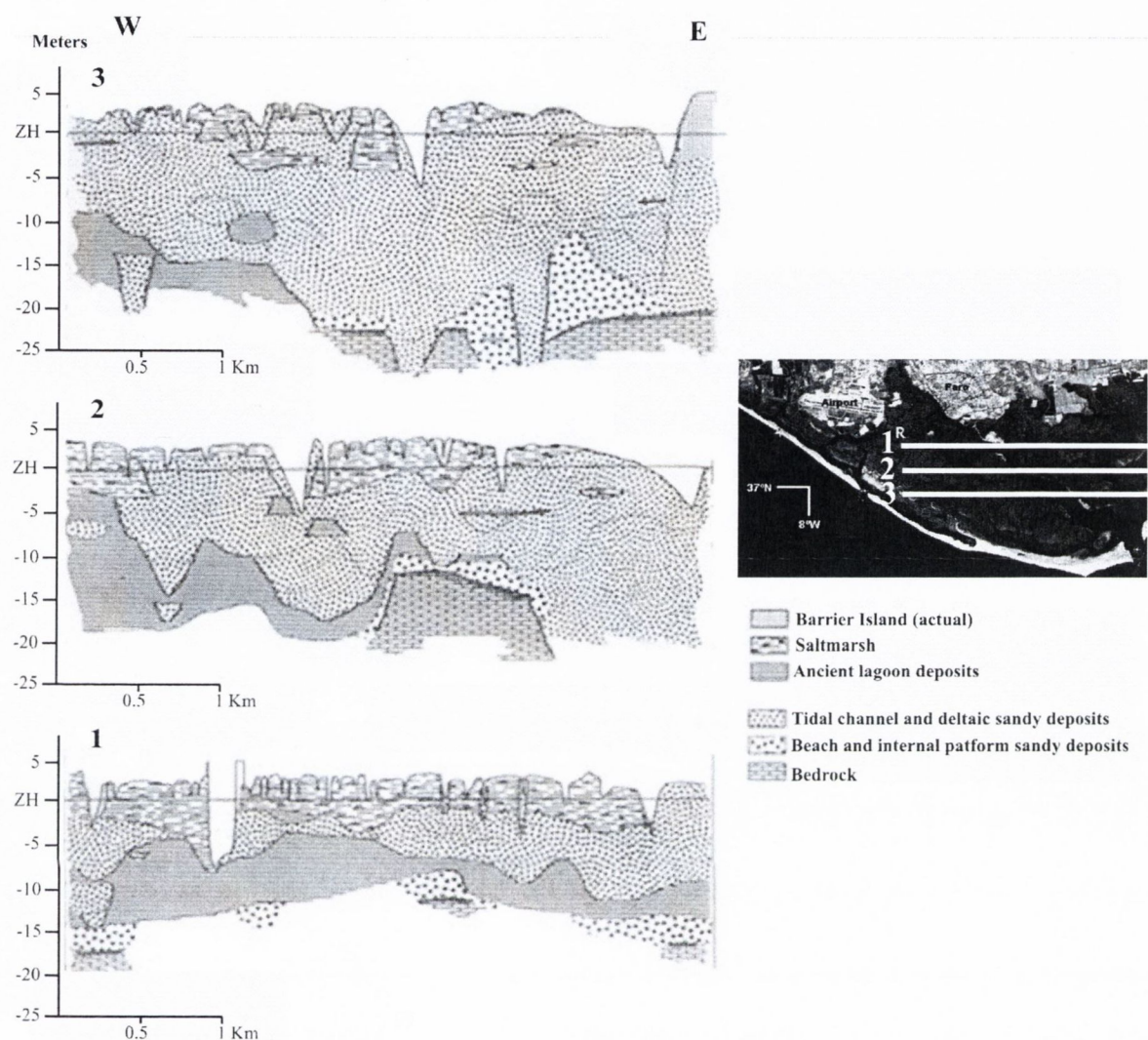


Figure II.2 Bettencourt's interpretation of a complete set of 30 m depth high resolution sediment cores taken at Ria Formosa. Note the homogeneity of the sandy deposits. Adapted from Bettencourt (1994).

The lagoon is surrounded in-land by an extensive coastal plain with the highest agriculture pressure in the region, the “campinas” (Alice Newton and Mudge 2005). Since the early 80s, the local vegetation cover was progressively substituted by olive trees, tomato farming and citric trees. The area of Campina de Faro (Northern to Faro) presents two main aquifers. The deeper aquifer is formed by Cretaceous limestones and the surficial formed by sands and gravels from the Plio-Quaternary. Both aquifers are connected through sub-horizontal Miocene sandy layers (Almeida et al. 2000). During the last decades, the aquifer system has been extensively exploited for irrigation and public water supply. In the early 80’s

Almeida and Silva (1987) identified heavy NO_3^- pollution in the surficial aquifer system (Miocenic and Plio-Quaternary sediments). Reported NO_3^- concentrations in the groundwater averaged 1.7 mM (n=28), with maximum values of 6 mM. The $\text{NO}_3^- / \text{SO}_4^{2-}$ ratios present in groundwater samples from the aquifer system indicated the origin of this NO_3^- pollution in the aquifer was from inorganic fertilizers used in agriculture areas (Almeida and Silva 1987). NO_3^- reaches the Plio-Quaternary aquifer by direct infiltration and polluting the underlying Miocenic strata through water interchange. As part of the policies aiming to control NO_3^- levels in the area, the Campina de Faro aquifer system was designated as a Nitrate Vulnerable Zone in 2001 in compliance with the European Union Nitrate Directive (91/676/ECC). In 2005, the the area covered was extended to the East to Campina da Luz aquifer system, which also surrounds the Ria Formosa coastal lagoon (Stigter et al. 2006).

II.4 Current status

Numerous problems related with an intense human activity have been reported in the lagoon during the last few years. Pollution related with fatty acids (Mudge et al. 1999), organic matter (Gamito 2008), microbiology (Dionisio et al. 2000), PCB's (Barreira et al. 2005), heavy metals (Bebianno 1995; Morgado and Bebianno 2005) and nutrients (Edwards et al. 2005; Newton and Mudge 2005) has been identified. This growing anthropogenic pressure has important impacts over the Ria Formosa ecosystem. For instance, human activities have changed the sedimentary balance of the lagoon marshes promoting erosive processes and loss of habitats (Arnaud-Fassetta et al. 2006). Padinha et al. (2000) also identified the decline in populations of marine phanerogams associated with an increase in the abundance of macroalgae. The lagoon was been documented as having a macroalgae bloom (*Enteromorpha/Ulvae* spp) during the winter since 1988 (Aníbal 2004). Other consequences related with the lost of ecological quality in the system can have a direct impact on the local economy. Aquaculture is affected by episodes of anoxia and by the winter bloom of green algae (Gamito 1997; Aníbal 2004). Human poisoning induced by the accumulation of biotoxins in clams has been also reported (Vale et al. 1999). Additional consequences such as a decline in clam's harvesting or extensive fish deaths due to seasonal anoxia have been also identified (Newton et al. 2003).

In order to improve water circulation and quality in the lagoon and the hydraulic competency of the two inlets located at each end of the sandy barrier, a project started in 1997 with direct implications for the morphology of Ria Formosa. The project included channel dredging, restructuration of dunes and beaches and relocation of the Ancão inlet to the West and the Fuzeta inlet to the East (Vila-Concejo et al. 2003; Vila-Concejo et al. 2004).

Local government, managing organizations and tourism promoters are among those responsible for the loss of environmental quality of the Ria Formosa (Ramos et al. 2003). Nevertheless, the nutritional status of the lagoon is still unclear. Several signs of eutrophication were identified (e.g. Ferreira et al. 2003; Newton and Mudge 2005). Dissolved Inorganic Nitrogen (DIN) levels are consistently higher inside the lagoon than at the surrounding coastal ocean (Newton et al. 2003), suggesting the lagoon is a net N source for the surrounding ocean. Work by Ferreira et al. (2003) identified urban and industrial sewage as a main known source of nutrients to the lagoon (421 ton N year⁻¹; 83 ton P year⁻¹). The authors also estimated, based on mass balance approaches between the lagoon's water levels at high and low tide, that there should be another N and P source to the lagoon accounting for 600 ton N year⁻¹ and 80 ton P year⁻¹. Mass balance approaches have some limitations when applied in Ria Formosa coastal lagoon waters due to the lack of a comprehensive knowledge on tidal flow patterns inside the lagoon and in the adjacent coastal sea. Recent research seems to show a more complex linkage between the lagoon's water and the adjacent coastal sea than what was previously assumed (Rocha, *pers. com.*). The different hydraulic competency of the six tidal inlets surrounding the lagoon could drive the reentrance of a large proportion of lagoon's water that exited the inlets at the previous ebb tide, thus increasing its residence time and therefore questioning previous nutrient mass balances for Ria Formosa (Rocha, *pers. com.*). Nevertheless, Stigter et al. (2006 and references therein) constated the predominant NW-SE groundwater flow in the Campina de Faro aquifer system, and suggested the direct discharge of groundwater and associated solutes from the Campina de Faro aquifer system to the lagoon. Due to the high NO₃⁻ pollution found at the Campina de Faro aquifer system, groundwater discharge could be a previously overlooked important source of nutrients to the lagoon.

II.5 Submarine Groundwater Discharge at Ria Formosa coastal lagoon

Submarine Groundwater Discharge (SGD) was first identified and quantified at Ria Formosa coastal lagoon during the execution of the research project “O-DOIS” (Leote et al. 2008). From November 2005 to January 2007, the authors performed a series of monthly sampling campaigns in the intertidal area of Ancão peninsula (Fig. II.1). Brackish porewater associated with high NO_3^- content was registered to discharge across the intertidal area to the lagoon. During the entire period of field sampling campaigns, salinities lower than those characteristic of the lagoon were systematically measured at the permanently saturated area of the intertidal, ranging from 16.8 to 37.7 salinity.

At the intertidal area, measured seepage rates were maximum during sediment exposure (up to 185 cm d^{-1} on average), being minimum or absent during high tide (Fig. II.3). The discharge showed a cyclic, reproducible pattern linked to tidal oscillation. Results showed that the tide is the main forcing factor modulating the rate and temporal extension of the discharge (Rocha et al. 2009). A perpendicular discharge gradient was verified at the permanently saturated seepage area. Maximum discharges were systematically registered at the lower intertidal, with minimum rates at the upper limit. Furthermore, porewater seeping out at the lower levels was characterized by low salinities, whereas in the upper intertidal levels, porewater salinity was consistently similar to that found in the lagoon (Leote et al. 2008). Averaged discharge per linear m of coast line was estimated to be $3.6 \text{ m}^3 \text{ d}^{-1}$.

NO_3^- content in the seeped water was inverse linearly correlated with salinity (Leote et al. 2008). Results demonstrated the presence of two water masses in the porewater seeping out of the sediment: one characterized by high salinity associated to low nutrient content, and another with low salinities and high NO_3^- concentration. Freshwater content in the porewater typically varied between 10 and 30%. Seasonal changes in the total discharge to the lagoon were correlated with rainfall in the basin, thus suggesting an in-land origin of the freshwater component. Based on the seasonal modulation of the discharge together with the high NO_3^- content the authors suggested the hydraulic connection of the seepage face with the surrounding aquifer systems in-land (Rocha et al. 2009).

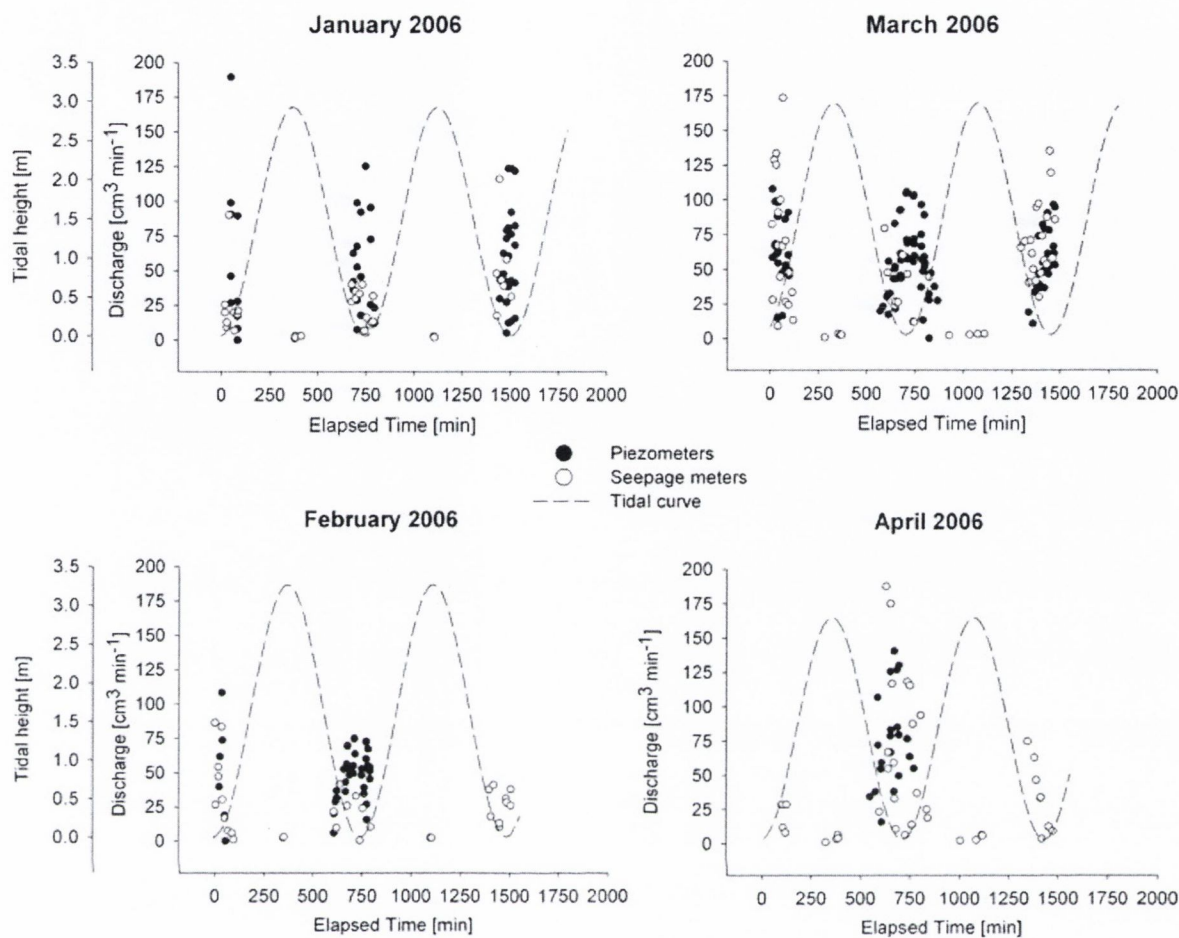


Figure II.3 Discharge rate and tide height as measured at the seepage face during January-April 2006 as measured with Lee-type seepage meters and piezometry. Adapted from Rocha et al. (2009).

SGD is revealed to be an important source of Dissolved Inorganic N (DIN) to Ria Formosa coastal lagoon (Fig. II.4). Calculated annual N fluxes per m of coast line were $36.2 \text{ mol m}^{-1} \text{ y}^{-1}$ (Leote et al. 2008). Furthermore, NO_3^- was the dominant DIN form in SGD fluxes to Ria Formosa throughout the year, with NH_4^+ representing 10% of the DIN fluxes. Two distinctive periods in terms of NO_3^- fluxes were registered. Higher DIN fluxes were observed during the second half of the period studied (Fig. II.4). This increase in DIN fluxes over the second half of the studied period compared to the first months was not exclusively related to the total volume of water discharged at the beach face, which depends on the hydraulic gradient at the seepage face (i.e. thus controlled by the tide and aquifer recharge dynamics). Instead, this annual pattern may reflect the periodicity in agricultural practices as identified in

the varying NO_3^- content in the groundwater throughout the year, further suggesting the hydraulic connection of the seepage face with in-land aquifers (Leote et al. 2008; Fig. II.4). Nevertheless, the magnitude of DIN fluxes to the lagoon characterizes SGD as an important, previously not recognized source of nutrients to Ria Formosa coastal lagoon.

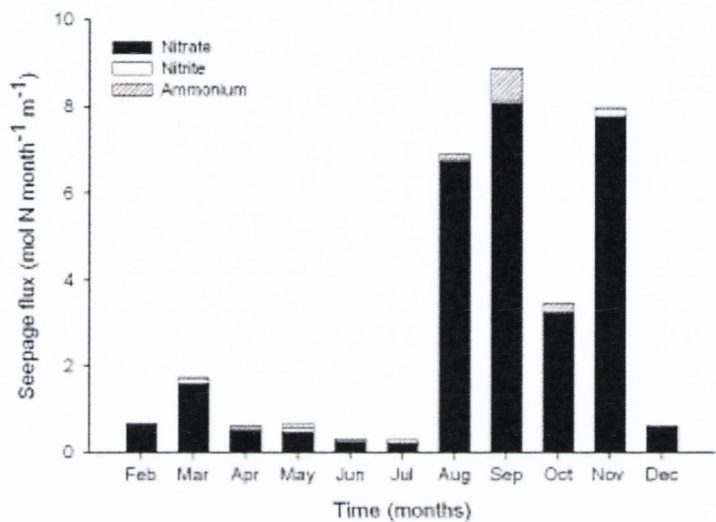


Figure II.4 Monthly DIN (NO_3^- , NO_2^- and NH_4^+) fluxes per linear m of coastline as measured with Lee-type seepage meters at the seepage face. Adapted from Leote et al. (2008).

Porewater movement during active discharge at the seepage face showed to have a predominant vertical direction. Several observations supported this argument. The good agreement between seepage rate determination with Lee-type seepage meters and nested piezometers (which only considers the vertical component of the pressure gradients) supports this view (Fig. II.3). In this sense, under saturation of the upper part of the beach during active seepage (i.e. at low tide) and the associated lowering of the freatic level inside the beach avoids significant horizontal hydraulic gradient to promote water movement from the upper levels to the main seepage area inside the unconfined aquifer. Furthermore, influence of the upper beach levels (chartacterized by salinity close to that found in the lagoon and low nutrient concentration) in the porewater solute distribution at the lower levels was not identified. Salinity profiles in the area affected by SGD were vertically homogeneous and

systematically lower than that found in the lagoon when seepage peaked (Rocha et al. 2009). Moreover, vertical NO_3^- distribution consistently peaked at the sediment-water interface, showing no dilution with the NO_3^- -poor porewater from the upper beach levels (Fig. II.5; Rocha et al. 2009).

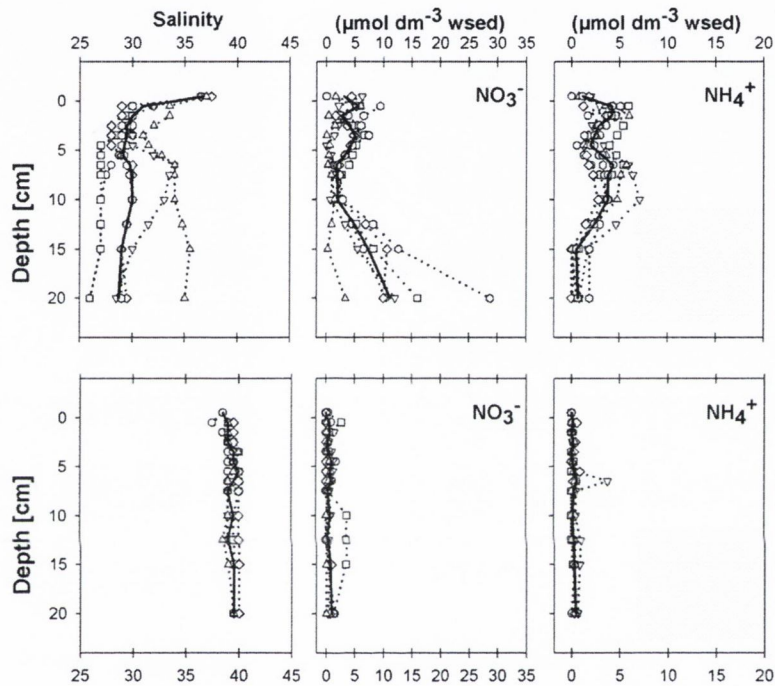


Figure II.5 Profiles depicting the variation of porewater levels of NO_3^- , NH_4^+ and salinity with depth for November 2005 during the period of active seepage (i.e. at sediment exposure), respectively for the area affected by SGD near the peak of the discharge(upper pannels), and the area above that affected by SGD (lower pannels) at the beach face. Solid lines depict the median of the concentration at each depth. Dotted lines represent individual profiles, to allow assessment of the range in variation measured throughout the sampling period (adapted from Rocha et al. 2009).

Preliminary analysis of porewater profiles collected in-situ at the seepage face (20 cm depth) showed the presence of significant vertical DIN concentration gradients during active seepage (Fig. II.6). Under dominant vertical movement of porewater during active seepage, observed vertical solute gradients can only be present due to dilution effect promoted by the mixing of both water masses present at the subterranean estuary and/or caused by benthic reactivity in place. Conservative DIN profiles (i.e. assuming no benthic reactivity over DIN fluxes) were calculated by using porewater salinity as mixing tracer (Fig. II.6; white dots).

Calculated non-reactive DIN profiles differed significantly from those measured at the seepage face. Thus, the presence of significant benthic reactivity in modulating DIN distribution in the porewater was confirmed (Rocha et al. 2009).

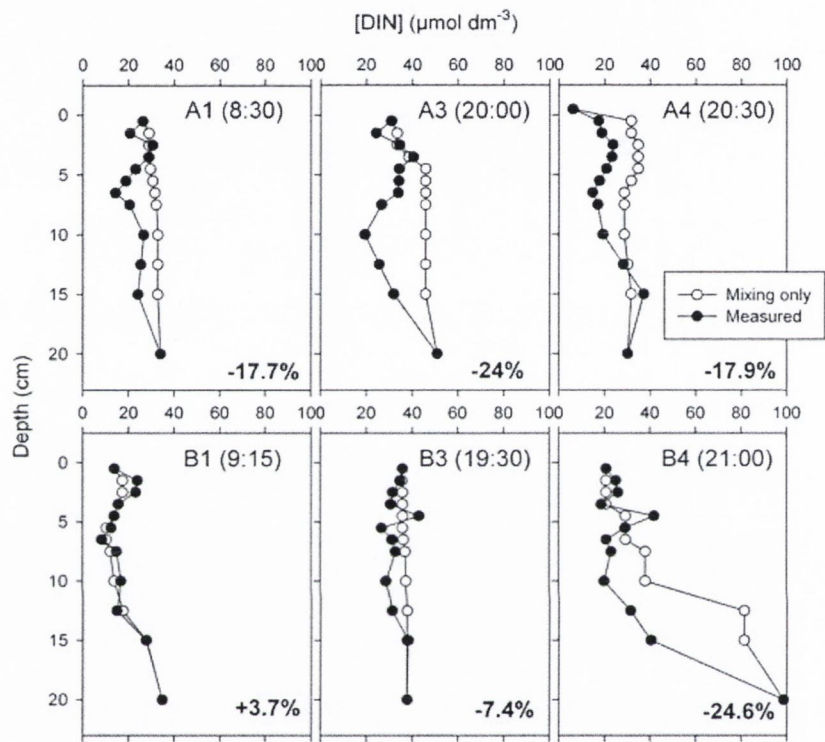


Figure II.6 Vertical porewater DIN distribution at the seepage face as measured in November 2005. Black dots represent in-situ measurements, whereas white dots represent calculated conservative curves, assuming no sediment reactivity over porewater DIN content. Upper panels represent the lower intertidal area (A), close to where discharge peaks. Lower panels represent the upper limit of the permanently saturated intertidal area. Profiles were taken at low tide, during active seepage. The time the profiles were taken are also shown. Adapted from Rocha et al. (2009).

Rocha et al. (2009) further explored benthic reactivity over SGD-derived DIN fluxes. Based on the predominant vertical porewater movement during active seepage, the authors analyzed vertical NO_3^- and NH_4^+ profiles through mass balance approaches over the studied domain (20 cm depth). Thus, the authors estimated NO_3^- reduction rates and concomitant NH_4^+ production rates for the studied period. Despite some concerns about mass balance approaches applied to an advective-dominated environment as discussed in Rocha et al.

(2009), preliminary results suggested the presence of alternative NO_3^- reduction pathways in mediating SGD fluxes at the seepage face.

Chapter III

Porewater sampling for NH_4^+ with Rhizon Soil Moisture Samplers (SMS): potential artifacts induced by NH_4^+ sorption

III.1 Abstract

Hydrophilic poly-ether-sulfone (PES) membranes (Rhizon SMS, Rhizosphere, The Netherlands) are increasingly used to sample soil and sediment porewater due to their versatility and easy adaptability to field studies in benthic biogeochemistry. Nevertheless, possible interference of membrane surface properties with filtered water composition raises uncertainties regarding the accuracy of resultant solute composition of the filtrates. In this study, we identify and describe ammonium sorption onto Rhizon SMS. Maximum adsorption capacity is associated with low operational temperatures and reduced filtrate salinity. Ammonium partition coefficients (K^*) higher than 8×10^{-2} cm were measured at 5°C and 0 salinity. However, K^* follows an exponential decay curve with increasing salinity and an inverse exponential decay with temperature. Ammonium desorption from the membranes is fast, although affects the first millilitre of filtered sample after internal volume removal. Based on our results, recommendations for improved practice are put forward to minimize the influence of membrane adsorption capacity on the ammonium composition of porewater samples when using Rhizon SMS. Thus, discarding of first filtered water after dead volume and collecting enough sample volume is necessary in order to obtain a filtrate representative of sample composition when salinity and/or temperature gradients are present.

III.2 Introduction

In biogeochemical studies involving the description of porewater solute composition to understand sediment-water transfer processes, porewater needs to be separated from the solid phase for subsequent analysis. Thus obtained, the porewater aliquot must be representative of in-situ solute concentrations to the highest degree of accuracy possible. Different methods exist for porewater collection, both ex-situ and in-situ. Common ex-situ methods include collection of sediment cores and subsequent separation of the water from the solid phase by squeezing, applying vacuum or centrifugation the sediment-water mixture (Watson and Frickers 1990; Bufflap and Allen 1995; Carr and Chapman 1995). Ex-situ sampling techniques have disadvantages associated to the potential modification of porewater chemistry both during core collection and porewater extraction (de Lange et al. 1992). Thus, in-situ techniques like peepers (Teasdale et al. 1995), modified piezometers (Duff et al. 1998; Martin et al. 2003) or gel probes (Krom et al. 1994) have been developed to overcome potential sampling and handling artifacts in different environmental circumstances. Recently, Rhizon Soil Moisture Samplers (SMS; Rhizosphere, The Netherlands), first used for agricultural and soil science, have been applied in porewater extraction on ex and in-situ sampling strategies supporting biogeochemical studies (Alberto et al. 2000; Song et al. 2003; Seeberg-Elverfeldt et al. 2005).

Rhizon SMS consist of a hydrophilic porous poly-ether-sulfone (PES) tube connected to a sampling port. The membranes are 10 or 5 cm long, with an external diameter of 2.4 mm and typical pore size of 0.1 μm . The use of PES membranes for porewater extraction and simultaneous filtration benefits from some operational advantages when compared with other porewater extraction methods: it is an inexpensive, easy to use and less time consuming way of filtering porewater, while maintaining the sample isolated from the enveloping environment throughout the process (Tiensing et al. 2001; Seeberg-Elverfeldt et al. 2005). Their small pore size (0.1 μm) improves sample preservation by reducing the number of microorganisms retained in the filtered samples to a considerable degree (Andersson 2003; Shen and Hoffland 2007) The small sampler diameter also allows porewater extraction with minimum disturbance of the sediment pore structure, a crucial characteristic in certain environments like rooted soils (Gribsholt and Kristensen 2002), or when looking at adsorption-desorption mechanisms

(Tiensing et al. 2001). It is also possible to sample porewater for dissolved gas analysis while avoiding contact with the atmosphere (Alberto et al. 2000). In addition, and most importantly for permeable sediment settings, the small diameter and pore size permits collection of in-situ depth profiles of porewater solute concentrations with minimum interference with preferential interstitial flow patterns induced by local natural pressure gradients (Song et al. 2003; Seeberg-Elverfeldt et al. 2005; Ibáñez et al. 2011). Thus, it is possible to obtain reliable porewater samples where interstitial water flow influences the sediment distribution of important biogeochemical compounds (Boudreau et al. 2001). Furthermore, the samplers can be left installed in the field and successive porewater extracts collected at different time intervals during the entire experimental run (Song et al. 2003; Rocha et al. 2009).

Previous testing of Rhizon SMS has been carried out in order to confirm their suitability in environmental studies. Alberto et al. (2000) showed their suitability for sampling dissolved methane by comparing their Rhizon-derived results with those obtained with methane traps. Gribsholt and Kristensen (2002) used them to explore DOM dynamics in the rhizosphere, concluding that for rooted soils, Rhizon SMS are a more reliable porewater extraction technique than centrifugation. Other tests have focussed on sampling for heavy metals (Spangenberg et al. 1997; Knight et al. 1998; Tiensing et al. 2001), dissolved reactive silicate (Seeberg-Elverfeldt et al. 2005), NO_3^- and SO_4^{2-} (Spangenberg et al. 1997), citrate (Shen and Hoffland 2007), amino acids (Andersson 2003) or NH_4^+ in freshwater sediments (Song et al. 2003). Even though the reported results were in generally good agreement with other, more established methods of porewater extraction, doubts on Rhizon SMS interference with porewater chemistry remain. PES membranes are commonly used for ultrafiltration purposes and they are known to be affected by fouling effects due to adsorption of different compounds on their surface (Liu and Kim 2009; Palencia et al. 2010). Surfactants, proteins and certain polymers are known to adsorb onto PES membranes (SalgIn et al. 2006; Liu and Kim 2009; Palencia et al. 2010). In natural sample filtration, van Haesenbroeck et al. (1997) identified PO_4^{3-} adsorption onto Rhizon SMS during soil core incubations, but they did not explore their results further.

To evaluate the performance of Rhizon SMS in the context of porewater extraction it is necessary to clarify potential interferences of the method with sample composition.

Preliminary tests confirmed the non interference of the samplers over NO₃⁻ and NO₂⁻ filtrate content (data not shown). By contrast, here we demonstrate previously undescribed ammonium adsorption properties of PES membranes (Rhizon SMS). We explore and quantitatively constrain ammonium sorption onto the membrane surface and, based on the results, suggest practical improvements to the operational use of this technique.

III.3 Methods

III.3.1 Ammonium sorption isotherms and isohalines

In order to study the sorption characteristics of Rhizon SMS, incubations of the samplers in solutions of differing ammonium and salinity levels were performed in the laboratory under controlled temperature (5, 10, 15, 20 and 25 °C). For the determination of NH₄⁺ sorption isotherms, two new Rhizon SMS (10 cm length, 2.5 mm diameter, Rhizosphere, The Netherlands) were placed for 2 hours in 15 mL solutions with different initial ammonium concentrations in milli-Q water (0, 7, 18, 36, 71, 143 and 357 (or 286) μM). During the incubation period, the internal volume (dead volume) of the ultrafiltration samplers was periodically flushed out with air by back-wash. By doing this, the establishment of an ammonium concentration gradient due to adsorption inside the samplers was prevented. On completion of the 2-hour period, the remnants of the incubation solution left within the samplers were washed out by filtering 40 mL of Milli-Q water stored at the same temperature as the ammonium solutions through them. The internal volume of the samplers was then re-emptied with air by back-wash. Ammonium adsorbed onto the sampler surface was then extracted with 10 mL of 2 M KCl solution for 2 hours (Rosenfeld 1979). The final concentration of the incubation solution was calculated by subtracting the total ammonium sorbed onto the samplers from the known concentration of the solutions at the beginning of the incubations. The entire procedure was performed in duplicate.

Ammonium adsorption isohalines on Rhizon SMS were determined following the same procedure taken for the isotherms. A series of new Rhizon SMS samplers were incubated in solutions of different ionic strength with varying ammonium concentrations at fixed temperature (10 °C). In this case, the ammonium solutions were made using aged

oligotrophic sea water mixed in different proportions with Milli-Q water in order to obtain a known range of salinities (0, 10, 20 and 35). Ammonium adsorption isotherms and isohalines were constructed by plotting sorbed ammonium relative to membrane surface (e.g. Penfold et al. 2007). Adsorption coefficients were calculated from the relationship between the ammonium sorbed per cm^2 of membrane surface and the final ammonium concentration of the incubation solutions.

III.3.2 Flow-through experiments

Ammonium desorption kinetics of Rhizon SMS was also studied. The samplers were first incubated in solutions with different ammonium concentrations in Milli-Q water for 2 hours at 10°C , as described above for the isotherms and isohalines. In parallel, a set of sampler incubations was performed in ammonium solutions ($\sim 390\mu\text{M}$) of different ionic strength (10 and 20 salinity). Each sampler was then flushed with 20 mL of Milli-Q water in order to ensure no incubation solution was left in the interior of the sampler. Subsequently, a peristaltic pump was used to filter oligotrophic sea water at a constant flow rate ($1.79 \pm 0.04 \text{ mL min}^{-1}$) through the samplers. Both the Milli-Q and the sea water solutions used were stored at 10°C in order to avoid ammonium desorption caused by temperature changes. Salinity was used as tracer to control mixing and residence time inside the samplers. The process was performed in triplicate for each incubation solution. Water samples were collected at fixed time intervals throughout the experiment and stored in the dark at 4°C until analysis.

III.3.3 Analytical techniques

Ammonium concentration in the samples taken during the laboratory experiments was determined in a Lachat Quickchem 8500 Flow Injection Analysis system following standard colorimetric methods (Grasshoff et al. 1983) as adapted for automated sequential analysis by the manufacturers. Salinity in samples taken during the flow-through experiments was measured with a hand-held refractometer using single-point calibration with distilled water.

III.4 Results

III.4.1 Ammonium sorption isotherms and isohalines

PES membranes adsorbed ammonium. Within the concentration range used in the incubations, ammonium adsorption onto Rhizon SMS surfaces followed linear isotherms (Fig. III.1A, $R>0.975$). Ammonium adsorption was inversely related to temperature. The partition coefficients at equilibrium (K^* , calculated from the isotherm slopes, $p<0.0005$) were higher at lower temperatures (Fig. III.1B), ranging from $8.14\times10^{-2} \pm 4.1\times10^{-3}$ cm at 5°C to $3.20\times10^{-3} \pm 2.00\times10^{-4}$ cm at 25°C. However, no linear relationship between $\log K^*$ and $1/\text{Temperature}$ was found. Instead, the NH_4^+ partition coefficient decreased following an inverse exponential decay relationship with temperature (dotted line, Fig. III.1B, $R=0.999$). Salinity also influenced the amount of ammonium adsorbed onto the membranes (Fig. III.2). Ammonium adsorption was highest when Milli-Q water was used for the incubations. K^* obtained from linear regression analysis of the isohalines followed an exponential decay curve with salinity (dotted line, Fig. III.2, $R=1$); K^* dropped by one order of magnitude from 0 to 10 solution salinity, and remained at similarly low values above the 10 salinity threshold.

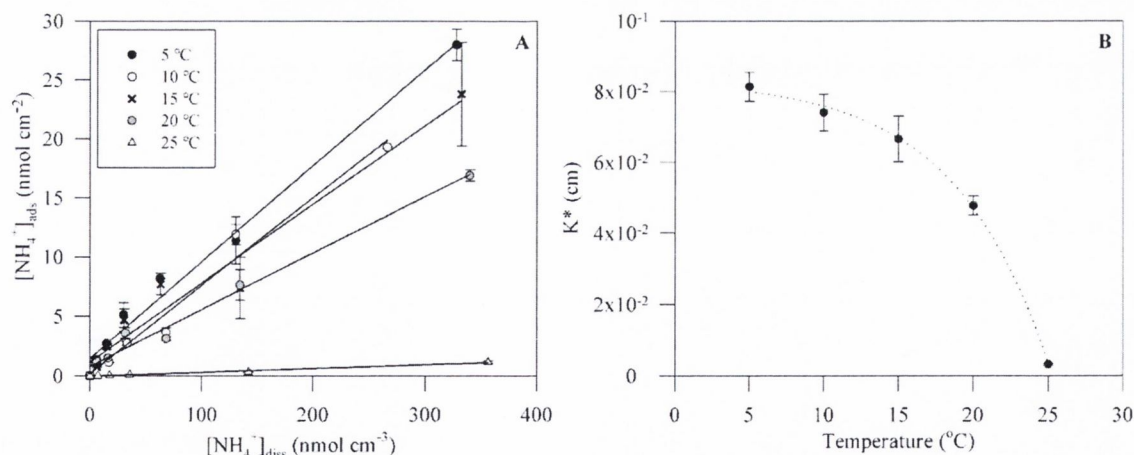


Figure III.1 A. Linear ammonium adsorption isotherms of polyethersulfone membranes at different temperatures. B. Ammonium partition coefficient at equilibrium (K^*) for the different isotherms, fitted with an inverse exponential decay equation ($K^* = 8.34\times10^{-2} - 1.6\times10^{-3} / \exp (-0.158 \times T)$; $R=0.9993$, dotted line).

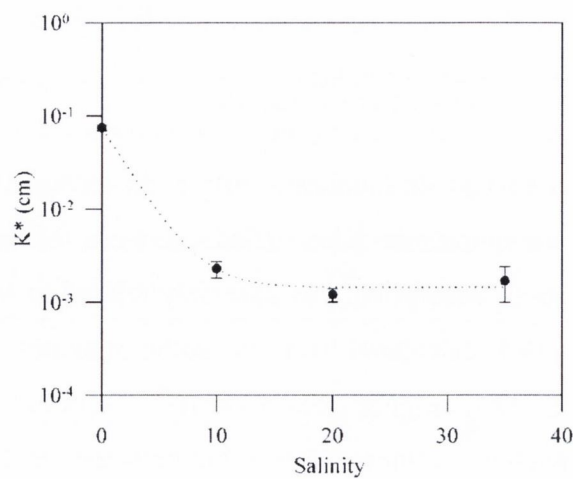


Figure III.2 Equilibrium partition coefficients (K^*) of ammonium adsorption onto Rhizon SMS at 10°C as a function of salinity. K^* was fitted to an exponential decay equation ($K^* = 1.443 \times 10^{-3} + 7.281 \times 10^{-2} \times \exp(-0.4475 \times S)$; $R=1$, dotted line). Note the logarithmic scale in K^* axis.

III.4.2 NH_4^+ sorption kinetics

Flow-through experiments showed fast desorption and subsequent release of ammonium from the membrane to the filtrate when increasing the salinity of the solution being filtered (Fig. III.3A). Most of adsorbed ammonium was desorbed within the first mL of filtrate after submerging into sea water, which corresponds to flushing of the dead volume in the membrane and associated sampler tubing. Extraction recovery efficiency was assessed: the total ammonium recovered from the membranes during flow-through experiments was compared with that expected from the 0 and 35 isohalines at 10°C. Good agreement between ammonium recovered and expected levels was confirmed ($NH_4^+_{\text{recovered}} = (1.02 \pm 0.00) \times NH_4^+_{\text{expected}} + (14.07 \pm 0.33)$, $R=1$). Recovery efficiency after the 4 mL of sea water were pumped through the samplers was 100%, meaning that all the ammonium previously sorbed onto the membrane surface was desorbed in the course of the reverse experiment. Nevertheless, ammonium desorption from the sampler was detected after removal of sampler's internal volume (i.e. after pumping the first mL of sea water; Fig. III.3B). A significant proportion of the ammonium previously sorbed onto the sampler's membrane was measured after reaching 35 salinity (8.4% for the samplers incubated with 69µM NH_4^+ in 0 salinity, 17.8% at 367µM NH_4^+ and 18.9% at 721µM NH_4^+).

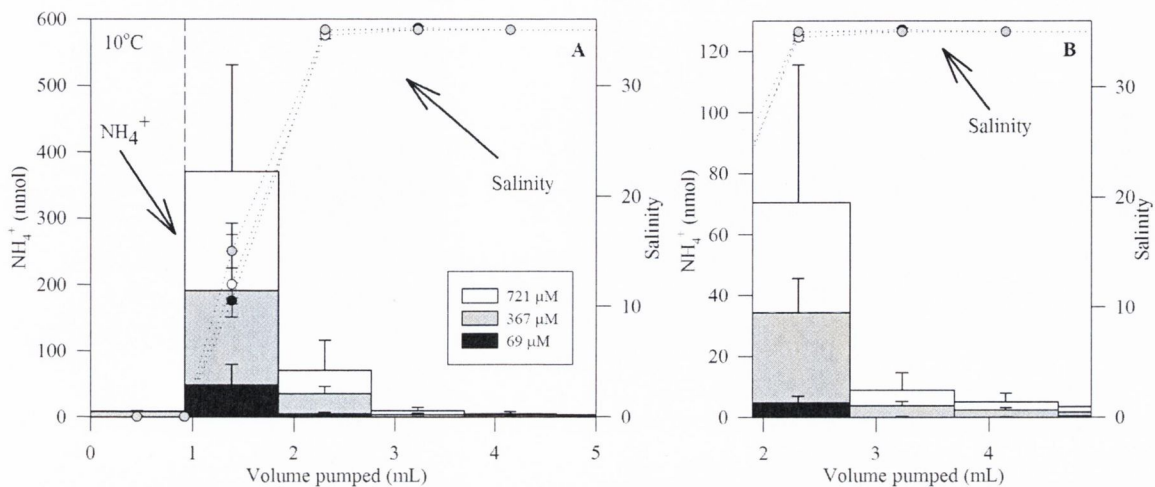


Figure III.3 A. Ammonium desorption from Rhizon SMS when changing salinity. Membranes were first incubated in distilled water solutions, cleaned and then a 35 salinity solution was filtered. B. Detail for the 3 mL pumped after recovery of 100% of the sea water solution. Associated salinity break through curves are also shown.

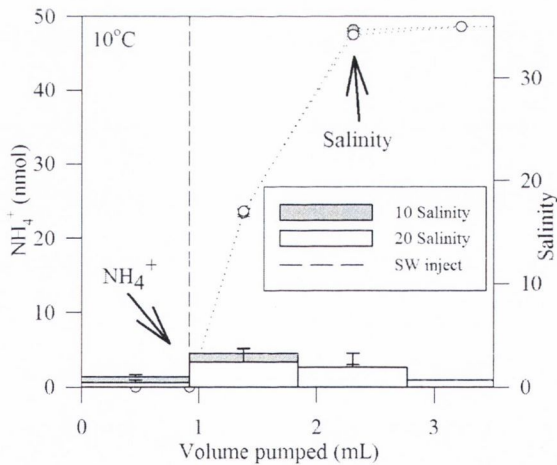


Figure III.4 Ammonium desorption from Rhizon SMS when changing salinity. Membranes were first incubated in ammonium solutions of different salinity, cleaned with distilled water and then a 35 salinity solution was filtered. Associated salinity break through curves are also shown.

Another set of flow-through experiments were carried out with samplers previously incubated in ammonium solutions (390 μM) prepared in different mixtures of Milli-Q water and sea water (Fig. III.4). Ammonium was desorbed from the samplers, following a similar

pattern than the flow-through experiments performed with samplers incubated at 0 salinity (Fig. III.3). Nevertheless, ammonium recovery was low, in accordance with the low partition coefficient measured in the 10 and 20 salinity isohalines.

III.5 Discussion

III.5.1 NH_4^+ sorption properties of Rhizon SMS

Ammonium adsorption onto hydrophilic PES membranes (Rhizon SMS) was described and quantified with respect to temperature and ionic strength of the filtered aliquots. Whereas in neutral to hydrophobic surfaces monolayer adsorption tends to be the norm, in hydrophilic surfaces, adsorption tends to be micellar, thus explaining the potentially high adsorption capability of hydrophilic materials (Grant et al. 2000). Hence the hydrophilic properties of Rhizon SMS can certainly explain their high ammonium adsorption capacity. Higher ammonium adsorption onto Rhizon SMS was confined to low temperature and low salinity, and found to decay exponentially with increasing salinities and following an inverse exponential decay with temperature. Adsorption from solutions is generally an exothermic process, unless an increase in temperature decreases solute solubility (Bartell et al. 1951). Therefore, the decay of ammonium adsorption partition coefficients (K^*) characteristic of Rhizon SMS membranes with increasing temperature is expected. Nevertheless, the exponential decay of K^* with temperature further suggests that Rhizon SMS surface is energetically heterogeneous, i.e. NH_4^+ affinity to the adsorbent surface depends on the place on the surface (von Gemmingen 2005). As in marine sediments, ammonium competition with other positive ions like Na^+ for adsorption sites in the membranes and ion pairing of anions with ammonium can explain the decay in K^* at increasing salinity (Gardner et al. 1991; Seitzinger et al. 1991). Since ammonium concentrations never exceeded 400 μM in our experiments to describe NH_4^+ sorption isohalines, active competition for membrane adsorption sites was mostly complete at 10 salinity, explaining the almost unchanged low levels of ammonium sorbed onto the membranes in the range of 10 to 35 salinity.

Results of the flow-through experiments showed fast ammonium desorption from Rhizon SMS membranes when sea water was filtered through the samplers previously

contaminated by sorbed NH₄⁺. The good agreement between the quantity of ammonium desorbed from the membrane during the experiments and that expected from the NH₄⁺ sorption isohalines confirms that most of the previously sorbed ammonium desorbs into the first few mL of filtrate. During removal of the sampler's dead volume (i.e. by discarding previous filtered Milli-Q water remaining inside the membrane + tubing in order to ensure no admixture with the new sample), the membrane surface was subject to an important salinity gradient, which in turn desorbed most of the previously sorbed ammonium. Nevertheless, ammonium was still being detected towards the end of the flow-through experiments, i.e. even after the dead volume was removed. A significant proportion of the ammonium previously sorbed onto the membrane surface (up to 18.9%) was recovered while the filtrate composition was already sea water (35 salinity). In those cases, ammonium desorption from Rhizon SMS can influence sample composition even after the sampler's dead volume is discarded.

III.5.2 Implications for porewater and surface water sampling

Rhizon SMS is an inexpensive, easy to operate and fast way to obtain filtered porewater, and it is now commonly used in fresh and sea water biogeochemical studies (e.g. Lu et al. 2000; Song et al. 2003; van den Berg et al. 2005; Leote et al. 2008; Ibáñez et al. 2011). However, NH₄⁺ adsorption onto Rhizon SMS surfaces may in fact influence the final NH₄⁺ composition of the filtered porewater aliquot.

Fig. III.5 shows potential influence of ammonium sorption characteristics of Rhizon SMS over filtered aliquot composition calculated on the basis of isotherm and isohaline curves. Different scenarios were simulated by assuming fast desorption of ammonium (within the first filtered 0.5 mL), no change in the ammonium concentration of the water passing through the membrane and ignoring the dead volume of the membranes (scenario occurring when the membranes are dry prior to re-use). The influence of the sorption capabilities of the samplers over filtrate ammonium composition due to temperature and salinity changes alone can be expressed in terms of percentage of ammonium added or removed to the filtrate relative to ammonium sample composition. At 20 °C in a freshwater environment (Fig. III.5A), an increase in temperature of 1 °C can induce an ammonium concentration increase on the filtrate

of up to 10% the ammonium content in the porewater, whereas a 5 °C temperature increase will induce an increase up to 74% in the ammonium concentration of the filtered sample. Therefore, temperature changes by themselves will alter the equilibrium between the ammonium sorbed onto the membranes and the ammonium in solution thus making the resulting filtrate not representative of the porewater ammonium concentration. For example, when filtering a porewater solution of 50 µM, an increase of 1 °C in the porewater will contaminate the sample with 10 nmol of NH_4^+ , thus producing a filtrate with an ammonium concentration of up to 55 µM, depending on the volume of sample being filtered. Moreover, at low porewater salinities (5, 10 °C, Fig. III.5B), small salinity gradients during sample filtration will also alter ammonium composition of the filtered aliquot due to changes induced in the portioning of ammonium between sorbed and solute fractions. In both cases, as shown in Fig. III.5, increasing the volume of sample filtered will reduce the membrane's influence over the ammonium composition of the filtrate, although this might still remain potentially important when high temperature or salinity gradients are in place. In either case, isohaline and isotherm sorption curves resulting from our work may be successfully used to correct the final analytical results and remove artefact influence.

Based on the results presented here, some operational good-practice protocols can be implemented in order to minimize ammonium contamination of filtered samples. Cleaning solutions with ionic strength matching that expected for the samples to be filtered should be prepared prior to the use of Rhizon SMS in the field or in the laboratory. A minimum ionic strength threshold equivalent to a salinity of 10 in the cleaning solution will desorb most of the ammonium adsorbed during previous uses of the samplers. By cleaning the membranes prior to any filtration with these solutions, the amount of ammonium sorbed onto Rhizon SMS is guaranteed to remain at a minimum due to the low K^* found at 10 salinity. Nevertheless, the opposite effect could happen, where adsorption onto Rhizon SMS could artificially lower resulting filtrate NH_4^+ composition. Changes in sample temperature alone will also induce a new sorption equilibrium, thus potentially influencing filtrate composition. Due to the fast sorption kinetics of PES membranes demonstrated in our experiments, equilibration of the membranes with the surrounding medium after insertion into the sediment is recommended before applying vacuum. Furthermore, our results showed that discarding sampler's dead volume plus at least the first mL of subsequently filtered sample aliquots is necessary for

NH₄⁺ sorption onto Rhizon Soil Moisture Samplers (SMS)

appropriate sample filtration. This set of procedures will minimize potential sampler interference over filtrate composition, occurring either because the concentration has lowered (on a flow field), or when the salinity/temperature of the sample is changed significantly and the same membrane is used. Furthermore, sampler influence over NH₄⁺ composition in the filtrate is also likely to be minimized when enough sample volume is taken (Fig. III.5).

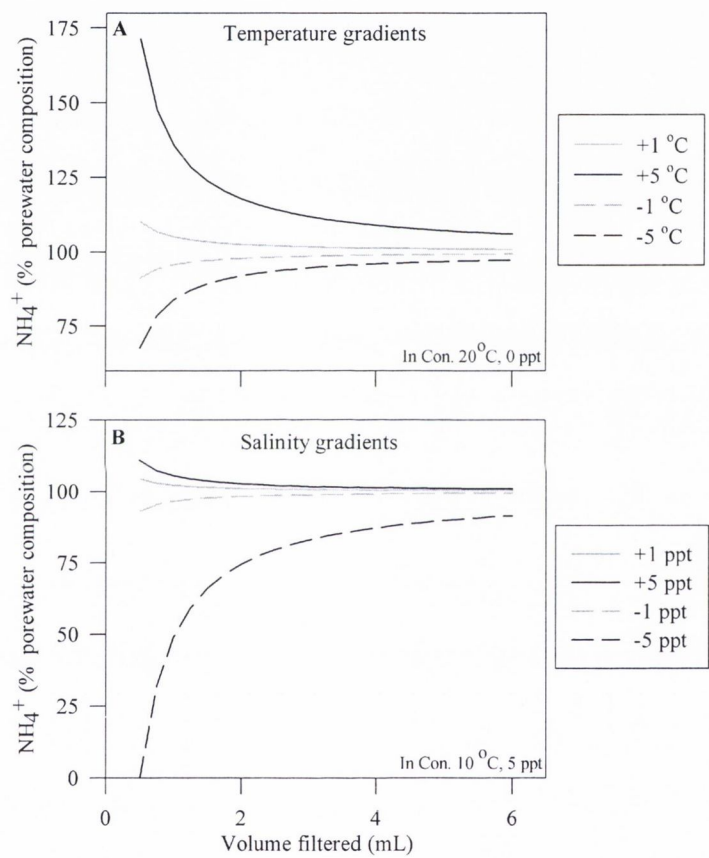


Figure III.5 Potential impact of ammonium sorption capacity of Rhizon SMS over filtered aliquot composition in the presence of environmental gradients: (A) Temperature and (B) salinity gradients. Simulations were performed assuming fast sorption kinetics (within the first 0.5 mL filtered), no change in sample ammonium composition and neglecting internal dead volume of the membranes. Initial conditions of the simulations are also shown.

Rhizon SMS also allows in-situ porewater samples and vertical profiles to be collected with minimum disturbance of pore structure and water flow (Seeberg-Elverfeldt et al. 2005). This characteristic is crucial when sampling permeable sediments, where vertical distribution

of relevant biogeochemical solutes is strongly dependant on porewater flow (e.g. (Shum and Sundby 1996; Falter and Sansone 2000; Boudreau et al. 2001; Rocha et al. 2005). When using Rhizon SMS for long-term field deployment in order to obtain porewater gradients from sediments subject to oscillations in porewater salinity and temperature, ammonium sorption onto the membranes can significantly influence sample composition (Fig. III.5). Coastal aquifers (e.g. Kim et al. 2005), submarine groundwater discharge seepage faces (e.g. Leote et al. 2008) and intertidal sediments (e.g. Rocha 1998) are likely to show strong temperature and/or salinity porewater oscillations. The reequilibrium of the ammonium adsorbed onto the membranes with changing environmental conditions may represent an important fraction of the ammonium present in the porewater aliquots at the analysis stage, depending on the volume of sample extracted in-situ (up to 100% of change in ammonium composition between porewater and filtrate composition; Fig. III.5). Nevertheless, due to the fast reequilibrium of the sorbed ammonium pool within the membranes as shown by our flow-through experiments, this influence will be restricted to the first few filtered millilitres. Therefore, discarding dead volume plus at least the first mL of sample and taking enough sample volume in these situations would help in collecting samples representative of ammonium in-situ conditions.

III.6 Comments and recommendations

Rhizon SMS are very useful and versatile membranes for in- and ex-situ filtration of water and porewater samples. Nevertheless, our results showed possible interference of these membranes over sample ammonium composition through its previously not identified ammonium sorption feature. By improving cleaning procedures, along with pre-stabilization of the membranes with the porewater solution, this effect is minimized. Nevertheless, when sampling environments subject to important temperature, salinity and NH_4^+ concentration gradients, discarding of first filtered water after dead volume and collecting enough sample volume is necessary in order to obtain a filtrate representative of sample composition.

Chapter IV

Porewater nitrate profiles in sandy sediments hosting Submarine Groundwater Discharge described by an Advection-Dispersion-Reaction (ADR) model.

IV.1 Abstract

In order to separate the effects of reaction from those of transport on vertical porewater concentration profiles of nitrate at an intertidal groundwater seepage site (Ria Formosa, Portugal), a free-boundary solution of an Advection-Dispersion-Reaction (ADR) model was used to describe the shape of NO_3^- concentration profiles collected in-situ. The model includes three sequential reaction layers, postulated with basis on the local distribution of the benthic organic C:N ratio and major identifiable changes in concentration gradients with depth. The advective nature of the system was used to propose a mass balance simplification to the constitutive equations permitting a free-boundary solution, which in turn allowed prediction of sediment-water fluxes of NO_3^- . Sensitivity analysis confirmed that in similarly advective-dominated environments, both the porewater concentration distribution and the interfacial fluxes are strongly dependant on seepage rate and benthic reactivity. The model fitted the measured profiles with high correlation (usually higher than 90%), and model-derived sediment-water NO_3^- fluxes were in good agreement to fluxes measured in-situ with Lee-type seepage meters (0.9948 slope, $R^2=0.8672$, $n=8$). Nitrate oxidation and reduction rates extracted from model fits to the data (10^{-2} - 10^0 mmol $\text{m}^{-2} \text{h}^{-1}$) agreed with literature values. Because dispersive effects are not included in direct mass balances based on the porewater concentrations, the model presented here increases the accuracy of apparent reaction rate estimates and geochemical zonation at Submarine Groundwater Discharge (SGD) sites. The results establish the importance of sandy sediments as reactive interfaces, able to modulate mass transfer of continental-derived contaminants into coastal ecosystems. We suggest that tools such as the one described here might be used to advantage in preparing further experimental studies to elucidate how benthic reactivity affects nitrate distribution and fluxes in sediments affected by SGD.

Nomenclature

Z	Depth [L]
$\hat{C}(z)$	Concentration of solute per sediment volume [M L ⁻³]
C_0	Concentration of solute per sed. vol. at the upper interface [M L ⁻³]
C_{aq}	Concentration of solute per sed. vol. at the lower interface [M L ⁻³]
D_{molec}	Molecular diffusion in water [L ² T ⁻¹]
D_{if}	Molecular diffusion in sediment [L ² T ⁻¹]
D_{isp}	Mechanical dispersion [L ² T ⁻¹]
D_{ef}	Effective dispersion [L ² T ⁻¹]
V	Advective velocity [L T ⁻¹]
K_n	Nitrification rate [M L ⁻³ T ⁻¹]
K_d	Nitrate reduction rate [T ⁻¹]
L_x	Layer depth limit [L]
P_e	Peclet number
D_{a1}	Damköhler number for nitrification
D_{a2}	Damköhler number for nitrate reduction
J	Flux per area of total sediment [M L ⁻² T ⁻¹]
φ	Porosity
θ	Tortuosity

IV.2 Introduction

Human activities now fix more atmospheric N into the global Nitrogen cycle than the remainder of Earth’s ecosystems (Vitousek et al. 1997). Hence, natural systems have become artificially enriched in N species, while coastal waters became a common depository for this new available N (Mackenzie et al. 2002). Consequences of this progressive increase in Nitrogen loading of coastal systems include local eutrophication, leading to loss of water quality and biodiversity (Herbert 1999; Rabouille et al. 2001).

Although riverine and point-source transport of nutrients have received much attention as pathways for the transport of excess N from land to sea, the role of other routes such as underground flow paths leading to coastal zone N loading have only been recognized recently (Moore 1999; Burnett et al. 2003). This knowledge gap is explained in part by the difficulty of identifying and directly measuring the discharge, because of its spatial and temporal

variability (Nowicki et al. 1999; Burnett et al. 2006; Taniguchi et al. 2006; Bowen et al. 2007). Based on similarities with estuarine mixing zones, Moore (1999) established the “subterranean estuary” concept applied to the mixing zone between seawater and groundwater. This concept is intertwined with the Submarine Groundwater Discharge (SGD) phenomenon, which refers to the discharge itself, defined as “any and all flow of water on continental margins from the seabed to the coastal ocean, regardless of the fluid composition or driving force” (Burnett et al. 2003).

Total N content of groundwater arriving at the coastal zone, with NO_3^- as the usually dominant form, is highly variable, depending on factors like in-land fertilizer application, sewage infiltration, waste landfill and nitrification at the origin (Slomp and Van Cappellen 2004). The subsurface transport and biogeochemical transformation occurring throughout the aquifer sediment, in particular heterotrophic denitrification, recognized so far as the predominant process depleting groundwater N, can also modulate the final NO_3^- concentration arriving at coastal seepage areas (Bowen et al. 2007; Rivett et al. 2008). However, heterotrophic denitrification is heavily dependant on electron-donor availability, and the lack of labile organic matter could be responsible for the usually high concentrations of oxidized nitrogen arriving at the seepage areas (Slomp and Van Cappellen 2004; Kroeger and Charette 2008; Rivett et al. 2008). Consequently, nitrate attenuation at the sea water-groundwater mixing zone could strongly depend on the amount and quality of electron donors fed from the surface, thus affecting the resulting NO_3^- seepage fluxes (Bowen et al. 2007).

The classical biogeochemical paradigm of sediment accumulation and diffusion-limited reaction has neglected the role of sands on energy and material cycling in coastal sediment beds (Boudreau et al. 2001; Rocha 2008). Low standing stocks of organic matter and mineralization by-products were used to justify this misconception. However, sandy sediments in general and those affected by SGD in particular have recently come under scrutiny as zones of important biogeochemical transformation (Ueda et al. 2003; Slomp and Van Cappellen 2004; Bowen et al. 2007; Kroeger and Charette 2008). Advective motion, characteristic of permeable sediments, enhances the difficulty of discriminating between the relative roles of transport and reaction processes affecting benthic porewater solute concentrations (Boudreau et al. 2001; Rocha 2008). Moreover, permeability of sands could enhance benthic reaction

rates (e.g. Huettel et al. 1998; Falter and Sansone 2000). Thus, the classical approaches to study the biogeochemistry of sediments based on diffusion-limited solute transport are invalid in sandy environments: field sampling needs to preclude interference with the porewater flow patterns (Seeberg-Elverfeldt et al. 2005), and fluxes and reaction rates cannot be accurately estimated from porewater chemical gradients based on Diffusion-Reaction models (Boudreau 1997; Roychoudhury 2001). Furthermore, mechanical dispersion, caused by transport of a solute through a spatially varying flow field coupled with molecular diffusion, could cause flawed direct mass balances if not considered: solute gradients cannot be directly or exclusively linked to benthic reactivity with significant dispersion in place (Roychoudhury 2001; Bijeljic and Blunt 2006). Within this paradigm, diagenetic modeling could nevertheless be an important tool to understand the biogeochemical role of sandy sediments at seepage sites (see Berner 1980; Boudreau 1997). Advection-diffusion and ADR modeling were already successfully applied in elucidating surface water-groundwater interactions. Numerical modeling packages MODFLOW and MT3D were extensively used in modeling groundwater flow and interactions in the hyporheic zones (e.g. Lautz and Siegel 2006). In SGD places, (Rapaglia and Bokuniewicz 2009) used analytical solutions of the general advection diffusion equation for salt to quantify dispersive mixing up to 4m depth. Moreover, Spiteri et al. (2008) used a numerical 2-D ADR model to quantify the main diagenetic processes in SGD sites up to 8m depth into the sediment. Nevertheless, strong diagenetic modulation of solute loads are likely to occur within the first few centimeters depth of SGD seepage faces (Rocha et al. 2009). Labile organic matter distribution can be restricted to the top 2-5 cm (Rocha et al. 2009) and thus can control the behavior of important heterotrophic processes mediating solute loads like denitrification (Slater and Capone 1987; DeSimone and Howes 1996). In this sense, ADR models may be significant tools helping to establish the main reactive zones within the surface benthic concentration gradient, by separating the contribution of advection and dispersion from the role of reaction in changing solute concentrations at different depths. This support may be crucial when sub sampling sediment to carry out laboratory studies using isotope ratios to evaluate reaction kinetics, since criteria underscoring which depths to sub-sample in order to guarantee dominance of a specific nitrogen-transforming microbial population would be clearer with the absence of the effect of transport phenomena over substrate concentration profiles.

Chapter IV

Here we describe the vertical distribution of NO_3^- concentrations in porewater up to 20cm depth of sandy intertidal sediments, subject to cyclic, nitrate-rich SGD on the permanently saturated zone with the aid of a mathematical model inclusive of mechanical dispersion. The main objective is to explain and predict the magnitude of observed interfacial fluxes at sediment exposure within an advective-dominated system and suggest preliminary explanations for the extension of different areas of benthic biogeochemical reactivity found in vertical succession at the permanently saturated seepage area.

IV.3 Methods

IV.3.1 Site description and sample collection

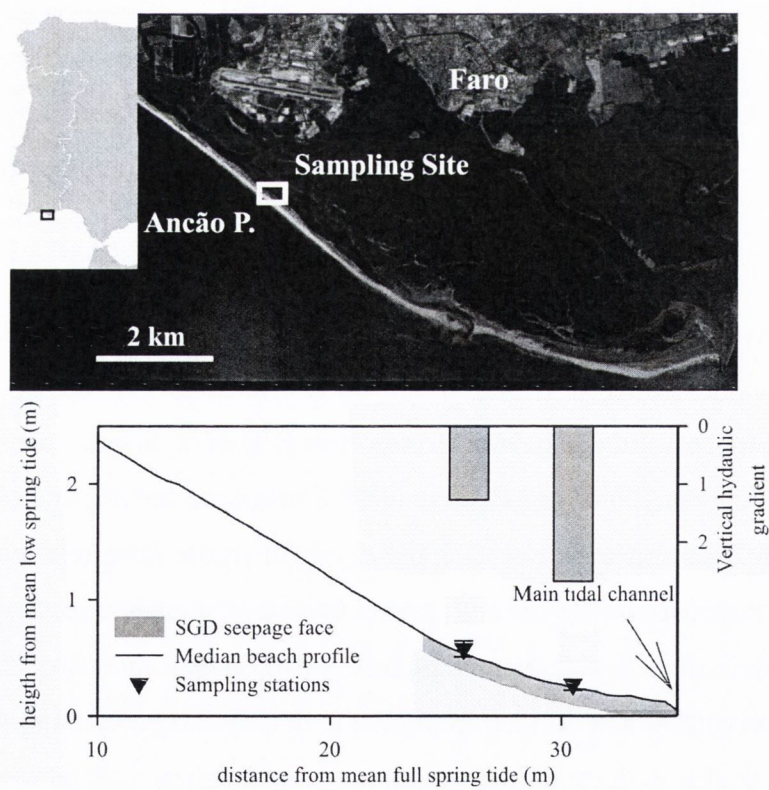


Figure IV.1 Sampling site location at the Ria Formosa Coastal Lagoon (South Portugal, image obtained from LandSat). Median beach profile and location of sampling station in the main seepage face as well as relative distance and height related with mean spring low tide are also shown. Bar charts show vertical hydraulic gradient between 40 and 30 cm depth into the sediment measured in April 2006 at low tide.

The data presented here was collected in the sediment of Praia de Faro (37°00'04''N, 7°88'57''W), one of the two sandy peninsulas of the Ria Formosa coastal lagoon, south Portugal (Fig. IV.1). Porewater concentration profiles of salinity and NO_3^- were measured monthly from November 2005 to January 2007 by using in-situ profilers deployed with poly-ether-sulphone (PES) membranes (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment, 0.1 μm pore size) at constant depths in the sediment. These were mounted on own design in-situ acrylic samplers, modified from the design described in Seeberg-Elverfeldt et al. (2005). The samplers were placed along the low-intertidal beach profile and porewater was collected by connecting vacuum tubes to the membranes. The acrylic sampler has the advantage of being left in place throughout the entire experiment causing minimal disturbance to the local benthic flow patterns (Cappuyns et al. 2004; Seeberg-Elverfeldt et al. 2005). The porewater collected this way was ready for analysis without the need of further preservation (Luo et al. 2003; Seeberg-Elverfeldt et al. 2005). Sample analysis was always performed within 2 weeks after collection. Profiles from March and April presented here correspond to the upper limit of the SGD seepage area, since for the other months the porewater profiles were taken within the seepage face, close to the peak discharge (Fig. IV.1). For direct measurements of advective velocities and fluxes from the sediment, between 2 and 3 Lee-type seepage meters were used at each sampling location, placed in line with the porewater profilers (Fig. IV.1; Lee 1977; Burnett et al. 2006; Taniguchi et al. 2006). The seepage meters consisted on a cylinder enclosing an area of surface sediment and connected to water collecting bags. Seepage rates can thus be determined by measuring the volume of water collected in the bags, and samples can be taken for nutrient analysis and subsequent flux determination (Lee 1977). Sampling started one tidal cycle after installation to permit system stabilization. Before sampling started, the two plastic collection bags connected to each seepage meter were left open to fully release any air trapped into the seepage meters or bags. Sampling started during the next sediment exposure phase (ebbing tide) and was carried out during a minimum of two subsequent tidal cycles. During sampling, continuous measurements of seepage velocity, salinity and temperature were performed in the water collection bags connected to the seepage meters, and aliquots were taken for NO_3^- determination. Operation of the seepage meters was performed following the precautions suggested by Cable et al. (1997). Plastic collection bags were kept at the same level as the seepage meters in order to minimize any interference of the bags with the

local hydraulic head. Due to logistic limitations, no pre-determined sampling times were set. Instead, sampling was performed by emptying the content of the bags when they were approximately $\frac{3}{4}$ full and registering the time of collection. No bag pre-filling was done due to the fast and frequent sampling needed to accomplish our sampling strategy, and to the existence of parallel nested piezometers (for vertical porewater pressure gradient determinations) that would either verify the assumption of essentially vertical flow (Fig. IV.1) or at the very least provide independent gauging of pore pressure allowing comparison. However, as explained in Leote et al. (2008) and further demonstrated in Rocha et al. (2009), this could lead to wrong measurements due to the Shaw-Prepas effect, related to the short term influx of water to the plastic bags after installation due to the mechanical properties of the collection bags (Shaw and Prepas 1989). Nevertheless, as also demonstrated by Shaw and Prepas (1989), this artifact is minimal when studying seepage rates higher than $0.5 \text{ L h}^{-1} \text{ m}^{-2}$. The “Shaw-Prepas” effect should therefore be minimal in our study due to the high recorded seepage rates (lowest recorded rate $0.7 \text{ L h}^{-1} \text{ m}^{-2}$; Leote et al. 2008). The possible sources of error affecting seepage rate measurements with this technique (i.e. potential alteration of natural hydraulic head when sea water level drops below seepage meters or due to the “Shaw-Prepas” effect) raise uncertainties about our measurements. However, seepage rate records obtained with Lee-type seepage meters at our site showed strong coherence as discussed in Leote et al. (2008) and Rocha et al. (2009). Seepage rates showed strong tidal modulation, with peak rates at sediment exposure concomitant with the lowest salinities. At high tide, minimum or absent seepage rates were measured. Also, seepage rates decreased from the permanently saturated area to the upper levels of the beach. The physical coherence of seepage rate records was further tested by performing independent measurements using sets of mini-piezometers at each seepage meter deployment station (Rocha et al. 2009). Seepage rates were thus calculated from piezometric head in-situ by applying Darcy’s law. Generally good agreement between seepage meter and piezometer data was confirmed in Rocha et al. (2009). Despite the potential source of error of the seepage meters used, the local use of independent assessment of hydraulic head together with the operation based on literature references (including studies directly evaluating the relative effect of different operational measures, adapted to local conditions), the potential source of error associated with operation of the seepage meters, under these conditions, was effectively minimized.

In order to complement the information obtained with seepage meters and sediment vertical profilers, sediment cores (25cm length, 3.5cm diameter) were taken by hand, and sliced on-site for analysis of water content and porosity. Water content was measured in each sub-sample as a percentage of weight loss after freeze-drying for 24h. Porosity, φ , was calculated by the equation described by Dullien (1992), using 2.65 g ml^{-1} as dry sediment density. Tortuosity, θ , was calculated assuming to obey Archie's law, $\theta^2 = \varphi^{1-m}$, where m is a fitting parameter (Boudreau 1997). Experimental fitting work revealed $m=2$ for sands and mud sediments with porosities lower than 0.7 (Ullman and Aller 1982), thus obtaining the formulation $\theta^2 = \varphi^{-1}$ for the tortuosity calculation. Salinity of porewater samples was measured with an ATAGO S/Mill-E hand-held refractometer, using single-point calibration with distilled water. Salinity and temperature of the seeped water were determined in-situ with an YSI 600 multi-parameter probe (YellowSpring Instruments). NO_3^- content in porewater and seeped water were determined by the spongy cadmium method (Jones 1984). A more complete description of the sampling strategy, methods, and site specifics can be found in Leote et al. (2008).

IV.3.2 Modeling approach

IV.3.2.1 Conceptual model

In intertidal sandy sediments affected by SGD, the pressure balance between the hydraulic head of the unconfined aquifer and tide could determine the mass exchange through the sediment-water interface (Johannes 1980). In mesotidal environments, tidal modulation could therefore be the main forcing factor determining water exchange and related solutes across the sediment-water interface (Robinson et al. 2007). Inside the sediment, tide can induce dispersive mixture of both end members (groundwater and sea water) and thus determine the composition of the seeping water (Rapaglia and Bokuniewicz 2009). Moreover, the tide can determine porewater advective velocity due to the changing hydraulic head caused by its rise and fall. Thus, tidal level oscillation can also control the extent and magnitude of solute mixing within the sediment due to mechanical dispersion (Roychoudhury 2001; Rapaglia and Bokuniewicz 2009). At this site, significant advective velocities were measured

in-situ, mainly during sediment exposure, when discharge peaked (Leote et al. 2008). The largest fluxes of NO_3^- were associated with groundwater discharge peaks. In effect, tidal dynamics have been identified as the main modulator of temporal patterns of SGD at the Ria Formosa coastal lagoon (Rocha et al. 2009). A cyclic and reproducible discharge pattern was observed and described in Leote et al. (2008) and Rocha et al. (2009), with peak rates at low tide and minimum or absent seepage during peak high tide. Thus, for the purpose of model formulation, advective transport of NO_3^- is assumed to be exclusively the result of the seepage of porewater, accepted as essentially vertical (Fig. IV.1; Horn 2002; Burnett et al. 2006 and references therein). In fact, the possible horizontal component of seepage can introduce uncertainties on the shape of nitrate gradients observed in the porewater and their response to discharge. However, several field observations at our site (Leote et al. 2008; Rocha et al. 2009) support the assumption of the seepage being predominantly vertical. Seepage at the upper levels of the beach is substantially different in terms of salinity and solute content when compared with the lower levels affected by SGD. Nevertheless, influence of the upper levels in the solute distribution at the lower levels was not identified. With significant horizontal water movement, the higher and unchanged salinity at the upper levels should promote salinity gradients in the lower levels, especially in the upper cm where, at the site, hydraulic conductivity is higher (see Rocha et al. 2009). Instead, salinity profiles in the area affected by SGD were vertically homogeneous when seepage peaked. Moreover, in this more permeable layer close to the sediment-water interface, the lower nitrate content of the upper levels would dilute the nitrate content in the lower levels. Instead, nitrate concentration significantly peaks at the sediment-water interface. Finally, the under saturation of the upper part of the beach when SGD is at maximum avoids significant hydraulic gradient in the upper levels to promote significant horizontal water movement (see Rocha et al. 2009 for a more extensive discussion of this point). Thus, under vertical seepage of salinity-uniform groundwater, vertical solute gradients are clearly linked to benthic reactivity.

Strong depth gradients of dissolved inorganic nitrogen ($\text{DIN}=\text{NO}_3^-+\text{NO}_2^-+\text{NH}_4^+$) and salinity were identified on porewater profiles taken during sediment exposure at low tide. Typical transient DIN profiles (November 2005) are shown in Fig. IV.2, together with the mean C/N molar ratio of organic matter (March 2006) at the same depth scale. Three different reactive zones may be identified by comparing the measured DIN profiles with the theoretical

mixing curve (using salinity as conservative tracer of mixing throughout the vertical distance sampled in-situ; Bao-dong and Brockmann 2005; Fig. IV.2) and with the bulk organic matter C/N ratio depth profile: a) The deeper layer (from 20cm to 15-12 cm depth) characterized by showing no significant deviation between mixing-only and measured DIN profiles. On that zone, the C/N ratio depth gradient remained constant, suggesting a relatively invariant refractory organic matter pool; b) An intermediate zone (from 15-12 cm to 3 cm depth) showing strong segregation between the measured DIN profiles and the theoretical distribution as calculated by the mixing curves. This intermediate layer was also distinguishable by a sharp change in the C/N depth gradient, coincident with the minimum nitrate concentration through the vertical profiles taken (see the results section); Finally, c) The top layer (between 3 cm depth and the sediment-water interface), characterized by DIN and nitrate distributions evidencing peaks against a uniform salinity gradient (see Fig. IV.2 and results section). Bulk organic matter C/N ratios persisted here at the lowest values found within the whole profile.

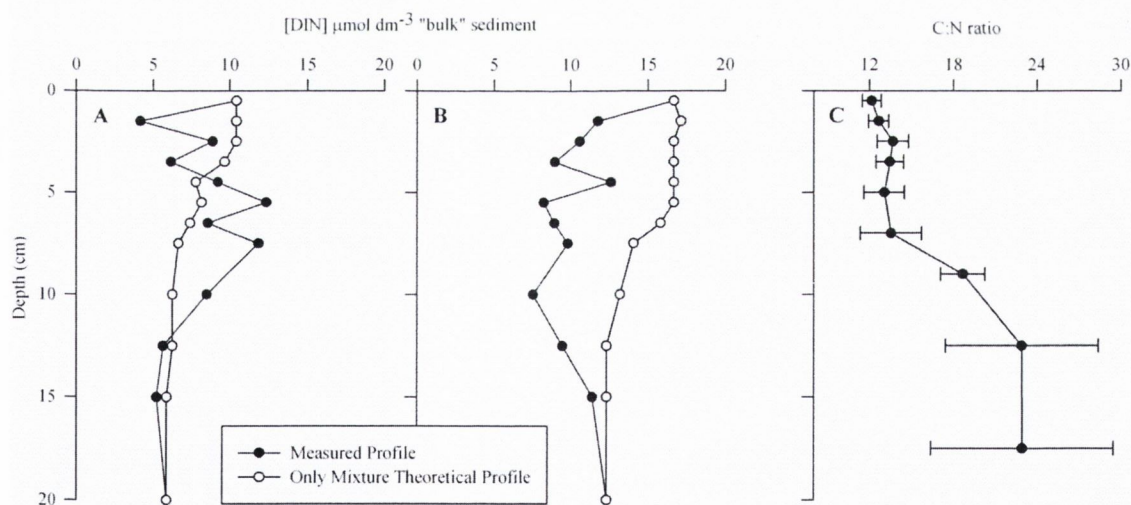


Figure IV.2 a and b. Dissolved Inorganic Nitrogen (DIN) distribution on porewater affected by Submarine Groundwater Discharge (SGD) measured on November 2005 (black) along with theoretical only-mixture profiles (white). c. Mean C:N molar ratio of organic matter into the sediment studied on March 2006, corresponding to four profiles taken. Sampled sediment is located at Praia de Faro, South of Portugal. See methods for sampled place description.

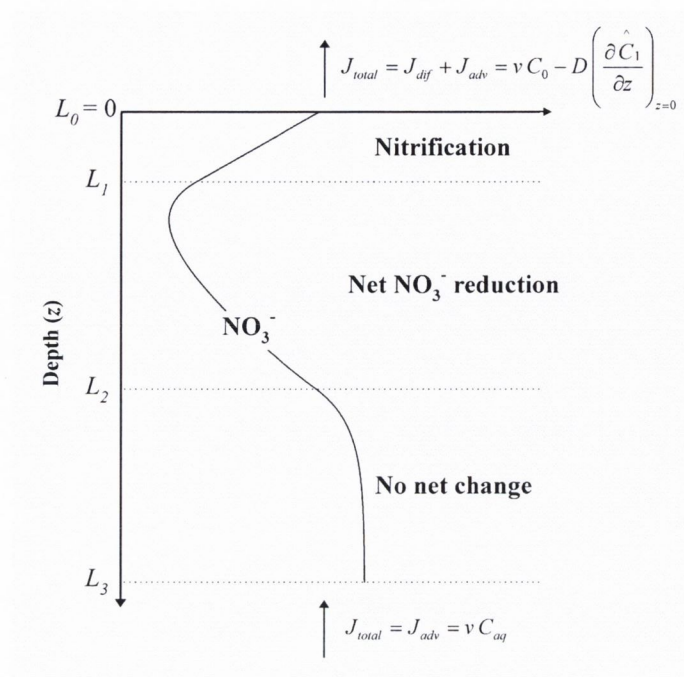


Figure IV.3 Schematic view of the three-layer model formulation resulting from the conceptual analysis; Distribution of layers, boundary fluxes and theoretical nitrate distribution on the porewater are shown.

Thus the conceptual reaction zonation to construct the model was based on the discrimination of the sediment reactivity areas into three depth horizons: a net nitrification layer (nitrogen oxidation) at the surface, a net nitrate reduction layer below that, and a third, non-reactive layer underlying the two (Fig. IV.3). Thus, L_3 represents the lower limit of the studied sediment horizon (in this case 20 cm depth). L_2 represents the lower limit of the net nitrate reduction layer (thus $L_3=L_2$ when the third, non-reactive layer was not included in the modeling procedure). Finally, L_1 represents the lower limit of the net nitrification layer. Thus, and based on the conceptual model constructed, L_2 was always considered deeper than L_1 .

IV.3.2.2 Model formulation

IV.3.2.2.1 General formulation and solution

The diagenetic problem was further simplified to a vertical-flow tubular reactor scheme. Transport processes included in the model formulation were molecular diffusion,

mechanical dispersion and advective transport. Molecular diffusion and mechanical dispersion were modeled as a single term, dubbed “effective dispersion”, and defined as the sum of the dispersive and the diffusive terms, viz: $D_{ef} = D_{if} + D_{isp}$ (Roychoudhury 2001). The NO_3^- diffusion coefficient in free water solution was calculated using the temperature-dependent parameterization suggested by Boudreau (1997). It was further corrected with the tortuosity in order to calculate the apparent molecular diffusion coefficient in the sediment, thus accounting for the presence of the solid phase ($D_{if} = D_{molec} / \theta^2$; Berner 1980). Because the modeled medium consisted mostly of sand, Archie’s Law was used to calculate the tortuosity from the porosity, thus obtaining $D_{if} = \phi D_{molec}$ for the apparent diffusion coefficient in the sediment. Mechanical dispersion was included in the model formulation by using the pore-scale solution described by Bijeljic and Blunt (2006, and references therein), $D_{isp} = P_e^{1.2}$, where $P_e = vL / D_{molec}$, P_e being the nondimensional Peclet number resulting from the ratio between the time-scales of advective and diffusive transport, and where v is the advective velocity and L is the characteristic pore length. According to Roychoudhury (2001), mean grain size diameter is a good proxy for characteristic pore lengths in sandy sediments. Since the simulations performed by Bijeljic and Blunt (2006) are designed at the pore scale (60x60 pores networks, 3x3cm in our case), they obtained identical results with longer pore networks (up to 4000 pores), thus being appropriate for the 20cm depth explored here.

For the purpose of this modeling exercise, advective transport of NO_3^- was assumed to be exclusively the result of the vertical seepage flow when the sediment was exposed (Horn 2002; Taniguchi et al. 2006; Rocha et al. 2009). This process is included on the model formulation as v , the velocity of the porewater with respect to the sediment particles, since the presence of the solid phase is accounted for by using the bulk NO_3^- concentration (see below). The reactive processes to be included were divided into nitrification and nitrate reduction. Nitrification was assumed to take place close to the surface, with zero-order kinetics in relation to the NO_3^- concentration. In order to simplify the formulation to a single species model, independence of nitrification rates from ammonium concentration was also assumed, based on the characteristic low K_m for nitrification found on controlled systems (see compilation made by Sheibley et al. 2003). The potential organic matter limitation of benthic

bacterial processes could also increase the likelihood of alternative metabolic pathways being present, including dissimilatory nitrate reduction to ammonium (DNRA), anaerobic ammonium oxidation (ANAMMOX) or oxygen-limited autotrophic nitrification-denitrification (OLAND) (Hulth et al. 2005; Bowen et al. 2007; Brandes et al. 2007; Kroeger and Charette 2008). For the purpose of this study, the term “nitrate reduction” describes any process responsible for the depletion of NO_3^- following first-order kinetics with respect to NO_3^- concentration. Finally, and since the distribution of organic matter and nitrate at our site suggests that constraints to nitrate reduction with depth are possible (Fig. IV.2, results section), we included a third model layer as an optional, non-reactive zone with respect to nitrate.

The porosity varies with depth, thus implying another depth-dependent parameter to be inserted into the model. This dependence (and thus the presence of the solid phase) was included by expressing the constitutive equations in relation to the “bulk” concentration of NO_3^- ($\hat{C}(z) = \phi(z)C(z)$, Boudreau 1997).

Based on this set of assumptions, the diagenetic equations for the three-layer model were established, based on the general formulation of Advection-Dispersion-Reaction (ADR) models, as follows:

Nitrification layer:

$$\frac{\partial}{\partial z} \left(D_{ef} \frac{\partial \hat{C}_1(z)}{\partial z} \right) - \frac{\partial}{\partial z} \left(v \hat{C}_1(z) \right) + K_n = \frac{\partial \hat{C}_1}{\partial t}$$

NO_3^- reduction layer:

$$\frac{\partial}{\partial z} \left(D_{ef} \frac{\partial \hat{C}_2(z)}{\partial z} \right) - \frac{\partial}{\partial z} \left(v \hat{C}_2(z) \right) - K_d \hat{C}_2(z) = \frac{\partial \hat{C}_2}{\partial t}$$

Non-reactive layer:

$$\frac{\partial}{\partial z} \left(D_{ef} \frac{\partial \hat{C}_3(z)}{\partial z} \right) - \frac{\partial}{\partial z} \left(v \hat{C}_3(z) \right) = \frac{\partial \hat{C}_3}{\partial t}$$

with K_n as the nitrification rate ($\text{nmol cm}^{-3} \text{ h}^{-1}$), K_d the NO_3^- reduction rate constant (h^{-1}) and z the sediment depth (cm).

The oscillatory nature of the system, along with its observed reproducibility, was used to postulate a pseudo-stationary state in order to solve the system of differential equations. After a temporal salinity gradient verified in the porewater seeping during the first stages of active seepage (i.e. during ebb tide), salinity of the seeped water became progressively uniform during low tide (Leote et al. 2008). The mixture of both end-members inside the subterranean estuary (i.e. sea water and groundwater) and the relative constant high seepage rate in place permitted to assume that the discharge remained nearly constant for the period of peak of discharge (i.e. around 3h during low tide). Therefore, and due to the fast water renewal at the top 20 cm of the sediment in the seepage face due to the high discharge rates, observed vertical NO_3^- gradients are stable over the time the discharge peaks. Thus, we assume that during peak discharge from the beach, a stationary state ($\partial C / \partial t = 0$) is reached. Due to the harmonic behavior of the tide, and consequent reproducibility of the seepage event, this behavior is verified every tidal cycle.

Amongst the possible boundary conditions for a finite sediment column, we chose to define the NO_3^- concentrations at the limits of the model domain (i.e., $\hat{C}_1(0) = C_0$ for the sediment-water interface and $\hat{C}_3(L_3) = C_{aq}$ for the concentration at the lower boundary). The continuity conditions imposed to solve the problem analytically were based on the identity of concentrations and fluxes across the interfaces of the domain:

$$\left. \frac{\partial \hat{C}_2(z)}{\partial z} \right|_{z=L_2} = \left. \frac{\partial \hat{C}_3(z)}{\partial z} \right|_{z=L_2} \quad \text{and} \quad \hat{C}_2(L_2) = \hat{C}_3(L_2) \quad \text{for } L_2$$

$$\left. \frac{\partial \hat{C}_1(z)}{\partial z} \right|_{z=L_1} = \left. \frac{\partial \hat{C}_2(z)}{\partial z} \right|_{z=L_1} \quad \text{and} \quad \hat{C}_1(L_1) = \hat{C}_2(L_1) \quad \text{for } L_1$$

where L_2 represents the depth boundary between the NO_3^- reduction and non-reactive layers (cm) and L_1 is the depth boundary between the nitrification and NO_3^- reduction layers (cm) (see Fig. IV.3). The analytical solution of the problem for each layer was obtained by solving the differential equations and using the continuity and boundary conditions in order to obtain the integration constants, viz:

Layer 1 (from $z=0$ to $z=L_1$):

$$\hat{C}_1(z) = \frac{1}{BvH} \left(2Ae^{\frac{L_1(v+\omega)}{2D_{ef}}} \left(-1 + e^{\frac{zv}{D_{ef}}} \right) v\omega + \right. \\ \left. B \left(K_n \left(-4D_{ef} \left(-1 + e^{\frac{zv}{D_{ef}}} \right) + zH - L_1v \right) - C_0v(v-H) + (K_nL_1 + C_0v) \left(e^{\frac{zv}{D_{ef}}} (v-\omega) + \omega \right) \right) \right)$$

Layer 2 (from $z=L_1$ to $z=L_2$):

$$\hat{C}_2(z) = \frac{1}{vBCH} \left(e^{\frac{z(v-\omega)}{2D_{ef}}} \left(2BC_{aq}e^{\frac{L_2(v+\omega)}{2D_{ef}}} v^2H - \right. \right. \\ \left. \left. A \left(2B + Cv \left(\left(e^{\frac{z\omega}{D_{ef}}} - e^{\frac{L_1(v+\omega)}{D_{ef}}} \right) (v-\omega) - \left(e^{\frac{L_1\omega}{D_{ef}}} - e^{\frac{L_1v+z\omega}{D_{ef}}} \right) (v+\omega) \right) \right) \right) \right)$$

Layer 3 (from $z=L_2$ to $z=L_3$):

$$\hat{C}_3(z) = \frac{1}{v^2 BCH} \left(\begin{array}{l} BC_{aq} v^2 H \left(C - \left(e^{\frac{L_3 \omega}{D_{ef}}} - e^{\frac{zv}{D_{ef}}} \right) (v - \omega) \right) + A e^{-\frac{L_2(v+\omega)}{2D_{ef}}} \left(e^{\frac{L_3 \omega}{D_{ef}}} - e^{\frac{zv}{D_{ef}}} \right) \\ B(v - \omega) + Cv \left(\begin{array}{l} 2D_{ef} K_d \left(-1 + e^{\frac{L_1 v}{D_{ef}}} \right) \left(e^{\frac{L_1 \omega}{D_{ef}}} - e^{\frac{L_2 \omega}{D_{ef}}} \right) + \\ v \left(e^{\frac{L_1(v+\omega)}{D_{ef}}} (v - \omega) - e^{\frac{L_2 \omega}{D_{ef}}} (H - v + \omega) \right) \end{array} \right) \end{array} \right)$$

With support constants defined as follows:

$$\omega = \sqrt{4D_{ef} K_d + v^2} ;$$

$$H = v - \omega + e^{\frac{L_1 v}{D_{ef}}} (v + \omega);$$

$$C = e^{\frac{L_3 v}{D_{ef}}} (v - \omega) + e^{\frac{L_2 v}{D_{ef}}} (v + \omega) ;$$

$$A = C e^{-\frac{L_1(v-\omega)}{2D_{ef}}} \left(e^{\frac{L_1 v}{D_{ef}}} (D_{ef} K_n - v(K_n L_1 + C_0 v)) - D_{ef} K_n \right) + C_{aq} e^{\frac{L_2(v+\omega)}{2D_{ef}}} v^2 H$$

and

$$B = 2vD_{ef} K_d \left(-1 + e^{\frac{L_1 v}{D_{ef}}} \right) \left(e^{\frac{L_2 v}{D_{ef}}} - e^{\frac{L_3 v}{D_{ef}}} \right) \left(e^{\frac{L_1 \omega}{D_{ef}}} - e^{\frac{L_2 \omega}{D_{ef}}} \right) +$$

$$v^2 \left(\left(e^{\frac{L_2(v+\omega)}{D_{ef}}} - e^{\frac{(L_1+L_3)v+L_1\omega}{D_{ef}}} \right) (v - \omega) + \left(e^{\frac{(L_1+L_3)v+L_2\omega}{D_{ef}}} - e^{\frac{L_2 v+L_1\omega}{D_{ef}}} \right) (v + \omega) \right)$$

IV.3.2.2.2 Free-boundary solution

To improve the predictive capabilities of the model, an additional boundary condition was used to derive an expression allowing for the prediction of the surface boundary concentration (C_0) by the model. This was obtained by simplification of the expression deriving the mass balance for the top layer (i.e., from $z=0$ to L_1). Assuming that the difference between the dispersive fluxes at both limits of the layer $D_{ef} \left(\partial \hat{C}_1(z) / \partial z \right)_0 - D_{ef} \left(\partial \hat{C}_1(z) / \partial z \right)_{L_1}$, are negligible by comparison with the advective and reactive terms when seepage is dominant (advective dominated system), the following expression for the concentration of NO_3^- at the sediment-water interface may be obtained:

$$C_0 \cong (1/v) \left(v \hat{C}_1(L_1) - K_n L_1 \right).$$

Substituting $\hat{C}_1(L_1)$ and solving for C_0 , we obtain a free-boundary solution for the problem, viz:

$$C_0 = \frac{BK_n \left(L_1(v - \omega) - 4D_{ef} \right) + 2v\omega \left(CK_n e^{\frac{L_1\omega}{D_{ef}}} \left(e^{\frac{L_1v}{D_{ef}}} (L_1v + D_{ef}) - D_{ef} \right) + v^2 HC_{aq} e^{\frac{(L_1+L_2)(v+\omega)}{D_{ef}}} \right)}{v \left(2v^2 \omega C e^{\frac{L_1(v+\omega)}{D_{ef}}} - B(v - \omega) \right)}$$

This approximation is only valid when advection is the dominant transport process for the domain (eg, during sediment exposure and when groundwater seepage is at its peak).

IV.4 Results

IV.4.1 Range of applicability of the free-boundary solution

In order to test the validity of the simplification for the free-boundary solution, the ratio between the extended mass balance for the surficial reactive layer and the simplified one (Ψ) was used:

$$\Psi = \frac{v \hat{C}_1(L_1) - K_n L_1}{v \hat{C}_1(L_1) - K_n L_1 + D_{ef} \left(\left(\partial \hat{C}_1(z) / \partial z \right)_0 - \left(\partial \hat{C}_1(z) / \partial z \right)_{L_1} \right)}$$

The range of parameters for which this ratio became close to unity, with a finite up-flow velocity, was explored. As an additional quality estimator for the results of the free-boundary solution, mass conservation was checked by systematically calculating the model-based mass balance for the whole domain.

Two important nondimensional parameters, the Peclet number, P_e , and the Damköhler number, D_a , were used both for the test of the free-boundary solution and for subsequent exploration of model results, at each of the modeled reaction domains. Damköhler numbers result from the ratio between the temporal scales of reaction and transport (e.g. Carleton 2002). We used the following expressions:

$$D_{a1} = \frac{K_n L_1^3}{D_{ef} \int_{L_1}^0 \hat{C}_1(z) dz}$$

for the Damköhler number representative of the nitrification layer, and

$$D_{a2} = \frac{K_d (L_2 - L_1)^2}{D_{ef}}$$

for the Damköhler number representative of the NO_3^- reduction layer.

The test results of the simplification applied to obtain the free-boundary solution showed high stability. No significant deviation (within 5%) of the function used as convergence criterion (Ψ) was found for the main tested parameters (data not shown). In fact, Ψ maintained values close to 1 whilst P_e , D_{a1} and D_{a2} were modified, implying the pertinence and physical coherence of our simplification. Some deviation was observed only for P_e values on the extreme low-end of the applied range (P_e around 1), in agreement with the premises of the modeled system, which stated that this was an advection dominated system (see above). This deviation becomes more obvious when manipulating the width of the nitrate reduction layer (distance between L_2 and L_1 , Fig. 4). Ψ became unstable for widths down to 2 cm when P_e number was in the extreme low-end of the transition zone between a diffusion dominated and a pure advective regime (Bijeljic and Blunt 2006). Exploring the instability at $P_e=1$, larger deviations were found for decreasing nitrate reduction rates (in the form of D_a numbers, Fig. 4). These deviations disappeared at higher P_e numbers and/or higher nitrate reduction rates.

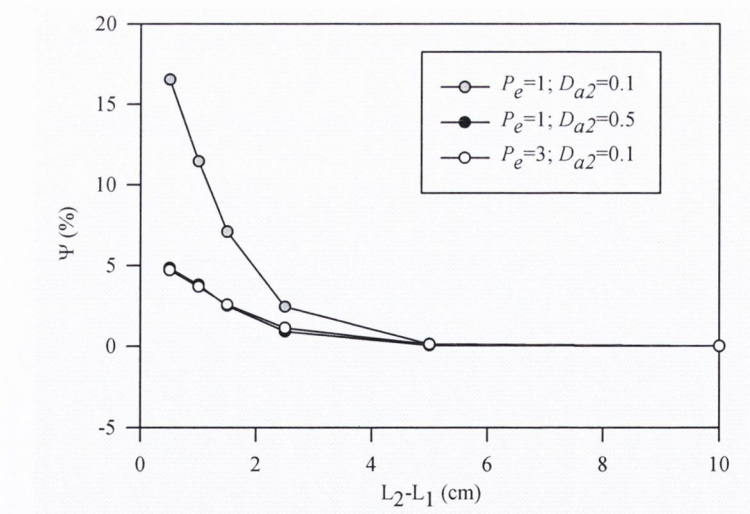


Figure IV.4 Results of the test performed (Ψ) over the free-boundary solution approximation: Stability of simplification within the nitrate reduction layer length is shown. Influence of advective regime (hydrological Peclet number) and nitrate reduction rates (in the form of dimensionless Damköhler numbers) are crucial at the lower end of the applicated range.

IV.4.2 Sensitivity analysis

A sensitivity analysis was performed to determine the influence of each different model input parameter both on the NO₃⁻ concentration gradients and on the derived surface fluxes. The results were used not only to identify the most important parameters to be determined during future on-site fieldwork, but also to obtain further information on the key variables controlling the NO₃⁻ distribution in the porewater and the magnitude of the NO₃⁻ seepage fluxes under the described conditions. Input parameters were varied across their realistic ranges, based on typical literature values and own data obtained in-situ, but excluding values outside the range of applicability of the model (see previous section).

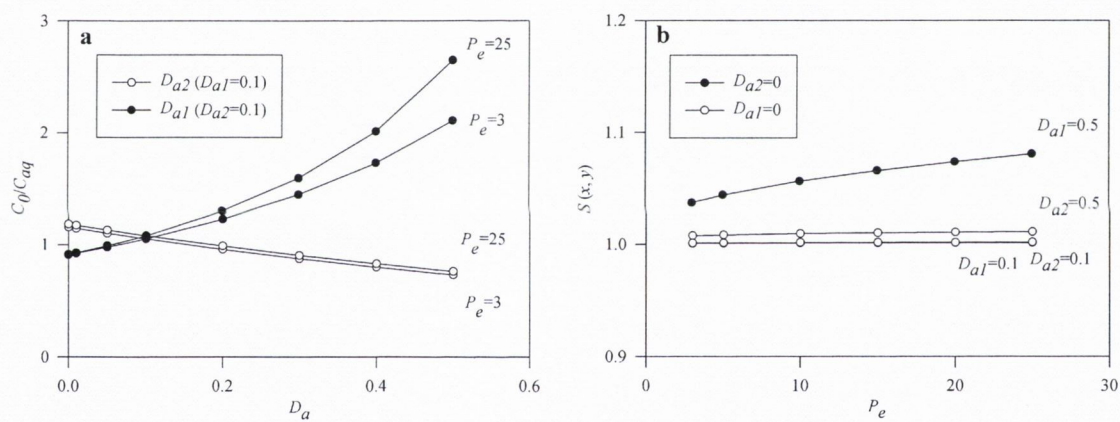


Figure IV.5 A. Influence of reaction rates (in the form of dimensionless Damköhler numbers) and advective regime (hydrological Peclet number) on net NO₃⁻ transformation capacity inside the model domain (C_0/C_{aq}). B. Sensitivity [$S(x,y)$] of the predicted interface fluxes calculated using the model at changing reaction rates (in the form of Damköhler numbers) and changing advective regime (in the form of Peclet number). Both Damköhler and Peclet numbers were used inside the model application range.

The nondimensional ratio between NO₃⁻ concentrations at both limits of the modeled domain, (i.e., C_0/C_{aq}), was used in order to determine the influence of each model parameter on the NO₃⁻ profile. The function $S(x,y) = (\partial x/x)/(\partial y/y)$ was used as an estimator of the sensitivity of the resulting upper-boundary fluxes (x) to changes imposed on each parameter (y) (Jørgensen et al. 1994; Schauser et al. 2006). The function $S(x,y)$ was used in its discrete

form. At each parameter value, 5% of change was induced to subsequently follow how upper-boundary fluxes reacted to those changes. This process was performed covering the whole parameter range inside the applicability of the model. Thus, this 5% induced change was performed at each discrete parameter value throughout the whole range of values for which the model can be applied. The use of non-dimensional D_a numbers allows the evaluation of the effect of changing reaction rates normalized to their most important physical constraints in-loco, i.e., spatial coverage of a given mechanism or relative weight of diffusion with regards to advection. Therefore, in order to minimize temporal and spatial interdependences of the model parameters in the sensitivity evaluation, the sensitivity analysis was performed using nondimensional D_a and P_e parameters.

The most important parameters controlling the NO_3^- distribution within the model domain were the reaction rates and the advective regime (Fig. IV.5A). Low reaction rates at low seepage velocities (D_a numbers lower than 0.2) can significantly change the NO_3^- concentrations inside the model domain (Fig. IV.5A). For example, setting D_{al} to a value close to 0.5 at low P_e values ($P_e=3$) can increment the nitrate concentration in the profile up to 100% from the initial amount of nitrate entering at the lower depth limit (C_{aq}). Advective velocity showed an important effect over those changes, by enhancing NO_3^- addition to the top layer by nitrification and increasing NO_3^- depletion by nitrate reduction (in terms of constant nondimensional D_a , Fig. IV.5A).

Analysis of the influence of the different model parameters in the resulting upper-boundary fluxes ($S(x, y)$, Fig. IV.5B) confirmed the crucial role of reaction rates and advective velocity; fluxes were always highly sensitive to changes in P_e ($S(x, y) \geq 1$), meaning that the upper boundary fluxes at least co-varied with changes in P_e . P_e and surface fluxes kept co-varying when reaction rates were low ($S(x, y) = 1$). This situation changes when higher D_a numbers are imposed, whereby changes to the P_e number induce larger changes on upper boundary fluxes ($S(x, y) > 1$, Fig. IV.5). Sensitivity analysis was also performed over the input nitrate concentration (C_{aq}) and effective dispersion. Sensitivity was always low for those parameters (lower than 0.5, data not shown).

IV.4.3 Model description of field data

IV.4.3.1 Model input parameters

Advective velocity was calculated from in-situ seepage meter data. The measured seepage rates were integrated throughout a one-hour time interval centered at the time at which the profile was sampled. As a result of the field work strategy, more than one seepage meter located beside each profiler produced data for subsequent use in the integrations. Temperature for the calculation of diffusion coefficients was also obtained by integrating temperature data obtained from the seepage meters during the same period. NO_3^- concentration of the water entering the model domain (C_{aq}) was fixed using the in-situ concentration measured at the lower end of each profile. The model was used to fit the in-situ NO_3^- concentration profiles and extract the reaction rates, layer lengths and fluxes. To ensure that NO_3^- gradients were mainly caused by reaction and the constant seepage of water, only profiles with a salinity depth-variance lower than 1 ppt, the error associated with hand-refractometer determinations of salinity, were used in this analysis. The average grain size was used to calculate P_e in accordance with Roychoudhury (2001). Model-derived porewater NO_3^- concentration profiles were fitted to the data, by minimizing the sum of squared differences between each other, with the aid of the Solver routine (Microsoft Excel). To check the quality of the adjustment, the linear correlation between the two data sets was used (Berg et al. 1998). Finally, in order to compare the quality of fit for different profiles, the standard deviation (s) of the fits was normalized to the integrated concentration of the whole domain, by using

$$U(\%) = \frac{\left(\int_{L_3}^0 \hat{C}(z) dz / L_3 \right) - \sqrt{s^2}}{\int_{L_3}^0 \hat{C}(z) dz / L_3} \times 100$$

All profiles used in this study were obtained in conditions fitting the range of applicability of the model. The selected profiles were chosen amongst all available profiles in order to show high temporal distribution and model response to different input parameters:

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profiles are differentiated by advective velocity (hence dispersion coefficient associated to the advective motion), porewater salinity and thus NO_3^- concentration entering in the domain (C_{aq} and associated flux), as well as apparent vertical NO_3^- gradients. They also differed on the output results in terms of apparent reaction rates, reactive layer length and number of layers employed in the fitting procedure (dependant on the inclusion or lack thereof of the optional non-reactive layer in depth).

IV.4.3.2 Fit Results

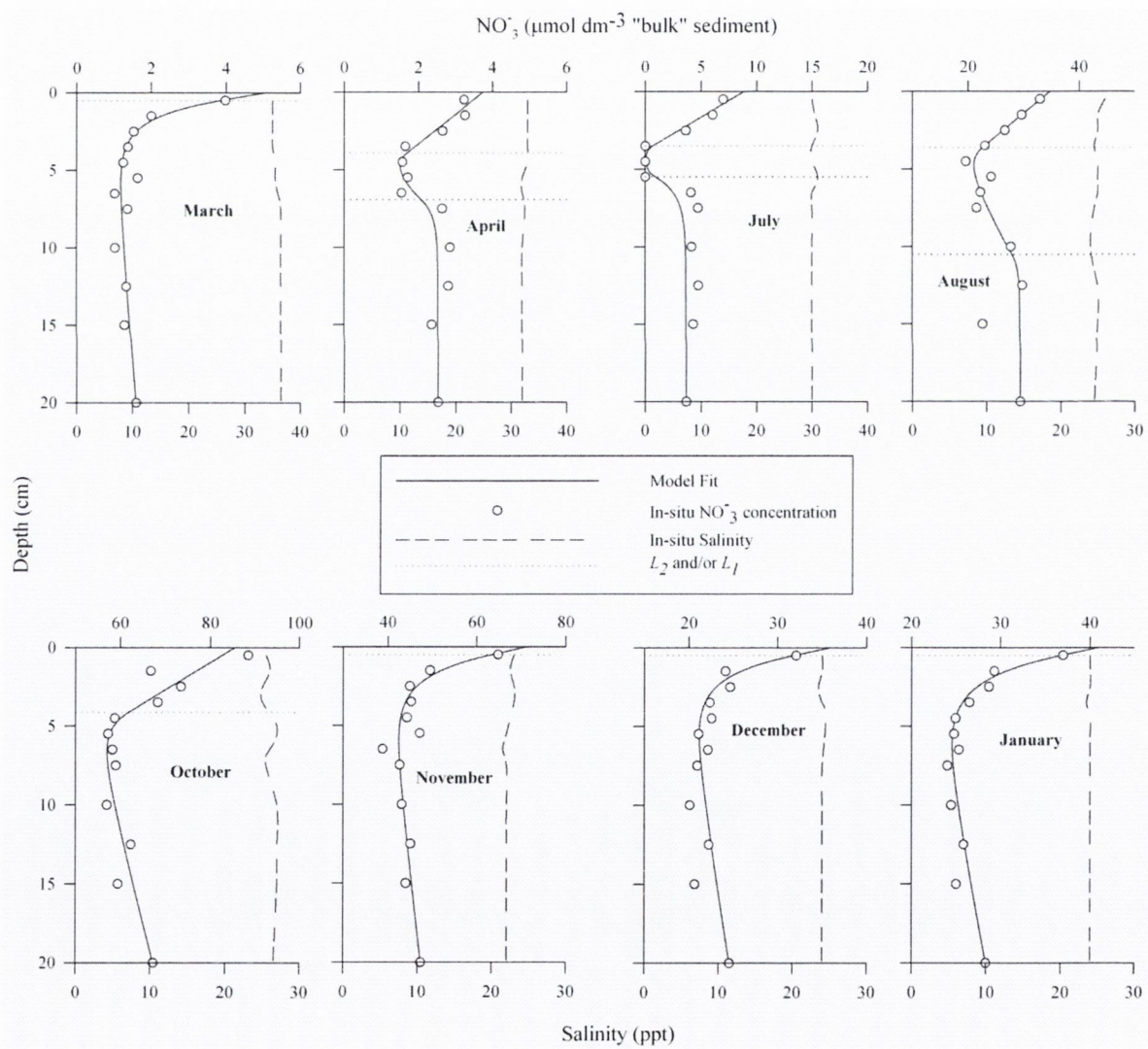


Figure IV.6 Measured NO_3^- profiles plotted together with modeling results. The concomitant measured salinity profiles are also shown.

The measured profiles, relevant salinity profiles, and associated model fits are shown in Fig. IV.6. Table IV.1 shows the values of the parameters used to quantify the goodness of model fits and the depth integrated standard deviation of the measured salinity profiles. The standard deviation of salinity in the profiles was always lower than 1 ppt (<0.7 ppt), thus meeting the criterion used to ensure a homogenized column in terms of mixing. The modeled NO₃⁻ profiles fitted the measured data with high correlation; both the linear correlation and the *U*(%) convergence criterion were always higher than 70%, and usually above 90%.

	Standard Deviation	Linear Correlation %	<i>U</i> (%) %	Salinity St. Deviation ppt
March	0.19	96.84	87.21	0.60
April	0.25	91.19	89.02	0.42
July	0.89	93.32	72.40	0.30
August	2.25	84.19	91.37	0.43
October	3.81	90.08	93.76	0.67
November	2.10	93.87	95.23	0.38
December	1.24	91.66	94.39	0.16
January	0.85	96.97	96.68	0.10

Table IV.1 Model fit results: Standard deviation and linear correlation (%) of the model fit to the measured NO₃⁻ distribution in the porewater, standard deviation of the fits normalized to the integrated concentration of the profile, *U* (%), and standard deviation of measured salinity porewater profiles used to ensure the homogeneous seepage of the mixed water.

	Profile Salinity	Seepage meters Salinity	Flux at <i>L</i> ₂	Modelled sed-water Flux	Measured sed-water Flux	Measured Flux Corrected by Salinity
mmol m ⁻² h ⁻¹						
March	36.12	29.49	-0.09	-0.11	-0.35	-0.10
April	32.26	33.68	-0.06	-0.08	-0.04	-0.09
July	30.11	31.90	-0.36	-0.60	-0.28	-0.47
August	24.76	26.44	-1.18	-1.27	-1.18	-1.55
October	26.42	23.62	-5.53	-6.35	-4.17	-3.37
November	22.16	30.38	-7.78	-7.80	-3.39	-7.08
December	24.00	33.23	-2.10	-2.00	-0.98	-2.25
January	23.98	23.24	-9.94	-9.22	-9.35	-11.68

Table IV.2 Comparison between measured and modeled fluxes: Integrated salinity over the measured profiles, salinity measured in the seeping water with the seepage meters, modeled fluxes of NO₃⁻ at the lower limit (*L*₂) and at the sediment-water interface, and fluxes of NO₃⁻ measured with seepage meters are shown. As result of the differences in salinity between the measured profiles and the seepage meters data, a salinity correction of the seepage meters data was performed allowing direct comparison with the modeled fluxes.

Sediment-water fluxes extracted from the model fits to the data (Table IV.2) were plotted with respect to those obtained from the seepage meters (Fig. IV.7). No direct 1:1 relationship between each other was found, mainly because integrated salinity profile values from seepage meters and profiles differed significantly (see Table IV.2). This might be explained by the dead volume inside the seepage meters leading to a delay between brackish water arrival at the chamber and sampling time, and the fact that the spatial heterogeneity effect on the seepage meter fluxes was minimized by using 2 or 3 chambers per position. However, significant linear correlation between NO_3^- concentration and salinity was evident on all our seepage meter data (Leote et al. 2008). This correlation was used to adjust the NO_3^- concentration at the seepage meters for that expected from each porewater salinity profile, allowing uniformization of the predicted and measured fluxes (Fig. IV.7). With this correction, a good linear regression ($R^2= 0.8672$) was obtained, with a slope close to unity (0.9948), thus convincingly linking the predicted boundary fluxes to the ones measured in-situ.

	P_e	D_{ef} $10^{-4} \text{ m}^2 \text{ h}^{-1}$	D_{a1}	Nitrification Rate $\text{mmol m}^{-2} \text{ h}^{-1}$	L_1 cm	D_{a2}	Nitrate reduction Rate $\text{mmol m}^{-2} \text{ h}^{-1}$	L_2 cm
March	4.76	6.52	0.26	0.06	0.50	0.45	0.04	20
April	2.15	2.52	0.76	0.05	3.91	0.82	0.03	6.93
July	7.50	11.24	1.71	0.79	3.49	20.58	0.55	5.49
August	2.93	3.66	0.42	0.49	2.86	0.42	0.39	10.50
October	6.64	9.71	0.32	1.90	4.13	0.22	1.09	20
November	13.99	23.72	0.12	1.27	0.50	0.17	1.25	20
December	8.03	12.19	0.12	0.34	0.49	0.23	0.44	20
January	29.78	58.73	0.11	1.42	0.50	0.23	2.14	20

Table IV.3 Advective velocity obtained from the seepage meters (in the form of the nondimensional P_e number) used in the modeling process as input parameter is shown. Effective dispersion coefficient calculated from diffusion coefficient and P_e number at each profile modeled is also shown, along with parameters obtained from the fit procedure: reaction rates in the form of reaction rates the nondimensional D_a numbers together with depth distribution of reaction layers.

The model-derived profiles accounted for the principal NO_3^- distribution patterns observed on the in-situ vertical profiles (Fig. IV.6). Transport input parameters used (P_e numbers and D_{ef}) along with reaction rates and reaction layer depths derived from model fits to the data are shown on Table IV.3. P_e numbers were in the range of the power law description of dispersion (Bijeljic and Blunt 2006), and together with the NO_3^- reduction layer length ($L_2 - L_1$), high enough to permit the use of the free-boundary approximation. Apparent

nitrification rates, derived from the model, ranged from 0.05 to 1.9 mmol m⁻² h⁻¹ and apparent NO₃⁻ reduction rates from 0.03 to 2.14 mmol m⁻² h⁻¹.

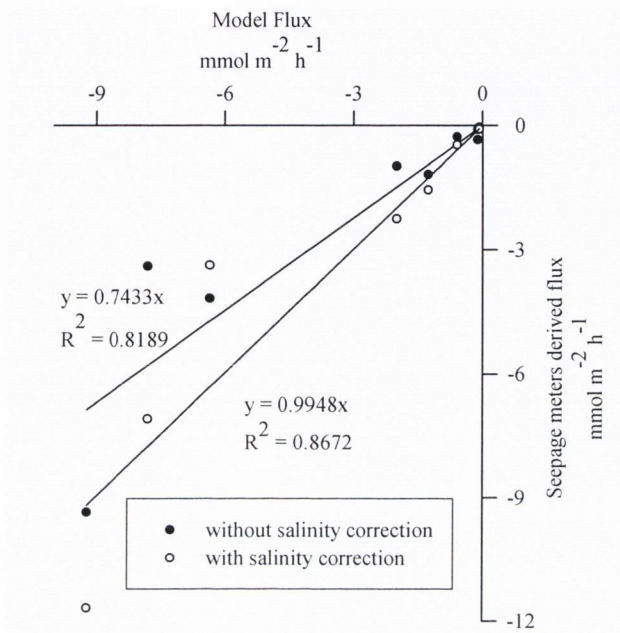


Figure IV.7 Model-predicted interfacial seepage fluxes plotted against measured fluxes on site with seepage meters (black dots). Salinity correction of field fluxes to be comparable with the exact depth-averaged salinity for the porewater profiles used in the modeling procedure are also shown (white dots).

Indication of the existence of limits to nitrate reduction at depth was also found on April, July and August, suggested by the unchanged vertical NO₃⁻ concentration at the deeper part of the profiles taken (requiring the inclusion of the third layer, Table IV.3). In these cases, the inclusion of a third, non-reactive layer could explain this unchanged NO₃⁻ distribution and help in constrain the spatial distribution of the net reactive horizons at the seepage face surficial sediment. At the profiles taken from these months, the simplified 2-layer model fit ($L_2 = L_3$; Fig. IV.8), always showed higher standard deviation to the measured NO₃⁻ profile than the 3-layer model fit. A one-tailed F test was used to test the statistical significance of the inclusion of the third layer on the model fits to the real data (Soetaert et al. 1996; Berg et al. 1998). The null hypothesis tested in this case was that the inclusion of the third, non-reactive layer did not significantly improve the model fit to the field-measured data, in comparison with the simpler two-layer model ($L_2 = L_3$). When applying the F-test between the 3-layer

model and the equivalent 2-layer model, statistical significance of the inclusion of the third layer was found on profiles from April and July, on a 99% confidence interval, while the more complex three-layer model application did not significantly improve the fit on August.

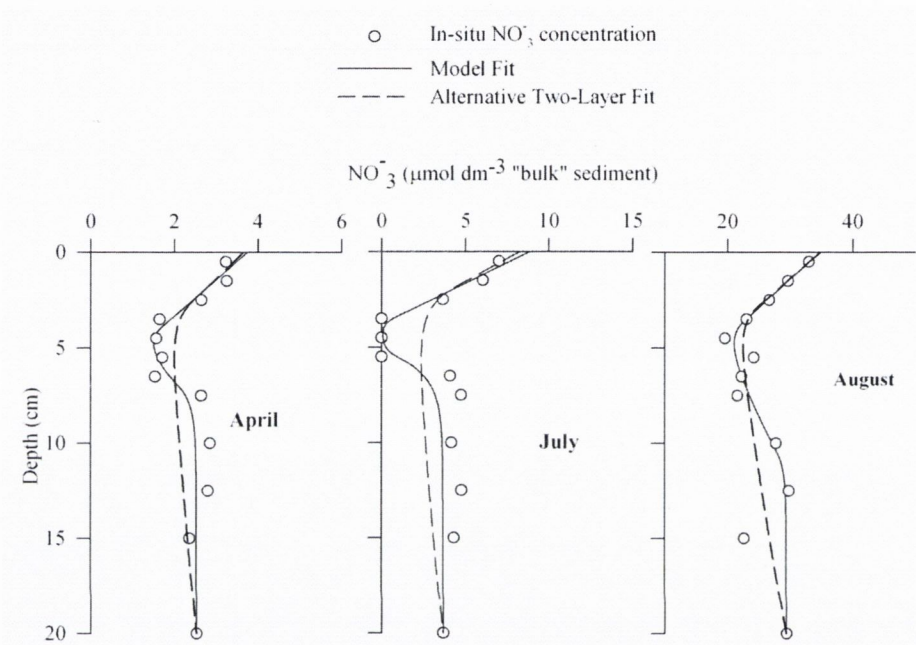


Figure IV.8 Model fits with and without inclusion of a non-reactive layer, when NO_3^- profiles evidence no gradient in concentration near the lowermost end of the domain; testing the statistical significance of inclusion of non-reactive layer on modeled field data.

IV.5 Discussion

The validity and applicability of the model with the free boundary solution approximation was tested via multiple methods. The mass balance performed within the entire domain of the model was always checked and always showed coherence. The results of testing the simplification (Ψ) showed the high stability of the primary assumption, e.g., an advection dominated system. Moreover, the free-boundary solution showed also high stability for different spatial scales, reactive rates, layer lengths and input parameters. Some instability of the free-boundary approximation at low P_e numbers (inside the lowest transition zone between

diffusive to advective dominated regime, i.e. $1 < P_e < 3$) was found. At this transition zone, the function Ψ deviated significantly for NO_3^- reduction layer lengths ($L_2 - L_1$) smaller than 2 cm. This deviation disappears at higher P_e numbers or when high reaction rates are imposed, which suggests the need of a minimum residence time of the water flowing through the reactive horizons for stability of the model, which correlates with the assumption of advective dominated system (P_e should be always higher than 1 for application of the model presented).

The performed sensitivity analysis showed that the main parameters controlling both the NO_3^- porewater distribution and seepage fluxes are the advective velocity and the reaction rates. Advective velocity is the forced parameter and thus the dominant factor of both the NO_3^- distribution in porewater and the cross-interface fluxes. The boundary fluxes depend strongly on the seepage rate and this dependence is well defined on this analysis. However, since the importance of the reaction rates in affecting the magnitude of the interfacial fluxes is shown, the NO_3^- concentration arriving at the domain (C_{aq}) could be a dominant parameter controlling the surface discharge of NO_3^- only when low benthic reactivity is present, but loses importance when higher potential reaction rates are possible. Thus, C_{aq} is an important parameter to characterize the system, but does not necessarily determine the solute flux to the external environment. Overall, analysis of the sensitivity of model output to different benthic reactivities, as well as discharge rates, suggest that the sandy environment can significantly change the final concentration and load of NO_3^- into the lagoon. Therefore, in order to predict the impact of SGD in the receiving environment, a good knowledge of benthic biogeochemistry at the seepage face is necessary, particularly near the sediment-water interface.

The description of measured benthic NO_3^- distributions shows the stability of the model and the pertinence of the assumptions underpinning its formulation. A variety of modeled profiles, characterized by different P_e numbers, salinities, C_{aq} concentrations and vertical NO_3^- gradients are shown. The model accounted for both overall shape and curvature, reproducing the measured depth profiles with high correlation. Even including the possible sampling artifacts like interference between sampled horizons, and the dynamism of the benthic environment, the high values of the fit correlations evidenced the quality of the model adjustment to the measured data. In addition, the comparison between the measured and

model-derived fluxes adds to the reliability and predictive capability of the model. The comparison between the modeled and measured fluxes offered a 1:1 relationship with a high linear regression coefficient even taking into account the potential artifacts associated with the operation of seepage meters, extensively discussed in the methodology section, thus validating the calculated fluxes.

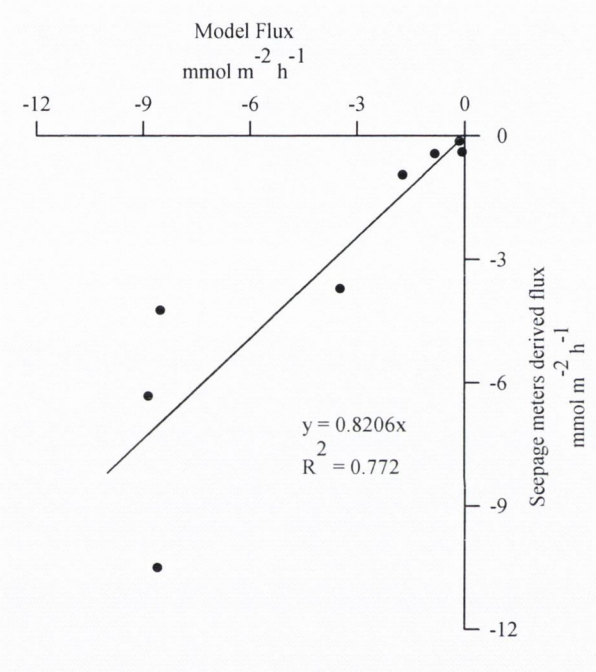


Figure IV.9 Pseudo-stationary state assumption tested; Model-predicted interfacial seepage fluxes plotted against fluxes measured on site with seepage meters during the whole sediment exposure period.

During the model development process we approximate the system to a pseudo-stationary state when sediment is exposed within an advective dominated environment caused by vertical submarine groundwater discharge. In order to test the pertinence of our assumption, the advective velocity and NO_3^- fluxes obtained with the seepage meters for the whole exposure period were integrated and checked against the profiles used. The resulting average advective velocity was used as input parameter for the model fit, and the resulting fluxes were plotted against those obtained from the seepage meters (Fig. IV.9). A deviation of almost 18% between modeled and measured data was observed. The considerations made on the comparison between modeled and measured fluxes during the main modeling effort are

ADR model for vertical NO₃⁻ distribution in SGD seepage faces

also applicable here. The deviation caused by the November data set, where the overall profile salinity was 22 ppt but the measured salinity on the seepage meters never dropped below 28 ppt during the entire sampled tidal cycle, was particularly noteworthy. Despite that fact, which shows an extreme case of the impact of spatial variability on seepage rates, good agreement between both data sets allowed confirmation of the physical verisimilitude of our pseudo-stationary state assumption during sediment exposure.

Place	Sediment type	Nitrification Rate mmol m ⁻² h ⁻¹	Method	Denitrification Rate mmol m ⁻² h ⁻¹	Method	Reference
Skidaway Island (USA)	Salt marsh	-0.023- 0.913 ^a	NH ₄ ⁺ incubations	0.195-3.228 ^a	Acetylene block technique	Dollhopf et al. 2005
Boston Harbor (USA)	variable (Subtidal)			0.01-0.412	Direct N ₂ flux	Nowicki et al. 1997
Marennes-Oléron Bay (France)	Intertidal mudflat	0.79-2.5 ^a	Acetylene block technique			Laima et al. 2002
Hiroshima Bay (Japan)	Subtidal, variable	0-0.021 ^a	Acetylene block technique	0-0.005 ^a (0-0.056) ^b	Acetylene block technique	Kim et al. 1997
Kertinge Nor estuary (Denmark)	Sandy sediment (0.5m depth)			0.002-0.12 ^a	Isotpe paring	Rysgaard et al. 1995
Finland estuary	variable (Subtidal)			0.007-1.26 ^a	Isotpe paring	Gran and Pitkänen 1999
Tama Estuary (Japan)	Subtidal fine sand and mud	0.246- 0.716	Mass balance	0.214-1.26 ^a	Isotpe paring	Usui et al. 2001
Sylt Island (Gernany)	Sandy and coarse sandy intertidal sediment	0.001- 0.026 ^a	Isotpe paring	0-0.011 ^a	Isotpe paring	Jensen et al. 1996
Sado estuary (Portugal)	Intertidal exposed sandy sediment	1.2	Mass balance	1.1	Mass balance	Rocha and Cabral 1998
Nauset Mars Estuary (USA)	Intertidal sand/silt flat ^c			0-0.096	Direct N ₂ flux	Nowicki et al. 1999
Tokio Bay (Japan)	Sandy beach ^c			0.00045- 0.018 ^{a, d}	Acetylene block technique	Ueda et al. 2003
Ria Formosa (Portugal)	Sandy sediment affected by SGD	0.05- 1.9 ^c	Model fit to real data	0.03-2.14 ^b	Model fit to real data	This study

^a Potential Nitrification/denitrification Rate

^b Nitrate reduction rate

^c Affected by nitrate rich groundwater

^d Minimal potential denitrification

Table IV.4 Typical benthic nitrification and denitrification rates on literature.

Apparent reaction rates (nitrification and nitrate reduction) extracted from the model fits were in the order of 10⁻²-10⁰ mmol m⁻² h⁻¹. These are compared to literature values in Table IV.4. Nitrification rates at our site are comparable with the potential rates found in muddy sediments by Laima et al. (2002) and Dollhopf et al. (2005). For sandy sediments, the mass balances published by Rocha and Cabral (1998) (at similar latitude) and Usui et al.

(2001) were also comparable. Model derived nitrate reduction rates were similar to denitrification rates found in marine muddy sediments (Dollhopf et al. 2005), subtidal sandy sediments (Gran and Pitkänen 1999; Usui et al. 2001) and intertidal sandy sediments at similar latitudes (Rocha and Cabral 1998). However, they are significantly higher than rates measured by Nowicki et al. (1999) and Ueda et al. (2003) on intertidal sandy sediments affected by nitrate-rich groundwater-borne discharge. This discrepancy can be explained by two facts: Firstly, rates determined by Ueda et al. (2003) were obtained from static core incubations (minimal potential denitrification). Because denitrification is usually identified as dependent on the amounts of NO_3^- provided to the reaction zone (first-order kinetics), its rate will depend on the NO_3^- loading of the reactive layer, thus being strongly conditioned by the advective flux. On the other hand, “nitrate reduction” is defined here as any process responsible for the depletion of porewater NO_3^- content in the direction of flow. For example, the work of Kim et al. (1997) showed that heterotrophic denitrification accounted for less than 10% of the total nitrate reduction rate. The environment studied here constitutes an interface link between nitrate-rich low organic content groundwater, benthic reactivity at the seepage zone, and oscillatory sea water infiltration into the subterranean estuary (Moore 1999). The dynamic biogeochemical zonation thus established could limit heterotrophic denitrification and enhance the potential for alternative metabolic pathways, like the autotrophic ANAMMOX or OLAND processes (Bowen et al. 2007; Brandes et al. 2007). Furthermore, the high temperatures found at the seepage face could also favor DNRA over denitrification due to the greater standard free energy change of ammonifiers when compared to denitrifiers under limited nitrate conditions (Dong et al. 2011). Apparent nitrate reduction rates derived from our model accounted for the observed net decay in NO_3^- concentration, but did not discriminate between potential pathways.

In natural porous media with advection dominating interfacial transport and hence solute distribution, the associated dispersivity, being orders of magnitude higher than diffusivity, can also affect the porewater distribution of the solutes (Roychoudhury 2001). Even including the advective motion in calculations based on the porewater nitrate gradients, direct (based on profile concentrations) mass balance approaches neglect the effect of dispersion. Thus, direct mass balances cannot separate the transport effect on NO_3^- gradient shape and reach from the gradients caused by biogeochemical reactivity. Here, mass balance

calculations were performed over the measured NO₃⁻ profiles. The minimum NO₃⁻ concentration found at each profile was postulated as the limit for each of the net reactive horizons (net NO₃⁻ reduction layer and net nitrification). For each net reactive sediment layer, measured advective velocity was used together with the NO₃⁻ concentration at the lower and upper limit of each sediment layer to calculate apparent benthic transformation. When compared with model outputs (Table IV.5), mass balance calculations lead to an underestimation of NO₃⁻ reduction potential and an overestimation of nitrification. More so, the direct mass balance approach might originate contradictory interpretation of the overall benthic mediating role over NO₃⁻ fluxes when compared with the model outputs (notice the contradictory overall sediment interference on NO₃⁻ fluxes calculated by mass balance and by modeling for December and January). The model successfully explained positive (production) solute gradients close to the sediment surface up to 6 cm depth with nitrification layer lengths of less than 1 cm width (Table IV.3). In this sense, modeling results show an interesting linkage between both reactive layers; even with significant advective up-flow transport, NO₃⁻ production in the nitrification layer can feed NO₃⁻ into the reduction layer below. Thus, due to the known dispersion effect over solute distribution in porous media (e.g. Roychoudhury 2001), mass balance approaches (which can not include this transport parameter) can lead to wrong interpretations of the net reactivity in place. Hence, the model not only incorporates the effects of advection and dispersion, but may also offer valuable information on the vertical biogeochemical zonation present at the time of profile collection.

	Mass balance approach				Current model			
	NO ₃ ⁻ Reduction	Nitrification	Overall transformation	L ₁	Nitrification Rate	NO ₃ ⁻ Reduction	Overall transformation	L ₁
	mmol m ⁻² h ⁻¹			cm	mmol m ⁻² h ⁻¹			cm
March	-0.03	0.16	0.127	6.50	0.06	-0.04	0.027	0.50
April	-0.02	0.04	0.017	6.50	0.05	-0.03	0.017	3.91
July	-0.36	0.68	0.324	3.50	0.79	-0.55	0.241	3.49
August	-0.39	0.54	0.142	4.50	0.49	-0.39	0.091	2.86
October	-0.82	2.52	1.699	5.50	1.90	-1.09	0.817	4.13
November	-1.40	4.20	2.804	6.50	1.27	-1.25	0.020	0.50
December	-0.38	1.01	0.629	10.00	0.34	-0.44	-0.096	0.49
January	-1.48	4.46	2.976	7.50	1.42	-2.14	-0.717	0.50

Table IV.5 Apparent reaction rates derived from simple mass balance approach over the NO₃⁻ profiles used in this study. Overall net transformation obtained by both simple mass balance and modelling approaches is also shown for comparison. Mass balances were performed by using the minimum NO₃⁻ concentration at each profile as the limit between both reactive layers (L₁ in the conceptual model performed).

The possible existence of different reactive pathways involving NO_3^- processing at benthic locations affected by SGD (Kroeger and Charette 2008), underpins uncertainties regarding the final role of the permeable sediments as modulators of land-derived nutrient inputs to the coastal zone (Rocha 2008; Rocha et al. 2009). In this sense, direct N_2 flux measurements and the use of stable isotope analysis on both natural samples and controlled systems would supply important information on the relative importance of each pathway, along with the interactions with other elements, biogeochemical kinetics and environmental factors controlling the system (Hamersley and Howes 2005; Pallud et al. 2007; Kroeger and Charette 2008). Identification of the main reactive zone along with the expected overall reactivity could help to improve the design of further laboratory studies, ensuring the dominance of a specific microbial population (Hamersley and Howes 2005; Pallud et al. 2007; Rocha 2008).

With the free-boundary solution presented here, the model is specifically designed for the sediment exposure period, an advective-dominated system. Moreover, it could be used without the proposed free-boundary solution for the sediment-water interface to fit NO_3^- profiles for the steady state diffusion-dominated transport case, when the sediment is flooded and no up-flow of porewater is affecting the system. The concentration at the interface (C_0) in this case is controlled by the overlying water (Vanderborght and Billen 1975). Thus, the solution presented here would be applicable to a variety of cases, crossing the transition from diffusion-dominated to advection-dominated environments, and beyond, to the pure advective transport case scenarios.

IV.6 Conclusions

We satisfactorily reproduce the porewater NO_3^- distribution in advective-dominated seepage zones by including mechanical dispersion within an ADR model with a free boundary solution. The stability of the model was demonstrated and constrained within the limits set by the applicability of the free-boundary solution. Based on the results of the sensitivity analysis, we show that NO_3^- porewater distribution and surface fluxes could be controlled primarily by the seepage velocity and benthic reactivity. Furthermore, the concentration of NO_3^- arriving at

the surface reaction zone might not define the resulting seepage fluxes when important biogeochemical reaction rates are present. Results obtained from the model fit to in-situ porewater profiles were analyzed and the reliability of the model outputs was demonstrated. The proposed model may increase the accuracy of reaction rate estimates and geochemical zonation at SGD sites, and be an important aid in preparing further experimental studies of local reaction kinetics affecting nitrate distribution in sediments impacted by groundwater discharge.

Chapter V

Seasonal enhancement of Submarine Groundwater Discharge (SGD)-derived nitrate loading into the Ria Formosa coastal lagoon assessed by 1-D modeling of benthic NO_3^- profiles

V.1 Abstract

The role of benthic sandy ecosystems in mitigating NO_3^- loads carried by Submarine Groundwater Discharge (SGD) to coastal marine ecosystems is uncertain. Benthic biogeochemical mediation of NO_3^- -rich submarine groundwater discharge was studied at the seepage face of a barrier island site in the Ria Formosa coastal lagoon (Southern Portugal). Preliminary analysis of NO_3^- porewater distributions at the seepage face during discharge indicated that benthic biogeochemical processes could significantly affect the fluxes of groundwater-borne NO_3^- into the lagoon. In order to discriminate between the relative contribution of transport and reaction processes to shape and concentration range evidenced by in-situ porewater NO_3^- gradients, an advection-dispersion-reaction (ADR) model of NO_3^- diagenesis was applied to describe NO_3^- porewater profiles obtained in March, June, September and December 2006. Good agreement between modeled and measured profiles was obtained. Model-derived apparent benthic nitrification and NO_3^- reduction rates ranged from 0.01 to 5.2 $\text{mmol m}^{-2} \text{h}^{-1}$, sufficient to explain gross observed changes in NO_3^- fluxes arriving at the seepage face (up to 70 % within the surficial 20 cm depth layer). Results of the analysis indicated that the upper limit of the seepage face promoted mitigation of NO_3^- fluxes to the lagoon throughout the year. In contrast, the lower limit of the seepage area promoted net amplification of the NO_3^- fluxes into the lagoon in June and September. These results will help constrain further work aiming to clarify the role of permeable sediments in mitigating nitrogen loading of coastal ecosystems.

V.2 Introduction

The discharge of groundwater to the coastal sea has been recently identified as an important source of fresh water and associated solutes to the coastal zone (i.e. Cable et al. 1997; Gallardo and Marui 2006; Taniguchi et al. 2006; Niencheski et al. 2007). The difficult identification due to its occurrence as diffusive flow rather than discrete spring flow, along with the spatial and temporal variability of the groundwater discharge explain the previous gap in knowledge about this fresh water source to the coastal zone (Burnett et al. 2003). Groundwater discharge to the coast occurs wherever a coastal aquifer is connected to the sea with a positive head related with the sea level (Johannes 1980). At the exit point, seawater can infiltrate the aquifer, and the resulting mixed water seeping out at discharge sites usually contains a large proportion of recirculated sea water. This phenomenon is termed Submarine Groundwater Discharge (SGD; Burnett et al. 2003). Global estimations suggest that SGD could represent 5-10% of the total fresh water discharge to the coast (Taniguchi et al. 2002; Slomp and Van Cappellen 2004). Since groundwater nutrient concentrations are generally higher than those found in surface waters, the groundwater contribution may rival riverine inputs in terms of nutrient transport to the coastal zone at a local level (Slomp and Van Cappellen 2004; Moore 2006a; Swarzenski et al. 2006). Because of its potential impact on the trophic status of receiving coastal waters, quantification of the contribution of groundwater-derived nutrients to the coastal budget is an important issue (Verhoeven et al. 2006; Bowen et al. 2007).

High NO_3^- concentrations in coastal aquifers are reported worldwide (Rivett et al. 2008). Intensification of agriculture since mid-20th century has lead to diffusive NO_3^- pollution of aquifers (Foster 2000). Other sources such as sewage and mains leakage, septic tanks, industrial spillages or the use of manure and sewage sludge inland can also contribute to the commonly observed high NO_3^- concentration in aquifers (Wakida and Lerner 2005). Since denitrification is assumed to be the main pathway in mitigating NO_3^- pollution within the aquifers (Rivett et al. 2008), limited NO_3^- removal is likely to occur prior to arrival at the coastal zone mainly due to a lack of labile dissolved organic matter (Slater and Capone 1987; DeSimone and Howes 1996). At SGD seepage faces, tidal and wave pumping and dispersion can promote a complex mixing pattern of sea water and groundwater in the sediment

(Cartwright et al. 2004; Robinson et al. 2007). Sea water infiltration can thus introduce oxygen and pelagic organic matter into the sediment and promote a complex physical and chemical zonation that in turn can control the resulting NO_3^- loads to the exterior (Moore 1999; Yamashita and Tanoue 2008; Santos et al. 2009). This mixing area is termed the subterranean estuary due to the analogy with surface estuaries (Moore 1999).

Subterranean estuaries have been recognized as zones of important biogeochemical reactions between the mixed waters and aquifer solids (e.g. Charette et al. 2005; Charette and Sholkovitz 2006). In particular, recent research has identified seepage faces as zones of strong nitrogen processing within the sediment prior to discharge (Ullman et al. 2003; Bowen et al. 2007; Kroeger and Charette 2008; Spiteri et al. 2008; Rocha et al. 2009). Due to the complex solute transport and biogeochemical zonation established at the seepage face, SGD sites have been identified as zones of potentially overlapping N reactive processes and pathways, including denitrification, anaerobic ammonium oxidation (ANAMMOX) or dissimilatory NO_3^- reduction to ammonium (DNRA) (Bowen et al. 2007; Kroeger and Charette 2008; Rocha et al. 2009). The possible overlapping of different reactive pathways with different end-products involving land-derived NO_3^- processing in SGD places raises uncertainties about the mediation role of the seepage face.

Here, seasonal nitrate benthic biogeochemistry is explored in a SGD seepage site by modeling nitrate distribution in the porewater. Mechanical dispersion, caused by the heterogeneous movement of the water in porous media (Roychoudhury 2001), can promote solute gradients that can be misinterpreted as caused by benthic reactivity. Therefore, an Advection-Dispersion-Reaction (ADR) model inclusive of a free-boundary solution is used in order to elucidate the relative contribution of transport and reaction processes in nitrate porewater distribution. The main objective of this study is to obtain preliminary seasonal explanations of the benthic reactivity and interfacial fluxes in nitrate-rich SGD seepage sites, and to estimate the mediation role of the benthic community in modulating land-derived N loads to the coastal area.

V.3 Methods

V.3.1 Field sampling

Field sampling was carried out at a site located in the inner intertidal face of the Ancão peninsula ($37^{\circ}00'04''\text{N}$, $7^{\circ}88'57''\text{W}$), one of the two sandy peninsulas framing the Ria Formosa coastal lagoon (see Fig. IV.1). Salinity and NO_3^- porewater profiles and seepage rate measurements and associated fluxes were carried out at the sampling site during March, June, September and December 2006. General sampling work was conducted during two consecutive tidal cycles at the intertidal seepage face during sediment exposure. Two sites in the permanently saturated sediment surface affected by SGD were selected, viz: A, located at the lowest part of the beach profile, close to the observed point at which the discharge peaks and B, at the upper limit of the discharge zone.

V.3.1.1 Porewater solute profiles

Porewater samples were taken in-situ using soil moisture samplers (Rhizon SMS-10 cm; Rhizosphere, $0.1\ \mu\text{m}$ pore size). The samplers were placed into the sediment at fixed depths via two in-situ acrylic profilers modified from the design of Seeberg-Elverfeldt et al. (2005), situated at each sampling location (Fig. IV.1). Each profiler was buried in-situ with 12 Rhizon membrane samplers located horizontally at vertical intervals of 1, 2.5 and 5 cm, covering the first 20 cm depth of the sediment column. The samplers provide the advantage of remaining in place throughout the entire field experiment, thus causing minimal disturbance over the local benthic flow patterns (Cappuyns et al. 2004; Seeberg-Elverfeldt et al. 2005). Porewater was collected at each depth by connecting a sterile, 11 ml vacuum flask (BH Vacutainer) to the Rhizon membranes through connection tubing protruding from the sediment surface. The first ml, corresponding to the dead volume inside the tubing and filtration device ensemble, was discarded to avoid contamination of consecutive samples (Seeberg-Elverfeldt et al. 2005). The final porewater aliquots (5-6 ml at each depth), sealed inside the vacuum tubes, were then stored at $4\ ^{\circ}\text{C}$ until analysis. The pore size of the filtration membranes together with the sampling storage in vacuum tubes permitted the collection of

porewater samples ready for analysis without further preservation (Luo et al. 2003; Seeberg-Elverfeldt et al. 2005). All parameters were analyzed within 2 weeks of sampling.

V.3.1.2 Seepage measurements

Three to five Lee-type seepage meters (Lee 1977) were deployed at the beach face (Fig. IV.1) to allow direct measurement of advective velocities and solute fluxes across the sediment-water interface (Lee 1977; Burnett et al. 2006; Taniguchi et al. 2006). These were deployed at low tide in alignment with the porewater profilers and sampling started on the subsequent low tide following the precautions suggested by Cable et al. (1997). Measurements of seepage velocity, salinity and temperature were performed in the water collected inside the connected bags. Water samples extracted at known time intervals from the collection bags were also taken for laboratory determination of NO_3^- concentrations.

V.3.1.3 Sediment properties

Sediment cores (20 cm length, 3.5 cm diameter) were taken by hand at the two locations in the low-intertidal beach profile (in alignment with the pore water profiler locations) for determination of benthic water content, porosity and sediment grain size distribution.

V.3.2 Analytical methods

Porewater and seepage water samples collected during each field run were analyzed for NO_3^- content, following the spongy cadmium method (Jones 1984), adapted previously to low (0.5 ml) aliquot volumes. Porewater salinity was measured with a hand-held refractometer using single-point calibration with distilled water. Salinity and temperature measurements on the seepage water were performed in-situ using an YSI 600 (YellowSpring instruments) multiparameter probe.

Water content was determined in each sediment core sub-sample as a percentage of weight loss at 60°C after 24-48h drying to constant weight. Porosity was calculated based on the water content (Dullien 1992), using 2.65 g ml⁻¹ (quartz) as the dry sediment density.

V.3.3 Model description

In-situ porewater NO₃⁻ distributions were described by fitting the diagenetic NO₃⁻ model described in detail in Chapter IV (see it for a complete description of the model including sensitivity tests), using the free-boundary solution developed therein. The main objective was to elucidate the relative contribution of transport and benthic reactivity over nitrate distribution in the porewater and thus explain and predict the magnitude of observed interfacial fluxes. Once this was achieved, the model outputs were used to suggest preliminary depth constraints for benthic biogeochemical reactions at the permanently saturated seepage area affected by SGD.

The model is an analytical solution of the general Advection-Dispersion-Reaction (ADR) equation. The main transport processes included in the model are molecular diffusion, mechanical dispersion and advective transport. Molecular diffusion and mechanical dispersion were modeled as a single term according to Fick's first law, and defined as the sum of the dispersive and the diffusive terms (Roychoudhury 2001). Calculation of the nitrate diffusion coefficient was based on the temperature-dependant parameterization presented by Boudreau (1997). Dispersion is included using the formulation presented by Bijeljic and Blunt (2006) for advection-dominated porous media. Advective transport of nitrate is accepted to be exclusively the result of the vertical seepage of porewater when the sediment is exposed (Horn 2002; Burnett et al. 2006; Rocha et al. 2009). It is introduced in the model formulation as the velocity of the water with respect to the sediment particles. The effect of the presence of the solid phase was introduced by using the "bulk" NO₃⁻ concentration (Boudreau, 1997):

$$\hat{C}(z) = \phi(z)C(z) \quad (1)$$

where $C(z)$ is the NO_3^- distribution in the porewater and $\phi(z)$ the porosity. Hence, all concentrations are expressed per whole sediment volume, thus automatically correcting for porosity changes within the domain.

Based on the local distribution of Dissolved Inorganic Nitrogen (DIN) and organic matter (Rocha et al., 2009; Chapter IV), the model allows for the existence of two main reactive zones defined in terms of the potential change in NO_3^- pools within the sediment: nitrification (NH_4^+ and NO_2^- oxidation, leading to NO_3^- accumulation) and NO_3^- reduction (leading to a reduction of the local NO_3^- pool). Nitrification is assumed to take place close to the surface, with zeroth order kinetics in respect to NO_3^- concentration. The NO_3^- reduction zone is included below the latter, at a greater depth, and considered as any process leading to a depletion of nitrate pools following first order kinetics with respect to nitrate concentration. Thus, this definition includes denitrification and other N reduction processes such as ANAMMOX, either in isolation or in combination under its broadest sense. Finally, a third layer in depth was included in the model as an optional, non-reactive zone with respect to nitrate. Its inclusion was based on the local distribution of organic matter and nitrate at the site that suggested potential constraints to nitrate reduction at larger depths (Chapter IV).

The reproducible oscillatory nature of the system was verified (Rocha et al., 2009). Furthermore, during ebb tide, salinity of the seeped water became progressively constant, both in time and in the top 20 cm depth into the sediment (Leote et al. 2008). The mixture of both end-members inside the subterranean estuary (i.e. sea water and groundwater) and the relative constant high seepage rate in place permits to assume that the discharge remained constant for the period of peak of discharge (i.e. around 3h during low tide). Therefore, and due to the fast water renewal of the top 20 cm of the sediment in the seepage face due to the high discharge rates, observed vertical NO_3^- gradients were assumed to be stable over the time the discharge peaked. This was used to postulate a pseudo-stationary state reached during peak seepage periods, i.e. during sediment exposure, and thus facilitate the solution of the mathematical problem by analytical means. In order to improve the model's predictive capabilities, the formulation includes a free-boundary solution at the sediment-water interface. The latter was obtained by accepting the system as being dominated by advection, thus permitting the

inclusion of a new boundary condition derived directly from a simplification of the mass balance between the upper and lower limits of the nitrification layer:

$$C_0 \cong (1/\nu) \left(\nu \hat{C}_1(L_1) - K_n L_1 \right) \quad (2)$$

where C_0 is the nitrate concentration at the sediment-water interface, ν is the advective velocity, L_1 is the nitrification layer depth limit, $\hat{C}_1(L_1)$ is the nitrate concentration at L_1 and K_n is the nitrification rate.

In this study, the model was used to explore the sediment exposure period, when SGD discharge rates and associated nitrate fluxes are at their highest. The free-boundary approximation described above and in Chapter IV could then be applied enabling the prediction of nitrate fluxes across the sediment-water interface.

V.3.4 Model input parameters

Advective velocity and temperature (for the calculation of diffusion coefficients) of the seeped water were obtained directly from in-situ seepage meter data. Measurements were integrated throughout a one-hour time interval around the time the porewater profile was taken. Data from between 2 and 3 seepage meters at each location in the beach profile were used for the integrations. NO_3^- porewater profiles were used to fit the model and thus extract reaction rates, reactive zone lengths and fluxes. All NO_3^- porewater profiles used in this study were obtained in conditions fitting the range of applicability of the model and the free-boundary approximation (see Chapter IV). NO_3^- concentration at 20 cm depth was used as C_{aq} (NO_3^- concentration at the lower limit of the model domain). To ensure that NO_3^- gradients were exclusively caused by biogeochemical reaction and the homogeneous seepage of water, only profiles with a salinity depth-variance lower than 1, the error of the hand-refractometer, were used for this study. The average grain size was utilized to calculate the adimensional Peclet number (P_e , ratio between the advective and diffusive transport time-scales), according

to Roychoudhury (2001). The modeled NO_3^- porewater distribution was fitted to the measured profile by minimizing the sum of squared deviations between each other with the aid of the Solver routine (Microsoft Excel). The quality of the adjustment was tested by using the linear correlation between field and modeled NO_3^- distribution in the porewater (Berg et al. 1998). The profiles used in this modeling study were characterized by different NO_3^- magnitudes. The standard deviation between each modeled and measured NO_3^- distribution was normalized to the integrated NO_3^- concentration at each profile, by using:

$$U(\%) = \frac{\left(\int_{L_3}^0 \hat{C}(z) dz / L_3 \right) - \sqrt{s^2}}{\int_{L_3}^0 \hat{C}(z) dz / L_3} \times 100 \quad (3)$$

where z is the sediment depth, L_3 is the lower sediment depth limit of the model domain and s^2 is the variance.

Since this normalization permits the direct comparison of the standard deviation of the different profiles, $U(\%)$ was used as another estimative of the goodness of the fits.

Finally, the coherence and reliability of the model outputs was ensured by permanently checking the model-derived mass balance for the entire sediment column studied. Thus, we checked if the difference between the NO_3^- fluxes at 20 cm depth and at the sediment-water interface can be fully explained by the reaction rates obtained. We assumed the mass balance is coherent when the difference between the net fluxes transformation and the balance for the reaction rates was less than $10^{-10} \text{ mmol m}^{-2} \text{ h}^{-1}$.

V.4 Results

V.4.1 In-situ porewater characterization for model implementation

Fig. V.1 shows the depth-integrated porewater salinity (for the 20 cm length of sediment), NO_3^- concentration at the lower boundary of the model domain (C_{aq}) and seepage velocity (in the form of Peclet numbers, P_e) used each month for the two stations. C_{aq} was inversely correlated with salinity ($r=-0.84$, $p<0.001$, $n=16$). Higher NO_3^- concentrations were

related to lower salinity values. Over the study period, September showed the highest seepage rates, concomitant with the lowest salinities in the seeped water. Peclet (P_e) numbers at both sampling sites were in the range of the power law description of dispersion (Bijeljic and Blunt 2006) used here. Hence, P_e numbers fall into the range of model applicability, and thus permitted the use of the free-boundary approximation developed in Chapter IV. All salinity profiles associated with NO₃⁻ porewater distribution used for this study had standard deviations lower than 1.0, the imposed condition to ensure homogeneous mixing throughout the model domain.

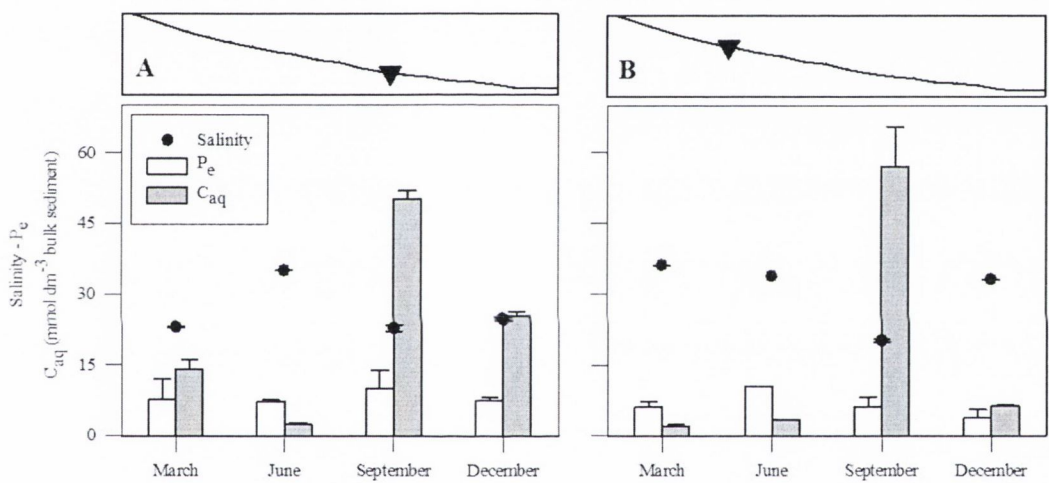


Figure V.1 Average model input parameters derived from in-situ data: salinity of the porewater integrated across the top 20 cm of the sediment column (black dots), NO₃⁻ concentration at 20 cm depth, used in the model as lower boundary (input) concentration (C_{aq} , grey bars) and Peclet numbers (P_e , white bars) are shown for the months under study. A and B represent the two sampling stations. An schematic view of the beach profile together with the relative location of each sampling site is also shown.

V.4.2 Model outputs

The model was able to replicate different NO₃⁻ porewater distributions measured under different dispersion regimes, hence characterized by different P_e numbers. Fig. V.2 shows selected NO₃⁻ profiles and concomitant salinity distributions from both sampling sites used in this study. Fig. V.2 also includes the model fits to the in-situ data for comparison. The model evidenced a high degree of versatility while reproducing different NO₃⁻ porewater

concentrations. Moreover, the model was able to reproduce the main NO_3^- gradients observed in situ, even with highly contrasting shapes and curvatures in the measured porewater profile.

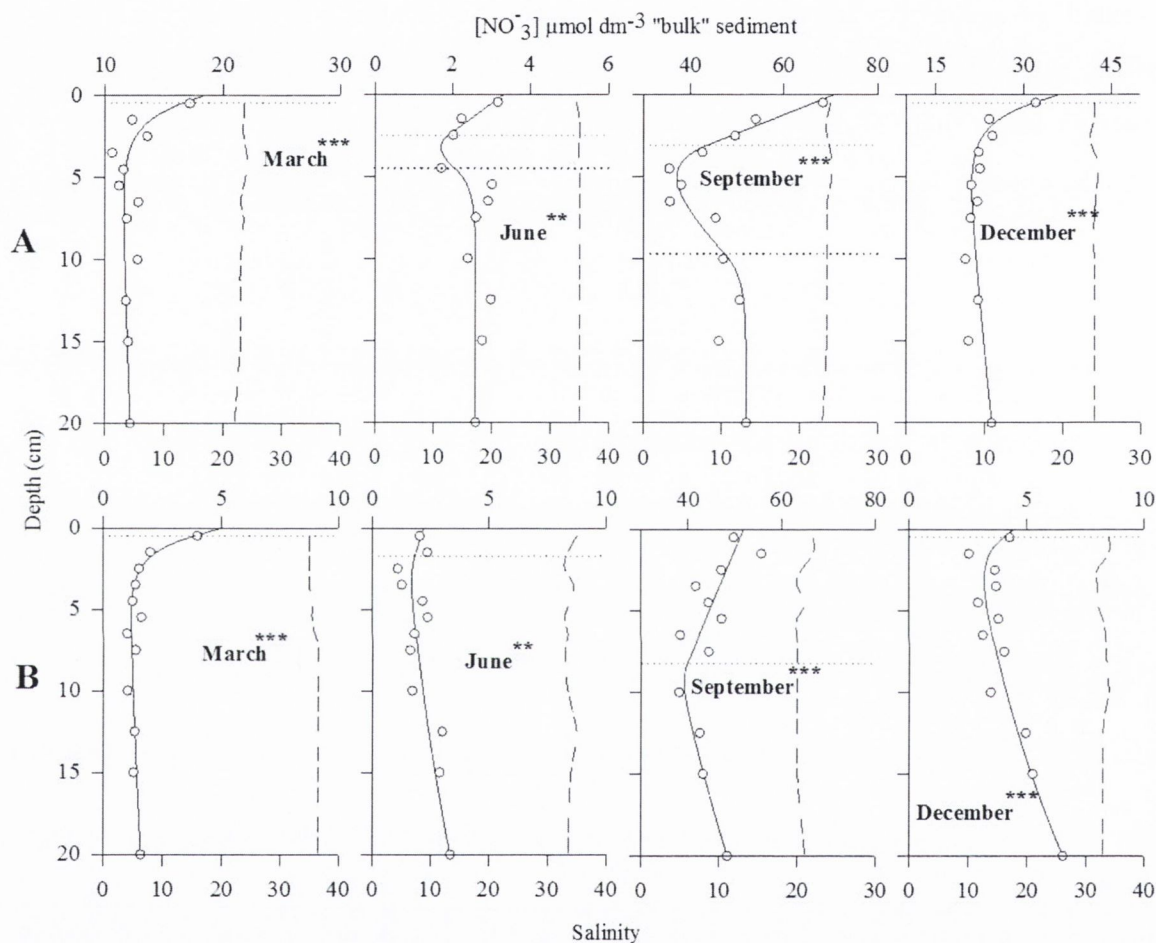


Figure V.2 Selected NO_3^- profiles measured in-situ with the model output superimposed for each studied month. p -value of the linear correlation coefficient is shown (*** $p<0.001$, ** $p<0.01$). Salinity profiles (dashed lines) measured in parallel with the NO_3^- concentrations are also plotted for demonstration of the well mixed nature of the sediment porewater across the sampled domain.

The modeled NO_3^- profiles fitted the measured data with high degree of correlation (Table V.1). The linear correlation coefficient between modeled profiles and field data was always higher than 75 % ($p<0.02$, Fig. V.2). Also, the standard deviation of the model fit to the measured data represented always less than 25% of the integrated NO_3^- concentration in the profile ($U (\%)>75\%$). The mass balance of the resulting modeled profiles was always checked and always showed coherence. The reaction rates obtained always explained the

calculated overall change in NO_3^- fluxes between 20cm depth and the sediment-water interface.

Surface boundary sediment-water fluxes obtained from modeling the porewater concentration profiles were comparable to those measured in situ with seepage meters (0.1 to $9 \text{ mmol m}^{-2} \text{ h}^{-1}$). Direct correlation between model-derived fluxes and seepage meter data has some restrictions since integrated salinity values from seepage meters and porewater profiles were significantly different. This is explained by the dead volume within the seepage meters, which produces a time lag between direct flux measurements and concentration distributions in the porewater. Due to the different means of measurement, direct comparison between modeled and measured fluxes over the time frame picked to select porewater velocity as an input parameter to the model is not feasible (see section 2.4). In addition, fluxes measured by the seepage meters are spatial integrations of the individual results at each meter, due to the necessity of avoiding spatial heterogeneity effects by using 2 to 3 seepage meters at each location. No direct 1:1 relationship between modeled and measured fluxes was consequently found under this paradigm (Fig. V.3). However, significant linear correlation between NO_3^- concentration and salinity was evident in the seepage meter data collected over a full year (Leote et al., 2008). These correlations allowed correction of the NO_3^- concentration for that expected at the salinity characteristic of the individual modeled profiles. Thus, the correction executed allowed recalculation of the field-derived fluxes to a normalized salinity in the sediment column, thus correcting for the time lag and spatial variability issues associated with seepage meter deployment. This analysis did not include results from September since the integrated salinity in the porewater profiles for this month (22.7 in station A and 20.1 in station B) was significantly lower ($p < 0.01$) than the lowest salinity measured in the water seeping through to the collection bags on the meters (26 in station A and 25 in station B). After performing the salinity uniformization of the modeled and measured fluxes, both data sets showed a good and significant correlation ($R^2 = 0.96$, $p < 0.0001$, $n = 12$), with a close to 1:1 relationship (0.82 ± 0.05), thus satisfactorily linking the measured and predicted boundary fluxes (Fig. V.3).

	A			B		
	Standard Deviation	Linear Correlation	U (%)	Standard Deviation	Linear Correlation	U (%)
		%	%		%	%
March	0.97±0.07	84.64±0.12	92.89±0.21	0.21±0.02	85.08±11.76	87.81±0.59
June	0.40±0.09	77.56±1.43	82.79±4.48	0.43	78.90	81.44
September	3.24±0.38	90.11±5.08	92.96±1.17	3.83±0.67	86.23±8.77	90.61±2.24
December	1.47±0.12	80.44±6.02	93.74±0.40	0.48±0.02	88.80±1.92	90.47±1.09

Table V.1 Results of the parameters used to check the goodness of the fit between the modeled and the measured NO₃⁻ profiles. Results shown are means discriminated by sampling position.

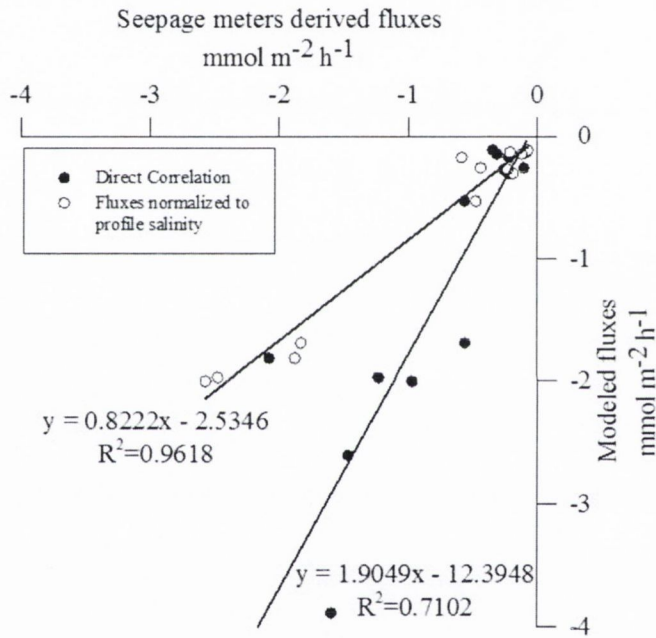


Figure V.3 Linear correlation between model-predicted interfacial seepage fluxes and fluxes measured on site with seepage meters (black dots; $p < 0.0001$, $n = 16$). White dots represent the relationship between the modeled and measured fluxes after salinity uniformization ($p < 0.0001$, $n = 12$). September was excluded from this analysis since porewater salinities were substantially lower than the lowest salinity measured with the seepage meters.

The model fits to the profiles collected at station A in June and September showed indication of the existence of constraints to net NO₃⁻ reduction at depth, denoted by the unchanged NO₃⁻ concentration in depth. The inclusion of the third, non reactive layer always resulted in better fit between modeled and measured porewater NO₃⁻ gradients as revealed by the lower standard deviation of the model fit to the measured NO₃⁻ distribution when

compared to the simpler two-layer model. A one-tailed F test was thus used to test the statistical significance of the insertion of the third layer on the model fits to the field data (Soetaert et al. 1996; Berg et al. 1998). The addition of the third, non-reactive layer significantly improved (95 % confidence interval) the model fit in the profiles showed in Fig. V.2 for June and September. In the other two profiles used for those months, while the inclusion of the third layer resulted in lower standard deviation of the fits, it was not statistically significant.

	A		B	
	Nitrification	NO ₃ ⁻ reduction	Nitrification	NO ₃ ⁻ reduction
mmol m ⁻² h ⁻¹				
March	0.15±0.08	-0.12±0.01	0.04±0.02	-0.06±0.03
June	0.16±0.01	-0.09±0.05	0.05	-0.26
September	2.93±1.64	-2.22±1.26	1.71±1.08	-2.89±2.30
December	0.30±0.04	-0.40±0.03	0.03±0.00	-0.12±0.11

Table V.2 Average reaction rates extracted from the modeling procedure, discriminated by position in the beach slope and month over the period studied.

Table V.2 shows the average reaction rates obtained in this study. The highest reaction rates were obtained for September, one order of magnitude higher than for the other months. Nitrification and NO₃⁻ reduction rates were in the order of -5.2-4.6 mmol m⁻² h⁻¹ and in the same range as interfacial NO₃⁻ fluxes (Fig. V.3). Higher reaction rates were related with higher advective velocities and C_{aq} (Fig. V.1) and with higher interfacial fluxes (Fig. V.4). Sampling site A generally hosted higher reaction rates than site B. In addition, nitrification rates were generally higher than NO₃⁻ reduction rates at Station A, in opposition with the higher limit of the seepage face (station B), which showed an apparent dominance of NO₃⁻ reduction pathways.

Influence of sediment reactivity on resulting fluxes was confirmed (Fig. V.4). Net changes in NO₃⁻ fluxes over the 20 cm sediment horizon ranged between -2.4 and 1.1 mmol m⁻² h⁻¹. Apparent benthic reactive mediation in the top 20 cm sediment depth promoted up to 70% absolute change of the NO₃⁻ loads out of the seepage face. Sediment mediation at the main seepage face area (Station A) resulted in enhancement of NO₃⁻ fluxes during June and

September. On the other hand, the upper limit of the permanently saturated seepage face generally played a mitigation role (Station B).

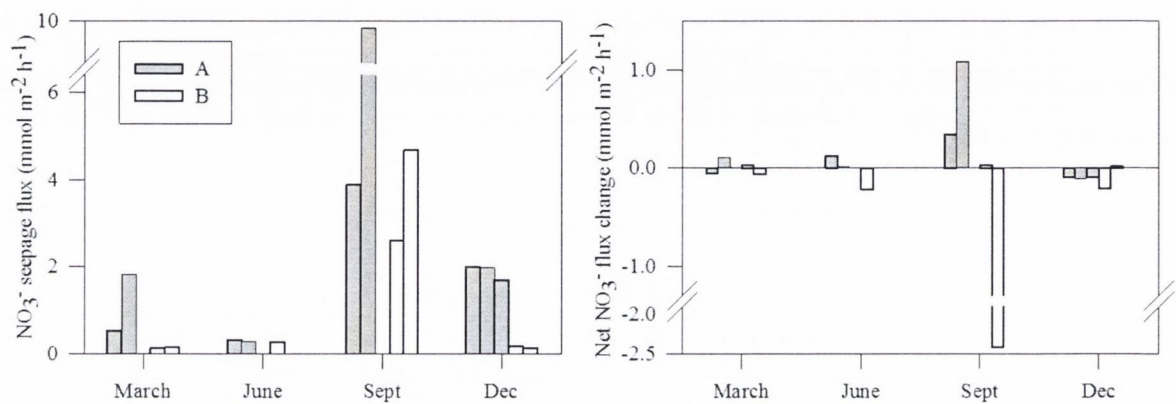


Figure V.4 Seepage NO_3^- fluxes obtained from the modeling procedure (left). Net benthic mediation over NO_3^- fluxes as result of the model fit to the measured porewater NO_3^- distribution (right). Negative values mean net decay in NO_3^- fluxes over the 20 cm sediment column studied, since positive values are linked to enhancement of the fluxes to the lagoon. Each column represents a single profile modeled.

V.5 Discussion

The restrictions imposed to the data obtained in the field work (advective regime, homogeneous salinity distribution in depth), the parameters used to check the quality and coherence of the model fits and the satisfactorily linked measured and calculated fluxes allowed us to ensure the quality of the model-derived outputs. The model successfully reproduced NO_3^- porewater distribution inclusive of advection and mechanical dispersion, thus permitting discrimination of the relative contribution of transport and benthic reactivity over NO_3^- gradients in the porewater. According to the obtained results, positive (NO_3^- buildup) solute gradients close to the sediment surface and down to 4 cm depth can be explained with active nitrification layer lengths of less than 1 cm. Here, organic matter mineralization and subsequent NH_4^+ production within the sediment and that produced and transported from the deeper sediment levels can feed the identified net nitrification found close to the sediment-water interface. Therefore, modeling results showed that, even with significant advective up-flow transport, NO_3^- production in the nitrification layer can transport NO_3^- into the reduction

layer below. Moreover, spatial discrimination of main reactive processes needs to take into account the advective and dispersive effects over solute porewater distribution (Roychoudhury 2001). In this sense, ADR modeling permitted a more accurate discrimination of the biogeochemical zonation present at the time the profiles were taken.

Model-derived reaction rates were in the order of published kinetics: nitrification rates derived from mass balance studies reported by Rocha and Cabral (1998) in exposed intertidal sandy sediments and by Usui et al. (2001) in submerged fine sandy sediments were similar to those obtained here. On the other hand, NO₃⁻ reduction rates calculated here were comparable to those obtained by Rocha and Cabral (1998) with mass balance approaches and also with denitrification rates measured with the isotope pairing technique by Gran and Pitkänen (1999) and Usui et al. (2001) in subtidal sediments.

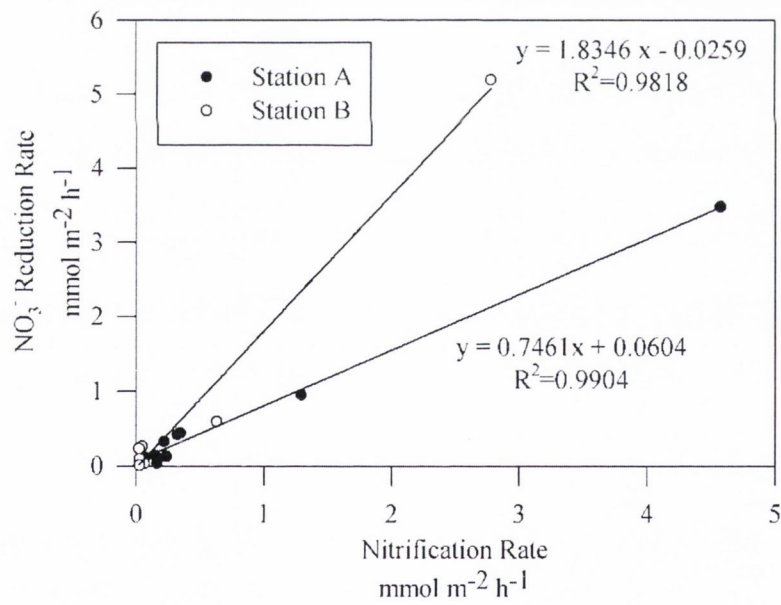


Figure V.5 NO₃⁻ reduction rates plotted against nitrification rates obtained from the model application for each single profile modeled. Results are discriminated by sampling station (Station A: $p < 0.0001$, $n = 9$; Station B: $p < 0.0001$, $n = 7$).

Reaction rates obtained from the application of the model to the measured NO₃⁻ profiles were usually in the same dimensional order as NO₃⁻ vertical interfacial fluxes. This

study identified net transformation rates of up to $2.4 \text{ mmol m}^{-2} \text{ h}^{-1}$ over benthic NO_3^- fluxes. Benthic reaction fluxes were at the same magnitude as NO_3^- interfacial fluxes and thus, strongly determined resulting NO_3^- fluxes at the sediment-water interface.

Reaction rates in permeable benthic systems may be strongly enhanced by the advective motion of porewater (i.e. Huettel et al. 1998; Falter and Sansone 2000). Reaction rates obtained here were highest in September, concomitant with the highest measured seepage rates and the likely highest water temperature. However, the strong correlation between nitrification and NO_3^- reduction rates at each place (Fig. V.5, $R^2=0.99$ $n=9$ $p<0.0001$ for station A and $R^2=0.98$ $n=7$ $p<0.0001$ for station B), indicates the covariance of both reactions. Advective velocity and NO_3^- concentration of the water seeping into the superficial layers of the seepage face may indeed strongly influence benthic modulation of NO_3^- loading of coastal waters by SGD.

The Nitrification/ NO_3^- reduction ratio differed appreciably between stations (Fig. V.5). The slope of the linear regression between the two processes at each station was notably different. The sampling station located within the main seepage face (station A), showed general dominance of nitrification over NO_3^- reduction (slope 0.746 ± 0.026 , Fig. V.5). On the other hand, NO_3^- reduction was dominant at the upper limit of the seepage (slope 1.835 ± 0.112), although the linear regression performed here is strongly influenced by a single point (Fig. V.5). Nevertheless, this opposite role in mediating NO_3^- -rich SGD was coherent with the analysis of the net influence of benthic reactivity over NO_3^- fluxes to the lagoon (Fig. V.4), where station B acted as a net NO_3^- consumption site (i.e. dominance of NO_3^- reduction over nitrification) and station A enhanced NO_3^- fluxes arriving to the seepage face during the summer (i.e. dominance of nitrification over NO_3^- reduction). Despite the variability between profiles, modeling results showed apparent seasonality in the groundwater-derived NO_3^- transformations into the seepage face; since results from March and December didn't show a clear pattern, during the warmer months (June and September) the upper limit of the seepage face was acting as a sink of NO_3^- and the lower seepage face was enhancing NO_3^- loads to the lagoon. Furthermore, benthic biogeochemical stratification was different in both places. In half of the profiles collected at the main seepage zone in June and September, the inclusion of the third, non-reactive layer did improve the model fit to the measured NO_3^- distribution in the

porewater significantly. The inclusion of this third layer in the model was based on the local distribution of organic matter and NO_3^- at the site (Chapter IV) and the results suggest that limitation of NO_3^- reduction in the main seepage face by organic matter availability is possible with depth.

The results presented here showed the high dynamism of the benthic community in mediating NO_3^- -rich SGD, both in time (differences between profiles taken at consecutive tidal cycles, denoted by the general low standard deviation of the modeling results at each month; see also Appendix A) and space (between the main seepage zone and the upper limit). The studied beach environment represents a link between NO_3^- -rich low organic content groundwater, benthic reactivity at the seepage face, and oscillatory sea water infiltration into the subterranean estuary (Moore 1999; Rocha et al. 2009). The apparent capacity of benthic community to change NO_3^- loads arriving to the seepage face by SGD was high. However, the expected role of the benthic community as net sink of NO_3^- due to denitrification (usually considered as the dominant process of NO_3^- reduction in coastal sediments; Herbert 1999) was not confirmed. Moreover, our modeling exercise showed that, in fact, enhancement of NO_3^- fluxes to the receiving waters could be possible, depending on factors like advective regime or organic matter load. Therefore, in order to accurately estimate the contribution of nitrogen loads through SGD to the receiving waters, benthic biogeochemical mediation at the seepage faces needs to be clarified (Slomp and Van Cappellen 2004).

The lack of significant amounts of labile organic carbon is usually identified as the major factor limiting denitrification rates in aquifers (i.e. Bradley et al. 1992; Rivett et al. 2008) and coastal sediments (i.e. Cornwell et al. 1999; Herbert 1999). Thus, it is also usually identified as the main factor controlling the groundwater NO_3^- concentration arriving to the coastal zone (Slomp and Van Cappellen 2004; Kroeger and Charette 2008). The dynamism of vertical biogeochemical zonation at the SGD seepage face could limit heterotrophic denitrification in depth, but also enhance the likelihood of alternative metabolic pathways, including autotrophic processes like ANAMMOX or Oxygen-Limited Autotrophic Nitrification Denitrification (OLAND) (Bowen et al. 2007; Brandes et al. 2007; Kroeger and Charette 2008). Furthermore, the high temperatures found at the seepage face could also favor DNRA over denitrification due to the greater standard free energy change of ammonifiers

when compared to denitrifiers under limited nitrate conditions (Dong et al. 2011). Thus, during the summer months, the potential presence of DNRA (and subsequent NH_4^+ production, thus recirculating N into bioavailable forms) together with organic matter mineralization at the seepage face and associated NH_4^+ production could feed nitrification at the upper levels and thus explaining the observed NO_3^- flux enhancement. Rocha et al. (2009), comparing NH_4^+ production and NO_3^- reduction rates obtained by simple mass balances over porewater profiles taken at our sampling site, showed the likelihood of co-occurrence of different reduction pathways involving NO_3^- processing.

These presented results, along with the possible existence of different reactive pathways involving NO_3^- processing at places affected by SGD (Kroeger and Charette 2008; Rocha et al., 2009), establish uncertainties about the final role of the permeable sediments as modulators of land-derived nutrient inputs to the coastal zone. In this sense, direct N_2 flux measurements and the use of stable isotopes analysis on both natural samples and controlled systems could give important information on the relative importance of each pathway, along with the interactions with other elements, biogeochemical kinetics and environmental factors controlling the system (Hamersley and Howes 2005; Pallud et al. 2007; Kroeger and Charette 2008). The main reactive zones identified here together with the apparent seasonal distribution could help to further work in controlled laboratory experiments where it can thus be possible to isolate the net reactive sediment layers to further explore the main processes involved in NO_3^- processing at the seepage face prior to discharge to the receiving waters. Thus, results about the expected overall reactivity and the main environmental factors controlling biogeochemical mediation could help to improve the design of further kinetics and stoichiometric studies in order to elucidate nitrate processing in SGD seepage faces (Hamersley and Howes 2005; Pallud et al. 2007; Rocha 2008).

V.6 Conclusions

Seasonal porewater NO_3^- distribution in a SGD seepage face was satisfactorily reproduced by using an ADR model with a free boundary solution approximation. The modeling procedure was able to identify main biogeochemical zonation in processing NO_3^- -

rich seepage. Sediment reactivity was able to significantly change NO_3^- porewater distribution and resulting fluxes to the receiving waters. Results of the analysis indicated that the upper limit of the seepage face promoted mitigation of NO_3^- fluxes to the lagoon throughout the year. In contrast, the lower limit of the seepage area promoted net amplification of the NO_3^- fluxes into the lagoon in June and September. The potential presence of DNRA (and subsequent NH_4^+ production, thus recirculating N into bioavailable forms) together with organic matter mineralization at the seepage face and associated NH_4^+ production could feed nitrification at the upper levels and thus explaining the observed NO_3^- flux enhancement. The mediation role of the permeable sediments at the exit points of SGD is thus uncertain. The results obtained from the modeling procedure may increase the accuracy of reaction rate estimates and geochemical zonation at SGD sites, and be an important support in preparing further experimental studies of local reaction kinetics affecting NO_3^- distribution in sediments impacted by groundwater discharge.

Chapter VI

Anthropogenic N pollution of coastal aquifers drives carbon loss from subterranean estuaries

VI.1 Abstract

Coastal aquifers are globally important organic Carbon (C) sinks due to their capacity to store organic matter and their role in delivering land-derived refractory organic compounds to the oceans. Nevertheless, information on how increasing Dissolved Inorganic Nitrogen (DIN) pollution of groundwater registered worldwide might affect organic matter storage in subterranean estuaries is currently unavailable. Here we present a series of flow-through experiments conducted with undisturbed sediment columns collected at a nitrate-rich Submarine Groundwater Discharge (SGD) seepage face in order to evaluate the role of land-derived DIN on organic C storage and dynamics at the subterranean estuary. Our results show that in the absence of DIN, the pace of benthic mineralization covaried with porewater flow rates. Furthermore, benthic mineralization promotes an increase on the refractory nature of the resulting Dissolved Organic C (DOC) fluxes, effectively increasing its residence time. Nevertheless, the presence of high concentrations of DIN and high discharge velocities act to catalyze the microbial breakdown of refractory organic material. Considered in their entirety, our results indicate that the availability of DIN within SGD increases the release of labile DOC, by enhancing the ability of extant microbes to process refractory organic matter. We conclude that DIN pollution of coastal aquifers drives C loss from the stored organic C pool promoting its release in more labile form to coastal waters, thus constituting a positive feedback into the global C cycle.

VI.2 Introduction

Benthic systems straddling the land-sea interface have historically been considered as important storage compartments, or alternatively, enhancers of the refractory nature of organic material originating on land, hence effectively prolonging its residence time in the ocean (Masiello 2007; Aller and Blair 2011; Keil 2011). Most of the research into land-sea organic Carbon (C) transfer has so far focused on riverine transport as the primary delivery pathway (Aller and Blair 2011; Keil 2011). Nevertheless, coastal aquifers are now recognized as important sources of fresh water and associated solutes to the coastal ocean, accounting for ~10% of the total fresh water input into the sea (Moore 1999). Due to the accumulation of solutes and metabolites originated from soil lixiviation (i.e. solute transport from soil to groundwater resulting from a wash-out process due to the action of rainfall or excess water) and microbial activity along the flow path, groundwater generally contains higher dissolved organic and inorganic C concentrations than the receiving surface waters. Thus Submarine Groundwater Discharge (SGD) can be an important transport vector of C to the coastal sea (Santos et al. 2009; Moore 2010).

Little information is available on the chemical composition of Dissolved Organic Matter (DOM) in aquifers (Longnecker and Kujawinski 2011). Humic and fulvic acids introduced by lixiviation of soils within the watershed and from microbial action on Sedimentary Organic Matter (SOM) seem to constitute a major fraction of DOM in groundwater (Artinger et al. 2000). SGD may hence contribute to the large refractory DOM pool with considerable residence time found in the oceans. Furthermore, despite the general low levels of SOM in aquifer sediments, the large volume of coastal aquifers and the ubiquitous presence of SOM suggest that these may yet constitute a C reservoir of global importance.

Human activities are now the main source of reactive Nitrogen (N) to Earth's ecosystems (Vitousek et al. 1997). Recent estimates suggest that 10% of the total 150 Tg N year⁻¹ human-derived reactive N applied on the land surface ends in groundwater reservoirs (Schlesinger 2009). Other anthropogenic N sources, including atmospheric deposition, sewage disposal or animal and industrial waste may also contribute to the observed increase in N pollution of groundwater worldwide (Wakida and Lerner 2005). Due to the high mobility of N compounds in the environment and the role N plays in microbial and primary producer

metabolism, human disruption of the global N cycle influences into the pace of other elemental cycles, particularly oxygen and carbon (Falkowski 1997; Mackenzie et al. 2002). Hence mobilization of organic C from high residence time reservoirs like peat bogs (Bragazza et al. 2006) and the arctic tundra (Mack et al. 2004) are being linked to anthropogenic N enrichment of terrestrial ecosystems. Although substantial research has focused on understanding natural NO_3^- attenuation processes within the groundwater flow path (Rivett et al. 2008), to our knowledge the impact of Dissolved Inorganic N (DIN) contamination on organic C transport and storage by coastal aquifers under marine influence has not been investigated.

The objective of this study is therefore to evaluate the role of land-derived DIN on organic C storage and mineralization dynamics at the subterranean estuary and SGD seepage face, where groundwater flow from land meets marine water intrusion. We performed a series of flow-through reactor experiments simulating active SGD seepage through undisturbed sediment slices taken from a NO_3^- -rich SGD seepage face at the Ria Formosa coastal lagoon, Southwest Iberia. A series of control experiments was carried out during October'10 and January'11 (Experiments 1 and 2) in order to study the vertical distribution of reactivity of benthic DOM in the absence of DIN in the porewater. Different seepage velocities were also imposed in parallel experiments to assess the influence of interstitial flow rate on benthic reactivity (Experiments 2 and 3). As a measure of control, glucose was added to the porewater at the higher end of the range of imposed interstitial flow rates to explore potential organic matter limitations on benthic reactivity. Finally, another series of experiments (Experiments 4-7) were carried out with amendments of NO_3^- and NO_3^- combined with NH_4^+ to the circulating porewater. These covered the range of concentrations found at the site (Rocha et al. 2009). The response of the benthic organic pool to the different treatments was assessed by following Dissolved Organic C (DOC) levels at both inlet and outlet of the experimental reactors and simultaneously assessing the makeup of the Chromophoric DOM (CDOM) pool by 3D-Excitation Emission Matrix (EEM) fluorescence and Parallel Factor (PARAFAC) analysis. Potential transformations occurring in both labile and refractory C pools during the experiments were subsequently evaluated by mass balance approaches.

VI.3 Materials and methods

VI.3.1 Study Site

The Ria Formosa (SW Iberian Peninsula; Fig. VI.1) is a 110 Km² coastal lagoon separated from the Atlantic Ocean by a multi-inlet sand barrier system composed by five islands and two peninsulas. The lagoon is surrounded by a 760 Km² semi-arid watershed that includes an intensively farmed coastal plain. The indiscriminate use of inorganic fertilizers led to heavy NO₃⁻ contamination of the main aquifer systems north of the lagoon (Almeida and Silva 1987). Following the EU nitrate directive, the Campina de Faro aquifer system (NW Ria Formosa) was designated Nitrate Vulnerable Zone in 2001, and this extended to the Campina da Luz aquifer system (NE Ria Formosa) in 2005. Preliminary estimates of N loading through Submarine Groundwater Discharge (SGD) suggest SGD to be the primary source of N to the lagoon (36.2 mol N year⁻¹ m⁻¹ of beach front; Leote et al. 2008).

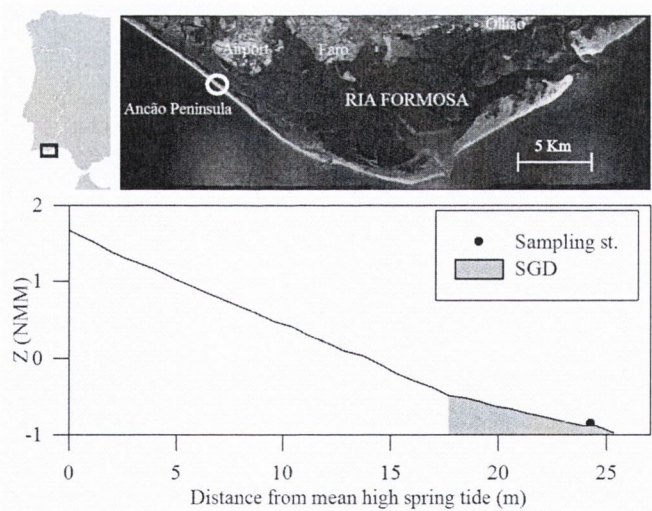


Figure VI.1 Sampling site location at Ancão peninsula (Ria Formosa coastal lagoon, SW Iberina peninsula). Beach profile measured during the period the study was performed along with the approximate area affected by SGD is also shown.

VI.3.2 Flow-through benthic reactor experiments

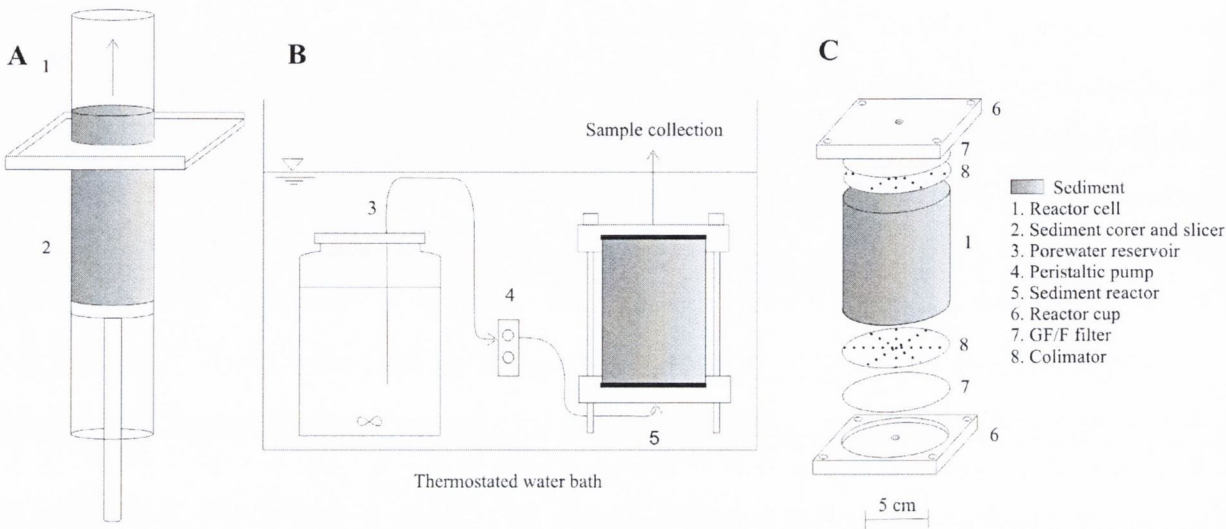


Figure VI.2 Schematic view of the experimental set-up: A. Sediment core slicing procedure. B. Flow-through reactor experiments performance. C. Detailed reactor scheme.

A series of sediment flow-through reactor experiments aiming to simulate the effect of active seepage in benthic C turnover was conducted from July 2010 to January 2011. The sediment used in the reactors was taken from the intertidal area affected by SGD on the inner part of Ancão Peninsula (37°00'04'' N, 7°88'57'' W; Fig. VI.1), composed mainly of medium-coarse sand (Rocha et al. 2009). High hydraulic conductivities measured by constant falling head permeametry ($1 \times 10^{-3} \text{ cm s}^{-1}$) underpin high seepage rates measured in-situ (up to $185.0 \text{ cm day}^{-1}$), essentially during sediment exposure at low tide (Leote et al. 2008). Fig. VI.2 shows a schematic view of the experimental set-up including the reactors we used. Undisturbed sediment cores (40cm length, 6.6 cm inner diameter) were collected with polycarbonate core liners in the lower intertidal area affected by SGD, close to the area where discharge peaks. These were collected at low tide and immediately transferred to the laboratory. An adapted HTH core slicer (Renberg and Hansson 2008) was used to transfer selected undisturbed vertical sediment slices onto Teflon flow-through reactor cells. The adapted HTH core slicer included a reactor holder on top of the core allowing the transfer of undisturbed sediment slices into the reactor cells while maintaining the pore structure (Fig.

VI.2A). The reactor cells were based on the design described in (Pallud et al. 2007). These included collimators and GF/F filters to ensure the even distribution of the water flow through the reactor's surface. The sediment flow-through cells were sealed and submerged into a temperature controlled water bath in the dark in order to prevent the possible influence of the microphytobenthos over DOM fluxes (Fig. VI.2B). Filtered sea water was then pumped through the reactor cells (bottom to top in order to simulate active seepage) at a constant flow rate for 4 h to adjust the system to constant temperature and interstitial flow rate prior starting the experiments with local porewater (Fig. VI.2C). Sediment flow-through experiments were carried out using filtered porewater (pumped from the bottom to the surface, simulating active seepage) collected in-situ with piezometers buried 40 cm into the seepage face. Porewater was amended with known concentrations of NO_3^- and NH_4^+ (Table VI.1). Oxygen concentration in the circulating porewater solution was controlled by purging the porewater mixture with helium and/or air prior to the experimental runs, the final working solution being sealed from contact with air by a layer of liquid paraffin. Flow-through experiments were performed during 9-18 h and samples taken at constant time intervals from the outflow into sterile tubes. These were stored at -20°C until further analysis, in order to avoid interference with both DOC and EEM fluorescence determinations (Otero et al. 2007).

VI.3.3 EEM Fluorescence of DOM and PARAFAC analysis

Three-dimensional EEM spectra of CDOM in porewater samples from the flow-through reactor experiments were measured on a Cary Eclipse Fluorescence spectrophotometer. Sample aliquots were placed in a temperature controlled bath at 20°C prior to analysis to ensure that changes to spectral intensities caused by temperature differences did not occur. Excitation and emission bandwidths were set at 5 nm and 10 nm, respectively. Emission wavelengths for analysis ranged from 280 to 560 nm with a 2 nm interval, while excitation intensities were recorded at 5 nm intervals spanning a wavelength range of 220 to 440 nm. Due to the low porewater DOC levels, inner filter effects (i.e. the absorption and re-emission of emitted energy at a longer wavelength by surrounding molecules) in our samples were assumed to be negligible. Daily recorded Milli-Q water blanks were subtracted from the sample spectra to eliminate water Raman scatter peaks. Subsequently

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EEMs were normalized to the integrated area of Milli-Q water Raman peaks determined daily as a calibration method for eventual instrument-dependent intensity factors (Lawaetz and Stedmon 2009). MATLAB with the DOMFluor Toolbox was used to handle first and second order Rayleigh scatter peaks and to perform the PARAFAC analysis of the samples (Stedmon and Bro 2008).

Exp.	Sol	Layer cm depth	n	T °C	S	O ₂ μ M	Velocity cm h ⁻¹	DOC μ mol L ⁻¹	DIN μM	NH ₄ ⁺ μM	CDOM composition (%)			
											Tryp	Tyr	Ter Humic	Mar Humic
October '10														
Exp. 1		0-2	2	18.5	36.1	225	15.1±0.2	293	< 2	< 2	24.7	45.6	11.1	18.6
Exp. 1		2-12	2	18.5	36.1	225	17.3±0.3	354	< 2	< 2	24.5	46.9	10.1	18.4
Exp. 1		12-32	2	18.5	36.1	225	17.1±0.0	319	< 2	< 2	24.6	46.3	10.6	18.5
Exp. 4		2-12	3	18.5	36.4	225	17.6±0.1	313	500	< 2	31.8	34.5	17.3	16.4
Exp. 5	A	2-12	3	18.5	36.4	37	18.8±0.1	302	101	< 2	25.5	47.2	10.5	16.8
	B				36.3	32		454	468	< 2	33.0	40.4	13.2	13.4
Exp. 6	A	2-12	3	18.5	35.7	29	17.9±0.7	346	1154	576	30.5	27.0	21.3	21.3
	B				35.7	44		433	1161	572	35.4	41.9	13.0	9.6
January '11														
Exp. 2	A B	0-2	3	14.5	35.4	249	15.5±0.4	290	< 2	< 2	30.6	31.0	14.4	24.0
Exp. 2		2-12	3	14.5	35.5	252	17.8±0.8	182	9	< 2	23.1	39.4	18.0	19.6
Exp. 2		12-32	3	14.5	35.5	248	17.6±0.2	> 2x10 ⁴	8	< 2	29.9	54.1	5.3	10.7
					35.5	245		183	< 2	< 2	26.4	43.4	11.1	19.2
Exp. 3a		2-12	3	14.5	34.7	245	6.7±0.1	156	8	< 2	68.5	2.6	20.1	8.8
Exp. 3b		2-12	3	14.5	35.6	248	3.7±0.1	178	9	< 2	51.7	24.9	14.6	8.8
July '10														
Exp. 7	A	2-12	3	24.5	36.9	78	17.5±0.2	348	124	< 2	22.7	43.1	13.8	20.4
	B				36.5	86		329	254	110	20.2	29.6	21.8	28.3

Table VI.1 List of relevant environmental and porewater conditions characterizing each of the flow-through experiments discussed in this study. Relative percentage of each of the four relevant fluorophores identified in the CDOM is also shown for each of the input porewater solutions. When more than one porewater solution was used in a single FTR experiment, these are denoted by A and B, thus showing the different initial parameters characterizing each solution.

PARAFAC modeling of EEM fluorescence has been extensively described elsewhere (Stedmon and Bro 2008; Andersen and Bro 2003). Briefly, PARAFAC analysis was performed over a large dataset obtained through different flow-through experiments carried out with sediments from the same location (1057 samples) and included the results presented here (346 samples). PARAFAC modeling allows decomposing the complex sample spectra into its individual fluorescence components (fluorophores) and quantification of scores obtained for each fluorophore present in each sample. These in turn are directly proportional

to the concentrations of individual components. The adequacy of the selected number of components and the uniqueness of the solution was tested by Split Half Analysis.

VI.3.4 Sediment properties

Additional sediment cores were sliced on-site with an HTH core slicer in order to evaluate relevant sediment properties. Water content was measured on every sub-sample taken this way as a percentage of the weight loss after drying at 90 °C for 48 h. Porosity used for benthic DOC fluxes estimation was then calculated based on the water content (Dullien 1992), using 2.65 g mL⁻¹ as the dry sediment density (quartz sand). Total organic matter content was determined for every dried subsample by Loss On Ignition (Dean 1974), and Total Organic Carbon (TOC) by combustion in a Vario EL Cube elemental analyzer after removal of inorganic C from bulk samples by exposure to concentrated HCl flumes for 6 h in a desiccator.

VI.3.5 Porewater chemistry

Oxygen concentration in the circulating porewater solutions was measured following the Winkler method adapted for low sample volumes (25 mL). Salinity and temperature were measured with a YSI 600 multi-parameter probe (YellowSpring Instruments). DIN concentration ($\text{NO}_3^- + \text{NO}_2^-$, NH_4^+) was determined in a Lachat Quickchem 8500 Flow Injection Analysis system following standard colorimetric methods (Grasshoff et al. 1983). DOC was determined in a Vario DOC Cube elemental analyzer, after acidification with a 2M HCl solution to ensure removal of dissolved inorganic C.

VI.3.6 Statistical analysis

Data taken from each individual experiment was compared to evaluate statistical significance of differences in measured quantities using the Student-t test. Two way ANOVA was performed on the data obtained at different porewater velocities to explore the significance of effects of porewater advection on the measured parameters. Pearson correlation

coefficients and the F-Fisher test were used for linear regression, after testing for normality, homogeneity and independence of the data.

VI.4 Results

VI.4.1 Sediment and porewater properties

During this study (July 2010-January 2011), the surficial sandy sediment from the unconfined aquifer locus of SGD at Praia de Faro peninsula (Ria Formosa, Southern Portugal) was characterized by low temporal variability in the vertical distribution of porosity. Highest porosity values were measured in the top 2 cm (0.37 ± 0.19 , $n=6$), remaining unchanged in the deeper sediment layers (0.31 ± 0.01 from 2 to 32 cm depth). Total organic matter measured by LOI showed a maximum in the top 2 cm, decreasing with depth (0.560 ± 0.058 % at 0-2 cm; 0.461 ± 0.034 % at 2-12 cm; 0.360 ± 0.016 % at 12-32 cm; $n=6$), whereas organic C content remained almost unchanged with depth (36.8 ± 6.3 ppt at 0-2 cm; 38.3 ± 4.5 ppt at 2-12 cm; 35.1 ± 2.1 ppt at 12-32 cm depth; see Chapter VII for high-resolution vertical distribution of TOC and TOM for October and January).

The sediment itself was characterized by low organic matter content. Even though slightly higher organic matter content was systematically restricted to the first 2 cm depth, total organic matter content as determined by LOI decreased to asymptotic levels below that depth. The progressive decrease in the total organic matter content together with the almost unchanged organic C content in depth suggests that organic matter at the surficial sediment at the seepage face becomes progressively enriched in carbon with depth. This suggests an increase in the refractory nature of bulk organic matter with depth as a feature of these sediments. The organic matter depth gradient therefore suggests that the fresher organic matter is accumulated in the sediment surface, thus suggesting the lagoon as a source of allochthonous particulate organic matter to the sediment column, through sea water infiltration during high tide.

In order to preserve the natural makeup of the DOM pool, porewater collected in-situ at 40 cm depth was used as input solution in the flow-through experiments. Collected

porewater consisted mainly of recirculated sea water characterized by permanently high salinity and low DIN content (Table VI.1). These characteristics allowed us to explore the effect of DIN addition on sediment-mediated DOM fluxes with no substantial DOC sorption effects caused by potential ionic strength gradients when porewater solutions started flowing through the sediment slice. DOC content in the porewater showed strong seasonal variability, with lower values found in January ($156\text{--}290\ \mu\text{mol C L}^{-1}$), whereas during July and October in-situ DOC concentrations were within the range of $293\text{--}495\ \mu\text{mol C L}^{-1}$ (Table VI.1).

VI.4.2 CDOM components identified with PARAFAC modeling

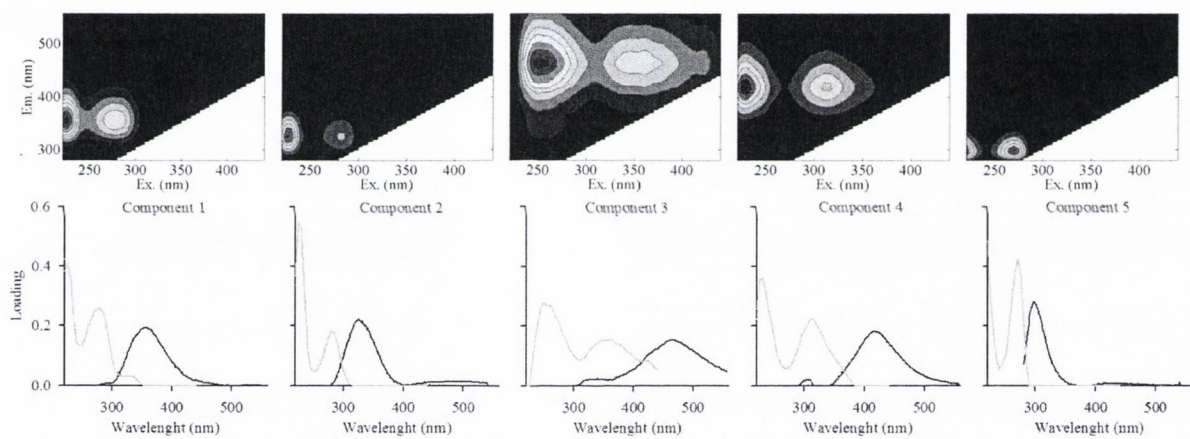


Figure VI.3 EEM spectra of the five fluorophores identified by PARAFAC modeling. Upper panels correspond to the EEMs contours of each component; lower panels show the excitation (gray lines) and emission (black lines) loadings for each component obtained after modeling our whole data set (1057 samples; solid lines), whereas dashed lines represent the PARAFAC results using the subset of samples explored in this study (346 samples).

Parallel Factor (PARAFAC) analysis was used to model our entire EEM fluorescence data set and to identify five main fluorophores within the organic matter pool (Fig. VI.3). Split-half analysis validation confirmed the adequacy and the uniqueness of the PARAFAC solution. Split-half analysis consists in dividing the data set into two random, equal sized groups and then running the PARAFAC analysis over each half. Thus, the loadings obtained

from each half should be the same since PARAFAC analysis solution should be unique (Stedmon and Bro 2008). In order to further evaluate the adequacy of the solution, an additional PARAFAC model was created by using the subset of samples collected in the current study (346). Despite the low DOC concentrations and therefore low signal to noise ratios in the measurements, good agreement was obtained between both models. However, since PARAFAC is based on the covariance of fluorophores, a larger data set (1057 samples) of similar experiments, including those derived from the experiments described here, gave a better explanation of the data and therefore its associated PARAFAC results were employed in subsequent interpretation.

Component	Ex/Em (nm)	Description and probable source	Reference
Component 1	225(275)/358	Tryp-like / Amino acids free or bound in proteins Peak T: 275/340 δ: 270-280/320-350 Pure Tryp: 278/340 C2: <250 (300)/368 C5: 280(<240)/368 C3: <250(285)/395 C5: <260/365	(Coble 1996) (Parlanti et al. 2000) (Kowalczuk et al. 2003) (Guo et al. 2011) (Stedmon et al. 2003) (Singh et al. 2010) (Santin et al. 2009)
Component 2	225(285)/326	Tyr-like / Amino acids free or bound in proteins Peak T: 275/340 Peak B: 275/305 δ: 270-280/320-350 γ: 270-280/300-320 Pure Tyr: 275/310 C4: 275/328 C7: 240(300)/338	(Coble 1996) (Coble 1996) (Parlanti et al. 2000) (Parlanti et al. 2000) (Kowalczuk et al. 2003) (Guo et al. 2011) (Murphy et al. 2008)
Component 3	250(345)/464	Terrestrial humic-like related to terrestrial particulate matter Peak C: 320-360/420-480 α: 330-350/420-480 C3: <250(380)/484 C3: 270(360)/478 C2: 270(370)/460 C1: <260(305)/439 C3: 260(370)/490	(Coble 1996) (Parlanti et al. 2000) (Guo et al. 2011) (Stedmon et al. 2003) (Singh et al. 2010) (Santin et al. 2009) (Murphy et al. 2008)
Component 4	230(315)/422	Marine microbial humic-like Peak M: 312/380-420 β: 310-320/380-420 C1: <250(335)/428 C4: 325(250)/416 C3: 320/388 C2: 315/418	(Coble 1996) (Parlanti et al. 2000) (Guo et al. 2011) (Stedmon et al. 2003) (Santin et al. 2009) (Murphy et al. 2008)
Component 5	<220(270)/298	Tyr-like minor component Peak B: 275/305 γ: 270-280/300-320 Pure Tyr: 275/310 C1: 275/<300 C4: 275/304	(Coble 1996) (Parlanti et al. 2000) (Kowalczuk et al. 2003) (Murphy et al. 2008) (Santin et al. 2009)

Table VI.2 Description of each of the five components identified with PARAFAC analysis in this study and comparison with previously identified marine and estuarine components in different studies

The five components identified through PARAFAC modeling can be separated into two groups according to their characteristic emission wavelength (emission wavelength associated to the maximum fluorescence intensity). Components 1, 2 and 5 (C1, C2, C5) have characteristic emission wavelengths of 358, 320 and 298 nm, whereas components 3 and 4 (C3, C4) have emission wavelengths of 464 and 422 nm (Fig. VI.3). Emission at longer wavelengths is associated with increased aromaticity or complexity in target molecules (Coble et al. 1998). C3 and C4 showed similar spectra to humic components identified in previous studies (see Table VI.2 for comparison with previously identified fluorophores). Studies by Clark et al. (2002) and Murphy et al. (2008) showed that peak C3 is a humic fraction of CDOM present in fresh water and deep sea waters, and strongly dependant on terrestrial sources to the sea, confirming its terrestrial origin (henceforth identified as Terrestrial Humic-like). Work by Parlanti et al. (2000) showed that peak C4 is a humic fluorophore specific to marine environments and correlated with salinity and microbial activity, i.e. freshly produced in the marine environment by microbial activity (henceforth identified as Marine humic-like). Components 1, 2 and 5 are similar to the fluorescence spectra of pure Tryptophan (C1) and Tyrosine (C2 and C5) (Coble 1996; Kowalczyk et al. 2003). Both are amino acids involved in protein synthesis. Due to the complex nature of DOM, identified fluorophores are more likely to represent co-varying groups of molecules with similar fluorescence rather than a single compound. Fluorescence spectra of C1, C2 and C5 are expected to differ in relation to the pure Trp and Tyr spectra since they can represent either amino acids bound in protein structures or peptides with both amino acids present. Nevertheless, these three components represent a labile, bioavailable DOM fraction, resulting from transport or in-situ production by microbial activity (Hudson et al. 2007). C5 represented a minor component of our dataset (<4% of the fluorescence scores) and was therefore excluded from the subsequent data analysis for the sake of simplicity. C1 and C2 are referred herein as Tryptophan-like (Tryp) and Tyrosine-like (Tyr) components respectively.

CDOM composition of the porewater used in the flow-through experiments was dominated by the labile components in terms of the scores obtained through PARAFAC analysis (Table VI.1). Significant linear correlation between all four components and DOC measured through our dataset was confirmed ($p < 0.05$, $n = 111$). Nevertheless, the Pearson correlation coefficient was generally low (Tryp $R = 0.6466$; Tyr $R = 0.5847$; Ter humic-like $R =$

0.6387; Mar humic-like $R=0.5459$), confirming non-conservative behavior of the four fluorophores within our reactors when subject to experimental conditions.

VI.4.3 Benthic mediation of CDOM and DOC fluxes

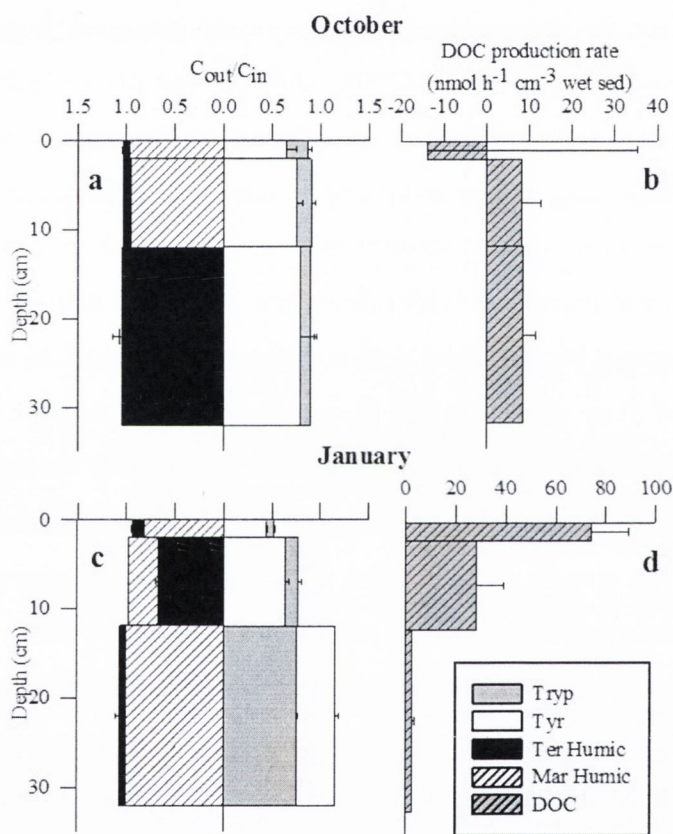


Figure VI.4 Vertical distribution of CDOM and DOC fluxes modulation by benthic reactivity measured in October 2010 (A and B) and January 2011 (C and D). Results correspond to time-integrated flow-through experiments carried out with local sediment and porewater collected in situ. The experiments were run under oxic conditions with no DIN addition to the porewater. Error bars represent SEM (n=2 in October and n=3 in January).

Control experiments performed under oxic conditions during October and January identified significant benthic modulation of both porewater CDOM and DOC flux (Experiments 1 and 2; see Table VI.1 for initial experimental conditions). Sediment mediation

of CDOM and DOC fluxes as measured in October are shown in Fig. VI.4a, b (the results of the experiments presented here are also shown in Table VI.3). Rates of transformation were quantified by integrating the relative change of each measured property over the experimental timeframe. In the absence of DIN (<2 μM) and at the given porewater advective velocity (16.5±0.5 cm h⁻¹), sediment acted as a net sink for the labile CDOM components (-10.5±4.1% Tryp-like and -22.7±13% Tyr-like as integrated for the 32 cm sediment column; Fig. VI.4a). No significant changes of the humic-like components of the CDOM pool were observed (C/C₀~1; p>0.2) in parallel. Measured sediment DOC production rates at the 2 to 12 cm depth layer were similar to those found between 12 and 32 cm depth, while the top 2 cm remained distinctive. The latter was characterized by highly variable DOC production/consumption rates, matching the highest consumption rates found for the labile fluorophores. Deeper sediment (2 to 32 cm depth) acted as a net source of DOC at a depth-integrated production rate of 2.61±1.14 mmol C m⁻² h⁻¹.

Experiment	Sol	Layer cm depth	n	Tryp C/C ₀	Tyr C/C ₀	Terr Humic C/C ₀	Mar Humic C/C ₀	DOC μmol L ⁻¹
October '10								
Exp. 1		0-2	2	0.87±0.04	0.65±0.10	1.03±0.02	0.96±0.04	-13.94±49.35
Exp. 1		2-12	2	0.91±0.04	0.75±0.07	1.03±0.00	0.95±0.04	8.05±4.65
Exp. 1		12-32	2	0.89±0.04	0.80±0.17	1.03±0.03	1.05±0.09	8.48±3.05
Exp. 4		2-12	3	1.08±0.02	1.23±0.05	1.09±0.02	1.03±0.01	23.90±3.22
Exp. 5	A	2-12	3	1.49±0.04	1.38±0.02	1.21±0.02	1.09±0.02	23.19±9.86
	B			0.90±0.05	0.83±0.07	1.13±0.02	1.14±0.01	-65.82±4.81
Exp. 6	A	2-12	3	1.60±0.21	1.77±0.33	1.41±0.14	1.10±0.04	20.16±16.77
	B			1.04±0.61	0.75±0.07	1.14±0.02	1.00±0.07	-39.38±6.96
January '11								
Exp. 2		0-2	3	0.52±0.05	0.44±0.05	0.95±0.02	0.83±0.03	74.28±14.96
Exp. 2		12-32	3	0.76±0.09	1.07±0.17	1.07±0.05	1.01±0.04	2.44±0.84
Exp. 2	A	2-12	3	0.77±0.05	0.63±0.09	0.67±0.03	0.98±0.02	28.49±10.49
	B			0.59±0.04	0.45±0.10	1.37±0.07	1.25±0.12	
Exp.3a		2-12	3	1.13±0.16	0.83±0.55	1.89±0.66	1.71±0.23	7.75±0.57
Exp. 3b		2-12	3	0.99±0.05		1.29±0.18	2.19±0.39	17.12±1.41
July '10								
Exp. 7	A	2-12	3	1.50±0.11	1.08±0.11	1.43±0.03	1.25±0.04	38.46±9.69
	B			2.95±0.29	3.25±0.43	1.22±0.04	1.16±0.10	68.89±11.80

Table VI.3 Benthic CDOM and DOC fluxes modulation as measured for each of the experiments. When more than one porewater solution was used in a single FTR experiment, these are denoted by A and B.

Consumption of the labile fluorophores in the absence of porewater DIN was confirmed in the control experiments carried out in January (Experiment 2, Table VI.1). Vertically integrated consumption rates of $-25.6\pm2.6\%$ for Tryp-like and $-5.7\pm3.8\%$ for Tyr-like CDOM were measured (Fig. VI.4c). Low depth integrated marine humic-like component consumption rates were also detected ($-6.0\pm3.7\%$), while no significant changes to the terrestrial humic-like fluorophore in the porewater CDOM ($1.1\pm1.0\%$) were observed, mainly due to the production occurring from 12 to 32 cm depth. As in October, the higher rates of CDOM consumption were restricted to the top 2 cm, as were the highest DOC production rates (72.2 ± 17.2 nmol C h⁻¹ cm⁻² wet sediment; Fig. VI.4d). Integrated DOC production rates measured during January in the deeper sediment column (from 2 to 32 cm depth; 3.43 ± 1.30 mmol C m⁻² h⁻¹) were higher than those measured in October, despite the lower temperature at which the experiments were conducted.

VI.4.4 Porewater advection effect over CDOM and DOC sediment production rates

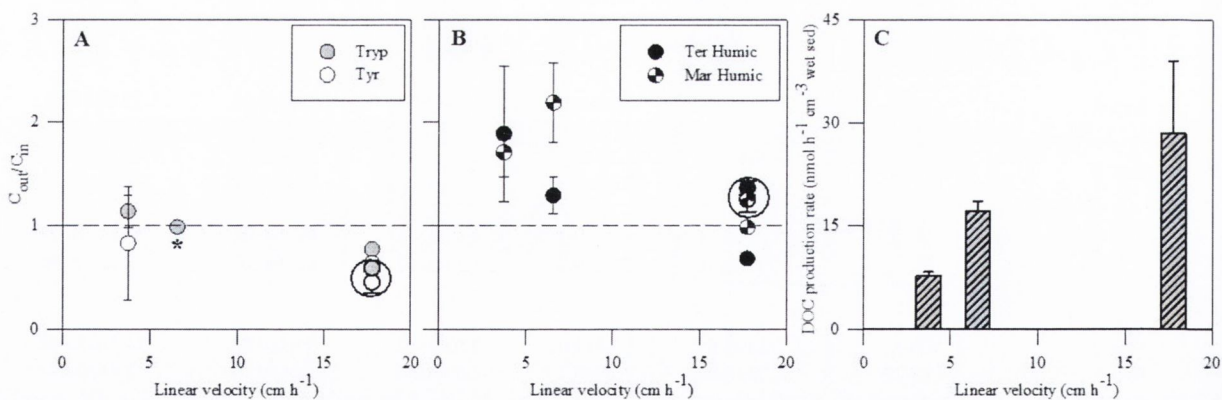


Figure VI.5 Sediment mediation of CDOM and DOC fluxes under different advective regimes for the selected sediment layer (2-12cm depth). Circled dots correspond to the extension of the experiment by glucose addition at ~20mM C. Error bars represent SEM (n=3).* data not available due to the extreme low content of the Tyr-like component in the input solution.

Porewater advective velocity influenced benthic reactivity of DOM (Fig. VI.5). At low porewater velocities (<10 cm h⁻¹) the sediment layer from 2 to 12 cm depth acted as a net

source of the two humic components identified in the CDOM pool, whereas the labile components remained unaffected ($C/C_0 \sim 1$; $p > 0.5$). This contrasted with benthic CDOM fluxes measured under a purely advective regime (Experiment 2; $17.8 \pm 0.7 \text{ cm h}^{-1}$), where sediment acted as a sink for the four fluorophores. The effect of porewater velocity on sediment reactivity is further illustrated by the DOC production rate resulting from each of the experiments (Fig. VI.5C). Increasing porewater advective velocity enhanced benthic DOC production rate ($p < 0.05$).

The experiment performed at the highest interstitial velocity ($17.8 \pm 0.7 \text{ cm h}^{-1}$) was extended by amending circulating porewater with glucose to a final concentration of $\sim 20 \text{ mmol C L}^{-1}$. This addition produced significant changes to CDOM reactivity (Fig. VI.5A, B circled dots; $p < 0.05$). In contrast to those measured in the absence of glucose (Tryp: $-23.1 \pm 4.5\%$; Tyr: $-36.4 \pm 3.4\%$), higher consumption rates of the labile components (Tryp: $-41.2 \pm 3.5\%$; Tyr: $-55.0 \pm 10.2\%$) were measured following amendment ($p < 0.05$). Furthermore, a shift in sediment reactivity with respect to humic fluorophores was observed: in the presence of glucose dissolved in porewater, the sediment acted as a net source of both humic components of the CDOM pool (Terrestrial: $36.5 \pm 6.6\%$; Marine: $25.2 \pm 11.9\%$), in contrast with net consumption observed before ($p < 0.05$).

VI.4.5 Sediment reactivity over CDOM and DOC fluxes after DIN addition to the porewater

A subsequent set of sediment flow-through experiments with different porewater DIN amendments was performed during October with sediment slices taken from 2 to 12 cm depth (Fig. VI.6; Experiments 4-6; Table VI.1). Under oxic conditions (Experiment 4), NO_3^- addition to the porewater ($500 \text{ } \mu\text{M}$ final concentration, representing the higher end of NO_3^- concentrations found at our site (Rocha et al. 2009) significantly stimulated both sediment labile and humic-like CDOM production rates when compared with the control experiment ($p < 0.05$). The sediment column acted as a source of all the four fluorophores ($C/C_0 > 1$), in contrast with the net consumption observed at the same depth in the control experiments. Benthic production of all four components of the CDOM pool in the presence of important NO_3^- pore water concentrations was concomitant with a statistically significant increase in the

sediment DOC production rate when compared with the control experiments performed during the same month ($24.2 \pm 3.3 \text{ nmol h}^{-1} \text{ cm}^{-3}$ wet sediment; $p < 0.05$).

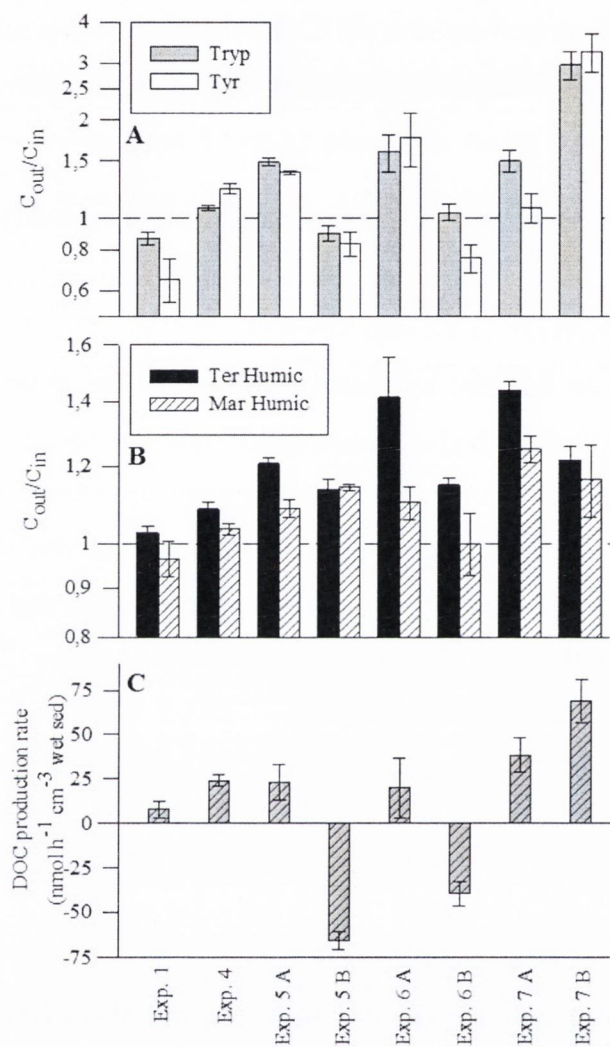


Figure VI.6 Compilation of the sediment reactivity over the CDOM and DOC pool measured in the FTR experiments performed with DIN addition to the porewater during October and July (see Table VI.1 for initial conditions). Results from the control experiment from October at the selected layer (2-12cm depth) are also shown for comparison. Sediment reactivity over CDOM is expressed as the integrated outflow porewater composition related to the inflow composition (C_{out}/C_{in}). DOC production/consumption rates integrated over the time the FTR experiments were run is also shown. Notice the logarithmic scale in the two upper panels corresponding with the results from the sediment CDOM mediation. Error bars represent SEM (n=3).

The effect of DIN addition on benthic DOM dynamics was further studied under suboxic conditions in a complementary set of experiments ($<50 \mu\text{M O}_2$; Experiment 5 and 6, Table VI.1). Enhancement of sediment CDOM production rates was confirmed after DIN addition to the porewater (Fig. VI.6A, B). Fluxes of all four major components in the CDOM pool were enhanced under those conditions, at a significantly increased rate by comparison to the behavior observed under oxic conditions ($p<0.05$). NO_3^- addition promoted an increase of the production of labile fluorophores by up to $49.0\pm4.5\%$ (Tryp-like) and benthic humic CDOM production by up to $20.9\pm1.8\%$ (Terrestrial humic-like). Addition of NO_3^- and NH_4^+ to the porewater (Experiment 6) resulted in an enhancement of labile CDOM fluxes by up to $77.1\pm33.5\%$ (Tyr-like) while simultaneously enhancing humic-like CDOM release by up to $41.4\pm14.1\%$ (Terrestrial humic-like). DOC production rates measured in both experiments were also higher than in those measured in the control experiments, but this difference was not statistically significant ($p>0.15$). Both experiments were further extended using porewater with naturally occurring higher DOC content. Under those conditions sediment became a net sink of DOC and the labile components of CDOM, whereas general release of the humic-like CDOM was still observed.

The effect of DIN addition to the porewater was also assessed during July 2010, at in-situ temperatures (24.5°C) and suboxic conditions (Experiment 7, Table VI.1). Two porewater solutions were used; first local porewater amended with NO_3^- and subsequently porewater amended with NO_3^- plus NH_4^+ . The results confirmed the observed patterns in the mediation of CDOM and DOC fluxes by the sediment. Porewater with added NO_3^- ($124 \mu\text{M}$) significantly enhanced the CDOM fluxes by comparison to control experiments carried out in October (Tryp $p<0.05$; Tyr $p=0.06$; humic-like $p<0.01$) and January (Labile $p<0.01$; humic-like $p<0.005$). DOC production rates ($38.5\pm9.7 \text{ nmol h}^{-1} \text{ cm}^{-3}$ wet sediment) were significantly higher than those observed in the control experiments of October ($p=0.05$), although not so when compared to results registered in January ($p=0.26$). On the second stage of the experiment (when NO_3^- plus NH_4^+ was added), enhancement of benthic CDOM fluxes was confirmed, with the highest recorded labile CDOM production rate ($p<0.05$). This was observed in parallel with the highest measured DOC production rate ($68.9\pm11.8 \text{ nmol h}^{-1} \text{ cm}^{-3}$ wet sediment; $p<0.05$), in itself higher than those measured during the first stage of the experiment, run with porewater containing NO_3^- only ($p=0.07$).

VI.5 Discussion

VI.5.1 Organic matter processing in the subterranean estuary

Despite the low standing stock of bulk organic matter in the sediment column (<0.6% weight), benthic DOC production measured in our control experiments indicated the microbial community was active processing organic matter. Generally, sediment consumption of CDOM was also observed. The results suggest that in the absence of significant DIN loading of the system, the microbial community consumed the labile components of the CDOM preferentially, while the humic-like fraction remained unaffected. Even so, if we exclude the top 2 cm of the sediment column, the underlying 2 to 32 cm depth may yet constitute an important source of organic C to the lagoon. Considering the porewater velocity imposed on flow-through reactors during our experiments as representative of the periods of active seepage (6h per day; Rocha et al. 2009), benthic organic matter mineralization during active seepage in winter may contribute between 15.7 and 20.6 mmol C m⁻² d⁻¹ to the lagoon. Apart from the refractory DOC that can be transported without attenuation or transformation from the coastal aquifer, microbial reactivity at SGD seepage faces can hence act as an important source of DOM to support heterotrophic metabolism in the lagoon's water column. Furthermore, due to the consumption of the labile components of the CDOM pool together with the net DOC production suggest the increase in the refractory nature of the resulting DOM fluxes, thus likely increasing its residence time in the receiving waters.

VI.5.2 Benthic organic matter processing controlled by porewater advection

Porewater advection is postulated to control not only solute distribution, but also microbial reactivity and therefore the size and makeup of organic and inorganic C pool in permeable sediments (Boudreau et al. 2001; Rocha 2008). Albeit mainly restricted to low tide when SGD rates are highest, peak advective porewater velocities in excess of 30 cm h⁻¹ were measured at our site during sediment exposure (Rocha et al. 2009). The range of velocities imposed in our sediment flow through reactor experiments was therefore a conservative representation of the local vertical advective porewater magnitudes measured during active seepage at our site.

A significant enhancement of the benthic DOC production rate with increasing porewater velocity was observed (Fig. VI.5). Consequently, higher seepage rates seem to enhance microbial-mediated organic matter mineralization, consistent with previous studies in permeable marine sediments (Boudreau et al. 2001; Rocha 2008). At the lower end of the imposed range of porewater velocities ($<10 \text{ cm h}^{-1}$), no significant change to the labile CDOM components was observed, while by contrast, at the highest end of the range, the benthic community became a net consumer of CDOM. Benthic mediation of the humic-like components, strongly linked to organic matter mineralization, showed a distinctive response to interstitial flow velocities. Whereas both humic-like components were released at low porewater velocities ($<10 \text{ cm h}^{-1}$), the sediment became a net sink of CDOM humic substances when porewater flow increased ($\sim 17 \text{ cm h}^{-1}$). This seems reasonable, since we accept that due to the high refractory nature of CDOM humic-like fluorophores, the microbial community will preferentially use other sources of organic C for their metabolic requirements. Therefore, we hypothesize that the change in benthic mediation of CDOM humic-like fluxes toward net consumption when porewater velocity increases occurs because both the organic matter composition and loading are limiting factors of benthic microbial metabolism. Our contention is further supported by the response of the benthic system to glucose amendment. Sudden availability of high quality organic C in the circulating porewater promoted the production of humic-like fluorophores by the benthic community, similarly to what was observed at lower porewater velocities. Furthermore, glucose availability increased the benthic consumption rate of the labile components of the CDOM pool. Benthic heterotrophic metabolism and subsequent DOC production in coastal aquifers and SGD seepage faces is therefore dependant on the volumetric seepage rate and on the quantity and quality of the available organic matter (both particulate and dissolved). Furthermore, the results indicate that under purely advective regime the preferential consumption of the labile CDOM fractions by the benthic microbial community increases the refractory nature of the leftover CDOM pool, thus in all likelihood increasing its residence time in the receiving water body.

VI.5.3 DIN-driven mobilization of stored organic matter in the subterranean estuary

In experiments performed with porewater amended with DIN, the DOC content seems to control benthic microbial activity. Porewater with higher content in DOC (Experiments 5B and 6B) drove benthic modulation of CDOM fluxes in a similar way to that observed under the presence of glucose, enhancing humic-like CDOM fluxes in parallel with net consumption of the labile components. Nevertheless, when sediment is exposed to lower DOC levels in the flowing porewater, DIN induced another shift in the way the benthos mediated CDOM fluxes, by comparison with appropriate controls. While in the absence of DIN the sediment acted as a net sink for the labile fraction of DOM, its presence stimulated production of the two labile components, under both oxic and suboxic conditions. Protein-like components, identified through analysis of EEM fluorescence of DOM, have been convincingly linked to the aromatic amino acid content in natural waters (Yamashita and Tanoue 2003). Production of amino acids, peptides and exogenous proteins by the microbial community in the presence of DIN counteracting previously identified labile CDOM components consumption in the absence of DIN hence suggests that under the identified organic C limitation, N is also a limiting substrate for local microbial metabolism. This view is further supported by observations of how the sediment mediates transfer of the humic-like fraction of CDOM. Whereas the humic fraction of CDOM in the porewater did not change significantly in the absence of DIN, a net increase of this fraction was measured in outflowing porewater after DIN was added to the circulating solution. In addition to the recognition of the potential source of the two identified humic-like components of CDOM as discussed earlier (marine and terrestrial) the fluorescence characteristics of each fluorophore offer valuable insights as to their diagenetic origin. When compared with the marine humic-like component, the blue shift of the terrestrial humic-like component suggests a more aromatic composition and therefore, indicates it originated from less reactive organic matter (Coble et al. 1998; Burdige et al. 2004). Benthic production of both humic-like fluorophores simultaneously therefore suggests that active microbial degradation of both freshly available and older, more refractory, organic matter is occurring. Furthermore, the origin of the organic matter being degraded may correspond to both marine and salt-marsh organic matter, as suggested by the origin of both humic components. High DIN concentrations increased benthic microbial metabolism and this in turn enhanced benthic C remineralization. This rational thread is supported by a significant enhancement of the

benthic DOC production rate observed (Fig. VI.6C) when porewater was amended with DIN. Hence the presence of inorganic N carried by SGD within the coastal aquifer subterranean estuary stimulates organic matter decomposition, by increasing the ability of extant microbes to process refractory organic matter. Furthermore, the enhancement of the labile CDOM fluxes in the presence of DIN suggests that this process constitutes a net source of labile, short residence time organic matter to the receiving coastal waters.

The presence of NH_4^+ was shown to have a distinct effect on microbial-mediated processing of CDOM. Even though temperature appears to be the main factor supporting the high DOC production rates measured during July with porewater containing only NO_3^- , supplementation with NH_4^+ enhanced both the DOC and labile CDOM production rate by the microbial community. Although NO_3^- is the most common main reactive form of N present in polluted coastal aquifers (Rivett et al. 2008), NH_4^+ may also be present as a result of land-derived waste inputs to groundwater, local organic matter mineralization or as resulting from microbial Dissimilatory Nitrate Reduction to Ammonium (DNRA). Previous studies have shown the possibility for this NO_3^- reduction pathway to exist in SGD seepage faces (Rocha et al. 2009). Therefore, the potential occurrence of DNRA in aquifer sediments may also constitute a positive feedback over the NO_3^- -driven organic matter mineralization as described before.

VI.5.4 Implications for C cycling in coastal aquifers

SOM is considered ubiquitous in aquifer sediments even if it is generally present at low concentrations. SOM is often considered the main reductant available in aquifer bodies, controlling for example the NO_3^- attenuation potential of the local microbial community (Rivett et al. 2008). Groundwater SOM oxidation rates are usually limited by C availability, suggesting that both its quality and quantity are important rate-controlling steps in mineralization sequences (Hartog et al. 2004). Our results are consistent with these findings, by revealing that in-situ oxidative processes were C limited when glucose was added to the experiments. However, they also suggest that under these conditions, N is a co-limiting factor for microbial activity. Increasing N pollution of coastal aquifers can therefore be a positive

feedback affecting benthic cycling of organic C. The observed enhancement of microbial mineralization rates driven by DIN, along with the significant discharge rates of SGD across the seepage face promote the microbial breakdown of highly refractory organic material. This results on the acceleration of the C cycle within the subterranean estuary, leading to a decrease in organic C storage potential. A fraction of the organic C stored as refractory material within the aquifer is transferred from a DOM pool with generally high residence times in the environment to near shore coastal waters in a more labile form through the SGD pathway. There, it becomes available to support heterotrophic growth.

Groundwater represents 97% of the total liquid planetary freshwater reserves (van der Leeden et al.1990). The large volume of these reservoirs coupled to the ubiquitous presence of SOM suggests that SOM in aquifer sediments may be a C reservoir of global importance. The impact of the mobilization of organic C stored in coastal aquifers to marine surface waters could therefore have a strong effect on the C cycle both locally and globally. Anthropogenic reactive N pollution of coastal aquifers could hence be an important positive feedback to climate change by enhancing the mobility and bioavailability of the organic matter pool stored as highly persistent C in the CDOM and SOM pool to nearshore surface waters.

Chapter VII

**Processing of Inorganic Nitrogen at a NO_3^- -rich subterranean estuary seepage face:
Processes and kinetics**

VII.1 Abstract

Subterranean estuaries, including the seepage face, are now recognized as important reactive interfaces mediating solute transport to coastal ecosystems through Submarine Groundwater Discharge (SGD). In this study, benthic biogeochemical mediation of SGD-borne inorganic N loading of the Ria Formosa lagoon (Portugal) was studied. NO_3^- reduction rates were obtained by kinetic modeling of the results of advective-dominated flow-through reactor experiments and ranged from 0.23 ± 0.11 to $1.42 \pm 0.02 \text{ mmol m}^{-2} \text{ h}^{-1}$. Good agreement with NO_3^- reduction rates previously estimated by diagenetic modeling of NO_3^- porewater vertical profiles at the same location was found. Short-term temperature sensitivity of local NO_3^- reduction rates was high ($Q_{10} = 3.5 \pm 0.2$). Porewater velocity (seepage rate) and the quality and availability of organic matter seem to be also main controlling agents modifying NO_3^- loading through SGD into the lagoon. Our results show that porewater velocity can enhance net NO_3^- reduction but also that the process might ultimately be limited by benthic organic makeup and content. Furthermore, our experiments indicate a degree of spatial overlap of nitrification with alternative NO_3^- reduction pathways, including Dissimilatory Nitrate Reduction to Ammonium (DNRA), hence raising uncertainties with regards to the ultimate function of the seepage face as a mediator of anthropogenic N loads to the coastal ocean through SGD.

Nomenclature	
K_{red}	Maximum NO ₃ ⁻ reduction rate (μmol h ⁻¹)
K_{half}	Half-saturation NO ₃ ⁻ reduction constant (μmol)
K_{red2}	Maximum NO ₂ ⁻ reduction rate (μmol h ⁻¹)
$K_{halfnitrite}$	Half-saturation NO ₂ ⁻ reduction constant (μmol)
f_{DNRA}	Factor of the total NO ₂ ⁻ reduced to NH ₄ ⁺
K_{nit}	NH ₄ ⁺ nitrification rate (h ⁻¹)
K_{nit2}	NO ₂ ⁻ nitrification rate (h ⁻¹)
K_{ads}	Rate constant of first order NH ₄ ⁺ adsorption-desorption (h ⁻¹)
NH_4^{+eq}	Total NH ₄ ⁺ adsorbed at equilibrium in the reactor (μmol h ⁻¹)
NH_4^{+ads}	Total NH ₄ ⁺ adsorbed in the reactor (μmol h ⁻¹)
K_{decay}	Hyperbolic decay inhibition factor
$K_{inhibition}$	Inhibition factor
I_{nh}	Hyperbolic decay inhibition property
K_{assim}	NH ₄ ⁺ assimilation rate (h ⁻¹)

VII.2 Introduction

Groundwater discharge to the coast occurs when a coastal aquifer is hydraulically connected to the sea with a positive head relative to sea level (Johannes 1980). In 1999, Moore established the concept of subterranean estuary applied to the subsurface mixing zone between groundwater and sea water due to the similarities of this mixing area with surface estuaries. The mixed water seeping out of the sediment to the coastal ocean is termed Submarine Groundwater Discharge (SGD; Burnett et al. 2003). Recent estimates suggest that groundwater accounts for over 5-10% of the total freshwater discharging to the coastal ocean (Taniguchi et al. 2002; Slomp and Van Cappellen 2004). As a consequence of the extensive use of fertilizers and subsequent nitrate pollution of aquifers registered worldwide (Rivett et al. 2008; Schlesinger 2009), SGD has been linked to the decline in water quality and trophic status of several coastal ecosystems (e.g. Johannes 1980; Rutkowski et al. 1999; Slomp and Van Cappellen 2004; Moore 2010; Waska and Kim 2011).

In order to quantify solute loads to the coastal ocean through SGD and thus constrain potential impacts to the receiving waters, research into the reactive role of the subterranean estuary has to be included in studies of land-ocean interaction. Recent research has identified subterranean estuaries as zones of active Dissolved Inorganic N (DIN) processing within the sediment prior to discharge (Nowicki et al. 1999; Ueda et al. 2003; Ullman et al. 2003; Bowen et al. 2007; Kroeger and Charette 2008; Spiteri et al. 2008; Rocha et al. 2009). Interactions with sediment surfaces, aquifer physical and chemical gradients due to heterogeneous mixture of subterranean estuary end-members or ion activity modulation by changing ionic strength can modify DIN content of SGD derived fluxes (e.g. Postma et al. 1991; Moore 1999; Charette et al. 2005; Charette and Sholkovitz 2006). Furthermore, the presence of denitrification at the seepage face has been shown to sometimes reduce NO_3^- fluxes associated to SGD (Nowicki et al. 1999; Ullman et al. 2003; Kroeger and Charette 2008).

Recent work has shown that the expected mitigation role of seepage faces over NO_3^- loads to the receiving waters as being carried out mainly through heterotrophic denitrification can be misleading, since other pathways reducing NO_3^- can coexist (Bowen et al. 2007; Kroeger and Charette 2008; Rocha et al. 2009; Chapter V). The presence of Anaerobic Ammonium Oxidation (ANAMMOX) and Dissimilatory Nitrate Reduction to Ammonium (DNRA) at subterranean estuary sediments has been suggested recently (Kroeger and Charette 2008; Rocha et al. 2009). Indeed, enhancement of SGD NO_3^- fluxes at the seepage faces can be possible through alternative NO_3^- reduction pathways and marine organic matter remineralization (Rocha et al. 2009; Chapter V). Preliminary diagenetic modeling of high resolution vertical porewater NO_3^- profiles at a seepage face in the Ria Formosa (Portugal) identified significant net NO_3^- reduction taking place (Chapter IV). Apart from the potential for NO_3^- flux modulation by the microphytobenthos community at the sediment surface during the day (e.g. Sundbäck et al. 1991; Thornton et al. 2007), microbial community within the sediment can significantly change SGD-derived NO_3^- loads to the receiving waters. Nevertheless, the potential for alternative NO_3^- reduction processes other than denitrification taking place at Ria Formosa seepage face questions the commonly accepted mitigation role played by the benthic community in mediating NO_3^- -rich SGD fluxes (Rocha et al. 2009; Chapter V).

Here, benthic biogeochemical reactivity over Dissolved Inorganic N (DIN) was studied at a Ria Formosa SGD seepage face. Flow-through reactor (FTR) experiments and kinetic modeling of the resulting temporal distribution of DIN compounds were used in this study. The objective was to evaluate and describe benthic biogeochemical processes involved in the modulation of NO_3^- -rich SGD fluxes and to constrain the influence of a set of environmental parameters over benthic reactivity.

VII.3 Materials and methods

VII.3.1 Study Site and sampling strategy

Sampling site was located at the intertidal area of the inner part of Ancão peninsula ($37^{\circ}00'04''$ N, $7^{\circ}88'57''$ W), one of the two sandy peninsulas surrounding the Ria Formosa coastal lagoon and within the area affected by SGD (see Chapter II for a general description of the area and Chapter VI for a description of the selected sampling site). Preliminary diagenetic modeling of in-situ NO_3^- porewater profiles allowed identification of the main environmental controls exerted over NO_3^- reduction at our site (Chapter V). Furthermore, it permitted to constrain main vertical biogeochemical zonation in processing NO_3^- -rich SGD seepage. Net NO_3^- production seems to be restricted to the top few cm, whereas NO_3^- reduction was always present in underlying layers. Limitation of NO_3^- reduction in depth was also identified in some of the benthic NO_3^- profiles (Chapter IV and V). The information obtained through preliminary modeling of in-situ porewater NO_3^- profiles at our site was used to constrain further experiments under controlled conditions. Based on the vertical zonation of benthic reactivity previously identified at our site, sediment slices from 2 to 12cm depth (excluding the net nitrification layer on top and avoiding possible net NO_3^- reduction limitation in depth as suggested by the modeling results showed in Chapter IV and V) were isolated to characterize the microbial processes involved in NO_3^- reduction within the seepage face. Furthermore, in-situ environmental parameters obtained at the seepage face were used to further constrain FTR experimental conditions and to study their influence on local reactivity.

VII.3.2 Sediment FTR experiments

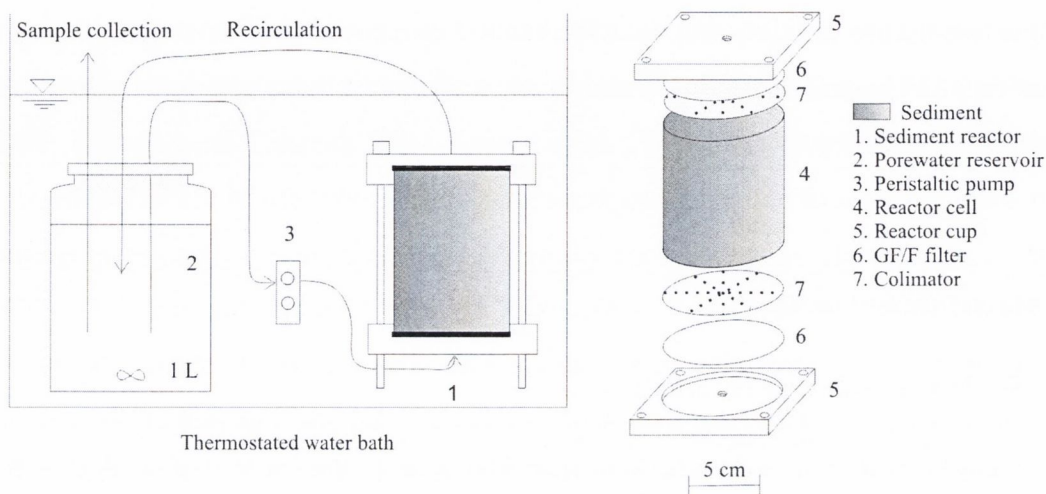


Figure VII.1 Schematic view of the experimental set-up for the flow-through reactor experiments run under recirculation mode.

In order to explore benthic NO_3^- reduction rates and kinetics, a series of sediment FTR experiments were conducted during October 2010 and January 2011 simulating active seepage of porewater. Undisturbed sandy sediment cores (40cm length, 6.6 cm inner diameter) were collected with polycarbonate core liners in the lower intertidal area affected by SGD, close to the maximum discharge zone. Sediment cores were collected at low tide and immediately transferred to the laboratory. There, an adapted HTH core slicer (Renberg and Hansson 2008) was used to transfer selected vertical sediment slices (from 2 to 12 cm depth) onto Teflon FTR cells. The FTR cells included collimators and GF/F filters to ensure even distribution of water flow through the reactor's surface (Pallud et al. 2007; Fig. VII.1). The adapted HTH core slicer included a reactor holder on top of the core, thus permitting transfer of undisturbed sediment slices into the reactor cells while maintaining the pore structure. Sealed FTR cells were submerged into a thermostated water bath in the dark in order to prevent the possible influence of the microphytobenthos over DIN fluxes. Filtered sea water was then pumped through the sediment from the bottom to the surface simulating active seepage at a constant flow rate for 4 h to re-equilibrate the system for temperature and flow rate. After re-

Processing of Inorganic Nitrogen at a NO₃⁻-rich seepage face

equilibration, FTR experiments were carried out using filtered porewater collected in-situ with piezometers at 40 cm depth into the seepage face.

Experiment	n	Treatment	T °C	S	O _{2-end} μM	Velocity cm h ⁻¹	NO ₃ ⁻ μM	NH ₄ ⁺ μM	DOC μmol C L ⁻¹
1	2		14.5	35.3	47.6±0.1	18.2±0.0	89±0	<2	532
2	3		18.5	35.5	10.3±0.0	19.1±0.3	93±0	<2	707
3	2		24.5	35.4	33.8±2.3	17.7±0.6	124±8	<2	553
4	2	Glucose	14.5	34.2	25.8±8.5	18.9±0.3	101±6	<2	>2x10 ⁴
5	2+1		14.5	34.3	44.3±27.3	3.7±0.1	93±2	<2	431
6	2		14.5	34.1	49.5	7.3±0.3	76±10	<2	530
7	2+1		14.5	34.2	13.3±1.7	18.1±0.2	85±4	49±0	471
8	2	Acetylene	14.5	34.9	-	16.7±1.8	107±4	63±7	-
9	2+1		18.5	35.0	-	17.8±0.1	73±0	<2	310
10	3	O ₂ saturation	18.5	35.1	Saturated	18.7±0.4	72±6	<2	430

Table VII.1 Relevant experimental conditions in the flow-through reactor experiments performed under recirculation mode. n is the number of replicates for each experiment. At experiments 5, 7 and 9, one of the replicates showed a distinctive output (thus showed as 2+1) despite the fact that the initial experimental conditions were identical.

Porewater collected in-situ was amended with known concentrations of NO₃⁻ and NH₄⁺ within the range of concentrations previously found at our site (Leote et al. 2008; see Table 1). Br⁻ (1 mM final concentration) was also added to the porewater as a tracer in order to infer reactor’s internal volume renewal. Oxygen concentration in the porewater solutions was controlled by purging the porewater mixture with helium and/or air, and the final solution (1L for each reactor) was placed into an sterile container and sealed with liquid paraffin to avoid contact with the atmosphere. Once the porewater solution started flowing through the reactor cells, FTR experiments were performed in open mode during time enough to renew the internal volume of the reactor cell with the new solution. Subsequently, the outflow stream was redirected to the porewater reservoir thus running the experiments in recirculation mode for 50-90 h (Fig. VII.1). Samples were collected periodically from the porewater reservoir into sterile tubes and stored at -20 °C until further analysis. A porewater solution similar to that used at the start of each experiment (refilling solution) was used to replace the volume of sample taken, thus maintaining the porewater volume in the reservoir constant.

VII.3.3 Sediment properties

Independent sediment cores were sliced on-site with an HTH core slicer in order to measure relevant sediment properties at the sampling site. Water content was measured in each sub-sample as a percentage of weight loss after drying at 90 °C for 48 h. Porosity was then calculated as described in Dullien (1992), using 2.65 g mL^{-1} as dry sediment density since sediment at the sample site consists mainly of quartz sand. Total organic matter content was determined at each dried subsample by Loss On Ignition (LOI; Dean 1974), and Total Organic Carbon (TOC) was determined by combustion in a Vario EL Cube elemental analyzer. Inorganic C was removed from the solid samples by exposure to concentrated HCl flumes for 6 h in a desiccator.

Sediment incubations in sea water amended with different NH_4^+ concentrations were performed in order to explore the heterogeneous equilibrium of NH_4^+ at the sediment horizon studied here (from 2 to 12 cm depth). Known quantities of dry sediment (2-3 g) collected at our site at 2-12 cm depth were incubated for 4 h at constant temperature (15 and 20 °C) in the NH_4^+ solutions (10 mL). On completion of the 4-hour period, sea water solutions were filtered and NH_4^+ adsorbed onto the sediment surface was then extracted with 10 mL of 2 M KCl solution for 2 h (Rosenfeld 1979b). The entire procedure was performed in duplicate. NH_4^+ partition coefficients (K^*) were calculated from the assumed linear relationship between the NH_4^+ sorbed per g of sediment and the final NH_4^+ concentration of the incubation solutions.

VII.3.4 Porewater chemical analyses

Oxygen concentration in the porewater solutions used in the FTR experiments was determined following the Winkler method adapted for low volumes of sample (25 mL). Salinity and temperature were measured with an YSI 600 multi-parameter probe (YellowSpring Instruments). NO_3^- , NO_2^- and NH_4^+ concentrations in the samples from the flow-through experiments were determined in a Lachat Quickchem 8500 Flow Injection Analysis system following standard colorimetric methods (Grasshoff, Erhardt, and Kremling 1983) as adapted for automated sequential analysis by the manufacturers. Br^- concentration

was determined in a Dionex ICS-1500 Ion Chromatography system coupled with an AS40 Automated Sampler.

VII.4 Kinetics modeling approximation

The experimental design of sediment FTR experiments is constrained by the biogeochemical reactivity in place and the fluid residence time within the reactor cell (e.g. Pallud et al. 2007). The fluid residence time, which depends on the reactor volume and the fluid velocity, needs to be large enough to be able to induce measurable differences between the input and the output concentrations of the target solute(s) promoted by the local benthic reactivity. Nevertheless, benthic reactivity in permeable sediments is modulated by the rate of porewater advection (Boudreau et al. 2001; Rocha 2008). Advective velocity can enhance important biogeochemical reactive pathways in permeable sediments (e.g. Janssen et al. 2005; Cook et al. 2007; Chapter V and VI). Thus, in advective-dominated SGD seepage faces, characterization of the local benthic reactivity needs to take into account the effect of porewater movement over reaction rates in order to be representative of field conditions (Chapter IV). High porewater velocities (up to $19.1 \pm 0.3 \text{ cm h}^{-1}$) were imposed in our FTR experiments due to the high seepage rates measured at our site (up to 185 cm d^{-1} ; Rocha et al. 2009). Nevertheless, this characteristic can make difficult to measure NO_3^- reduction rates with FTR due to the short residence time of the flowing porewater. Furthermore, the potential overlapping of different microbial processes involved in the NO_3^- processing at the seepage face can highlight uncertainties on the operating N benthic processes when they are assessed via instant, steady-state reaction rates. In order to preclude with those constraints, experiments aiming to elucidate NO_3^- processing at the seepage face were run under recirculation mode. The outflow stream was diverted back to the porewater reservoir after passing through the reactors. Sediment reactivity was evaluated by following the change in composition of the porewater reservoir over time.

Assuming that there are not any microbial biomass gradients or solute gradients established inside the reactor cell, FTR experiments run under the porewater recirculation approach mixed-flow reactor behavior (Levenspiel 1999). Under these assumptions, the

steady-state reaction rate of a microbial process can be simply calculated by mass balance between the input and the output solution compositions (eq. 1; Roychoudhury et al. 2003).

$$R = \frac{\Delta C Q}{V} \quad (1)$$

where R is the reaction rate, ΔC is the change in the concentration of the solute between the input and the output points, Q is the flow rate and V the reactor's volume. Further assuming that the time scale for steady-state (i.e. no significant change over a period of time in the DIN distribution inside the reactor) to be reached in the reactor is smaller than the time scale of changes within the porewater reservoir, reaction rates may be estimated from the observed changes of the porewater reservoir composition. As the volume of porewater in the reservoir remained constant throughout each experiment, measured concentrations were transformed into quantities by multiplying the concentrations by the reservoir volume, and the observed changes in the porewater reservoir were directly related to the volume of the sediment reactor cell.

In order to obtain a mechanistic explanation of the reactor's behavior and the kinetics parameters of NO_3^- reduction at our site, simple kinetics models were built based on the empirical, non-linear Monod equation (Monod 1949) and zero-order reaction kinetics (see Table 2). NO_3^- reduction was modeled as a two step process including the NO_3^- reduction to NO_2^- and NO_2^- reduction (eq. 2 and 3). In a similar manner, a Michaelis-Menten-type equation with a DNRA factor term (f_{DNRA} , eq. 4) was used to describe the kinetics of NO_2^- reduction to NH_4^+ . For the modeling of nitrification we followed two different approaches. Nitrification was modeled as following a zeroth-order reaction kinetics when no NH_4^+ was added to the porewater, since no NH_4^+ accumulation was registered throughout the course of the experiments (eq. 5 and 6). When NH_4^+ was added to the circulating porewater (Experiment 7), nitrification was modeled as following first-order kinetics related to NH_4^+ concentration (eq. 7 and 8) In both cases, nitrification was simplified to a one step reaction due to the fact that accumulation of NO_2^- was not observed (oxidation of NH_4^+ to NO_3^-). Kinetics of sediment NH_4^+ adsorption/desorption was assumed to follow the Lagergren pseudo-first order kinetics equation (eq. 9; Ho and McKay 1998). Adsorption equilibrium was assumed to be reached within 2h ($K_{\text{ads}}=0.5 \text{ h}^{-1}$; Rosenfeld 1979; Fitzsimons et al. 2006). Experiment 8 was performed

with porewater amended with acetylene to a final concentration of $30\mu\text{M}$. Acetylene is known to irreversibly inhibit the NH_4^+ monooxygenase enzyme in aerobic ammonia-oxidizing bacteria at concentrations higher than $4.1\mu\text{M}$ (Berg et al. 1982). Therefore, in the experiments performed in the presence of Acetylene, NH_4^+ consumption was assumed to be the result of net assimilation by the microbial community, and modeled following zero-order reaction kinetics (eq. 10).

(2) NO_3^- reduction to NO_2^-	$\frac{d\text{NO}_3^-}{dt} = -K_{red} \frac{\text{NO}_3^-}{K_{half} + \text{NO}_3^-}$
(3) NO_2^- reduction	$\frac{d\text{NO}_2^-}{dt} = -K_{red2} \frac{\text{NO}_2^-}{K_{half\text{nitrite}} + \text{NO}_2^-}$
(4) DNRA	$\frac{d\text{NH}_4^+}{dt} = f_{DNRA} K_{red2} \frac{\text{NO}_2^-}{K_{half\text{nitrite}} + \text{NO}_2^-}$
(5) Nitrification – NH_4^+ zeroth-order	$\frac{d\text{NH}_4^+}{dt} = -K_{nit}$
(6) Nitrification – NO_3^- zeroth-order	$\frac{d\text{NO}_3^-}{dt} = K_{nit}$
(7) Nitrification – NH_4^+ first-order	$\frac{d\text{NH}_4^+}{dt} = -K_{nit} \frac{\text{NH}_4^+}{K_{half\text{ammonium}} + \text{NH}_4^+}$
(8) Nitrification – NO_3^- first-order	$\frac{d\text{NO}_3^-}{dt} = K_{nit} \frac{\text{NH}_4^+}{K_{half\text{ammonium}} + \text{NH}_4^+}$
(9) Adsorption	$\frac{d\text{NH}_4^+}{dt} = -K_{ads} (\text{NH}_4^+_{eq} - \text{NH}_4^+_{ads})$
(10) Assimilation	$\frac{d\text{NH}_4^+}{dt} = -K_{assim}$
(11) Exponential decay function	$\frac{\partial I}{\partial t} = \frac{Inh}{Inh + K_{decay}}$
(12) Competition/inhibition terms	$1 - \left(\frac{Inh}{Inh + K_{inhibition}} \right); \quad \left(\frac{Inh}{Inh + K_{inhibition}} \right)$

Table VII.2 Equations used for kinetics modeling of the sediment FTR experiments performed under recirculation mode.

Initial inhibition of NO_3^- reduction processes in reactor's studies has been justified as an adjustment for the microbial community to start processing NO_3^- fluxes (André et al. 2011). NO_3^- concentration in the porewater reservoirs remained unchanged or even increased during the first stages of the FTR experiments performed here. A general inhibition/competition term

was postulated during the modeling procedure, accounting for the succession of nitrification/ NO_3^- reduction over time. For this, a general exponential decay function (I) ranging from 1 to 0 ($I=1$ at $t=0$) was used as an undetermined inhibition property (eq. 11). This was used to postulate a competition term between nitrification and NO_3^- reduction over time (eq. 12), or simply a transitional inhibition affecting NO_3^- reduction during the first stage of the FTR experiments (e.g. Beg and Hassan 1987).

For each experiment, the system of first-order ordinary differential equations (ODE) was solved numerically using the lsoda method included in the ReacTran 1.3 package (Soetaert and Meysman 2010) for R software (R Development Core Team 2009). Fitting procedure was performed using the minimizing method described by Nelder and Mead (1965) and included in the FME package for R software (Soetaert and Petzoldt 2010). The method implemented finds a local minimization of the sum of squared residuals (SSR). Due to the large number of parameters, the fitting procedure was performed following a series of steps. First, a preliminary estimate of nitrification and NO_3^- reduction rates was obtained through mass balance approaches over the temporal NO_3^- distribution. With these preliminary values, and assuming that there is no inhibition of NO_3^- reduction towards the end of each experiment (thus avoiding overestimation of NO_3^- reduction rates), the inhibition parameters were subsequently obtained and fixed. Finally the fitting algorithm was used to adjust the NO_3^- and NO_2^- (and NH_4^+) distribution over time and to obtain the kinetic reaction parameters. The fitting procedure was performed until good convergence of the model with the measured data was achieved ($p<0.001$).

VII.5 Results

VII.5.1 Sediment and porewater properties

During this study (October 2010, January 2011), porosity measured on independent sediment cores taken at the seepage face was characterized by low temporal variability (Fig. VII.2). Porosity profiles displayed a classic vertical distribution, with a maximum close to the sediment-water interface, becoming progressively constant with depth (e.g. Bahr et al. 2001). Nevertheless, vertical porosity distribution showed a minimum between 4 and 12 cm depth, in

accordance with the profiles measured by Rocha et al. (2009) at the same location. The sediment was characterized by low standing stocks of particulate organic matter during both months. Slightly higher organic matter content was registered during January, although remaining at very low levels (< 0.8%). As discussed in Chapter VI, the almost unchanged TOC levels in the sediment along with the decayment in total organic matter content with depth as measured by LOI suggest the increase in the C content of the total organic matter pool in depth. This is interpreted as an increase in the refractory nature of the total organic matter pool with depth. Therefore, the results suggest the lagoon as a source of allochthonous particulate organic matter to the sediment column, through sea water infiltration during high tide, becoming accumulated in the surficial sediment.

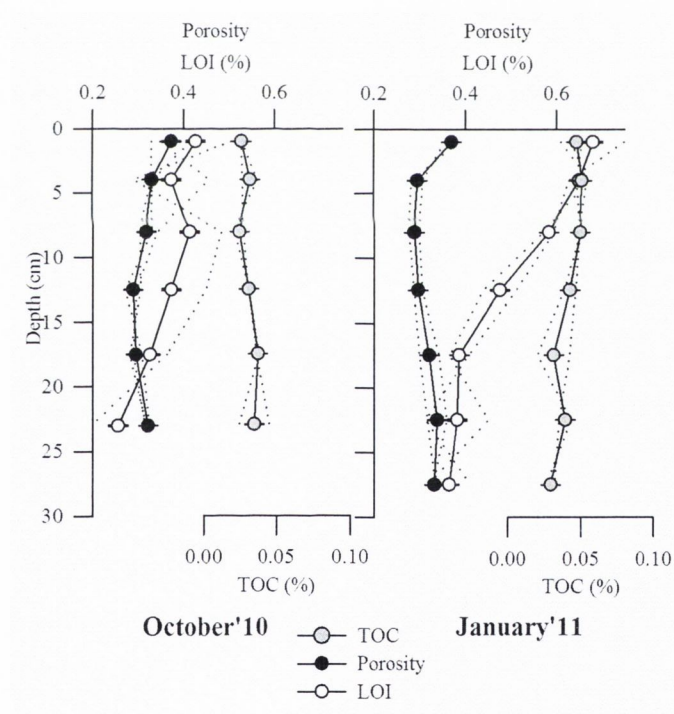


Figure VII.2 Vertical distribution of relevant sediment properties measured in October 2010 and January 2011 at Ancão peninsula, Ria Formosa. Sediment cores were collected within the area affected by SGD, close to the peak discharge.

In order to simulate close to in-situ conditions, porewater collected in-situ at 40 cm depth was used as input solution in the flow-through reactor experiments. Collected porewater

consisted mainly of recirculated sea water, characterized by permanently high salinity (Table VII.1). DOC content in the porewater at the start of the experiments remained at relative stable values (between 400 and 600 $\mu\text{mol C L}^{-1}$), with the exceptions of experiment 2 (707 $\mu\text{mol C L}^{-1}$) and experiment 9 (310 $\mu\text{mol C L}^{-1}$).

Br^- concentration in the reactors' outflow before diverting it back to the porewater reservoir (thus starting recirculation) indicated in all cases >95% renewal of internal volume before diverting the outflow stream to the porewater reservoir (i.e. outflow stream was in all cases >95% the porewater solution introduced; data not shown).

Steady-state NH_4^+ sorption characteristic of the sediment studied here from (2 to 12 cm depth) was explored in the laboratory at controlled temperatures (15 and 20°C). NH_4^+ adsorption isotherms are shown in Fig. VII.3. For the sake of simplicity, NH_4^+ adsorption isotherms were assumed to be linear for the range of NH_4^+ concentrations found at the site. NH_4^+ partition coefficients obtained from the slope of the adsorption isotherms ranged from 0.050 to 0.059 $\text{cm}^3 \text{ g}^{-1}$. NH_4^+ adsorption at our site was revealed to be very low when compared to NH_4^+ partition coefficients found in marine sandy sediments with similar median grain size (Raaphorst and Malschaert 1996; Rocha 1998).

In order to include the heterogeneous equilibrium between the NH_4^+ in solution and that sorbed onto the sediment surface in the modeling procedure, partition coefficients were scaled to the reactor's size. For this, partition coefficients were corrected by:

$$K = K^* \rho V_{\text{reactor}} (1 - \varphi) / V_{\text{reservoir}} \quad (11)$$

where K^* is the measured partition coefficient, ρ is the sediment density (assumed 2.65 g L^{-1}), V_{reactor} and $V_{\text{reservoir}}$ are the volume of the reactor and the porewater reservoir respectively and φ is the sediment porosity. K was then used to calculate the total NH_4^+ sorbed onto the sediment surface within each reactor ($\text{NH}_4^+_{\text{ads}}$; equation 7).

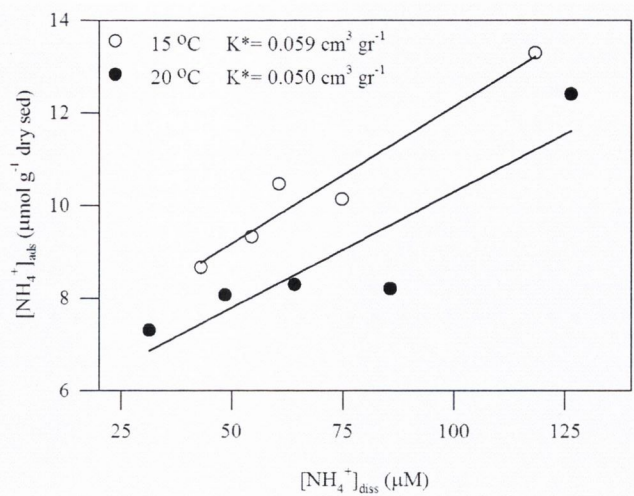


Figure VII.3 Linear NH_4^+ adsorption isotherms at 15 and 20 °C characteristic of the vertical sediment horizon studied (from 2 to 12cm depth).

VII.5.2 FTR experiments

The course of NO_3^- , NO_2^- and NH_4^+ (when NH_4^+ concentrations were above the quantification limit) over the time for the FTR experiments are shown in Fig. VII.4. Significant changes of the DIN content over time in the porewater reservoir were confirmed under the different treatments applied to the FTR experiments. Furthermore, a temporal succession of two distinctive sediment mediation roles over flowing porewater solutes was identified in all the experiments: first, a period where NO_3^- content in the porewater reservoir remained unchanged or even increased, followed by a period of net NO_3^- removal. In some cases, the observed net NO_3^- removal by the sediment community led to complete depletion of porewater DIN content. NO_2^- accumulation was observed only in parallel with net NO_3^- consumption. In general, no accumulation of NH_4^+ throughout the course of the experiments in the absence of NH_4^+ addition was observed.

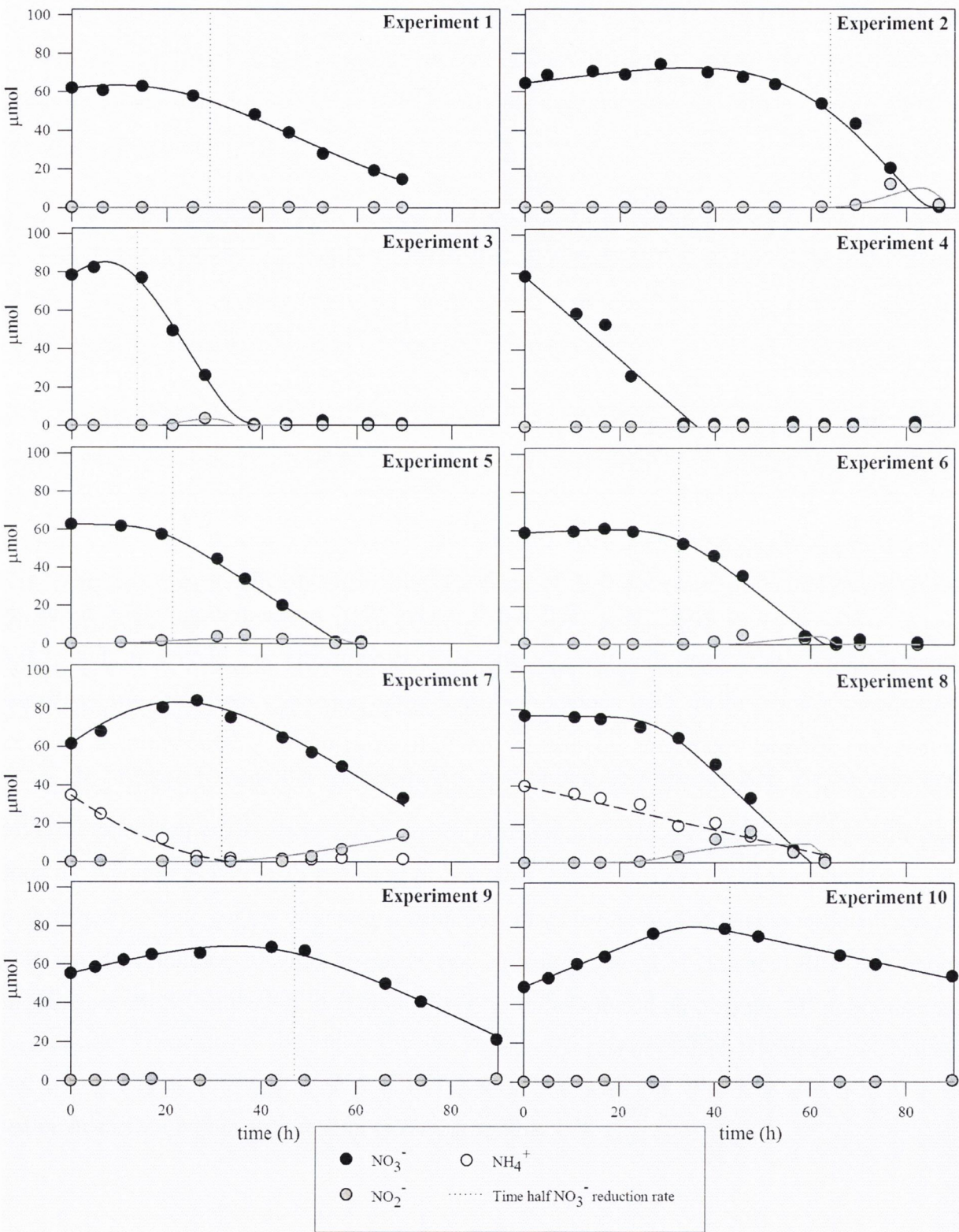


Figure VII.4 Representative measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Measured and modeled NH_4^+ temporal distribution is also shown in the experiments with NH_4^+ addition to the porewater (in the other cases NH_4^+ concentration was always below the quantification limit).

VII.5.3 Kinetics modeling approximation

VII.5.3.1 Sensitivity analysis

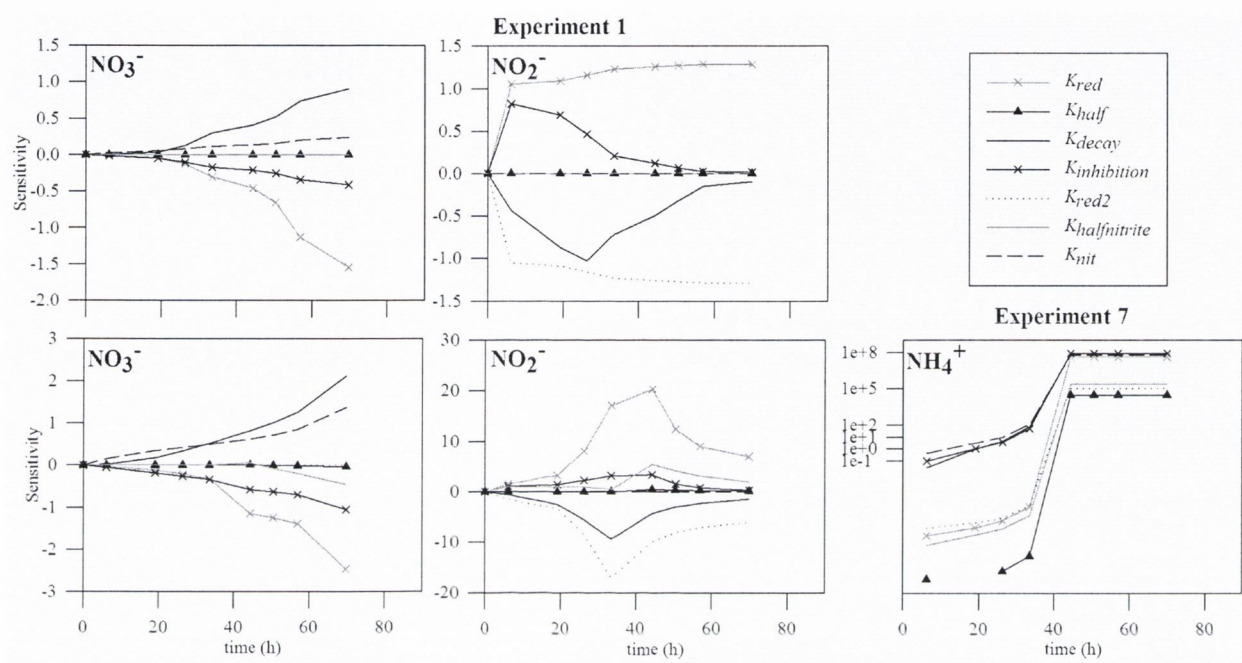


Figure VII.5 Sensitivity analysis performed at each modeled experiment. Results from experiment 1 (upper panels) and 7 (lower panels) are shown as examples. Sensitivity results from experiment 7, NH_4^+ are shown in absolute values and logarithmic scale for easier representation.

The best-fit solution of the mathematical models and the experimental data is shown in Fig. VII.4. A local sensitivity analysis was performed over each fitted model to the data in order to elucidate the relative importance of each parameter on the model outcome (Soetaert and Petzoldt 2010). The normalized, scaled sensitivity analysis performed permitted to evaluate, at each data point, the scaled derivative of the SSR promoted by changes on a selected model parameter (i.e. $\partial \text{SSR} / \partial \text{Parameter}$). Specifically, the importance of each parameter (reaction rate constants, half saturation constants, adsorption, etc) in the final modeling result was evaluated by considering how the model fit to the data deviates (SSR) after changing each parameter. Fig. VII.5 shows the sensitivity analysis results from two selected examples, experiment 1 (upper panels) and 7 (lower panels). Reaction rates and the parameters included in the inhibition/competition function are the parameters with higher

sensitivity regarding NO_3^- and NO_2^- temporal distribution. This means that reaction rate constants and the inhibition term are the parameters which primary determine the model fit to the data. On the other hand, half-saturation constants from the Monod reaction models showed little impact on the overall fit, gaining importance only at low solute concentrations. In the experiments where complete depletion of NO_3^- was not observed, the low impact of K_{half} on the modeled NO_3^- effectively suggests that NO_3^- reduction at our site follows close to zero-order reaction kinetics at the given NO_3^- concentrations:

$$\frac{d\text{NO}_3^-}{dt} = -K_{red} \frac{\text{NO}_3^-}{K_{half} + \text{NO}_3^-} \approx -K_{red}; \text{ when } \text{NO}_3^- \gg K_{half} \quad (13)$$

Sensitivity analysis on the NH_4^+ temporal distribution showed similar patterns when compared with NO_3^- (Fig. VII.5, lower panels). Reaction rates and the inhibition function showed to be the most sensitive parameters affecting the fit. Only when NH_4^+ was depleted from the porewater reservoir aid the other parameters gaining importance.

VII.5.3.2 Modeling results

Averaged standard error (ASE) of modeled temporal distribution of the inorganic N species at each experiment is shown in Table VII.3. The Monod and zero-order reaction kinetics model replicated the temporal distribution of the analyzed species in the FTR experiments with high correlation. As denoted by the low SE, model-derived temporal distribution of DIN species accounted for the main DIN distribution patterns observed during the course of the FTR experiments. Furthermore, the modeling procedure satisfactory linked DIN species analyzed through the reactive pathways included in the primary modeling assumptions (Fig. VII.4). Transient NO_2^- accumulation registered in the FTR experiments was satisfactorily identified with NO_2^- as being an intermediary compound in the two step NO_3^- reduction model. Moreover, experiment 10, run under constant oxygen saturation of the porewater in the reservoir, showed a similar behavior to the other experiments, with a first stage where NO_3^- concentration increased followed by net NO_3^- consumption over time.

Experiments 7 and 8 confirmed the potential for nitrification in the studied sediment layer. NH_4^+ added to the porewater in experiment 7 was satisfactorily linked to the build-up of

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NO₃⁻ through nitrification, with no NO₂⁻ accumulation, whereas after acetylene addition to the porewater (thus blocking the first oxidation step in the overall nitrification process) NO₃⁻ remained unchanged.

Reaction rates obtained from the modeling procedure ranged from -2.33±1.06 to -14.17±0.22 μmol dm⁻³ h⁻¹ for NO₃⁻ reduction and from 0 to 7.46±1.32 μmol dm⁻³ h⁻¹ for nitrification. Maximum NO₃⁻ reduction rates were registered in the experiment performed at the highest temperature (24 °C), whereas maximum nitrification rate was obtained from the experiment with porewater amended with NH₄⁺ (experiment 7). Experiment 8 (performed with nitrification blockage) permitted estimation of NH₄⁺ assimilation by the microbial community (2.04±0.00 μmol dm⁻³ h⁻¹).

Experiment	ASE			NO ₃ ⁻ reduction	NO ₂ ⁻ reduction	Nitrification	Assimilation	Time half rate
	NO ₃ ⁻	NO ₂ ⁻	NH ₄ ⁺	μmol dm ⁻³ h ⁻¹	μmol dm ⁻³ h ⁻¹	μmol dm ⁻³ h ⁻¹	μmol dm ⁻³ h ⁻¹	
1	3.2±1.2	-	-	-4.63±0.24	<<-4.63	0.76±0.76	-	41.2±12.2
2	2.9±0.1	0.3±0.2	-	-11.76±0.33	-7.80±1.68	3.03±1.99	-	61.4±1.7
3	9.6±2.3	3.2±1.2	-	-14.17±0.22	-10.59±0.38	3.79±3.79	-	12.3±1.5
4	3.8±0.5	0.8±0.8	-	-5.94±0.38	<<-5.94	-	-	-
5	0.9±0.1	1.0±0.2	-	-4.06±0.67	<<-4.88	0	-	19.8±1.5
6	1.1±0.6	0.9±0.5	-	-6.02±0.04	-4.88±0.23	0.72±0.35	-	33.7±1.4
7	3.4±0.2	0.9±0.3	1.5±0.0	-4.03±0.58	-3.26±0.03	7.46±1.32	-	31.4±0.2
8	1.8±0.7	2.0±1.5	2.8±0.2	-5.69±1.20	-6.23±0.00	0	2.01±0.26	38.2±10.6
9	1.8±0.4	-	-	-2.33±1.06	<<-2.33	2.96±1.14	-	37.8±9.3
10	1.9±2.0	-	-	-2.66±0.64	<<-2.47	1.54±0.69	-	51.3±4.8

Table VII.3 Results obtained from the Monod and zero-order reaction kinetics modeling of FTR experiments. The goodness-of-fit represented by the averaged standard error (ASE) and obtained reaction rates are shown, together with the time where the rate of NO₃⁻ reduction was half the maximum rate identified.

VII.5.4 Temperature dependence of NO₃⁻ reduction rates

Temperature effect over NO₃⁻ reduction rates obtained from the modeling of FTR experiments is shown in Fig. VII.6A. Experiments 1-3 and 7 were included in the analysis, covering the range of porewater temperatures found at our site (Chapter V). Experiment 9 was

excluded from this analysis due to the significantly lower DOC content in the porewater when compared to all the other experiments. At the higher porewater velocity imposed ($> 16 \text{ cm h}^{-1}$; Table 1), NO_3^- reduction rates increased with porewater temperature ($p<0.01$). $\text{Ln}Q_{10}$ (Q_{10} ; the relative increase in a metabolic rate when temperature increases 10°C) was calculated from the slope of the relation $\text{Ln}(K_{\text{red}}/K^*_{\text{red}})$ and $(T - T^*)/10$ (Van't Hoff 1898). K^*_{red} and T^* are the reference NO_3^- reduction rate and temperature, in this case those obtained at 18.5°C (experiment 2). In a similar way, activation energy (A_e ; the change in the potential energy necessary to start a metabolic process) can be calculated from an Arrhenius plot of the same modeling results (Fig. VII.6B). Calculated Q_{10} was 3.5 ± 0.2 ($p<0.005$), whereas A_e was $0.92\pm0.19 \text{ eV}$ ($p<0.005$).

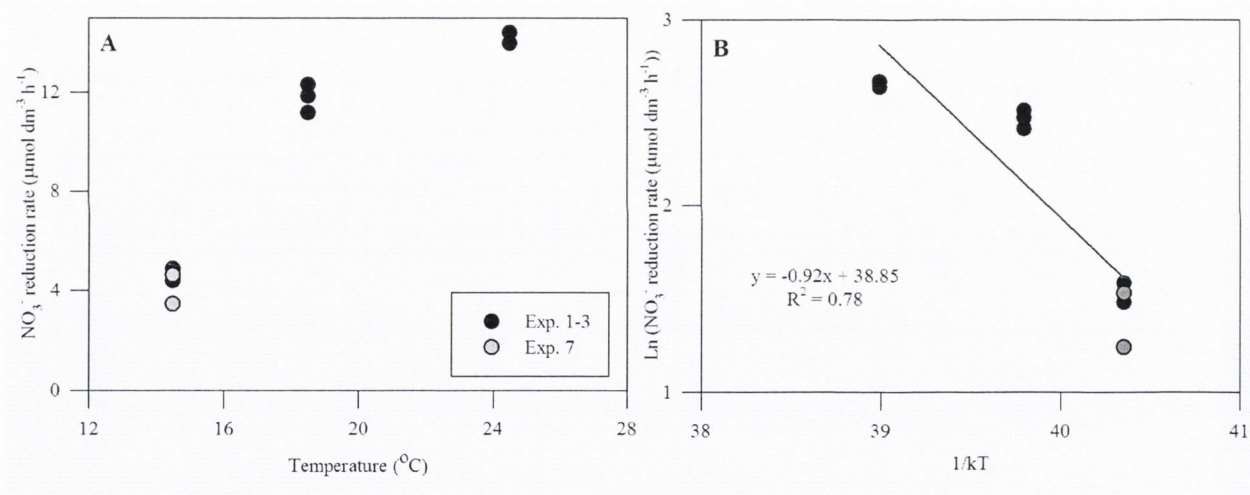


Figure VII.6 A. Sediment NO_3^- reduction rates obtained through the modeling procedure at different porewater temperatures for the selected sediment layer (2-12cm depth). The Arrhenius plot of the modeling results is also shown (B) (k is Boltzmann's constant, $8.62\times10^{-5} \text{ eV K}^{-1}$, and T is absolute temperature).

VII.5.5 Advective velocity and organic matter modulation of NO_3^- reduction

Imposed porewater advective velocity was shown to modulate the rate of NO_3^- reduction obtained through the modeling procedure of FTR experiments (Fig. VII.7A). At 14°C , the increase in porewater velocity from experiment 5 ($3.7\pm0.1 \text{ cm h}^{-1}$) to 6 ($7.3\pm0.3 \text{ cm h}^{-1}$) promoted a significant increase in the rate of NO_3^- reduction (from 4.06 ± 0.67 to 6.02 ± 0.04

$\mu\text{mol dm}^{-3} \text{ h}^{-1}$; $p < 0.05$). Further increasing advective velocity (experiments 1 and 7; $> 17 \text{ cm h}^{-1}$) did not enhance NO_3^- reduction rates when compared with the experiment at the lowest velocity.

Glucose addition to the porewater ($18.9 \pm 0.3 \text{ cm h}^{-1}$; experiment 4) stimulated NO_3^- reduction rates (Fig. VII.7A; white dots; $p < 0.05$). NO_3^- reduction rates obtained with porewater amended with glucose were significantly higher than those obtained at the lower velocity ($p = 0.05$), although not significantly different to those obtained at $7.3 \pm 0.3 \text{ cm h}^{-1}$ linear velocity.

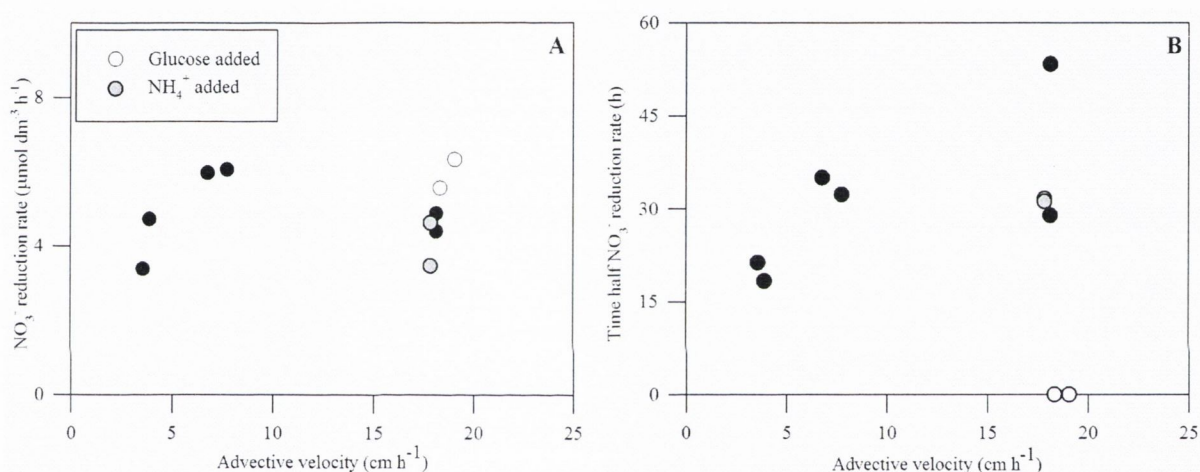


Figure VII.7 A. Sediment NO_3^- reduction rates obtained through the modeling procedure under different advective regimes for the selected sediment layer (2-12cm depth). **B.** Time during the course of each experiment where NO_3^- reduction started to dominate over inhibition processes.

The inhibition/competition term was introduced in the model to account for the observed changes in NO_3^- reduction rate with time throughout the FTR experiments. Thus, the inhibition term was introduced as an unknown property responsible for the observed change in net sediment mediation over NO_3^- fluxes, from net NO_3^- enhancement on the beginning of the experiments to net NO_3^- consumption towards the end of the experiments. Potential factors responsible for the observed change of net sediment mediation over NO_3^- fluxes in the FTR experiments performed include initial adaptation of the microbial NO_3^- reduction community to start processing NO_3^- fluxes (André et al. 2011). Other factors like initial inhibition of NO_3^- reductase activity by oxygen (Bonin and Raymond 1990; Herbert 1999; Brandes et al. 2007;

O'Connor and Hondzo 2008) or initial organic matter limitation of heterotrophic NO_3^- reduction (e.g. Bradley et al. 1992; Cornwell et al. 1999; Herbert 1999; Slomp and Van Cappellen 2004) could also be responsible for the observed patterns. Thus, the inhibition term was included in the modeling procedure as any process accounting for the observed temporal succession of dominance of benthic NO_3^- flux enhancement followed by net NO_3^- consumption. The time where NO_3^- reduction started to dominate over the inhibition processes (i.e. instant NO_3^- reduction rate equals half the maximum NO_3^- reduction rate) was used as indicator for the degree of inhibition observed at each experiment. Advective velocity was shown to affect the observed NO_3^- reduction inhibition (Fig. VII.7B). Minimal limitation of the NO_3^- reduction rate was obtained for the lowest porewater velocity ($p < 0.05$), and increased at higher velocities. Addition of glucose to the porewater at the highest advective velocity removed the observed inhibition pattern. In this experiment, the more complex kinetics model inclusive of the inhibition term was compared with a simpler model not considering inhibition. A one-tailed F test was used to test the statistical significance of the inclusion of the inhibition term on the model fit to the data (Soetaert et al. 1996; Berg et al. 1998). The more complex model accounting for inhibition did not significantly improve the fit to the measured data on a 99% confidence interval (data not shown). Thus, the experiment with glucose addition to the porewater was the only experiment not showing initial inhibition of NO_3^- reduction rates.

VII.5.6 NH_4^+ production

During the course of the FTR experiments, three individual reactors corresponding to experiments 5, 7 and 9 (Fig. VII.8) showed distinctive DIN reactivity when compared with the mediation role of the equivalent replicates (Fig. VII.4). These, anomalous individual reactors, showed the accumulation of NH_4^+ over time concomitant with the net consumption of NO_3^- . Results obtained from these individual reactors were explored in order to elucidate possible alternative NO_3^- processing within the selected sediment layer. NH_4^+ production in these reactors was postulated as being originated from the reduction of NO_3^- , with NO_2^- as an intermediary compound. Thus, the kinetics of the postulated two-step process of NO_3^- reduction to NH_4^+ was explored. The low standard error of the model fit to the experimental data confirmed the appropriateness of the mechanistic model proposed (Table VII.4). In those

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three reactors, the observed NO₃⁻ reduction over time was successfully linked to the NH₄⁺ produced through Monod kinetics, pointing to NO₂⁻ as an intermediary species.

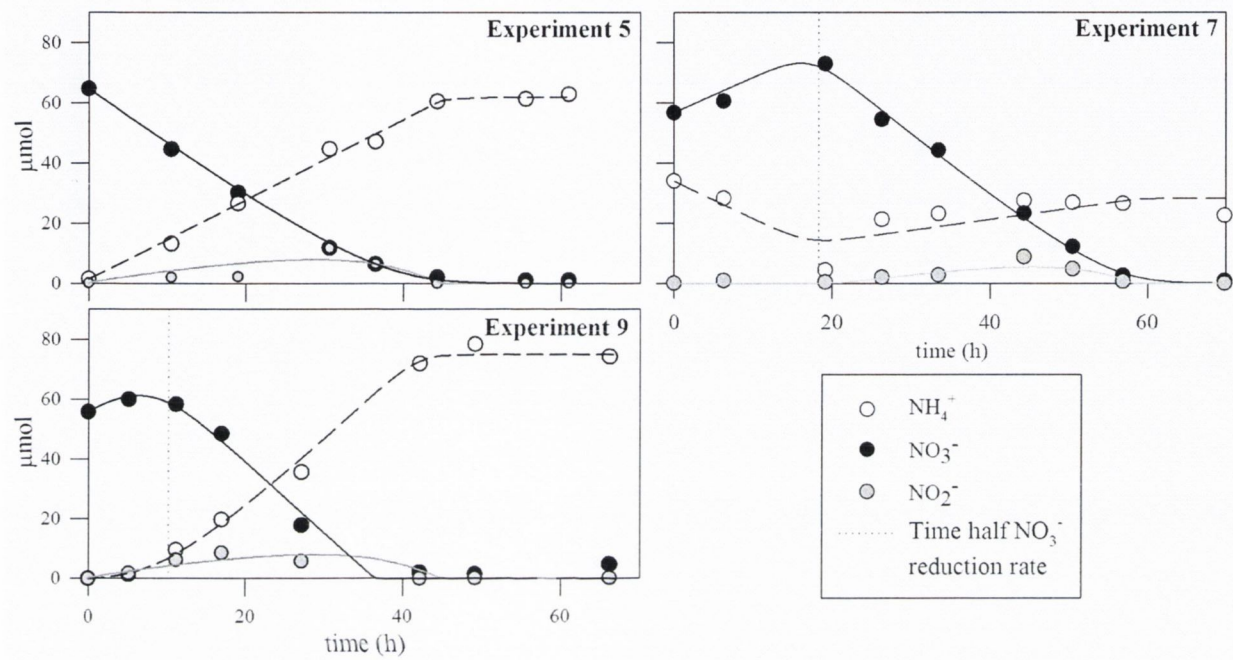


Figure VII.8 Single FTR experiments that showed production of NH₄⁺ during the course of the experiments. The modeling procedure satisfactorily linked NO₃⁻ reduction with the build-up of NH₄⁺ in the porewater reservoir, along with the identification of NO₂⁻ as intermediary compound.

Experiment	SE			NO ₃ ⁻ reduction μmol dm ⁻³ h ⁻¹	NO ₂ ⁻ reduction μmol dm ⁻³ h ⁻¹	Nitrification μmol dm ⁻³ h ⁻¹	DNRA %	Time half rate H
	NO ₃ ⁻	NO ₂ ⁻	NH ₄ ⁺					
5	0.9	2.2	1.5	5.75	4.19	-	97	-
7	2.5	1.3	4.8	6.73	5.21	3.98	22	18.3
9	2.7	1.8	2.2	7.03	8.38	5.59	112	10.2

Table VII.4 Results obtained from the Monod and zero-order reaction kinetics modeling of FTR experiments that showed NH₄⁺ production. The goodness-of-fit represented by the standard error (SE). Obtained reaction rates are shown, together with the percentage of NO₃⁻ reduction vs NH₄⁺ production and the time where the rate of NO₃⁻ reduction started to dominate over the inhibition processes.

The ratio of NO₃⁻ reduction vs NH₄⁺ production (f_{DNRA}) was high for the replicates from experiments 5 (97%) and 9 (112%), whereas in the reactor from experiment 7 was 22% (Table

VII.4). NO_3^- reduction rates identified in these anomalous three individual reactors were in all three cases higher than those found at the remaining reactors from each experiment. Furthermore, inhibition denoted by the time where NO_3^- reduction rate started to dominate over the inhibition processes was in the three cases lower than in the associated FTR replicates with DNRA presence not identified (Experiments 5, 7 and 9).

VII.6 Discussion

VII.6.1 Benthic reactivity at the seepage face

With the combination of FTR experiments run under porewater recirculation and kinetic modeling it was possible to obtain a mechanistic explanation for the reactivity at the seepage face and to extract the in-situ reaction rates. Preliminary diagenetic modeling of 20 cm depth benthic NO_3^- profiles obtained at our sampling site identified apparent nitrification and NO_3^- reduction rates ranging from 0.01 to 5.2 $\text{mmol m}^{-2} \text{h}^{-1}$ (Chapter V). Maximum net NO_3^- reduction rates obtained through kinetic modeling of FTR experiments with sediment columns from 2 to 12 cm depth were in the range of -0.23 ± 0.11 to $1.42 \pm 0.02 \text{ mmol m}^{-2} \text{h}^{-1}$, and maximum net nitrification ranged from 0 to $0.75 \pm 0.13 \text{ mmol m}^{-2} \text{h}^{-1}$ (Table VII.3; note the change in units for comparison with net reaction rates estimated in Chapter IV and V). Although NO_3^- reduction rates obtained in this study were lower than those calculated from in-situ NO_3^- vertical profiles, experimental results fit within the range framed by diagenetic modeling. Furthermore, results from FTR experiments correspond to 10cm sediment columns whereas modeled porewater profiles were sampled until 20cm depth. Unless in those profiles where limitation of NO_3^- reduction rates in depth was suggested (Chapter IV and V), the spatial extension of the net NO_3^- reduction layer in the modeled profiles was several cm higher than the 10 cm column used in the FTR experiments, thus explaining the higher net NO_3^- reduction rates estimated from the profiles. Thus, results from FTR experiments are in good accordance with model-derived reaction rates estimated for our site. NO_3^- fluxes measured with Lee-type seepage meters at the same location were also within the same order of magnitude of measured reaction rates in place (Chapter IV and V). Surficial sediment

reactivity at the seepage face can therefore significantly affect NO_3^- loading of the lagoon through SGD.

The presence of nitrification in the sediment column was demonstrated by the experiments including NH_4^+ addition to the porewater. At high seepage rates, NH_4^+ addition to the porewater stimulated nitrification rates, further suggesting that nitrification is limited by the availability of NH_4^+ at the seepage face. NH_4^+ assimilation by the microbial community was measured after acetylene blockage of nitrification (Experiment 8; e.g. Sloth et al. 1992; Herrmann et al. 2007). The obtained NH_4^+ assimilation rate was $2.01 \pm 0.26 \mu\text{mol dm}^{-2} \text{ h}^{-1}$, almost four times lower than potential nitrification measured at equivalent porewater velocity ($7.46 \pm 1.32 \mu\text{mol dm}^{-2} \text{ h}^{-1}$). Results suggest that ammonium produced by remineralization of organic matter within the sediment could be fast nitrified, depending on several factors like light, oxygen or the redox conditions (e.g. Kemp et al. 1990), thus potentially competing with microbial NH_4^+ assimilation. Furthermore, the lack of NH_4^+ accumulation in the experiments carried out without NH_4^+ addition to the porewater and the increase in NO_3^- content suggests a coupling between remineralization of organic matter and the subsequent nitrification of the NH_4^+ produced this way.

VII.6.2 Environmental parameters controlling benthic reactivity at the seepage face

VII.6.2.1 Temperature

NO_3^- reduction sensitivity to temperature was estimated as Q_{10} (3.5 ± 0.2) and A_e (0.96 ± 0.35 eV) for the range of temperature found in-situ. Despite the large number of microbial groups capable of using NO_3^- as electron acceptor, temperature sensitivity of NO_3^- reduction found here was comparable with that reported from similar ecosystems in the relevant literature. Jørgensen et al. (2009) calculated values of 1.8 and 0.43 eV for Q_{10} and A_e respectively in a Danish sandy aquifer. Also from sandy aquifer sediments, Smith et al. (1991) found much higher Q_{10} values (12.6). Rysgaard et al. (2004) reported temperature sensitivity of 2.2 (Q_{10}) and 0.54 eV (A_e) for denitrification and 2.4 (Q_{10}) and 0.65 eV (A_e) for ANAMMOX in permanently cold sediments from Greenland. Laverman et al. (2006) found

similar Q_{10} values (2.5-3.6) to those reported here from sediments from a coastal lake in The Netherlands.

The results obtained through these experiments highlighted the importance of short-term porewater temperature changes over NO_3^- reduction rates at the seepage face. Furthermore, the projected groundwater temperature increase due to climate change (IPCC 2008) could enhance the likelihood of alternative NO_3^- reduction processes like DNRA (Dong et al. 2011). In this sense, the potential increase in groundwater temperature and the aquifer thermal inertia (e.g. Banks et al. 1996) could have an important impact on the mitigation capacity of the benthic community in mediating NO_3^- -rich SGD.

VII.6.2.2 Seepage rate

In permeable sediments, porewater advection controls solute distribution, microbial reactivity and catabolic processes (Falter and Sansone 2000; Boudreau et al. 2001; Rocha 2008; Chapter VI). Porewater velocities used in the FTR experiments were representative of the local vertical advective velocity magnitudes measured during active seepage at our site (Rocha et al. 2009). Advective velocity was shown to control NO_3^- reduction rates at our site. At the lower end of the velocities imposed in our experiments, porewater movement enhanced NO_3^- reduction rates. Further increases of the velocity resulted in a decay of NO_3^- reduction rates concomitant with a temporal increase in the observed inhibition patterns. Seepage rate constrains not only the loading of NO_3^- to the sediment reactive horizons but also metabolic relevant compounds like DOM or oxygen, thus modulating benthic reactivity in mediating NO_3^- -borne SGD.

SGD seepage rate is controlled by the pressure balance between the aquifer and the surrounding coastal sea and the hydraulic conductivity of the aquifer sediment (Johannes 1980). In coastal areas with enough tidal range, tide is often identified as the main forcing factor modulating SGD seepage rate (e.g. Robinson et al. 2007). Tide was previously identified as a main controlling factor over solute distribution and SGD seepage rate at Ria Formosa coastal lagoon (Rocha et al. 2009). Thus, by shaping the advective field in the porewater, tide can control benthic reactivity in mediating NO_3^- fluxes associated to SGD.

VII.6.2.3 Organic matter

Glucose addition to the porewater (experiment 4) significantly enhanced NO_3^- reduction rates (Fig. VII.7). Results characterized NO_3^- reduction at our site as C-limited. This conclusion is further supported by results from experiment 2 and 9 (Table VII.1). Lower natural-occurring DOC content in the porewater (experiment 9) resulted in lower NO_3^- reduction rates ($p < 0.001$; Table VII.3). Results suggest that the dissolved organic matter (DOM) pool carried through and/or produced inside the subterranean estuary can modulate benthic reactivity at the seepage face.

The experiments carried out with porewater amended with glucose were the only experiments not showing limitation of NO_3^- reduction at the onset of the experiment. The results suggest that the benthic community at the selected sediment layer presented NO_3^- reduction capacity at the time of sediment collection. They further suggest that the organic matter content drives the observed inhibition patterns. Thus, lack of organic matter in the sediment and the circulating porewater could be the responsible for the observed initial absence of net NO_3^- reduction in all the remaining FTR experiments. In subterranean estuaries, tide can introduce oxygen and both dissolved and particulate organic matter with marine origin into permeable sediments that will ultimately drive the biogeochemistry of the seepage face (Santos et al. 2009). Despite the low standing stock of bulk organic matter within the sediment (Fig. VII.2), active organic matter processing by the microbial community can act as a net source of DOM within the seepage face (Chapter VI). Furthermore, DIN pollution of coastal aquifers and high discharge velocities act to catalyze the microbial breakdown of refractory organic material within the subterranean estuary seepage face. The availability of DIN within SGD increases the release of labile DOC, by enhancing the ability of extant microbes to process refractory organic matter (Chapter VI). Therefore, enhanced refractory organic matter processing driven by DIN pollution of coastal aquifers could act as a positive feedback to microbial NO_3^- reduction processes by feeding heterotrophic processes with labile organic matter produced in-situ.

VII.6.2.4 Oxygen

With the only exception of experiment 10, FTR experiments were carried out under suboxic conditions (see Table VII.1). Although anoxic nitrification and oxic denitrification have been described (Mortimer et al. 2004; Gao et al. 2009), O_2 is generally considered a main environmental parameter controlling both microbial processes. Denitrification is generally considered to be restricted to suboxic/anoxic conditions due to electron acceptor competition and enzymatic inhibition of the denitrification process by O_2 (Bonin and Raymond 1990; Herbert 1999; Brandes et al. 2007; O'Connor and Hondzo 2008). Nevertheless, according to the results of experiment 10, NO_3^- reduction under oxygen saturation of porewater is possible at the seepage face.

The development of sediment microenvironments due to high respiration rates in discrete organic-rich particles can explain simultaneous O_2 and NO_3^- reduction in the presence of oxic porewater (Jahnke 1985). In permeable sediments subject to porewater advection, media heterogeneity produces flow divergence on the particles thus establishing a differential flow field when compared with the observed macroscopic advective velocity. As a consequence, the transport of a solute in a spatially varying flow field together with molecular diffusion is what is called mechanical dispersion (Roychoudhury 2001; Bijeljic and Blunt 2006). On the micro scale, the potential for the establishment of microenvironments subject to lower or even absent water flow and the inclusion of organic matter particles could permit the development of suboxic or anoxic micro niches within the pore structure.

For the simplest case, an spherical microenvironment with an anoxic centre (i.e. organic matter particles or pore structures with limited porewater ventilation), Jørgensen (1977) found the following relationship:

$$R = (6DC/J)^{1/2} \quad (14)$$

where R is the radius of the particle/microenvironment, D is the O_2 diffusion coefficient, C is the O_2 content of the surrounding porewater and J is the O_2 consumption rate. Considering the case of the sediment studied here (respiration rates in place as measured in Chapter VIII, temperature range measured in the field for D calculation and assuming O_2 saturation in the porewater) a pore with a diameter of around 2.4 cm would be required (considering sea water

diffusivity, i.e. an sphere consisted of porewater). A large microniche of 2.5cm is unlikely to happen at the seepage face since median grain size at our site is 0.5mm. Therefore, the formation of microniches with anoxic centers will depend on other factors, like larger respiration rates or smaller internal diffusivity, as well as on the O_2 concentration in the surrounding porewater (Stockdale et al. 2009). For permeable shelf sediments, Rao et al. (2007) showed that O_2 concentration in the porewater ranging roughly from 100 to 200 μM can permit the formation of anoxic microniches explaining the simultaneous presence of oxic respiration and anoxic denitrification. Since FTR experiments were performed with sediment columns of 10 cm length, bulk oxic respiration will lower O_2 concentration inside the reactor. Thus, O_2 concentration in the upper part of the reactors will fit the concentration range proposed by Rao et al. (2007) for the formation of microniches, thus explaining the occurrence of NO_3^- reduction under oxic porewater conditions.

VII.6.3 Alternative NO_3^- reduction pathways in SGD seepage faces

Subterranean estuaries are now recognized as zones of important NO_3^- processing within the sediment prior to discharge (Nowicki et al. 1999; Ueda et al. 2003; Ullman et al. 2003; Bowen et al. 2007; Kroeger and Charette 2008; Spiteri et al. 2008; Rocha et al. 2009; Chapter IV and V). Nevertheless, the potential for alternative NO_3^- reduction pathways like ANAMMOX (Kroeger and Charette 2008) or DNRA (Rocha et al. 2009) taking place at the seepage face establishes uncertainties on the final role played by the benthic community in mitigating NO_3^- pollution through SGD. Based on mass balances over in-situ vertical porewater profiles taken at our site, Rocha et al. (2009) suggested the presence of alternative NO_3^- reduction pathways in mediating SGD. By analyzing NH_4^+ production and NO_3^- reduction rates and including stoichiometric calculations based of the organic matter composition, the authors suggested the simultaneous presence of denitrification and DNRA at the seepage face.

Three out of the 25 FTR corresponding to 10 experiments modeled for this study showed a distinctive modulation of porewater DIN fluxes. NO_3^- reduction was satisfactorily linked to NH_4^+ accumulation in the porewater reservoir through kinetic modeling, with NO_2^-

as intermediate compound. NH_4^+ production rate/ NO_3^- reduction rate characteristic of canonical denitrification ranges from 0.19 to 0.03, depending on the composition of the organic matter being remineralized (thus explaining certain NH_4^+ production concomitant with denitrification; Laverman et al. 2006). The rate of NH_4^+ production to NO_3^- reduction (f_{DNRA}) indicative of DNRA at our site has been suggested as 1.04, considering the composition of the organic matter present at the seepage face (i.e. 104%; Rocha et al. 2009). Thus, two of the anomalous replicates where NH_4^+ accumulation over time was identified fitted within this content, thus suggesting the presence of DNRA. In addition, the lack of NH_4^+ accumulation in the remaining reactors (i.e. even considering only organic matter mineralization) and the successful kinetics linkage of NH_4^+ production to NO_3^- reduction in those three reactors suggest DNRA is present in the three reactors. The anomalous reactors from experiments 5 and 9 showed the temporal dominance of NO_3^- reduction to NH_4^+ , at a rate of 97% and 112% of the NO_3^- reduced over time (Table VII.4). On the other hand, the temporal NH_4^+ production observed in the anomalous replicate from experiment 7 explained 22% of NO_3^- reduction. In this case, results suggest the coexistence of DNRA with other NO_3^- reduction processes like for example canonical denitrification.

Necessary conditions for the occurrence of DNRA and denitrification are similar in the sense that both processes need anoxic conditions and nitrate and organic matter availability. Although DNRA is a poorly understood microbial process, research has suggested that it can prevail over denitrification under highly reducing conditions and organic-rich environments (Fazzolari et al. 1998; Tomaszek and Rokosz 2007; Schmidt et al. 2011). High temperature can also favor DNRA over denitrification due to the greater standard free energy change of ammonifiers when compared to denitrifiers under limited nitrate conditions, and due to the higher affinity of ammonifiers for nitrate at low concentrations (Dong et al. 2011). Although not constrained, FTR experiments seemed to show the presence of DNRA at the SGD seepage face under high imposed discharge rates. Furthermore, results suggest that DNRA can be the dominant NO_3^- reduction process in place when present. Thus, DNRA could happen at high rates under very low organic matter availability in the sediments. The potential presence of DNRA at our site establishes uncertainties about the factors favoring the occurrence of this microbial process.

Whereas end products of denitrification and ANAMMOX are N_2O and N_2 , thus making N unavailable for most of microorganisms, the final product of DNRA is NH_4^+ . Thus, NH_4^+ production by DNRA implies the recirculation of N through the microbial community, retaining N into bioavailable forms in the porewater. NH_4^+ thus produced, together with that originated from organic matter mineralization at the seepage face can feed NH_4^+ -limited nitrification on the sediment upper levels and thus explaining the observed seasonal NO_3^- fluxes enhancement by the benthic community, ultimately reaching the receiving waters where becomes available for primary production.

VII.7 Conclusions

Benthic reactivity at a SGD seepage face was successfully explored with a combination of FTR experiments and kinetics modeling. Results validated preliminary estimations of local reactivity through diagenetic modeling of in-situ NO_3^- profiles from the seepage face. Furthermore, the modulation capacity of benthic reactivity over SGD NO_3^- fluxes was confirmed. Higher temperature, seepage rate and organic matter content (both particulate and dissolved) and lower oxygen availability in the circulating porewater were identified as main environmental parameters enhancing microbial NO_3^- reduction at the seepage face. The spatial overlap of DNRA and nitrification within the main NO_3^- reduction sediment layer was suggested and could explain the observed seasonal NO_3^- fluxes enhancement at our site (Chapter V). NO_3^- arriving to the seepage face transported by groundwater can recirculate through different, bioavailable oxidation states, which together with the NH_4^+ produced by organic matter mineralization and subsequent oxidation to NO_3^- could explain the potential for NO_3^- fluxes enhancement by the benthic community. Results raise uncertainties with regards to the ultimate function of the seepage face in mediating anthropogenic N loads to the coastal ocean through SGD.

Chapter VIII

Organic matter reactivity at a NO_3^- -rich subterranean estuary seepage face: Implications for benthic modulation of Dissolved Inorganic N loads

VIII.1 Abstract

Lack of labile organic matter in coastal aquifer sediments is often identified as being responsible for the arrival of significant oxidized N originated from land to the coastal seepage areas. At the mixing zone, cyclic infiltration of sea water into the subterranean estuary can develop a distinctive biogeochemistry due to the associated marine organic matter supply to the benthic community. A combination of Flow-Through Reactor (FTR) experiments were performed with local sediment from Ria Formosa seepage face (Portugal) in order to elucidate organic matter processing within the sediment and its influence on benthic reactivity over SGD NO_3^- fluxes. Despite the low benthic organic matter content, significant aerobic respiration rates were measured on the top 32 cm at the seepage face ($4.92 \pm 0.57 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$). Advective velocity seemed to enhance respiration rates, although under high imposed seepage rates benthic respiration is ultimately limited by organic C. Nevertheless, during active vertical seepage, local respiration rates were sufficient to develop an oxygen minimum zone near the sediment-water interface, thus favoring the occurrence of NO_3^- reduction processes. In these conditions, and during NO_3^- processing within the sediment, benthic reactivity showed to produce labile Dissolved Organic Matter (DOM). Results show the potential establishment of an internal positive feedback between benthic organic matter processing and NO_3^- reduction within the seepage face by counteracting previously identified organic matter limitation of NO_3^- reduction rates. Nevertheless, benthic enhancement of SGD N fluxes to the receiving waters is possible due to the presence of Dissimilatory Nitrate Reduction to Ammonium (DNRA) concomitant with the enhancement of organic N fluxes to the exterior.

VIII.2 Introduction

The indiscriminate use of fertilizers and other anthropogenic N sources like waste water infiltration are responsible for NO_3^- pollution of coastal aquifers registered worldwide (Foster 2000; Wakida and Lerner 2005). As a consequence, coastal aquifers hydraulically connected with the sea are now recognized as an important source of Dissolved Inorganic N (DIN) to the coastal ocean (Ullman et al. 2003; Slomp and Van Cappellen 2004; Bowen et al. 2007; Leote et al. 2008). DIN transported through Submarine Groundwater Discharge (SGD; Burnett et al. 2003) to the receiving coastal waters has been identified as being responsible for the loss of environmental quality and eutrophication processes in several ecosystems (e.g. Finkl and Krupa 2003; Hwang et al. 2005; Bowen et al. 2007; Moore 2010).

Heterotrophic denitrification is assumed to be the main biogeochemical pathway in mitigating NO_3^- content in aquifers (Rivett et al. 2008). Nevertheless, organic matter limitation of heterotrophic denitrification in the aquifer sediment is often responsible for the high NO_3^- concentrations in the groundwater arriving at the exit points (Slater and Capone 1987; DeSimone and Howes 1996). At the seepage face, cyclic seawater infiltration into the coastal aquifer can transport important biogeochemical compounds like oxygen and labile organic matter (Santos et al. 2009), which in turn can modulate benthic reactivity in mediating NO_3^- fluxes to the coastal ocean (Bonin and Raymond 1990; Herbert 1999; Slomp and Van Cappellen 2004; Brandes et al. 2007; O'Connor and Hondzo 2008). Recent research has characterized subterranean estuaries as zones of intense biogeochemical reactivity in mediating land-sea fluxes of matter and energy (e.g. Moore 1999; Ueda et al. 2003; Ullman et al. 2003; Windom and Niencheski 2003; Charette et al. 2005; Bowen et al. 2007; Spiteri et al. 2007; Kroeger and Charette 2008; Rocha et al. 2009; Florian 2012). Benthic community at SGD exit points has been identified as capable of significant modulation of DIN loads to the receiving waters (Kroeger and Charette 2008; Rocha et al. 2009; Chapter IV, V and VII). Nevertheless, the expected mitigation role of the benthic community at the seepage face is uncertain. Enhancement of SGD-derived N loads to the coastal water is possible through organic matter mineralization and alternative NO_3^- reduction pathways like Dissimilatory Nitrate Reduction to Ammonium (DNRA) at the seepage face (Chapter V and VII).

Coastal permeable sediments are now recognized as zones of fast organic matter turnover enhanced by the advective movement of porewater, thus explaining the general low organic matter content present at these benthic environments (Boudreau et al. 2001; Rocha 2008). Nevertheless, the recently recognized high organic matter processing in permeable sediments (including SGD seepage faces) seems to consume preferentially the labile fraction of the organic matter pool (Chipman et al. 2010). Recent research showed that the presence of high discharge rates and high DIN quantities in permeable SGD seepage faces can act to catalyze the microbial breakdown of refractory organic material (Chapter VI). Thus, DIN pollution of subterranean estuaries can enhance organic matter mineralization at the seepage face and promote the release of labile Dissolved Organic Matter (Chapter VI). The presence of labile organic matter within the seepage face can feed benthic heterotrophic processes including heterotrophic denitrification, thus potentially controlling benthic mediation over SGD-derived N fluxes. Nevertheless, little information is available on organic matter dynamics at SGD seepage faces and its control over benthic reactivity in mediating DIN fluxes to the coastal ocean.

Here, a series of Flow-Through Reactor (FTR) experiments were performed with undisturbed sediment cores and local porewater in order to elucidate organic matter dynamics inside the coastal aquifer sediment and its influence on benthic reactivity over DIN loads transported by SGD prior to discharge. The working hypothesis is that marine organic matter supply through sea water infiltration together with DIN-driven organic matter degradation enhancement act to favor the occurrence of microbial NO_3^- reduction processes at the seepage face.

VIII.3 Materials and methods

VIII.3.1 Study Site

Sampling site was located at the intertidal area of the inner part of Ancão peninsula (37°00'04'' N, 7°88'57'' W), one of the two sandy peninsulas surrounding Ria Formosa coastal lagoon and within the area affected by SGD (see Chapter II for general description of the area and Chapter VI for description of the selected sampling site). Undisturbed surficial

sandy sediment cores were taken at the intertidal seepage face (0.5 mm average grain size; Chapter IV), close to the peak of the discharge (Fig. VI.1).

VIII.3.2 FTR experiments

Sediment FTR experiments were run with sediment and porewater collected in-situ in order to characterize organic matter processing at the seepage face. Local porewater was collected with the aid of push-pull piezometers (MHE, USA) inserted at the seepage face (40 cm depth). Undisturbed sandy sediment cores (40cm length, 6.6 cm inner diameter) were collected with polycarbonate core liners in the lower intertidal area locus of SGD. An adapted HTH core slicer (Renberg and Hansson 2008) was used to transfer selected vertical sediment slices (from 2 to 12 cm depth) onto Teflon FTR cells (see Chapter VI and VII for in-depth description of FTR set-up). Thermostated filtered sea water was then pumped through the sediment from the bottom to the surface simulating active seepage at a constant flow rate for 4 h to re-equilibrate the system for temperature and flow rate. The porewater solution was placed into sterile containers and stored in the temperature controlled water bath for temperature equilibration of the solution. Experiments were performed in the dark in order to prevent the possible influence of the microphytobenthos community over DOM and Oxygen fluxes.

VIII.3.2.1 Benthic respiration rates

FTR experiments aiming to elucidate benthic aerobic respiration dynamics were run in open mode (discarding the outflow after sampling, i.e. plug-flow reactors; Roychoudhury et al. 2003) during October 2010 and January 2011. After equilibration of the porewater mixture and the reactor cells, experiments started by pumping the porewater solution through the reactor cells. Experiments were run for 9-24 h and the porewater solution was periodically sampled from the reservoir and the outflow stream. In order to measure benthic oxygen consumption, the outflow stream was periodically diverted to the bottom of a glass container sealed with liquid paraffin thus avoiding contact between the porewater solution and the

atmosphere. Collection of porewater in the glass containers was stopped after the recovery of enough sample volume for oxygen determination (typically 60mL).

Filtered porewater collected at the sampling site was amended with Br^- (1 mM final concentration was added) in order to evaluate the homogeneous distribution of the water flowing through the entire reactor diameter and as a tracer of the reactor's internal volume renewal. Dissolved oxygen saturation of the porewater used as input solution was ensured by constant aeration of the mixture throughout the time the experiments were performed.

VIII.3.2.1.1 Flow regime in benthic reactors

For an advective dominated system and applying appropriate boundary conditions, van Genuchten and Parker (1984) suggested the following truncated solution for the general one dimensional advection-dispersion differential equation describing unreactive solute transport in porous media:

$$\frac{C_{out}}{C_0} = \frac{1}{2} \left(\text{erfc} \left(\frac{L-vt}{2\sqrt{Dt}} \right) + \exp \left(\frac{Lv}{D} \right) \text{erfc} \left(\frac{L+vt}{2\sqrt{Dt}} \right) \right) \quad (1)$$

where C_{out} and C_0 are the solute concentration in the outflow and the inflow respectively, L is the length of the sediment column, v is the advective velocity, D is the dispersion coefficient and t is the time. Br^- breakthrough curves obtained from FTR experiments aiming to measure benthic respiration rates were modeled by using eq. 1 in order to test for the appropriate functioning of the reactors (i.e. radial homogeneous distribution of the flow inside the reactor). Fitting procedure was performed using the Gauss-Newton algorithm included in the package *stats* version 2.11.1 for R software (R Development Core Team 2009).

VIII.3.2.1.2 Benthic aerobic respiration measured in FTR experiments

Flow regime and inflow porewater composition was kept constant throughout the course of the FTR experiments run in open mode. After ensuring complete reactor's internal volume removal and stabilization of the outflow oxygen concentration (i.e. steady state

reached), oxygen consumption at each reactor was calculated by simple oxygen mass balance between the inflow and the outflow. For this, the following expression was used (e.g. Pallud et al. 2007):

$$R = \frac{\Delta C Q}{V} \quad (2)$$

where R is the reaction rate, ΔC is the change in the concentration of the solute between the input and the output solutions, Q is the flow rate and V the reactor's volume.

VIII.3.2.2 Sediment DOM processing associated to benthic DIN reactivity

A series of FTR experiments were performed under recirculation of porewater during October 2010 and January 2011 to elucidate DIN reactivity and associated DOM processing within the sediment. In order to explore DOM dynamics associated with NO_3^- processing at the seepage face, the main NO_3^- reduction layer previously identified at the seepage face was isolated and used in the FTR experiments. Based on preliminary diagenetic modeling of NO_3^- vertical profiles taken at the seepage face (Chapter IV; V), sediment slices corresponding to 2-12 cm depth were used (Chapter VII). Porewater collected in-situ was amended with known concentrations of NO_3^- and NH_4^+ fitting the range of concentrations previously found at our site (Leote et al. 2008; see Table VII.1). Oxygen concentration in the porewater solutions was controlled by purging the porewater mixture with helium and/or air, and the final solution (1L for each reactor) was placed into an sterile container and sealed with liquid paraffin to avoid contact with the atmosphere. Except for Experiment 10, which was run under constant oxygen saturation of the porewater flowing through the reactors, oxygen concentration at the beginning of the experiments was always lower than 20% saturation. Once the porewater solution started flowing through the reactor cells, FTR experiments were performed in open mode (discarding the outflow stream) during time enough to renew the internal volume of the reactor cell with the new solution. Subsequently, the outflow stream was redirected to the porewater reservoir thus running the experiments in recirculation mode during 50-90 h (see Fig. VII.1). Samples were collected periodically from the porewater reservoir into sterile tubes and stored at -20°C until further analysis. Sample preservation in this way ensures no

interferences with DOC/TN and EEM fluorescence determinations (Otero et al. 2007). The porewater volume inside the reservoir was kept constant by replacing the volume of sample taken by a solution similar to that from the start of each experiment.

VIII.3.3 Porewater chemical analyses

The Winkler method adapted for low volumes of sample (25 mL) was used for oxygen concentration determination in porewater solutions used in FTR experiments. Salinity, temperature and initial oxygen concentration in the porewater solutions were measured with an YSI 600 multi-parameter probe (YellowSpring Instruments). Br⁻ concentration was determined in a Dionex ICS-1500 Ion Chromatography system coupled with an AS40 Automated Sampler. Dissolved Organic Carbon (DOC) and Total Nitrogen (TN) were determined in a Vario DOC/TN Cube elemental analyzer, after acidification with a 2M HCl solution in order to remove dissolved inorganic C. Dissolved Organic Nitrogen (DON) was calculated as $DON = TN - DIN$.

VIII.3.4 EEM Fluorescence of DOM and PARAFAC analysis

Three-dimensional EEM spectra of Chromophoric DOM (CDOM) in samples taken from the porewater reservoir from the recirculation FTR experiments were measured on a Cary Eclipse Fluorescence spectrophotometer. Sample aliquots were conditioned at 20 °C prior to analysis to avoid temperature-driven spectral intensity changes. Excitation and emission bandwidths were set at 5 nm and 10 nm, respectively. Emission wavelengths for analysis ranged from 280 to 560 nm with a 2 nm interval, while excitation intensities were recorded at 5 nm intervals spanning a wavelength range of 220 to 440 nm. Due to the low porewater DOC levels, inner filter effects in our samples were assumed to be negligible. Daily recorded Milli-Q water blanks were used to eliminate water Raman scatter peaks and to normalize EEMs following the method presented by Lawaetz and Stedmon (2009). MATLAB with the DOMFluor Toolbox was used to handle first and second order Rayleigh scatter peaks and to perform the PARAFAC analysis of the samples (Stedmon and Bro 2008).

Parallel Factor (PARAFAC) analysis (Andersen and Bro 2003; Stedmon and Bro 2008) was performed in Chapter VI over a large dataset obtained through different FTR experiments carried out with sediments from the same location (1057 samples) inclusive of the results presented here (257 samples). PARAFAC modeling allows decomposing the complex three-way fluorescence signals into its tri-linear fluorescence components (fluorophores) and quantification of scores obtained for each identified fluorophore present in each sample. When successfully performed, PARAFAC analysis solution is unique (Bro 1997), and the obtained scores are directly proportional to the concentrations of each individual component (e.g. Stedmon and Bro 2008). Non-negatively constraint over PARAFAC parameters was used since spectral parameters are known to be non-negative (Stedmon and Bro 2008). The adequacy of the selected number of components and the uniqueness of the solution was verified by Split Half Analysis of residuals and loadings (Stedmon et al. 2003).

Exp.	N	Treatment	T °C	S	Velocity cm h ⁻¹	DON μmol N L ⁻¹	DOC μmol C L ⁻¹	CDOM composition (%)				
								Tryp	Tyr	Ter-humic	Mar-humic	Labile
1	2		14.5		18.2±0.0	TN~DIN	532	23.0	12.2	12.2	21.6	14.1
2	3		18.5		19.1±0.3	37	707	24.0	14.5	14.5	22.9	20.7
3	2		24.5		17.7±0.6	15	553	22.7	13.6	13.6	23.9	18.2
4	2	Glucose added	14.5		18.9±0.3	TN~DIN	>2x10 ⁴	27.4	4.9	4.9	7.8	4.7
5	2+1		14.5		3.7±0.1	TN~DIN	431	38.1	11.3	11.3	9.2	6.5
6	2		14.5		7.3±0.3	35	530	26.6	8.0	8.0	11.3	6.6
7	2+1	NH ₄ ⁺ added	14.5		18.1±0.2	66	471	26.1	13.0	13.0	18.2	10.7
8	2	NH ₄ ⁺ and Acetylene	14.5		16.7±1.8	TN~DIN	-	33.5	12.1	12.1	6.1	3.8
9	2+1		18.5		17.8±0.1	88	310	18.3	19.0	19.0	26.3	13.5
10	3	O ₂ saturation	18.5		18.7±0.4	45	430	22.4	16.4	16.4	26.4	6.1

Table VIII.1 Relevant initial experimental conditions in the FTR experiments performed under recirculation mode.

Five fluorophores were identified through PARAFAC analysis of FTR EEM fluorescence data set (Chapter VI). Identified fluorophores are likely to represent co-varying groups of molecules with similar fluorescence rather than a single compound, due to the complex nature of the chromophoric DOM (CDOM) pool in natural ecosystems. Nevertheless, analysis of their spectral characteristics and comparison with fluorophores previously

identified in the literature permitted to constraint the nature of each fluorophore (Chapter VI). Three components (C1, C2 and C5; Chapter VI) with fluorescence spectra similar to that of pure Tryptophan and Tyrosine amino acids were identified (referred herein as Tryp, Tyr and Labile components). These three components represent a labile, bioavailable DOM fraction (Hudson et al. 2007). The other two components (C3 and C4) are characterized with emission at longer wavelengths (associated with increased aromaticity or complexity in target molecules; Coble et al. 1998), characteristic of chromophoric humic substances. Clark et al. (2002) and Murphy et al. (2008) showed that C3 is a humic fraction of CDOM originated from terrestrial sources and present in fresh water and deep sea waters (Terrestrial humic-like component). C4 is a humic fluorophore specific to marine environments and produced by microbial activity (Marine humic-like component; Parlanti et al. 2000).

VIII.3.5 Statistical analysis

Two-way ANOVA was performed on the calculated DOM production/consumption rates to explore the significance of effects of porewater advection and temperature on the measured parameters. Pearson correlation coefficients and the F-Fisher test were used for linear regression, after testing for normality, homogeneity and independence of the data. Wilcoxon rank-sum test was used in order to test for the statistical significance of sediment reactivity over CDOM and the temporal differences found in each reactor.

VIII.4 Results

VIII.4.1 Flow regime in the FTR

The truncated analytical solution for the advection-dispersion equation in porous media (eq. 1) fitted Br^- breakthrough curves obtained from the FTR experiments with high correlation (Fig. VIII.1). Dispersion obtained at each reactor from the fitting procedure was always higher than calculated diffusion coefficient (Boudreau 1997). As expected, dispersion increased with increasing porewater velocity, fitting the same order of magnitude as that calculated from the power law description of dispersion suggested by Bijeljic and Blunt

(2006). The Br^- breakthrough curves did not show indications for preferential flow inside the reactors used. The goodness of the fits confirmed that the flow was radially homogeneous inside the reactors.

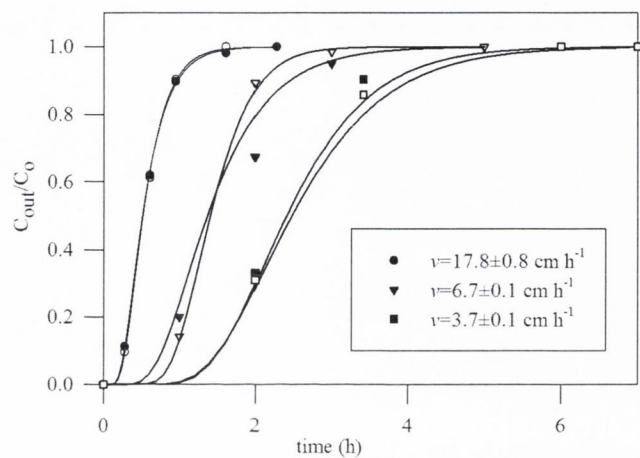


Figure VIII.1 Examples of Br^- breakthrough curves obtained at different advective regimes for the sediment layer from 2 to 12 cm depth. Lines represent best-fit solution of equation (1) to the experimental data (symbols).

VIII.4.2 Benthic respiration rates

Significant oxygen consumption by the benthic community at the seepage face sediment-water interface was confirmed under pure advective regime and in-situ temperature ($18.0 \pm 0.3 \text{ cm h}^{-1}$ linear velocity; Fig. VIII.2). Vertical zonation of aerobic respiration rates was observed. Higher respiration rates were restricted to the top 2 cm ($p < 0.01$), then decreasing with depth. Aerobic respiration rates ranged from $8.63 \pm 1.88 \text{ nmol O}_2 \text{ cm}^{-3} \text{ h}^{-1}$ in the deepest sediment slice studied (12–32 cm depth) to $53.55 \pm 7.93 \text{ nmol O}_2 \text{ cm}^{-3} \text{ h}^{-1}$ in the top 2 cm. Despite the different incubation temperature between the experiments performed during October and January, respiration rates were not significantly different between months ($p > 0.1$).

Porewater velocity effect over benthic respiration rates was explored during January 2011 at the intermediate sediment layer studied (from 2 to 12 cm depth). Advective velocity

showed to modulate respiration rates at the selected sediment horizon (Fig. VIII.3). Higher advective velocities were concomitant with higher respiration rates. Respiration rate enhancement was registered when increasing advective velocity from $3.7\pm0.1\text{ cm h}^{-1}$ to $6.7\pm0.1\text{ cm h}^{-1}$ ($p=0.07$), whereas no significant differences were found when further increasing velocity ($p>0.1$). Instead, an exponential rise to maximum function successfully explained the relationship between respiration rate and advective velocity ($R=0.99$, Fig. VIII.3).

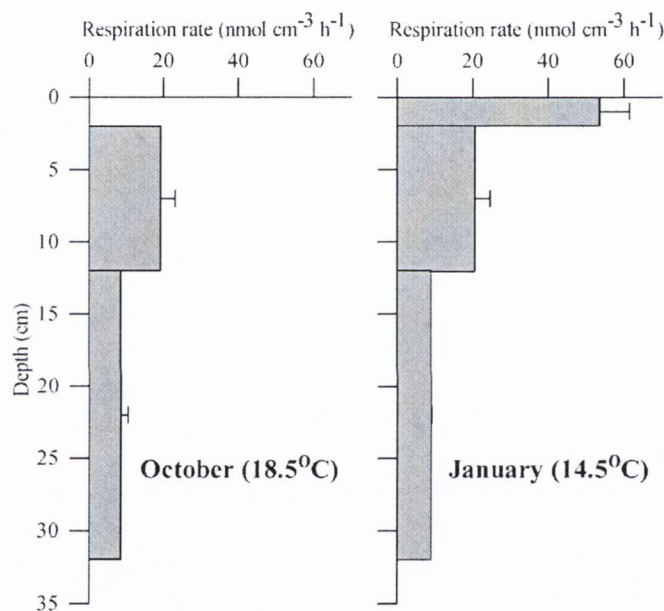


Figure VIII.2 Vertical distribution of benthic respiration rates measured in flow-through reactor experiments with intact vertical sediment slices during October and January ($n=3$) at in-situ temperatures. Results correspond to time-integrated flow-through experiments carried out with undisturbed sediment slices and porewater collected in situ. Imposed porewater velocity was $18.0\pm0.3\text{ cm h}^{-1}$ linear velocity. The experiments were run under aerobic conditions with no DIN addition to the porewater. Error bars represent SEM.

The experiment performed at the highest porewater velocity ($17.8\pm0.8\text{ cm h}^{-1}$ linear velocity) was further extended with filtered porewater collected in-situ and amended with glucose at $\approx 20\text{ mM C}$ final concentration (Fig. VIII.3 white dot). Glucose addition to the porewater significantly increased benthic respiration rates ($p<0.01$). Furthermore, respiration rates obtained after glucose addition were significantly higher than those obtained at lower

porewater velocities ($p<0.05$). Benthic respiration rates obtained after glucose addition to the circulating porewater permitted to obtain a linear relationship between benthic respiration and porewater velocity ($R=0.99$; Fig. VIII.3).

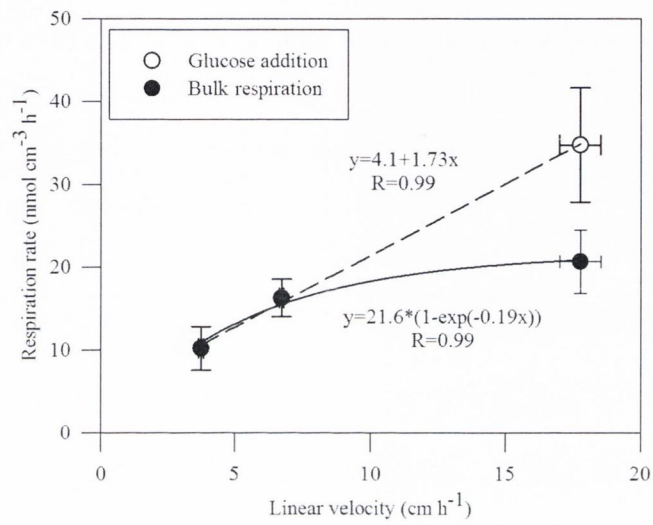


Figure VIII.3 Sediment respiration rates under different advective regimes for the selected sediment layer (2-12cm depth). White dot corresponds to the results gathered at the highest porewater velocity after extension of the experiment with glucose addition to the porewater at ~20mM C. Error bars represent SEM (n=3).

VIII.4.3 DOC and DON dynamics associated to DIN processing

A set of sediment FTR experiments with different porewater DIN amendments (Chapter VII) was performed with sediment slices taken from 2 to 12 cm depth at the seepage face. Microbial processes and kinetics of sediment reactivity over porewater DIN fluxes were explored in Chapter VII. Fig. VIII.4 shows the associated temporal evolution of sediment reactivity over DOC and DON fluxes. Rates of transformation promoted by benthic reactivity were quantified by integrating the relative change of each measured property over the experimental timeframe (see Table VIII.1 for initial conditions of the experiments). Temporal-integrated sediment reactivity in mediating porewater DOC and DON fluxes is summarized in Table VIII.2. Unless in experiment 9 and 10 (characterized by the lowest DOC content in the porewater; Table VIII.1), selected sediment layer acted as a net sink of DOC and a source of

DON. This differential sediment mediation over DOC and DON resulted in the temporal increase of the N/C molar ratio of the DOM pool.

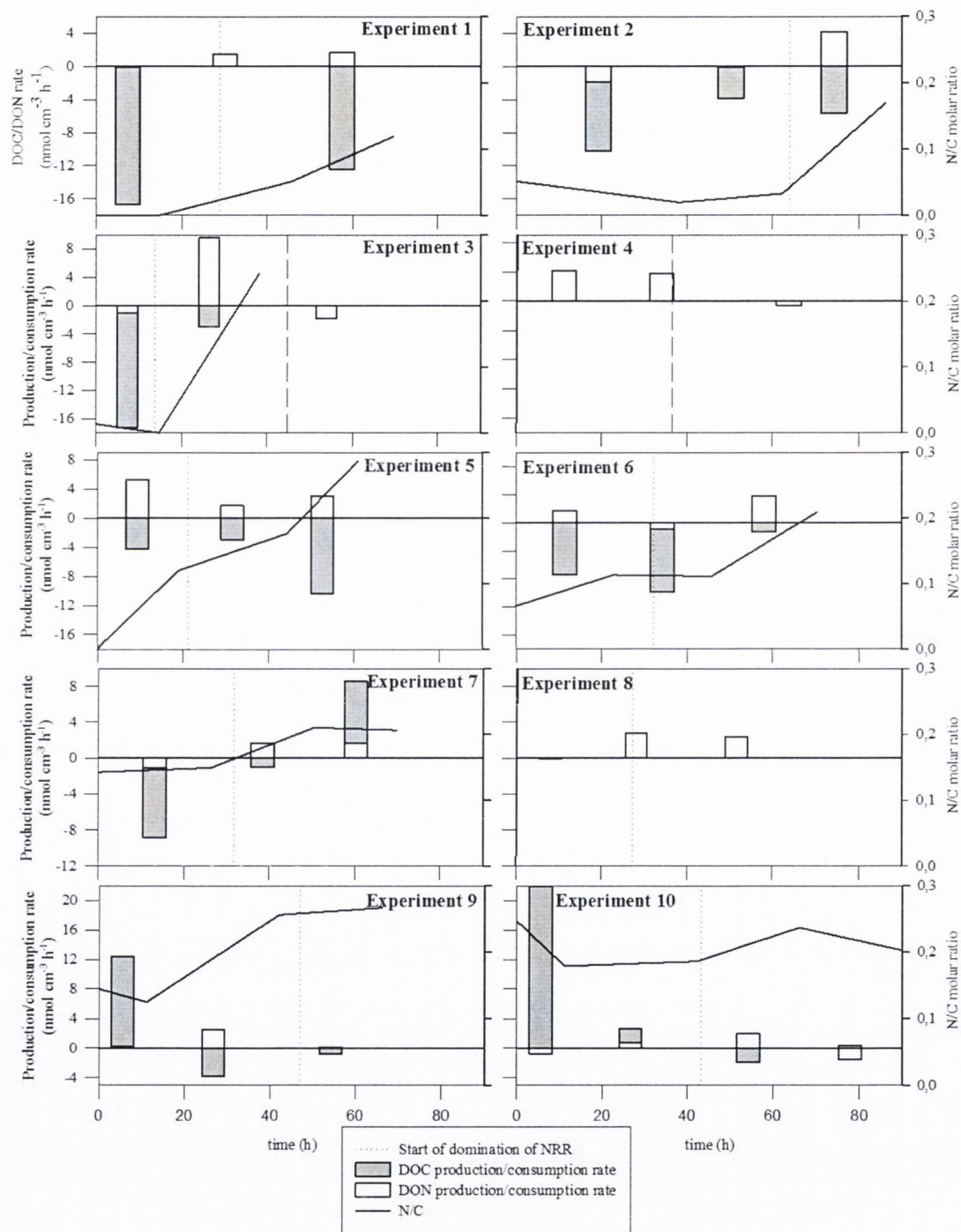


Figure VIII.4 Temporal evolution of sediment mediation over DOC and DON fluxes at FTR representative of each performed experiment. Temporal evolution of N/C composition of resulting DOM pool is also shown.

Experiment	DOC rate	DON rate	C/N ratio
	nmol cm ⁻³ h ⁻¹		
1	-6.2±1.5	0.8±0.4	13.1±7.3
2	-6.4±0.4	0.8±0.2	6.0±0.4
3	-8.0±0.5	2.2±0.0	4.5±0.4
4	-	2.2±0.5	-
5	-3.42±2.0	2.6±0.6	4.4±1.2
6	-4.9±1.1	1.8±0.2	6.3±2.2
7	-2.4±1.1	0.3±0.4	5.3±0.45
8	-	1.3±0.6	-
9	0.6±0.0	0.2±0.4	4.4±0.4
10	0.1±1.8	0.3±0.4	4.7±0.2

Table VIII.2 Sediment DOC and DON fluxes integrated over the time the experiments with porewater recirculation were run (60-90h). C/N ratio of the resulting DOM at the end of each experiment is also shown. Error values are represented by SE.

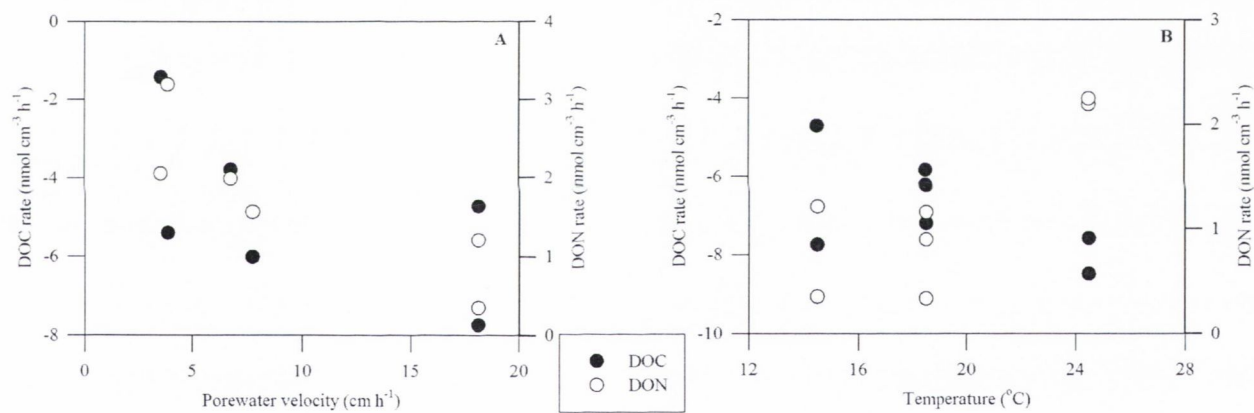


Figure VIII.5 Porewater velocity (A) and temperature (B) effect over sediment reactivity in mediating DOC and DON fluxes.

Porewater velocity and temperature showed to modulate sediment reactivity in mediating DOM fluxes at the selected sediment horizon (Fig. VIII.5). Both temperature and advective velocity seemed to influence the final C/N composition of the resulting DOM pool. Whereas the temperature and advective velocity did not promote statistically significant changes in the resulting sediment mediation over DOC ($p>0.1$), DON production rate decreased linearly with advective velocity ($p<0.05$), resulting in the concomitant decrease of

the final N/C ratio in the DOM pool. On the other hand, temperature showed to enhance DON production rates ($p < 0.05$) thus resulting in an increase in the N/C ratio of the porewater DOM.

VIII.4.4 Sediment reactivity in mediating CDOM fluxes

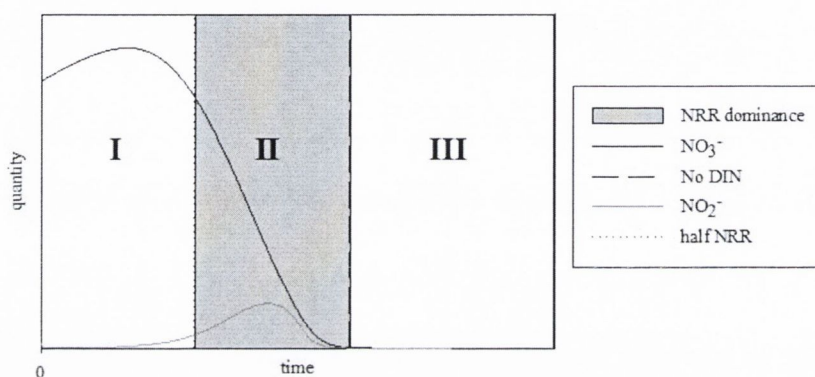


Figure VIII.6 Schematic view of the course of DIN in FTR experiments run under porewater recirculation. Three main distinctive periods were identified: I. Characterized by observed inhibition of NO_3^- reduction rates. II. Dominance of NO_3^- reduction rates (NO_3^- reduction rate $\geq \frac{1}{2}$ maximum NO_3^- reduction rate). III. Course of FTR experiments after completely depletion of DIN.

In Chapter VII, a Monod and zero-order kinetic model was constructed in order to find a mechanistic explanation for the observed sediment reactivity over DIN fluxes and to obtain relevant reaction rates in place (Chapter VII). The postulated model accounted for the observed succession of nitrification- NO_3^- reduction over time. For this, an inhibition/competition term was included in the model. The inhibition term modulates the Nitrate Reduction Rate (NRR) over time, from complete inhibition at the beginning (NRR=0) to no inhibition towards the end of each experiment (maximum NRR). The results obtained from the course of DIN at each reactor were successfully modeled and presented in Chapter VII. In order to explore the associated CDOM dynamics at the selected sediment layer, the course of each FTR was subdivided into three different periods based on the identified differential reactivity over time (Fig. VIII.6). Experiments started by a first period characterized by the dominance of inhibitory processes over NRR, i.e. instant NRR lower than

half the maximum NRR at each experiment (period I; Fig. VIII.6). After that, a second period was present where NO_3^- concentration at the porewater reservoir decreased at the highest rate, concomitant with transitional NO_2^- accumulation (period II). Finally, a third period was occasionally identified (experiments 3 and 4), after complete porewater DIN consumption by the benthic community was achieved (period III).

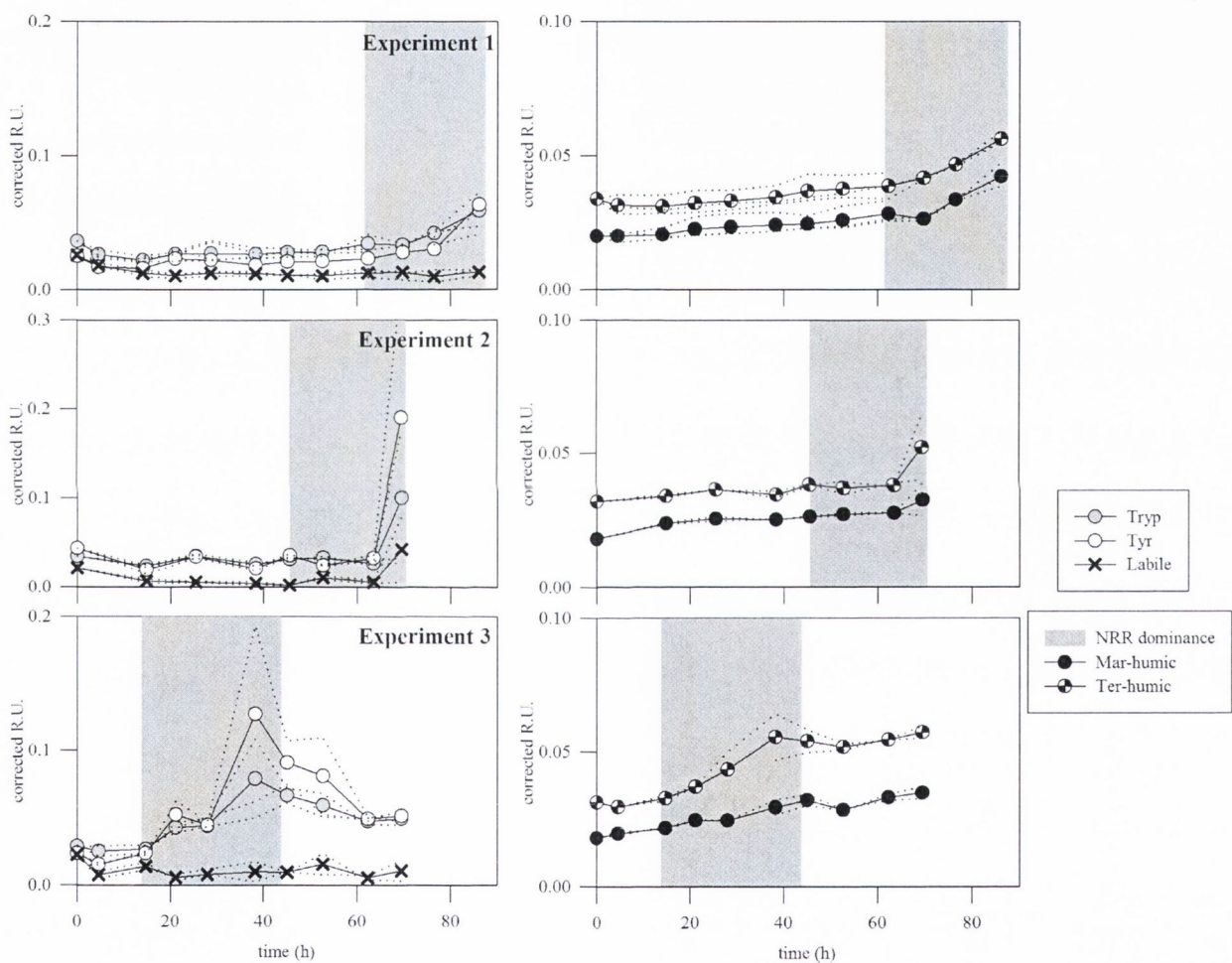


Figure VIII.7 Averaged temporal course of the five identified fluorophores in the CDOM pool through PARAFAC modeling of EEM's from the FTR experiments performed under recirculation of porewater amended with DIN. Experiments 1, 2, and 3 are shown as examples. Dotted lines correspond with each individual replicate. Identification of the averaged NO_3^- reduction area (period II) is also shown in dark background.

The course of the five fluorophores identified in the CDOM pool through PARAFAC modeling of the EEM's data corresponding to experiments 1-3 is shown in Fig. VIII.7 as example. Since the scores of the fluorophores are proportional to their concentration (Stedmon and Bro 2008), these were corrected by the porewater volume inside each reservoir, thus making them comparable among experiments. The high resolution of EEM's measurements permitted to explore the temporal sediment mediation over the five CDOM components. Differential sediment reactivity over the five fluorophores over time was identified. The course of the experiments was subdivided into the three different periods based on sediment reactivity over DIN fluxes, as discussed before (e.g. Fig. VIII.7). Benthic rates of transformation were calculated by integrating the relative change of each measured property over the experimental timeframe, and expressed as percentage of change in respect to porewater composition at the start of each experiment per $\text{dm}^{-3} \text{h}^{-1}$.

Sediment reactivity over CDOM fluxes at period I and II are compared in Fig. VIII.8A-C. Benthic reactivity at the selected sediment depth modulated all five CDOM flux components. During the first stages of the FTR experiments run with recirculation of porewater amended with DIN (i.e. during dominance of inhibition over NRR, period I), and despite the different experimental conditions imposed over the benthic community (see Table VIII.1), sediment acted as a net sink of two labile components of the CDOM pool, although the differences found for Tryp-like component were not statistically significant (Tyr-like and Labile: $p < 0.001$; Tryp-like: $p = 0.0616$; Fig. VIII.8A; B). Furthermore, sediment reactivity significantly enhanced CDOM Marine humic-like fluxes ($p < 0.05$). On average, benthic reactivity further acted as a source of the terrestrial humic-like component, although with low statistical significance ($p = 0.0570$). During the period of dominance of NRR (period II), a shift in sediment mediation over CDOM fluxes was registered. Benthic reactivity acted as a source of both CDOM humic-like components ($p < 0.01$; Fig. VIII.8C). Furthermore, during NRR dominance over inhibition processes (period II), sediment reactivity significantly increased all three CDOM labile components production rates when compared with period I ($p < 0.01$), becoming a net source to the circulating porewater ($p < 0.01$; Fig. VIII.8A, B). Finally, after complete consumption of porewater DIN (period III), another shift in sediment reactivity over CDOM fluxes was observed (Fig. VIII.8D). Here, all replicates acted as a sink of the two main

labile components (Tryp and Tyr-like), whereas humic-like component content in the porewater did not suffer significant changes ($p>0.05$).

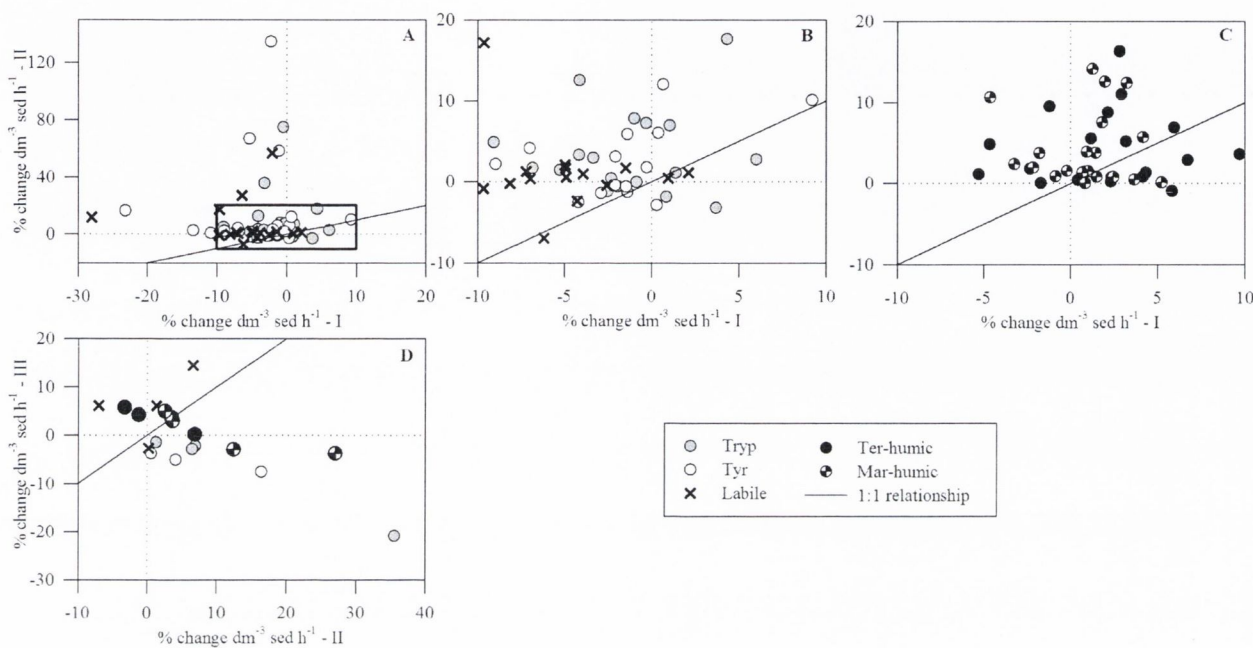


Figure VIII.8 Calculated sediment reactivity over the five CDOM components at each of the three distinctive periods regarding DIN processing at each FRT. A. Sediment reactive rates over the three labile components of the CDOM pool at period I and II. B. Detail at smaller scale of the area where most of the values were registered. C. Sediment reactivity over humic fluorophores at period I and II. D. Sediment reactivity over all fluorophores at period II and III (experiments 3 and 4).

VIII.4.5 DOM processing associated to Dissimilatory Nitrate Reduction to Ammonium (DNRA)

Three FTR replicates from experiments 5, 7 and 9 showed the presence of DNRA in processing NO_3^- porewater fluxes (Chapter VII). The specific reactivity of those three replicates in mediating DOM fluxes was explored (Fig. VIII.9). Benthic community acted as a net source of DON, whereas DOC processing showed to be highly variable. DON consumption was only registered at the beginning of the replicates from experiment 7 and 9, concomitant with the period of time where dominance of inhibition processes over NRR happened (Fig. VIII.9). Consequently, N/C molar ratio of the DOM pool generally increased with time, on a similar fashion than the remaining FTR experiments.

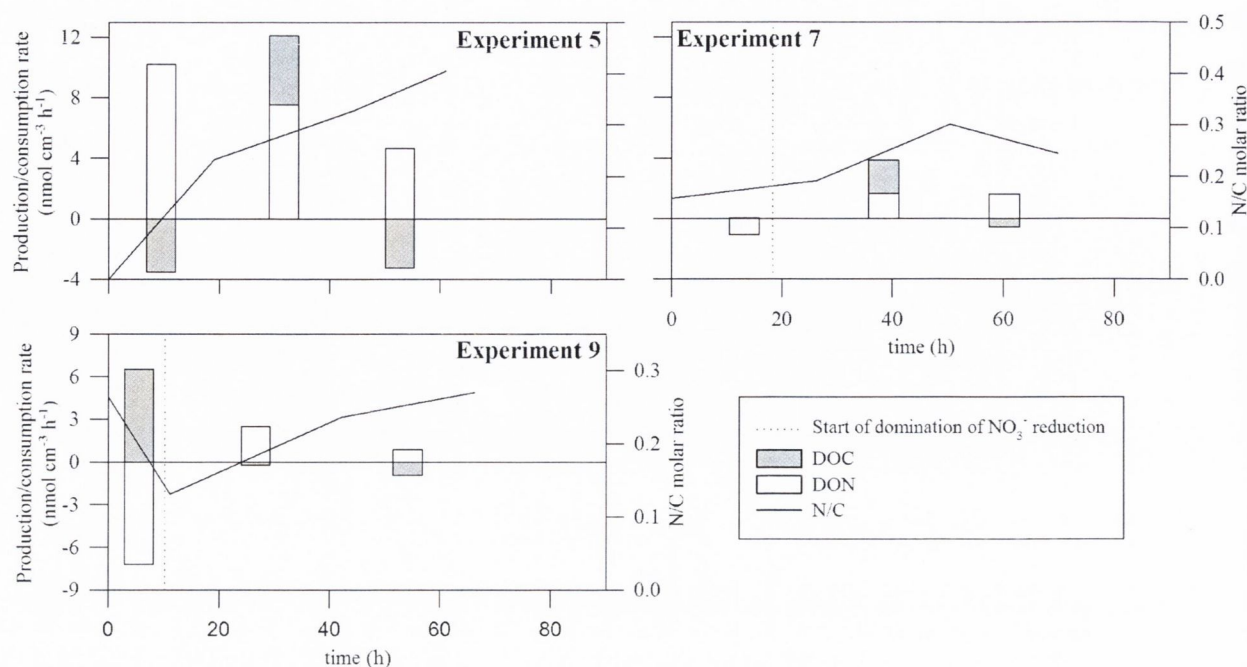


Figure VIII.9 Temporal evolution of sediment mediation over DOC and DON fluxes at individual FTR where the presence of DNRA in processing porewater NO_3^- fluxes was identified. Temporal evolution of N/C ratio of the DOM pool is also shown.

Sediment reactivity over CDOM fluorophores at the FTR with the presence of DNRA revealed to be similar to the the remaining experiments performed (Fig. VIII.10). During the dominance of NRR (period II) CDOM fluxes enhancement was registered ($p<0.01$), with the exception of the “Labile” component. Furthermore, registered sediment enhancement of CDOM fluxes in the reactors with the presence of DNRA was always higher than that measured in the remaining reactors from experiments 5, 7 and 9 ($p<0.01$).

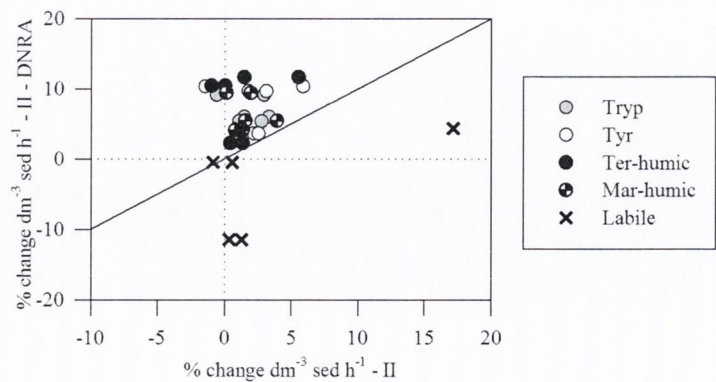


Figure VIII.10 Comparison of sediment mediation of CDOM fluxes in FTR with identified presence of DNRA with equivalent replicates from each experiment.

VIII.5 Discussion

VIII.5.1 Aerobic organic matter mineralization at the seepage face

The assumption that important benthic standing stocks of organic matter are necessary for active benthic biogeochemical reactivity has neglected the role of permeable sediments in modulating matter and energy transfers between land and sea until the last two decades (e.g. Boudreau et al. 2001; Rocha 2008). Despite the low particulate organic matter content within the sediment (Chapter VII), significant oxygen consumption due to aerobic heterotrophic processes was verified at the seepage face. Vertical distribution of aerobic respiration rates followed that of particulate organic matter at the seepage face, with maximum rates at the sediment-water interface concomitant with higher Particulate Organic Matter (POM) content (Chapter VII). Despite the different temperature imposed in the respiration experiments between October and January, respiration rates found at both months were not significantly different. This contrasts with the high temperature sensitivity found for NRR at the same site ($Q_{10}=3.5$; Chapter VII). Nevertheless, input porewater solutions used for the respiration measurements presented here were characterized by significantly lower DOC content (270 ± 40 μM DOC) when compared to the experiments run for NRR determination (>400 μM DOC; Table VIII.1). Furthermore, glucose addition to the circulating porewater almost doubled respiration rates during the experiments run in January. Thus, results suggest that, as in the case for NRR, DOM transported by the porewater could promote important limitations over respiration rates. Thus, the low DOC levels of the porewater used as input solutions in the respiration measurements could explain the apparent temperature unsensitivity of respiration at our site. Therefore, DOC can largely constrain heterotrophic reaction rates at the seepage face. At the imposed conditions (DOC concentration, advective velocity), and despite the potential for important organic C limitation of the heterotrophic community, integrated respiration rates for the studied 32 cm sediment column were 4.92 ± 0.57 $\text{mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ (January). Measured aerobic respiration rates were in the same order of magnitude as those measured with microsensors and sediment incubations in coarse sandy sediment ($4.38\text{--}7.29$ $\text{mmol m}^{-2} \text{ h}^{-1}$, Beer et al. 2005), or those measured with benthic chambers in different permeable sediments ($0.33\text{--}1.47$ $\text{mmol m}^{-2} \text{ h}^{-1}$; Janssen et al. 2005), both of which occurred in the North Sea.

Advective velocity is a major modulator of benthic biogeochemistry in permeable sediments (e.g. Shum and Sundby 1996; Huettel et al. 1998; Falter and Sansone 2000; Janssen et al. 2005; Rocha 2008). Advective velocity was shown to enhance DOC production rates (Chapter VI), NRR and inhibitory effects over NRR (Chapter VII) at the studied site. Results presented here demonstrated that advective velocity (seepage rate) enhances aerobic respiration rates at the seepage face (Fig. VIII.4). Aerobic respiration rates increased non-linearly with advective velocity. Nevertheless, glucose addition to the porewater significantly enhanced aerobic respiration rates at the highest advective velocity imposed ($17.8 \pm 0.8 \text{ cm h}^{-1}$ linear velocity; Fig. VIII.3). A significant linear relationship between aerobic respiration rates and advective velocity was then found after glucose addition to the experiment run at the highest porewater velocity (Fig. VIII.3). Those findings together with the identified vertical distribution of aerobic respiration rates suggest that, at the imposed seepage rate, aerobic heterotrophic processes at the seepage face are ultimately constrained by organic matter availability.

Allochthonous organic matter sources to the seepage face are that transported by the cyclic sea water infiltration into the aquifer body, mainly restricted to high tide, and DOM transported through the coastal aquifer sediment from land. Due to the expected refractory nature of DOM from the aquifer (Artinger et al. 2000), sea water infiltration is expected to be the main source of allochthonous fresh organic matter to the sediment (Santos et al. 2009), apart from contributing to the humic fraction observed at the seepage face. Low standing stocks of particulate organic matter within the sediment (Chapter VII) together with measured aerobic respiration rates suggest a fast turnover of organic matter within the seepage face. Hence, heterotrophic metabolism at the seepage face may be dependent on the organic matter transported from the adjacent coastal sea. Thus, benthic biogeochemistry at the seepage face is linked to primary production in the surrounding waters.

Despite the general low organic matter content, coastal aquifers and seepage faces are zones of active biogeochemical reactivity in mediating solute transport to the coastal sea (Slomp and Van Cappellen 2004; Moore 2010). In these conditions, modulation of SGD-derived anthropogenic nitrogen loads to the receiving waters by the benthic community has been demonstrated (Kroeger and Charette 2008; Rocha et al. 2009; Chapter IV, V and VII).

Although aerobic denitrification has been reported in marine sediments (Gao et al. 2009), it is generally accepted that oxygen concentration controls denitrification by competing with NO_3^- as electron acceptor in microbial catabolism and through oxygen inhibition of certain enzymes involved in the denitrification process (Bonin and Raymond 1990; Herbert 1999; Brandes et al. 2007; O'Connor and Hondzo 2008). Oxygen concentration in the coastal aquifer can be highly variable and will depend on the oxygen levels in the coastal aquifer end-members (sea water and groundwater), the mixing ratio of both water masses and oxygen consumption by the benthic community. Porewater collected in-situ at the seepage face and used as input solution in the FTR experiments was characterized by salinities near to those found in the lagoon's water (Table VIII.1). Although salinity is not a good mixing tracer between sea water and groundwater in areas subject to potentially important evaporation processes at the beach face (thus potentially changing salinity in the porewater), porewater characterization suggests that it is largely composed by recirculated sea water. Thus, oxygen concentration inside the seepage face is likely to be relatively high (by assuming oxygen saturation in the lagoon's water). An oxygen saturated porewater solution seeping out of the sediment at similar conditions as those imposed in the respiration experiments (14.5°C , $\sim 18 \text{ cm h}^{-1}$ seepage rate) represents an oxygen flux of $14 \text{ mmol m}^{-2} \text{ h}^{-1}$. Furthermore, oxygen consumption during seawater infiltration and inside the aquifer sediment are likely to decrease oxygen content in the porewater prior to arrival to the studied sediment-water interface. Thus, measured oxygen consumption at the sediment-water interface may significantly decrease oxygen concentration of the water seeping out of the sediment and could develop an oxygen minimum zone near the sediment surface. Furthermore, the heterogeneous movement of the porewater flowing through a porous media, which macroscopic effect is the mechanical dispersion of solutes (Roychoudhury 2001; Bijeljic and Blunt 2006), can help in increasing the spatial extent of suboxic conditions due to the development of anoxic microenvironments (Jahnke 1985). The potential for the establishment of an oxygen minimum zone near the sediment-water interface during active seepage along with the transient accumulation of marine organic matter at the first cm depth in the seepage face establishes the conditions for the occurrence of significant NRR prior to porewater discharge.

VIII.5.2 DOM dynamics during DIN processing at the seepage face

Organic matter content is a major constrain for heterotrophic denitrification in aquifers (Bradley et al. 1992; Rivett et al. 2008), marine sediments (Cornwell et al. 1999; Herbert 1999) and SGD seepage faces (Slomp and Van Cappellen 2004). The usually low POM content in aquifer sediments (Hartog et al. 2004) and the common refractory nature of the transported DOM (Artinger et al. 2000) can explain the high concentrations of oxidized nitrogen arriving at SGD seepage areas (Slomp and Van Cappellen 2004; Kroeger and Charette 2008; Rivett et al. 2008). Near the sediment-water interface, where marine POM can accumulate due to cyclic sea water infiltration, benthic organic matter content was shown to modulate NRR at our site (Chapter VII). Sediment FTR experiments performed under porewater recirculation showed significant sediment modulation over porewater DOC and DON fluxes concomitant with benthic reactivity over DIN fluxes. Although accumulation of metabolites in the porewater reservoir could promote changes in sediment reactivity (Roychoudhury et al. 2003; Pallud et al. 2007), results are in good agreement with those obtained in Chapter VI with plug-flow reactors (FTR without porewater recirculation; Roychoudhury et al. 2003). Sediment acted as a sink of DOC when porewater DOC levels were sufficiently high, whereas at lower DOC concentration (experiment 9) benthic community acted as a net source of DOC, concomitant with the lowest NRR observed (Chapter VII).

Sediment reactivity acted differentially on DOC and DON fluxes, resulting in an increase in the apparent lability of the DOM pool (decrease of the DOM C/N ratio). Lower seepage rates and higher temperatures seemed to enhance the tendency towards increasing DOM lability registered in all recirculation FTR experiments performed with porewater amended with DIN (Fig. VIII.5). N pollution is known to enhance microbial mineralization of highly refractory organic matter in certain natural ecosystems (Mack et al. 2004; Bragazza et al. 2006). In coastal aquifer seepage faces, the availability of significant DIN concentrations in the porewater along with high seepage rates may enhance the ability of the benthic community to process refractory organic matter (Chapter VI). As a consequence, microbial breakdown of refractory organic matter increases the lability of the released DOM. Results from FTR experiments run under recirculation of porewater amended with DIN further

support these findings. Since heterotrophic denitrification is known to be constrained by the composition of the organic matter pool (e.g. Her and Huang 1995; Sobieszuk and Szewczyk 2006; Yang et al. 2012), increasing DOM lability promoted by benthic reactivity can act as internal feedback over NRR.

Analysis of the temporal evolution of the five identified fluorophores in the local CDOM pool permitted to further constrain sediment mediation over porewater DOM concomitant with DIN reactivity. Throughout the time the porewater reservoir contained significant amounts of DIN (periods I and II), sediment reactivity generally acted as a net source of the humic components. Despite the different treatments imposed in the FTR experiments (Table VIII.1), benthic production of both humic-like fluorophores suggests the active microbial degradation of both freshly available and older, more refractory, organic matter (Chapter VI). Furthermore, after complete consumption of porewater DIN (period III), benthic reactivity did not change humic-like CDOM content in the circulating porewater. Thus, the observed net ability of the benthic community in process humic-like CDOM fluxes in the presence of DIN in the porewater ended after complete DIN consumption. These results suggest that the presence of DIN in the porewater increase the ability of the benthic community in processing refractory organic matter, thus acting in support of the hypothesis of DIN-driven organic matter processing enhancement as suggested in Chapter VI.

Benthic reactivity further modulated labile CDOM fluxes to the receiving waters. As shown in Chapter VI, sediment acted as a sink of the labile components during the initial performance of experiments with relative high DOC concentration was present ($> 400 \mu\text{M C}$), on a similar way as the sediment mediation registered after complete DIN consumption in the experiments. Nevertheless, benthic mediation over labile CDOM components shifted when concomitant NO_3^- reduction was performed at its maximum rate. Results suggest a direct linkage between the production of labile CDOM and NO_3^- reduction at our site. Identified labile CDOM components are representative of the aromatic amino acid content in natural waters (Yamashita and Tanoue 2003). Thus, results suggest the production of amino acids and/or exogenous proteins concomitant with active NO_3^- reduction by the benthic community. As a result, microbial reactivity in mediating NO_3^- -rich SGD promotes the progressive

increase in the lability of the resulting DOM fluxes, thus establishing a direct internal feedback between the production of labile DOM and NRR.

At low tide, when SGD happens at its maximum rates, porewater movement has a predominant vertical component at the seepage face (Rocha et al. 2009). Due to the mobility of DOM, seepage rate can modulate benthic heterotrophic processes at the seepage face by constraining the availability of organic matter. The increase in the ability of microbes in processing refractory organic matter due to anthropogenic N pollution of coastal aquifers and the production of labile DOM by the benthic community can develop a succession of internal feedbacks upstream. Microbial metabolic end-products can feed benthic reactivity at higher sediment horizons.

Organic matter limitation of heterotrophic NO_3^- reduction can increase the likelihood for alternative metabolic pathways in mediating NO_3^- -rich SGD fluxes (Kroeger and Charette 2008; Rocha et al. 2009). Three of the FTR replicates corresponding to experiments 5, 7 and 9, showed the presence of DNRA in processing NO_3^- loads prior to discharge (Chapter VII). Associated DOM and CDOM processing showed similar patterns to those identified in the remaining replicates. Differential sediment reactivity over DOC and DON led to the progressive increase in the N/C ratio of the circulating DOM, whereas production of labile CDOM concomitant with active NRR were observed. Furthermore, the presence of DNRA was shown to increase the N/C ratio of the resulting DOM pool, thus enhancing the tendency towards production of labile organic matter within the sediment. As a result, SGD N fluxes enhancement can be possible due to the NH_4^+ production by DNRA and the concomitant organic N production identified.

VIII.6 Conclusions

Organic matter reactivity at a SGD seepage face was explored with a combination of FTR experiments in order to elucidate its influence over NO_3^- processing within the seepage face. Aerobic respiration rates enhancement by advective velocity was confirmed, although respiration is ultimately limited by organic C availability. Despite the low benthic organic matter content, local respiration rates can develop an oxygen minimum zone within the top cm

Organic matter reactivity at a subterranean estuary seepage face

at the seepage face during active, vertical SGD seepage, thus favoring the occurrence of NO_3^- reduction. During DIN processing within the sediment, benthic reactivity enhanced the degree of lability of the DOM. Results show the establishment of an internal feedback between the organic matter processing and reactivity over DIN within the seepage face by counteracting previously identified organic matter limitation of NRR. Nevertheless, the occurrence of DNRA at the seepage face together with the identified increase of the lability of concurrent DOM demonstrates that the seepage face can enhance SGD N loads to the receiving waters.

Chapter IX

Final considerations

IX.1 General discussion

IX.1.1 Technical concerns

Methodological approaches for the study of sandy sediment biogeochemistry need to account for the intrinsic permeability of the deposits, which in turn determines porewater solute distribution and associated fluxes (e.g. Huettel et al. 1998; Falter and Sansone 2000; Rapaglia and Bokuniewicz 2009). The combination of field sampling techniques and experiments in controlled conditions implemented in this study of N biogeochemistry at the Submarine Groundwater Discharge (SGD) seepage face were selected following this constrain. The sampling techniques employed, enabled the acquisition of in-situ porewater NO_3^- distribution concomitant with seepage rate determination without significant interference with prevalent flow paths (Leote et al. 2008; Rocha et al. 2009; Chapters III-V). Furthermore, Flow-Through Reactor (FTR) experiments permitted the reproduction of advective-dominated porewater flow under controlled conditions. The combination of FTR with the collection of local porewater and the use of an adapted HTH corer (Renberg and Hansson 2008) for undisturbed sediment core slicing provided conditions necessary to perform experiments close to in-situ conditions (Chapters VI-VIII). Finally, deterministic modeling techniques were employed for the description of benthic reactivity and flow transport (Chapters IV, V and VII).

Appropriate tests were performed over the techniques used here in order to ensure the quality and reliability of the data thus obtained. For instance, seepage rate measurements used as input parameter in the diagenetic model developed in Chapter IV were confirmed by two independent estimations, Lee-type seepage meters and piezometry (Rocha et al. 2009). The appropriate functioning of sediment reactors constructed based on the design of Roychoudhury et al. (1998) and Pallud et al. (2007) (i.e. radial flow distribution of the porewater flow, no preferential flow paths) was confirmed by fitting measured Br^- breakthrough curves to the truncated solution of the 1-D unreactive advection-dispersion equation found by van Genuchten and Parker (1984) (Chapter VIII). Nevertheless, the performed tests over the implemented methodology identified previously not described NH_4^+ adsorption onto Rhizon Soil Moisture Sampler (SMS) membrane surface (Chapter III). Potential influence of the adsorptive characteristics of the poly-ether-sulfone membranes on sample composition was demonstrated. Therefore, in order to ensure that samples taken with

Final considerations

Rhizon SMS were representative of in-situ porewater composition, NH_4^+ adsorption onto Rhizon SMS was quantified and constrained. Results showed that NH_4^+ adsorption onto the sampler's surface was dependent on porewater ionic strength and temperature. Changes in these properties promoted a change in the NH_4^+ partition coefficient between the sampler's surface and the surrounding waters, thus potentially influencing filtrate composition. Since SGD seepage faces are subject to cyclic temperature and salinity oscillations induced by the pressure balance between the tide and the coastal aquifer, NH_4^+ adsorption characteristics of Rhizon SMS could influence the composition of the filtrate sampled in these systems. Nevertheless, NH_4^+ adsorption onto Rhizon SMS surface was shown to be fast and restricted to the first mL of sample taken. Thus, improved cleaning procedures and operational protocols were suggested in order to minimize the influence of Rhizon SMS' NH_4^+ sorption over sample composition.

IX.1.2 Inorganic N processing at the seepage face

SGD-derived NO_3^- loads to the receiving waters can be significantly modulated by the seepage face benthic reactivity. A diagenetic model was successfully developed for the description of in-situ vertical NO_3^- porewater profiles taken at the seepage face, in order to discern in between transport and reaction rates effect over observed vertical NO_3^- porewater gradients (Chapter IV). After ensuring the appropriateness of the assumptions made, the model successfully reproduced NO_3^- profiles obtained in-situ. Significant reaction rates (both nitrification and NO_3^- reduction) were obtained through the modeling procedure ($0.01\text{--}5.2 \text{ mmol m}^{-2} \text{ h}^{-1}$). Reaction rates were in the same order of magnitude as measured SGD-derived NO_3^- fluxes to the receiving waters, thus confirming the modulation capacity of the benthic community over SGD N fluxes (up to 70% of change in the top 20 cm at the seepage face). Vertical stratification of apparent reaction rates was postulated. Dominance of the nitrification process showed to be restricted to the top cm whereas NO_3^- reduction always occurred underneath, providing further insight of NO_3^- reduction limitation with depth observed in some porewater profiles (Chapters IV and V). Temporal analysis of benthic reactivity showed that despite the significant NO_3^- reduction rates in place, seasonal enhancement of NO_3^- fluxes to the lagoon was possible (Chapter V). The upper limit of the permanently saturated seepage

face mitigated NO_3^- loads throughout the study period. However, NO_3^- flux enhancement was registered during the warmer months (June and September) at the main seepage area, where discharge peaked. The potential presence of alternative NO_3^- reduction pathways, particularly Dissimilatory Nitrate Reduction to Ammonium (DNRA) which implies the recirculation of N through a NO_3^- reduction-nitrification internal loop, was suggested. DNRA together with organic matter mineralization within the sediment (thus acting as net DIN source to the circulating porewater) and subsequent nitrification of the NH_4^+ produced were suggested explaining the observed NO_3^- flux enhancement at the seepage face.

Modeling results were used in order to design and constrain FTR experiments. The main NO_3^- reduction sediment layer was isolated based on the identified vertical reactive zonation at the seepage face (Chapters IV and V). The range of advective velocities imposed in FTR experiments was based on measured seepage rates used as input parameters during the modeling procedure. NO_3^- and NH_4^+ concentrations used in porewater solutions were within the concentration range found at the study site (Leote et al. 2008). Thus, and by using local porewater collected at the seepage face, FTR experiments were representative of in-situ conditions. FTR experiments confirmed the magnitude of NO_3^- reduction rates at the seepage face (Chapter VII), thus validating the Advection-Dispersion-Reaction model developed in Chapter IV and confirming the modulation capacity of the seepage face in mediating NO_3^- -rich SGD. Nevertheless, coexistence of NO_3^- reducers and nitrifiers at the main NO_3^- reduction layer (from 2 to 12 cm depth) was verified. Other processes such as assimilation, NH_4^+ adsorption and organic matter mineralization, identified in the FTR experiments, contributed to the observed inorganic N processing within the seepage face.

Nitrification dominated the first hours of the FTR experiments. O_2 and NH_4^+ availability seemed to be the main parameters controlling nitrification rates. Other environmental factors like redox potential may also control nitrification rates (e.g. Kemp et al. 1990), although this parameter was not controlled in the FTR experiments performed. The lack of NH_4^+ accumulation in the porewater (apart from those experiments where the presence of Dissimilatory Nitrate Reduction to Ammonium (DNRA) was suggested) and the identified NH_4^+ limitation of nitrification rates suggest the coupling between organic matter mineralization and subsequent oxidation of the NH_4^+ produced. Assimilation by the microbial

community could compete with nitrification for the NH_4^+ produced during organic matter mineralization, although potential nitrification rates were significantly higher than measured assimilation rates.

NO_3^- reduction rates (NRR) at the seepage face seemed to be primarily controlled by environmental parameters such as temperature and advective velocity, O_2 distribution within the pore structure and organic matter availability and composition (Chapter VII). The temporal succession of nitrification- NO_3^- reduction dominance in the FTR experiments appeared to be controlled by initial inhibition of NRR. Acetylene addition to the porewater during FTR experiments, which is known to irreversibly inhibit the NH_4^+ monooxygenase enzyme in aerobic ammonia-oxidizing bacteria at concentrations higher than $4.1 \mu\text{M}$ (Berg et al. 1982), successfully banished initial NO_3^- production by the benthic community. Nonetheless, despite successful net nitrification blockage with Acetylene, NO_3^- reduction was not registered at the start of the experiments, with NO_3^- content remaining unchanged in the circulating porewater. Despite this, glucose addition to the circulating porewater effectively enhanced NRR concomitant with no initial net NRR inhibition registered. At the imposed conditions, organic matter availability and composition was suggested as the main inhibitor over NO_3^- reduction at the seepage face. Other factors like temperature and O_2 concentration showed to modulate NO_3^- reduction at the seepage face. As a microbial-mediated process, net NRR was shown to be modulated by temperature. FTR experiments permitted to characterize the influence of temperature over NRR for the range of in-situ porewater temperatures. Q_{10} values of 3.5 ± 0.2 were obtained. Furthermore, O_2 concentration in the porewater appeared to affect NRR. Nevertheless, even at porewater O_2 saturation, net NRR were measured. The development of microenvironments within the pore structure where O_2 consumption would permit the establishment of the conditions necessary for NO_3^- reduction was suggested.

IX.1.3 Kinetics of the processes involved in N processing at the seepage face

In Chapters IV and V a diagenetic model of the NO_3^- porewater distribution at the surficial seepage face sediment was developed as a tool for preliminary exploration of the apparent benthic reactivity over NO_3^- fluxes. There, net NO_3^- reduction was considered as any

process responsible for the decayment in the NO_3^- concentration in the direction of porewater flow, following first-order kinetics over porewater NO_3^- concentration. Thus, it was assumed the vertical, pseudo-stationary state distribution of NO_3^- reduction as dependant on the available NO_3^- concentration in the porewater. On the other hand, net nitrification was considered as any process responsible for the increase in NO_3^- concentration in the direction of the porewater flow, close to the sediment-water interface. In the diagenetic model, net nitrification was considered as following zeroth-order kinetics, i.e. at a rate independent to the available metabolites in the porewater. The applicability of the model was constrained, validated and implemented in Chapter V in order to explore the apparent seasonal net benthic reactivity over SGD-derived NO_3^- fluxes observed at the seepage face.

Benthic reactivity was further explored with the use of FTR (Chapter VII). A kinetic model was then constructed in order to explore the results obtained from the FTR experiments conducted with sediment slices from the main NO_3^- reduction sediment layer previously identified in Chapters IV and V at the seepage face (from 2 to 12 cm depth). Here, net NO_3^- reduction was modeled assuming it follows Monod kinetics. Net nitrification was also observed in the beginning of the FTR experiments at the same sediment layer. Here, net nitrification was generally modeled as following zeroth order kinetics, i.e., the observed increase in NO_3^- concentration in the beginning of the FTR experiments was assumed to follow a constant rate over time, thus independent of the concentration of metabolites in the circulating porewater. Nevertheless, when NH_4^+ was added to the porewater, net nitrification was modeled assuming Monod kinetics of the oxidation of NH_4^+ to NO_3^- , instead of zeroth order models (i.e. nitrification rate over time was assumed as dependant of the available NH_4^+ concentration in the circulating porewater).

Kinetics modeling of net nitrification in the FTR experiments performed in Chapter VII confirmed the coherence of the modeling approach used during the diagenetic modeling of in-situ vertical NO_3^- profiles from the seepage face (Chapters IV and V). During the FTR experiments where no NH_4^+ accumulation in the porewater was registered, results suggested that net nitrification was coupled with benthic organic matter mineralization, thus explaining the lack of NH_4^+ accumulation in the porewater (Chapter VII). Other processes like NH_4^+ assimilation by the benthic community or NH_4^+ adsorption onto the sediment particles would

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also control the amount of NH_4^+ available for nitrification. Sediment NH_4^+ adsorption isotherms indicated the low adsorptive capacity of the local sediment. Therefore, benthic organic matter mineralization and benthic NH_4^+ assimilation would be the main processes in controlling the amount of NH_4^+ available for nitrification. Only when sufficiently high NH_4^+ concentrations were added to the porewater, observed nitrification was following a kinetic rate dependant of the available NH_4^+ in the circulating porewater, concomitant with the highest apparent nitrification rates registered (Chapter VII). Results suggested that nitrification at the sampling site was NH_4^+ limited, thus dependant mainly on the NH_4^+ supply by the net balance between organic matter mineralization and benthic NH_4^+ assimilation, which approach zeroth order kinetics (note the top 2 cm of the sediment where the microphytobenthos were concentrated was excluded from the FTR experiments). Therefore, the net nitrification rate at the seepage face identified in both in-situ vertical porewater profiles and in the FTR experiments performed under controlled conditions could be controlled by the NH_4^+ supply from the microbial reactivity over organic matter.

Net NO_3^- reduction process at the seepage face was modeled using two different approaches throughout this study (Chapters IV, V and VII). Whereas in the diagenetic model (Chapters IV and V) net NO_3^- reduction was included as following first order kinetics related to NO_3^- porewater concentration, it was assumed to follow Monod kinetics during the FTR experiments. Two distinctive areas can be identified in general Monod kinetic models, depending on the solute concentration (NO_3^-) and the half-saturation constant (K_{half}) (see as example the eq. 2 in Chapter VII). Monod models approach zeroth order kinetics when $\text{NO}_3^- > K_{half}$ (as shown in eq. 13 in Chapter VII), then behaving as first-order kinetic models when the substrate concentration (NO_3^-) is lower than K_{half} (e.g. Bekins et al. 1998). K_{half} in the kinetic modeling of FTR experiments was not successfully determined in most of the experiments due to the fact that NO_3^- was still present in the circulating porewater at the end of those experiments. When determined, K_{half} was generally low for NO_3^- reduction to NO_2^- , but still within the range of concentrations found in the NO_3^- profiles modeled at the upper limit of the seepage face in Chapters IV and V and in some months in the main seepage area. Thus, in those cases, both modeling approaches may support the content that NO_3^- reduction at our sampling site behave as following first-order kinetics related to NO_3^- concentration in the porewater (i.e. at a rate dependant on the NO_3^- concentration in the porewater).

Direct comparison of both methodological approaches for kinetic exploration of NO_3^- reduction at the seepage face has some limitations. Modeling of in-situ NO_3^- vertical profiles were performed assuming that a pseudo-stationary state was reached at the seepage face, i.e. assuming that the seepage rate remained almost unchanged during the discharge peak and the vertical NO_3^- gradients were stable over time. Furthermore, FTR experiments comprised the temporal evolution of the sediment reactivity over NO_3^- fluxes from the time the porewater amended with DIN was supplied. Initial inhibition of NO_3^- reduction rates at the sediment slice used in the FTR experiments was observed, which in turn constrained reaction rate determination at high porewater NO_3^- concentration. Thus, in the FTR experiments, observed kinetics showed a more complex pattern rather than simpler zeroth order rates as expected from pure Michaelis-Menten kinetics (e.g. Bekins et al. 1998). Thus, under high porewater NO_3^- concentration (i.e. at the start of the FTR experiments), results are not directly comparable with the situation observed in the field. Results from the FTR experiments further suggest that the microbial community could need an initial adaptation to high NO_3^- loads supply (i.e. an increase in the seepage rate and NO_3^- concentration in the circulating porewater, the situation when active SGD seepage starts) for NO_3^- reduction to dominate NO_3^- processing at the seepage face.

IX.1.4 Organic matter reactivity at the seepage face

Despite the low standing stocks of organic matter within the sediment column, active heterotrophic metabolism was observed at the seepage face (Chapters VI and VIII). Significant respiration rates were measured at the top 32 cm depth in the seepage face ($4.92 \pm 0.57 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$). Furthermore, significant sediment modulation over Dissolved Organic Matter (DOM) fluxes was registered both under oxic and suboxic conditions, concomitant with important biogeochemical reactivity over Dissolved Inorganic N (DIN) fluxes (Chapters VII and VIII). Nevertheless, results showed that, under pure advective regime, heterotrophic reactivity at the seepage face is ultimately limited by organic C availability. Both aerobic respiration and NRR were significantly enhanced after glucose addition to the porewater in the FTR experiments. Results indicated the rapid turnover of the organic matter within the seepage face, thus characterizing the biogeochemistry of the seepage

face as dependent on the cyclic transport of allochthonous organic matter into the sediment through sea water infiltration.

Cyclic sea water intrusion modulated by the pressure balance between the sea and the coastal aquifer can transport important biogeochemical metabolites into the subterranean estuary, like labile organic matter and oxygen (Santos et al. 2009). Despite the fact that aerobic respiration was shown to be organic C-limited, measured rates were sufficient for the potential establishment of an oxygen minimum zone near the sediment-water interface (Chapter VIII), and thus favoring the occurrence of NO_3^- reduction close to the sediment surface.

The results presented here showed the impact of DIN pollution of coastal aquifers over the organic matter pool (Chapter VI). Along with the identified organic C limitation over benthic heterotrophic metabolism, results characterized the microbial community within the seepage face as N-limited. As a consequence, DIN pollution of coastal aquifers seemed to enhance microbial catabolism by increasing the ability of microbes to process refractory organic matter. Thus, anthropogenic DIN pollution of coastal aquifers drives C loss from the stored organic matter pool, and promotes the increase in the lability of the resulting DOM fluxes (Chapters VI and VIII). Furthermore, production of labile Chromophoric DOM (CDOM) during active benthic NO_3^- reduction was confirmed. Since NRR can be modulated by the composition of the organic matter present (e.g. Her and Huang 1995; Sobieszuk and Szewczyk 2006; Yang et al. 2012), benthic processing of organic matter can promote an internal feedback between the production of labile DOM and NRR (Chapter VIII).

IX.1.5 Advective modulation of sediment biogeochemistry

In organic-rich cohesive sediments, electron acceptor exposure and time generally modulates heterotrophic microbial metabolism rather than organic matter supply to the reactive horizons (Rocha 2008). Nevertheless, intrinsic permeability of sandy sediments permits the water movement through the pore structure. Advective velocity is a major modulator of benthic biogeochemistry in permeable sediments (e.g. Shum and Sundby 1996; Huettel et al. 1998; Falter and Sansone 2000; Janssen et al. 2005; Rocha 2008). Advection

could introduce organic matter and electron acceptors into the sediment (e.g. Huettel and Rusch 2000; Santos et al. 2009) and remove resulting metabolic end-products (e.g. Huettel et al. 1998). As a result, permeable sediments are now recognized as environments of accelerated C turnover and important associated biogeochemical reactivity (e.g. Boudreau et al. 2001; Rocha 2008).

In several SGD sites, advection (seepage) is the dominant solute transport process during active SGD (Taniguchi et al. 2002; Moore 2010). Advective solute transport was shown to be orders of magnitude higher than diffusive transport at Ria Formosa seepage face (Leote et al. 2008; Rocha et al. 2009; Chapters IV-V). Advective porewater movement (seepage rate) showed to enhance benthic reactivity at the studied site (Chapters V-VIII). DOC production rates (Chapter VI), NRR (Chapter VII) and aerobic respiration (Chapter VIII) indicated dependance on the porewater velocity at the seepage face. Furthermore, seepage rate seemed to act together with anthropogenic N pollution of the coastal aquifer as catalyzing the microbial breakdown of refractory organic matter and subsequent increase in the lability of the resulting DOM fluxes (Chapter VI and VIII). Nevertheless, together with reaction rate enhancement, advective velocity seemed to also increase the likelihood and importance of inhibition and substrate limitation over heterotrophic processes. For instance, at the highest advective velocities imposed in FTR experiments, both NRR and aerobic respiration showed to be C-limited (Chapters VII-VIII). Thus, and in contraposition to cohesive sediments where the supply of oxidants to the reactive zones is usually identified as the limiting factor for heterotrophic metabolism (e.g. Boudreau et al. 2001), enhanced heterotrophic metabolism at the seepage face becomes limited on the reductant's supply.

Advection drives the transport and retention of particulate organic matter within the seepage face pore structure. Higher particulate organic matter content was therefore consistently registered at the sediment surface (Rocha et al. 2009; Chapter VII). In depth, heterotrophic metabolism relies therefore on the DOM supply from groundwater transport and sea water infiltration. Thus, the mobility of the DOM (Zsolnay 2003) within the subterranean estuary acts together with the advective movement of the porewater in constraining sediment reactivity in depth.

IX.1.6 Vertical biogeochemical stratification at the seepage face

Organic matter distribution and dynamics at the seepage face were suggested as responsible for the establishment of the observed vertical biogeochemical zonation at the seepage face (Chapters IV-VI, VIII). Vertical gradients in heterotrophic benthic metabolism were identified. Respiration rates showed a similar distribution to particulate organic matter within the sediment, with maximum rates at the sediment surface (Chapter VIII). Furthermore, accumulation of organic matter together with enhanced oxygen consumption near the sediment surface establishes conditions for the occurrence of heterotrophic NO_3^- reduction. This hypothesis was further supported by benthic biogeochemical zonation identified through diagenetic modeling of in-situ vertical NO_3^- profiles (Chapters IV-V).

DIN pollution of coastal aquifers appeared to enhance refractory organic matter mineralization resulting in the increase in the lability of the resulting DOM fluxes (Chapter VI). Labile DOM produced in depth can feed C-limited aerobic respiration upwards thus increasing the likelihood for the development of the oxygen minimum zone near the sediment-water interface (Chapter VIII). Furthermore, labile DOM can also act as an electron donor for heterotrophic NO_3^- reduction. Therefore, during active vertical seepage at low tide (Rocha et al. 2009), labile DOM production by the benthic community could feed heterotrophic processes within the flow direction.

IX.1.7 Alternative NO_3^- reduction pathways at the seepage face

Low standing stocks of organic matter within the seepage face were deemed a possible limiting factor over heterotrophic denitrification, thus enhancing the likelihood for alternative NO_3^- reduction pathways in processing SGD-derived NO_3^- fluxes prior discharge (Bowen et al. 2007; Brandes et al. 2007; Kroeger and Charette 2008; Rocha et al. 2009). Anaerobic Ammonium Oxidation (ANAMMOX) (Kroeger and Charette 2008) and Fe-driven NO_3^- reduction (Postma et al. 1991) were previously identified in subterranean estuaries. Furthermore, the presence of Dissimilatory Nitrate Reduction to Ammonium (DNRA) was suggested by Rocha et al. (2009) at a Ria Formosa seepage face. The results presented here confirmed the presence of DNRA at the seepage face. Furthermore, FTR experiments showed

that, when present, DNRA could be the dominant NO_3^- reduction process in place (Chapter VII). Reducing conditions, high organic matter content and high NO_3^- concentration in the porewater were postulated as favoring this NO_3^- reduction process over denitrification (Fazzolari et al. 1998; Tomaszek and Rokosz 2007; Schmidt et al. 2011). Nevertheless, our results showed that although DNRA can be the dominant process under organic-poor conditions, it was uncertain which conditions were necessary for the occurrence of DNRA.

DNRA reduces NO_3^- to NH_4^+ , with NO_2^- as intermediary compound (Schmidt et al. 2011). The presence of DNRA implies the recycling N through the microbial population in bioavailable forms. NH_4^+ produced through DNRA at the seepage face could therefore feed NH_4^+ -limited nitrification in the sediment column, be assimilated by the microbial community or become available for primary production in the receiving waters.

Other NO_3^- reduction processes could potentially take place at Ria Formosa seepage face. Abiotic processes like Fe or Mn-driven NO_3^- reduction to N_2 could take place at the subterranean estuary, although these processes are dependent on the local geology (Postma et al. 1991; Zee et al. 2003; Jørgensen et al. 2009). Sulfide-driven autotrophic denitrification could also potentially happen inside the subterranean estuary (Shao et al. 2011). Due to the coupling of this process with sulfate reduction and the periodical oxygenation of the seepage face by cyclic sea water intrusion into the coastal aquifer, this process would be potentially restricted to the salinity gradient at the groundwater-brackish water front. Another autotrophic NO_3^- reduction process, ANAMMOX, could potentially happen at the seepage face. ANAMMOX microorganisms produce N_2 from the 1:1 reduction of NO_2^- concomitant with the oxidation of NH_4^+ (Dalsgaard and Thamdrup 2002). As an autotrophic process, ANAMMOX could be favored over denitrification due to the identified C-limitation of NO_3^- reduction at the seepage face (You et al. 2009). The ANAMMOX process is known to have higher sensitivity to O_2 inhibition than canonical denitrification (Strous et al. 1997; Dalsgaard et al. 2005). Nevertheless, the potential for the formation of suboxic microenvironments within the pore structure (Jahnke 1985) together with the development of an Oxygen Minimum Zone at the seepage face surficial sediment (Chapter VIII) could establish the conditions necessary for the ANAMMOX process. At the seepage face, NH_4^+ can be produced by the microbial community through organic matter mineralization and the identified presence

of DNRA. Furthermore, transient accumulation of NO_2^- during NO_3^- reduction (including DNRA) at the seepage face was also identified (Chapter VII). Thus, benthic reactivity can develop the conditions necessary for the presence of ANAMMOX at the seepage face.

IX.2 N processing at the seepage face

Two distinctive periods could be identified in terms of water and solute transport at the seepage face. These were: a) active porewater seepage out of the sediment and b) seawater infiltration into the seepage face. The cyclic oscillation of the hydraulic gradient between the coastal aquifer and the lagoon promoted by the mesotidal regime at Ria Formosa determines the succession of these two distinctive periods. Sea water infiltration into the seepage face was considered as mainly restricted to high tide periods, followed by active seepage of porewater out of the beach face, confined to the periods of low tide. Although the research conducted here mainly focused on the elucidation of the main biogeochemical processes at the seepage face during active porewater seepage and associated solute transport to the receiving waters, both periods may help in understanding the reactivity of the seepage face in mediating N loads to the lagoon. Throughout this study, sea water infiltration was assumed to be the main source of labile organic matter and oxygen to the coastal aquifer. Despite this, high NO_3^- loads were assumed to be transported by groundwater (Fig. IX.1). Mixture of both water masses into the coastal aquifer sediment develops the subterranean estuary.

NO_3^- content at the subterranean estuary was previously linked to that transported by groundwater arriving at the subterranean estuary mixing zone from the aquifer systems surrounding Ria Formosa (Leote et al 2008; Rocha et al. 2009). However, vertical distribution of total organic matter and total organic C at the seepage face surficial sediments were in support of the assumption that sea water infiltration during high tide may be the main source of allochthonous labile organic matter to the sediment. Higher total organic matter content and apparent higher lability of the organic matter pool was systematically restricted to the surficial sediment layers (Chapters IV, VI and VII). Nevertheless, other sources of organic matter could also largely contribute to the observed organic matter budget and distribution at the seepage face. The microphytobenthos and benthic macroalgae community at the seepage face could be

an important source of both particulate and dissolved organic matter to the subterranean estuary. Winter macroalgae blooms (*Enteromorpha/Ulvae* spp) had been identified in the intertidal areas of the lagoon since 1988, inclusive of the areas locus of SGD (Anibal 2004). Furthermore, phytobenthic biomass and external polymeric sacarides production could be important sources of benthic organic matter which could be buried in the sediment during sea water infiltration and by the benthic fauna (e.g. Huettel et al. 1996; Bergamasco et al. 2003; Beer et al. 2005).

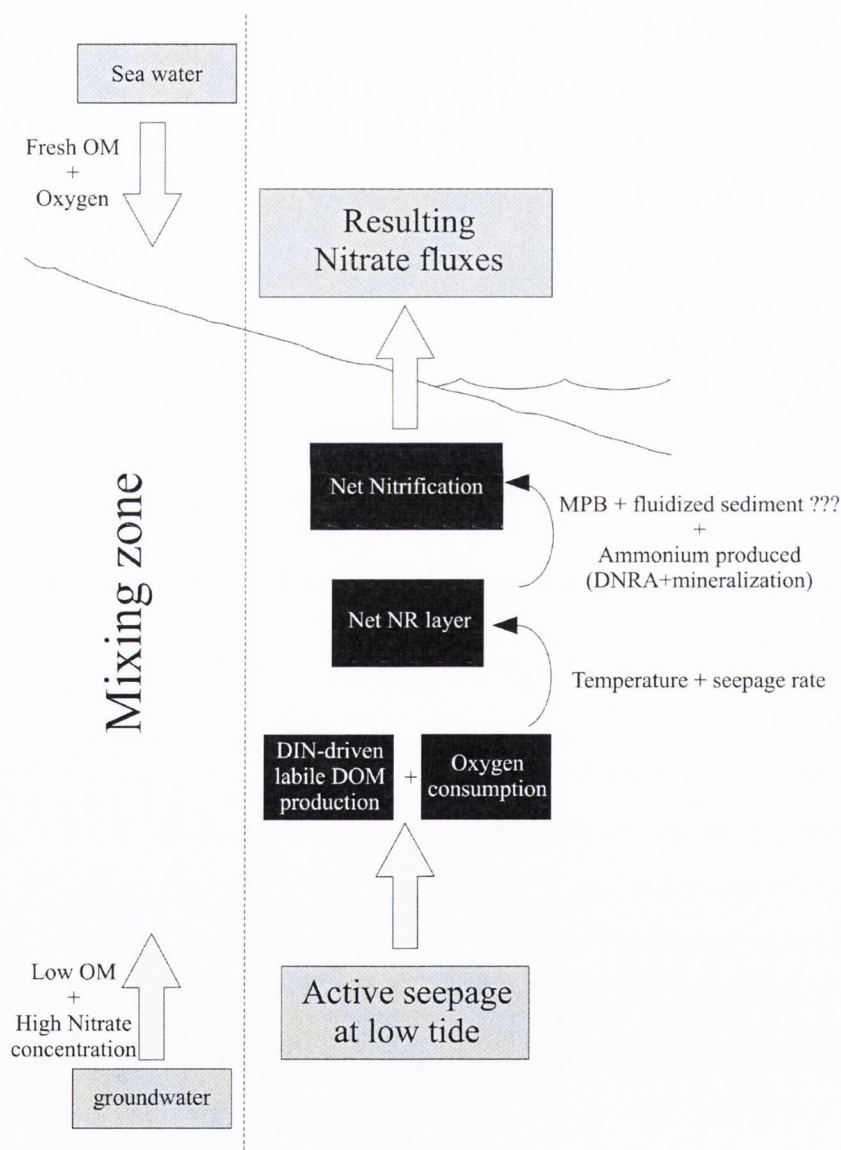


Figure IX.1 Schematic view of the conceptual model used to explain the NO_3^- processing at the seepage face. Characteristics of the two end members in the subterranean estuary are shown (left side), together with the main processes (dark boxes) and environmental parameters controlling NO_3^- processing at the seepage face.

Lack of labile organic matter within the coastal aquifer sediment (thus limiting denitrification along the groundwater flow path) was assumed to be responsible for the high NO_3^- content in the groundwater arriving at the seepage areas during the field campaigns of 2005-2007 (Chapters IV and V; Rivett et al. 2009). Thus, reductant supply and not oxidant availability might ultimately control heterotrophic metabolism at the coastal aquifer before entering into the subterranean estuary zone. Reductant supply in the form of both dissolved and particulate organic matter by sea water infiltration into the sediment could thus fuel the biogeochemistry of the subterranean estuary (Santos et al. 2009) and determine the final SGD-derived NO_3^- fluxes to the receiving waters. Organic matter supply to the subterranean estuary towards the seepage face might, therefore, be one of the main factors contributing to the significant sediment reactivity found at the first 20 cm depth surficial sediments in the seepage face (up to 70% change of SGD-derived NO_3^- fluxes).

As discussed in section IX.1.4, organic matter availability and composition might be one of the most crucial parameters in controlling the local reactivity over NO_3^- fluxes (see also Chapters VII and VIII). In fact, during active SGD, i.e. under pure vertical advective porewater transport, the heterotrophic community appeared to be largely limited by the availability of organic C. Nevertheless, other factors were identified as constraining the observed net NO_3^- reduction rates found at the seepage face (Chapter VII). Increasing advective velocity (seepage rate) and temperature seemed to enhance benthic net NO_3^- reduction rates (Chapter VII). However, seepage rate and temperature-driven net NO_3^- reduction enhancement might be ultimately controlled by the availability of the required organic matter for benthic heterotrophic metabolism (Chapter VII). O_2 concentration seemed to be also an important parameter in controlling the final benthic net reactivity over SGD NO_3^- fluxes, favoring the occurrence of NO_3^- reduction processes at lower porewater O_2 concentration (Chapters VII and VIII). Nonetheless, benthic heterotrophic O_2 respiration is also intimately linked to the availability of electron donors (organic matter) within the sediment. Thus, organic matter supply by seawater infiltration during high tide, together with that produced in-situ at the sediment surface by primary producers and transported in depth can control the likelihood and extension of net NO_3^- reduction rates at the seepage face (Fig. IX.1).

In the absence of DIN in the groundwater arriving at the seepage face, the benthic microbial metabolism seemed to be both C and N limited at the seepage face (Chapter VI). Under these circumstances, the supply of high quantities of DIN to the microbial community through the transport of polluted groundwater appeared to enhance the ability of the microbes to process refractory organic matter (Chapter VI). As a result, microbial activity over refractory organic matter can produce labile DOM which can then feed heterotrophic NO_3^- reduction processes in the direction of porewater flow. The enhancement of refractory organic matter processing within the sediment and the associated production of labile DOM in the presence of important DIN content in the porewater together with the accumulation of particulate organic matter at the top 20 cm in the seepage face could increase the likelihood for important O_2 respiration in the seepage face surficial sediment thus lowering porewater O_2 content in the direction of porewater flow. Moreover, the production of labile DOM together with O_2 consumption near the sediment-water interface could aid the development of an active sediment NO_3^- reduction layer by developing local O_2 minimum zones and counteracting organic C limitation over heterotrophic NO_3^- reduction processes (Fig. IX.1).

Other factors not constrained in the series of experiments presented here like redox conditions or the role of microphytobenthos could also be of importance in controlling the final NO_3^- flux mediation of the seepage face (Fig. IX.1; Sundbäck et al. 1991; Thornton et al. 2007; Rivett et al. 2008). Redox conditions in the porewater could control the occurrence and prevalence of NO_3^- reduction and nitrification (e.g. Kemp et al. 1990; Rivett et al. 2008). Furthermore, redox potential oscillation in the porewater had been suggested as a factor enhancing important benthic biogeochemical processes like organic matter remineralization in benthic systems (Aller 1994; Sun et al. 2002). At the seepage face, the cyclic alternacy of sea water infiltration and brackish water seepage induced by the tide suggest that these systems were likely to be subjected to important porewater redox oscillations. Thus, the potential for organic matter remineralization enhancement due to redox potential oscillation in the porewater could promote an increase in the labile DOM fluxes and thus counteract the observed organic matter limitation of the heterotrophic NO_3^- reduction processes at the seepage. Another important factor not included in this study was the potential influence of the benthic primary producers in mediating sediment-water NO_3^- fluxes at the seepage face. Although microphytobenthos and primary producers assimilate preferentially NH_4^+ for their N

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requirements, NO_3^- could also be assimilated under DIN limited conditions through the production of Nitrate reductase enzymes (e.g. Berges and Harrison 1995; Lartigue and Sherman 2005). Thus, under light conditions, benthic primary producers could potentially modulate the final SGD-derived NO_3^- fluxes to the receiving waters.

Despite the important NO_3^- reduction rates identified at the seepage face, the overall mitigation capacity of the benthic community in mediating SGD-derived NO_3^- fluxes to the receiving waters remain uncertain. The potential benthic NH_4^+ production through DNRA the enhanced organic matter mineralization at the seepage face could feed NH_4^+ -limited net nitrification rates at the sediment water interface and thus increasing NO_3^- fluxes to the receiving waters (Chapters IV, V and VII). Furthermore, the identified presence of nitrifiers within the main NO_3^- reduction sediment layer (Chapter VII) suggest that microbial community composition alone could not explain the vertical reactive zonation observed in the field, with net nitrification close to the sediment-water interface and a net NO_3^- reduction zone below that (Chapters IV and V). Photosynthesis at the sediment-water interface by the phytobenthos community could affect the oxygen and redox conditions distribution at the sediment surface (Fig. IX.1). Furthermore, as shown by the diagenetic model in Chapters IV and V, even with an upward porewater flow, photosynthesis at the sediment-water interface can affect the first cm into the sediment, potentially developing the conditions necessary for nitrification to dominate benthic DIN processing. Other factors like sediment fluidization during active vertical seepage could also help in explaining the observed reactive zonation at the seepage face (Fig. IX.1). The altered effective sediment weight at the sediment surface promoted by the porewater upflow at the beach face could have important implications for sediment transport processes (e.g. Turner and Nielsen 1997; Horn 2002). Sediment fluidization could also have important biogeochemical implications at the seepage face. For example, the development of oxygen minimum zones due to the establishment of anoxic microniches within the pore structure, as suggested in Chapter VII and VIII, would be completely vanished at the fluidized sediment zone due to the increase in the porosity of the sediment during active seepage, thus favoring nitrification over NO_3^- reduction. Nevertheless, research is needed in order to clarify the factors determining the transition from a net NO_3^- reduction sediment layer to a net nitrification layer in the direction of porewater flow at the seepage face.

IX.3 Implications for N and C biogeochemical cycling at the seepage face

The research conducted here demonstrated the active biogeochemical reactivity of the surficial permeable sediments at SGD seepage faces in mediating C, O and N fluxes between land and sea. The seepage face was characterized by the fast turnover of organic matter within the sediment, further enhanced by anthropogenic N pollution of coastal aquifers (Chapters VI and VIII). Furthermore, benthic-mediated increase of the lability of resulting DOM fluxes further accelerated organic C cycling at the subterranean estuary and the receiving waters. Nutrient regeneration from enhanced organic matter remineralization together with the increase in Dissolved Organic N (DON) fluxes and the potential presence of DNRA (thus retaining DIN into bioavailable forms) characterized the seepage face as a potential net source of bioavailable N to the receiving waters.

Surface estuaries are often considered as a net sink of atmospheric CO₂, due to organic matter accumulation in tidal marshes and export and subsequent burial in the shelf and open ocean (Cai 2011). The registered increase of organic matter fluxes to the sediments due to enhanced nutrient fluxes to estuaries could act together with sea level rise and sediment accretion to further increase the stored organic matter in estuarine marshes (Craft 2007). In the case of coastal aquifers, due to their large volume together with the ubiquitous presence of Sedimentary Organic Matter (SOM), stored organic C could be a reservoir of global importance. Nevertheless, anthropogenic N pollution of coastal aquifers enhanced microbial heterotrophic metabolism and promoted the mobilization of the stored organic C pool (Chapter VI). Thus, the introduction of anthropogenic N into coastal aquifers could therefore have an important impact on the global C cycle.

Surface and subterranean estuaries represent main transport routes of anthropogenic N between land and the coastal sea. 10% of the 150 Tg N year⁻¹ of anthropogenic N applied at land surface percolates to groundwater. Furthermore, 23% of the total reactive N introduced in the ecosystems was calculated to enter to the fluvial transport (Schlesinger 2009). Thus, surface and subterranean estuaries were major transport pathways for the calculated 48 Tg N year⁻¹ anthropogenic N fluxes to the coastal ocean (Schlesinger 2009). Aquifers and estuaries have been identified as zones of intense denitrification and therefore consumption of newly available anthropogenic N introduced to ecosystems (e.g. Slater and Capone 1987; Seitzinger

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et al. 2006; Bowen et al. 2007). Nevertheless, recent research has shown that, at least in tropical surface estuaries, DNRA and not denitrification can be the dominant NO_3^- reduction process (Dong et al. 2011), thus recirculating N into bioavailable forms and potentially enhancing ecological effects of reactive N enrichment of aquatic ecosystems (Vitousek et al. 1997; Galloway et al. 2003; Camargo et al. 2005; Camargo and Alonso 2006; Rabalais et al. 2009). Results presented here demonstrated that SGD-derived N fluxes enhancement by the seepage face reactivity was possible. Thus, these results raise uncertainties about the assumed mitigation role of sedimentary ecosystems in the context of actual revision of our understanding of the global N cycle (Brandes et al. 2007; Galloway et al. 2008; Gruber and Galloway 2008).

IX.4 Future directions

Throughout the research discussed here, evidence for the presence of alternative NO_3^- reduction pathways at the SGD seepage face was presented, and the presence of DNRA was demonstrated. Furthermore, results showed an intrinsic relationship between the benthic organic matter pool and DIN processing prior to discharge. Nevertheless, further research is needed in order to constrain the final mediation role of the seepage face in mediating NO_3^- -rich SGD.

The use of labeled N compounds can help in tracking main processes involved in NO_3^- processing within the sediment. This technique was previously used in order to elucidate the pathways involved in N processing in different aquatic systems (e.g. Lehmann et al. 2007; Mazéas et al. 2008; Rao et al. 2008). Nevertheless, the possible overlapping among the different pathways give rise to uncertainties regarding the application of this technique when based exclusively on the analysis of resulting N compounds. For instance, the isotope pairing technique was proposed for analysis of the relative contribution of heterotrophic denitrification and ANAMMOX over the observed NO_3^- consumption in natural systems (Risgaard-Petersen et al. 2003). This technique was based on the performance of different experiments with labeled NO_3^- and NH_4^+ together with the analysis of resulting N_2 produced from the two processes. Since both processes use different N compounds, the isotopic

composition of the produced N_2 gas can be related with the responsible process. Although this technique was successfully used in different environments (e.g. Rysgaard and Glud 2004; Trimmer et al. 2006; Hietanen and Kuparinen 2008; Rao et al. 2008; Minjeaud et al. 2009) uncertainties arrive when other processes like DNRA can also be present (Spott and Stange 2007). Nevertheless, the use of specific inhibitors together with labeled N compounds could help in discerning the main pathways involved in the N processing within the seepage face (Jensen et al. 2007).

Excitation-emission Matrix (EEM) fluorescence and PARAFAC analysis of the CDOM used in combination with FTR experiments offered new insights into the relationships between DOM and DIN reactivity at the seepage face (Chapters VI and VIII). In this sense, NMR spectroscopy could be used to identify specific organic compounds within the organic matter pool in order to evaluate their relationship with DIN reactivity and thus evaluate their suitability as target organic compounds in laboratory experiments (Lam and Simpson 2008; Abdulla et al. 2010; Simpson et al. 2011). Therefore, main organic and inorganic N compounds previously identified could be used isotopically enriched in laboratory experiments in order to follow the transformations driven by the benthic community and the different pathways present.

Finally, characterization of the microbial community at the molecular level could bring valuable information about the microorganisms responsible for the DIN processing within the seepage face (e.g. Dong et al. 2009; Jaeschke et al. 2009). These techniques could bring important information about the potential presence of microbial patchiness with specific reactivity within the sediment and the vertical and lateral distribution of the microbial population at the subterranean estuary (Brad et al. 2008; Sun et al. 2011).

Appendix A

Porewater profiles modeled in Chapter V

Appendix A

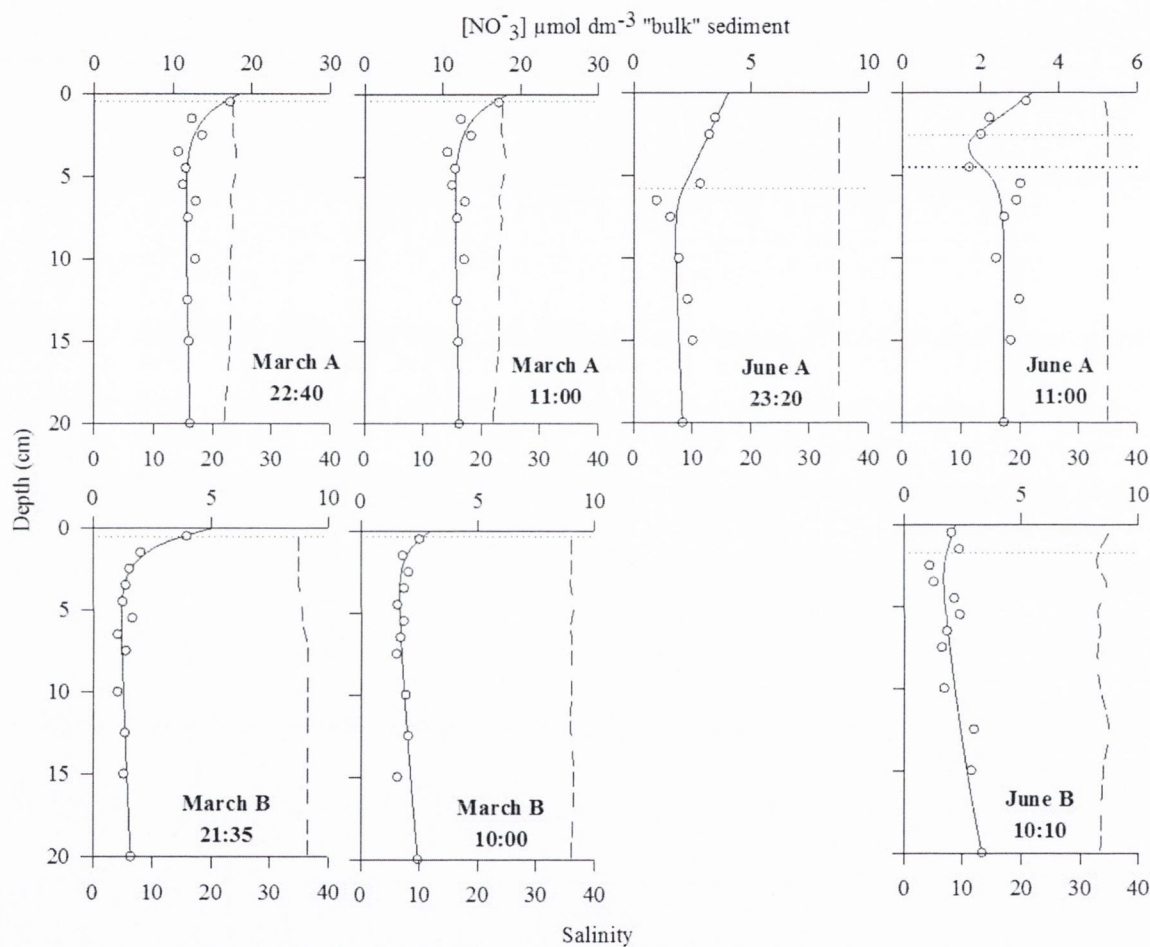
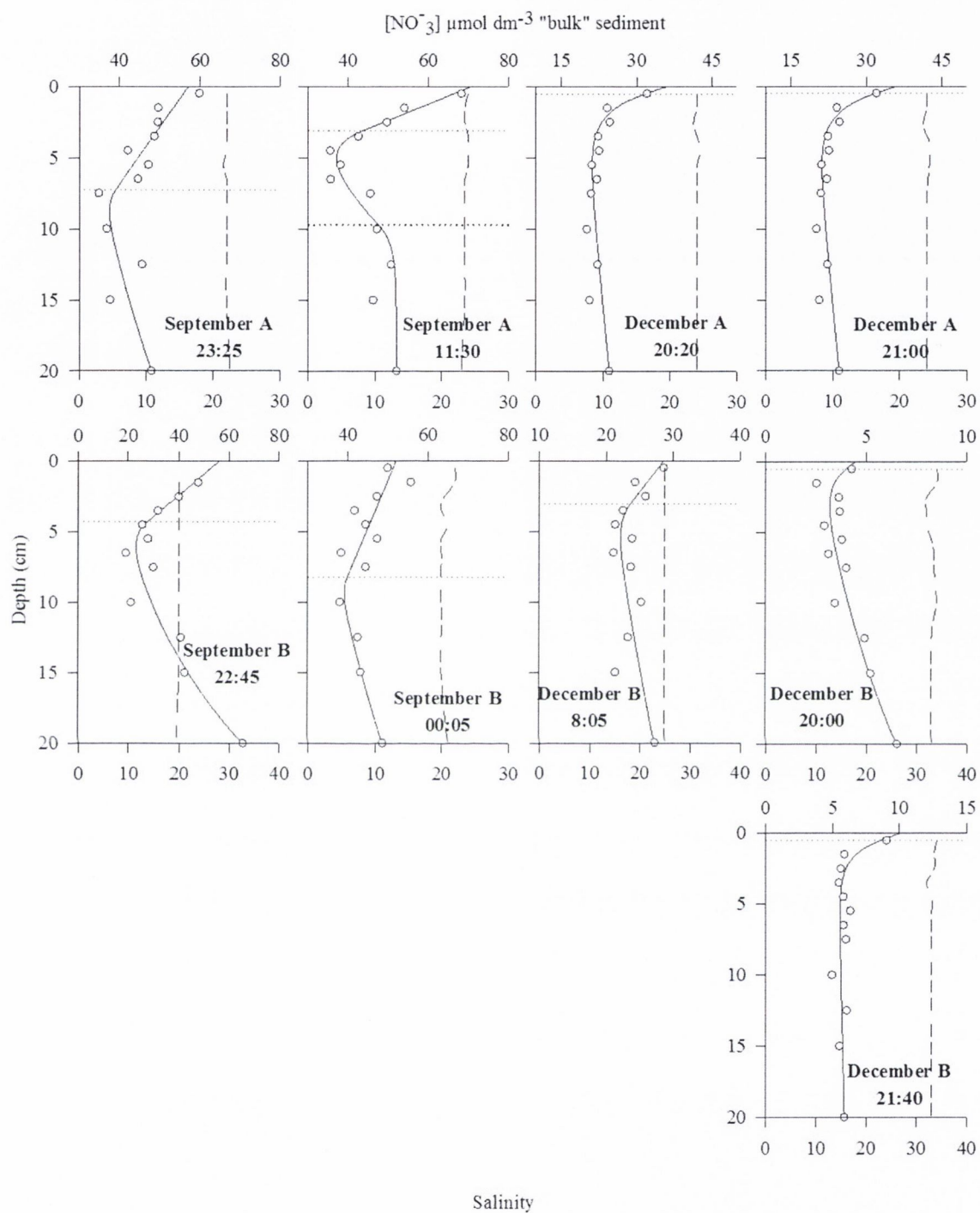


Figure A.1 NO_3^- profiles measured in-situ during March and June 2006 with the model output superimposed for each studied month (Chapter V). Salinity profiles (dashed lines) measured in parallel with the NO_3^- concentrations are also plotted for demonstration of the well mixed nature of the sediment porewater across the sampled domain.

Porewater profiles modeled in Chapter V



FigureA.2 NO_3^- profiles measured in-situ during September and December 2006 with the model output superimposed for each studied month (Chapter V). Salinity profiles (dashed lines) measured in parallel with the NO_3^- concentrations are also plotted for demonstration of the well mixed nature of the sediment porewater across the sampled domain.

Appendix B

**Flow-through reactor experiments. Kinetics modeling and associated DOC/DON fluxes
(Chapters VII and VIII)**

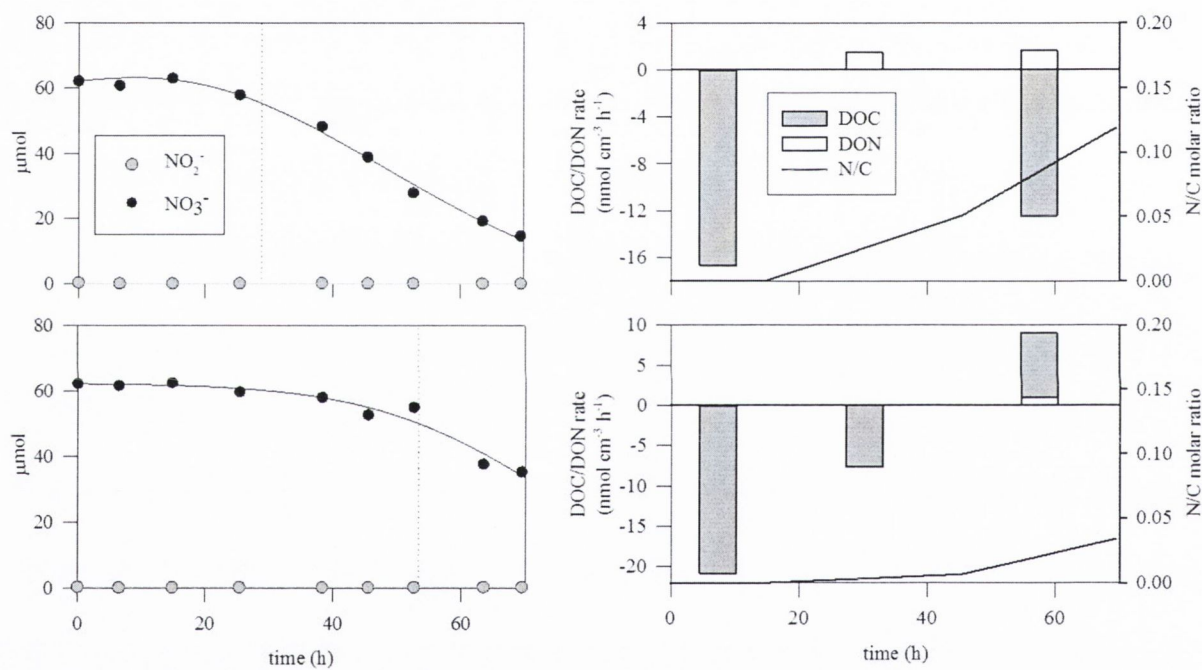


Figure B.1 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 1 (see Table VII.1 and VIII.1).

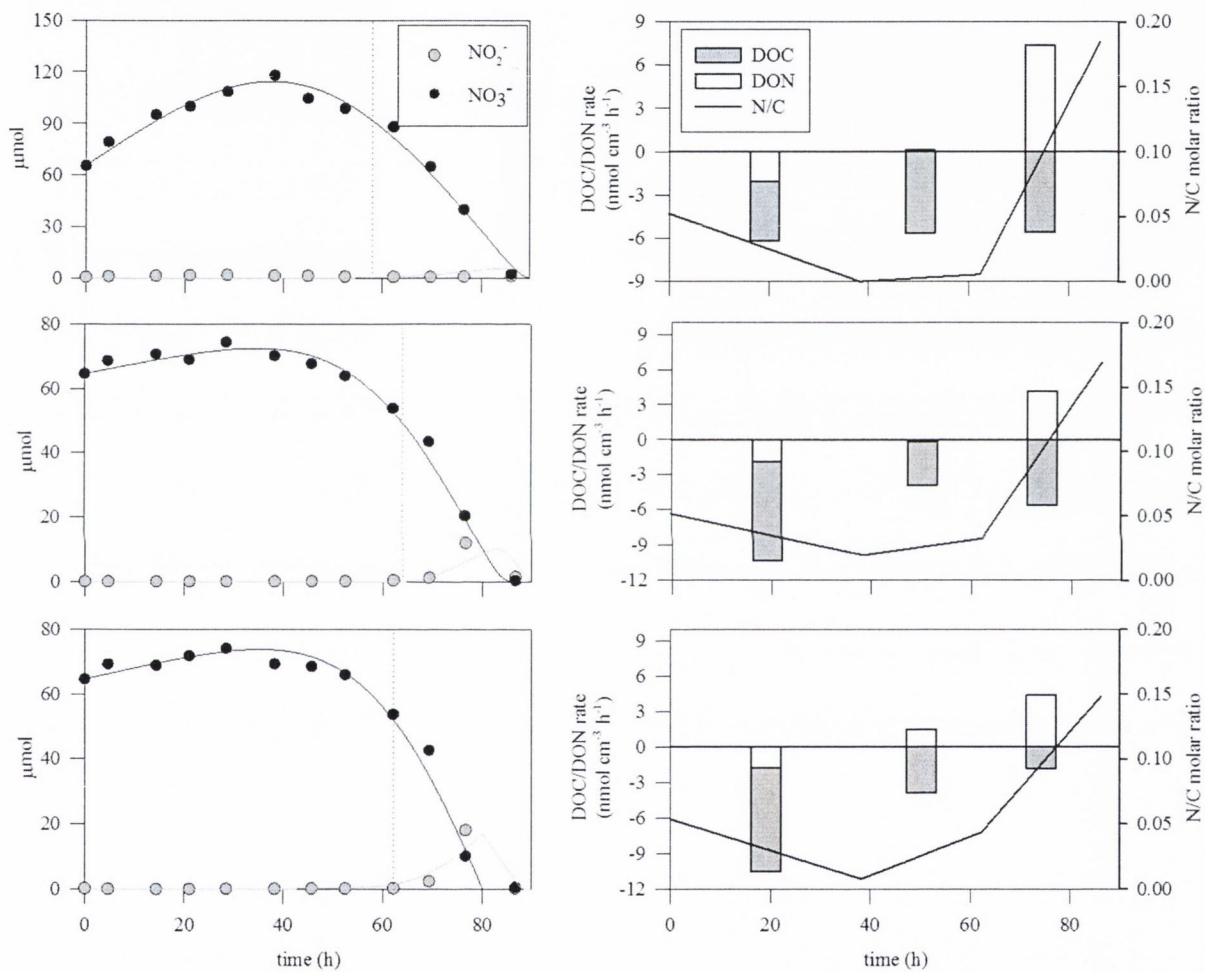


Figure B.2 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 2 (see Table VII.1 and VIII.1).

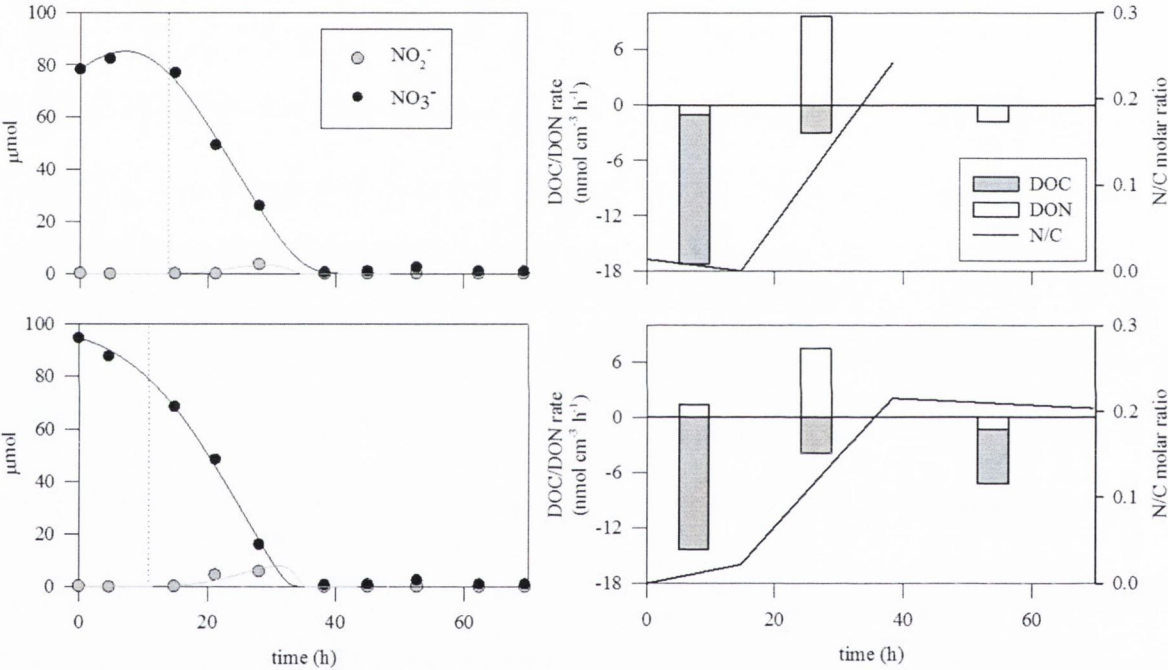


Figure B.3 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 3 (see Table VII.1 and VIII.1).

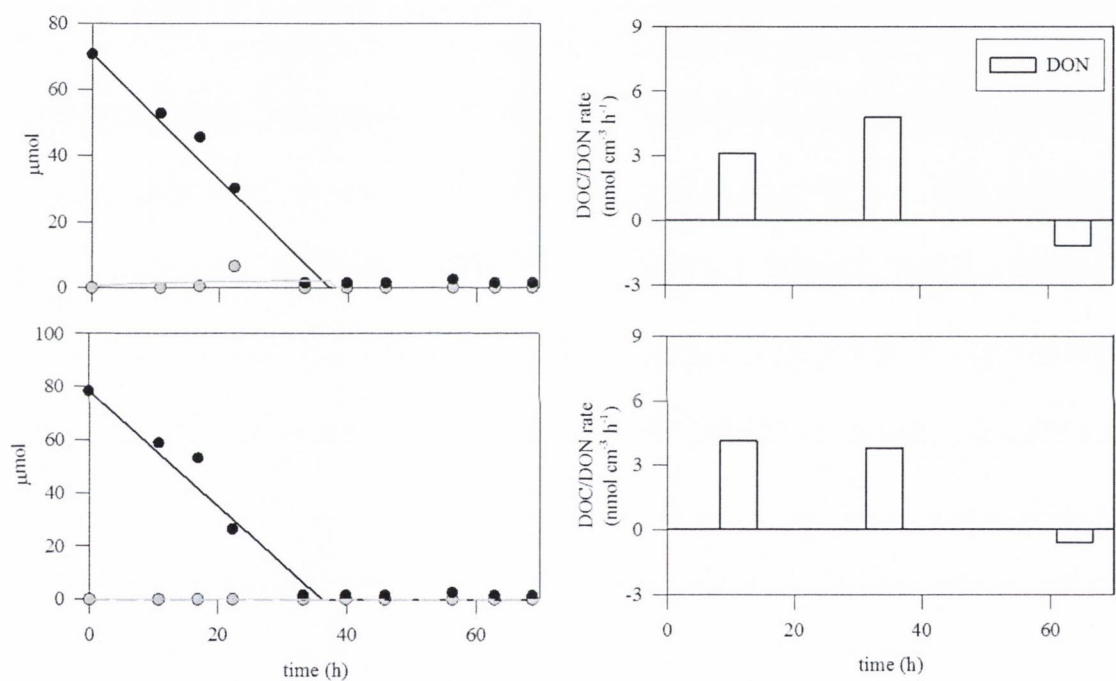


Figure B.4 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 4 (see Table VII.1 and VIII.1).

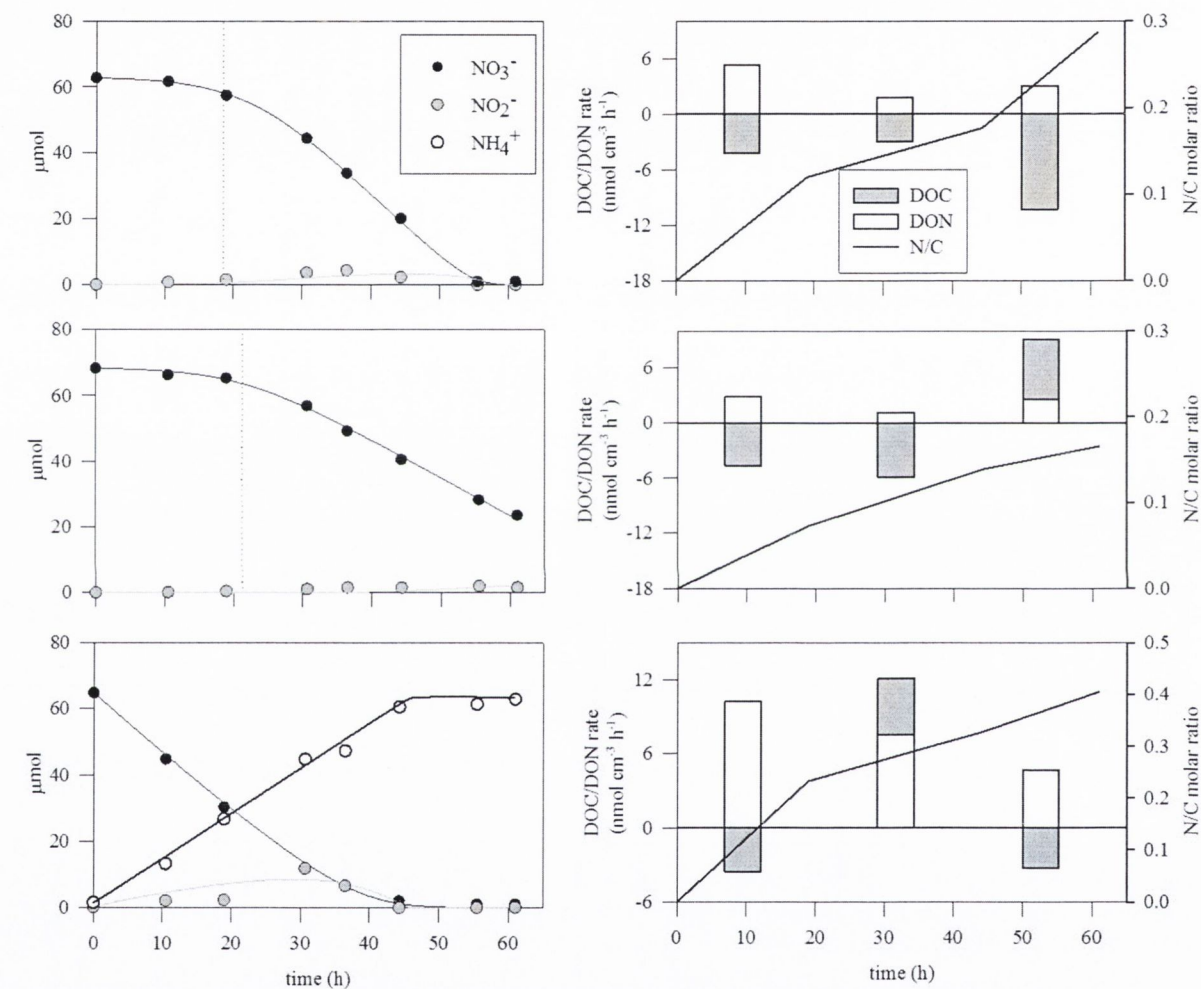


Figure B.5 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 5 (see Table VII.1 and VIII.1).

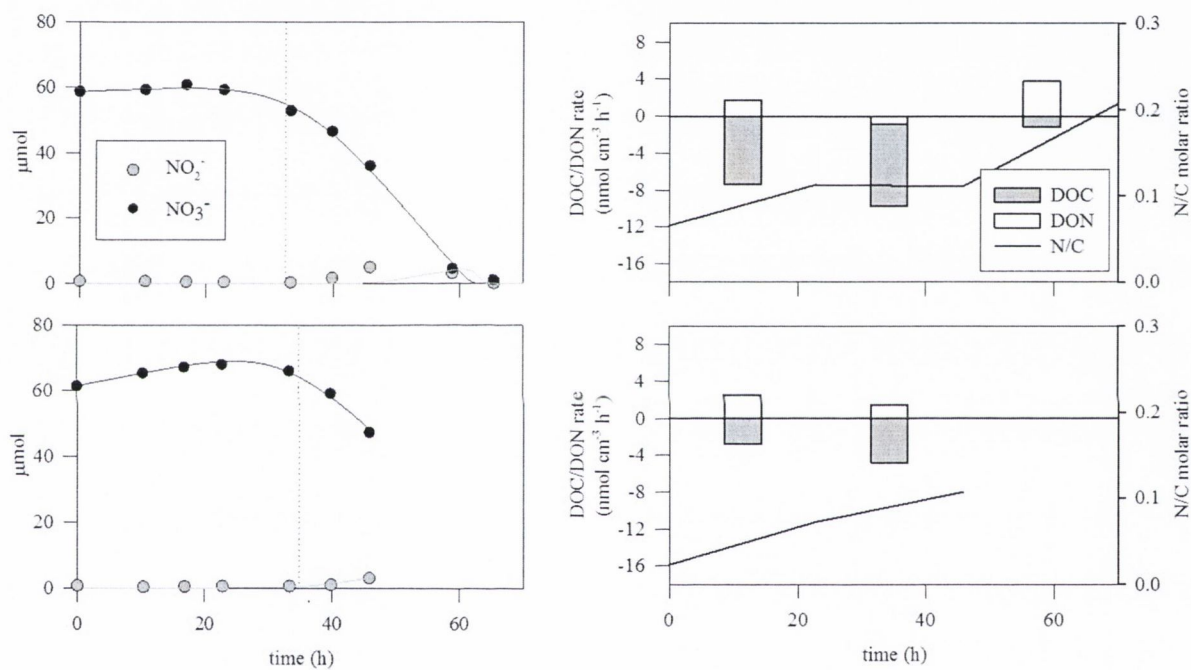


Figure B.6 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 6 (see Table VII.1 and VIII.1).

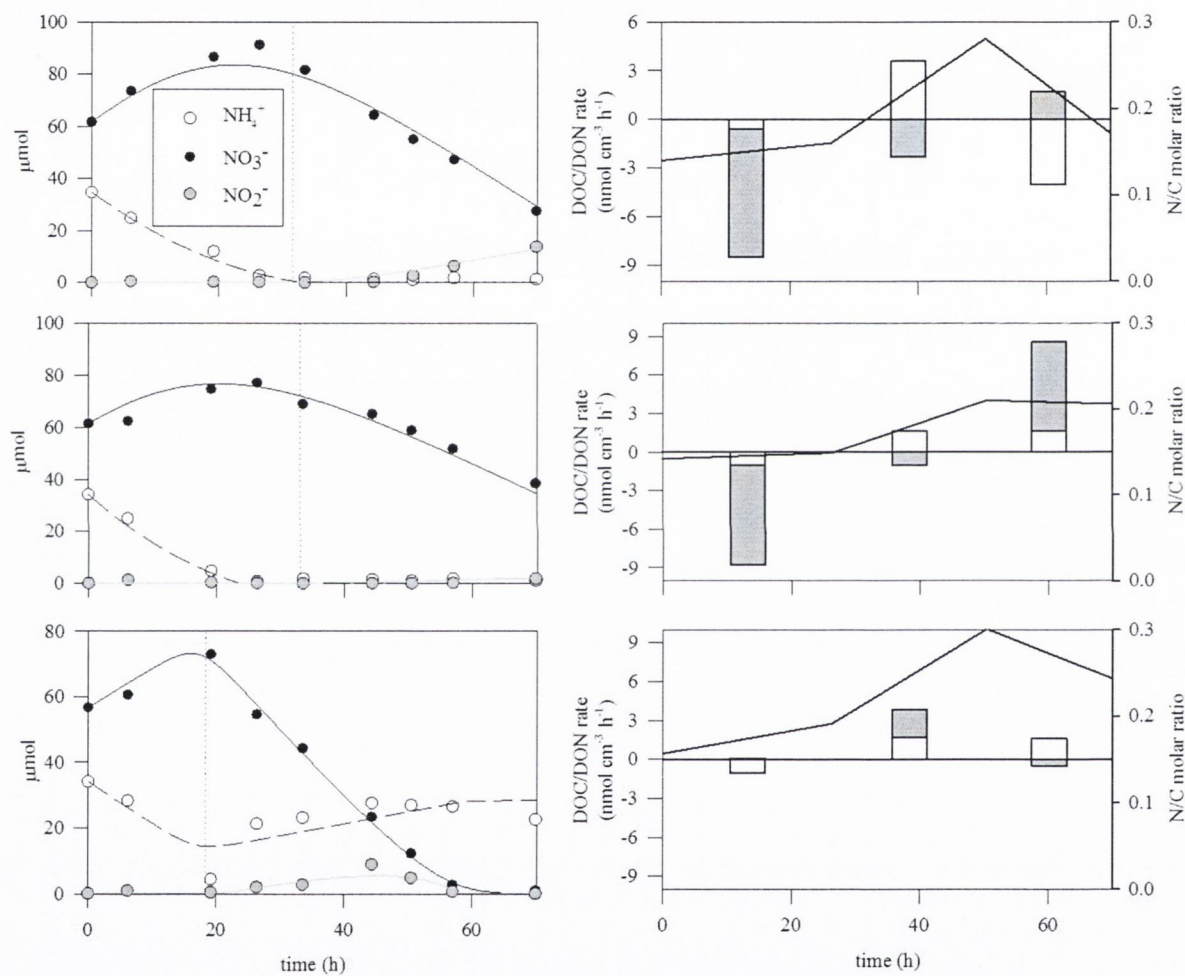


Figure B.7 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 7 (see Table VII.1 and VIII.1).

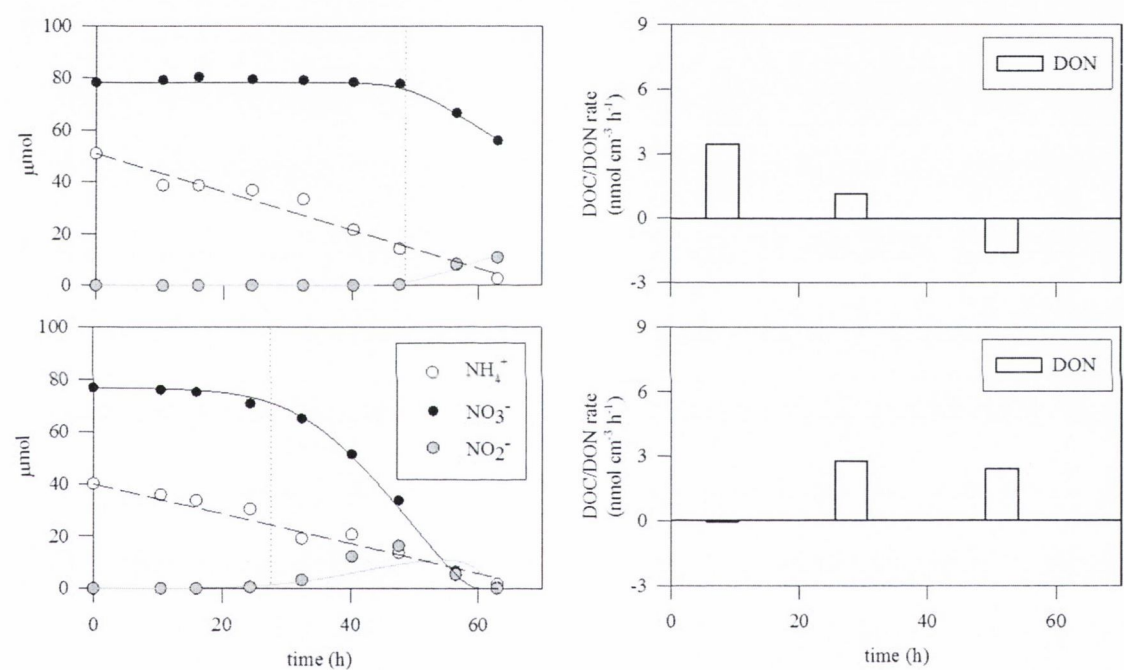


Figure B.8 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 8 (see Table VII.1 and VIII.1).

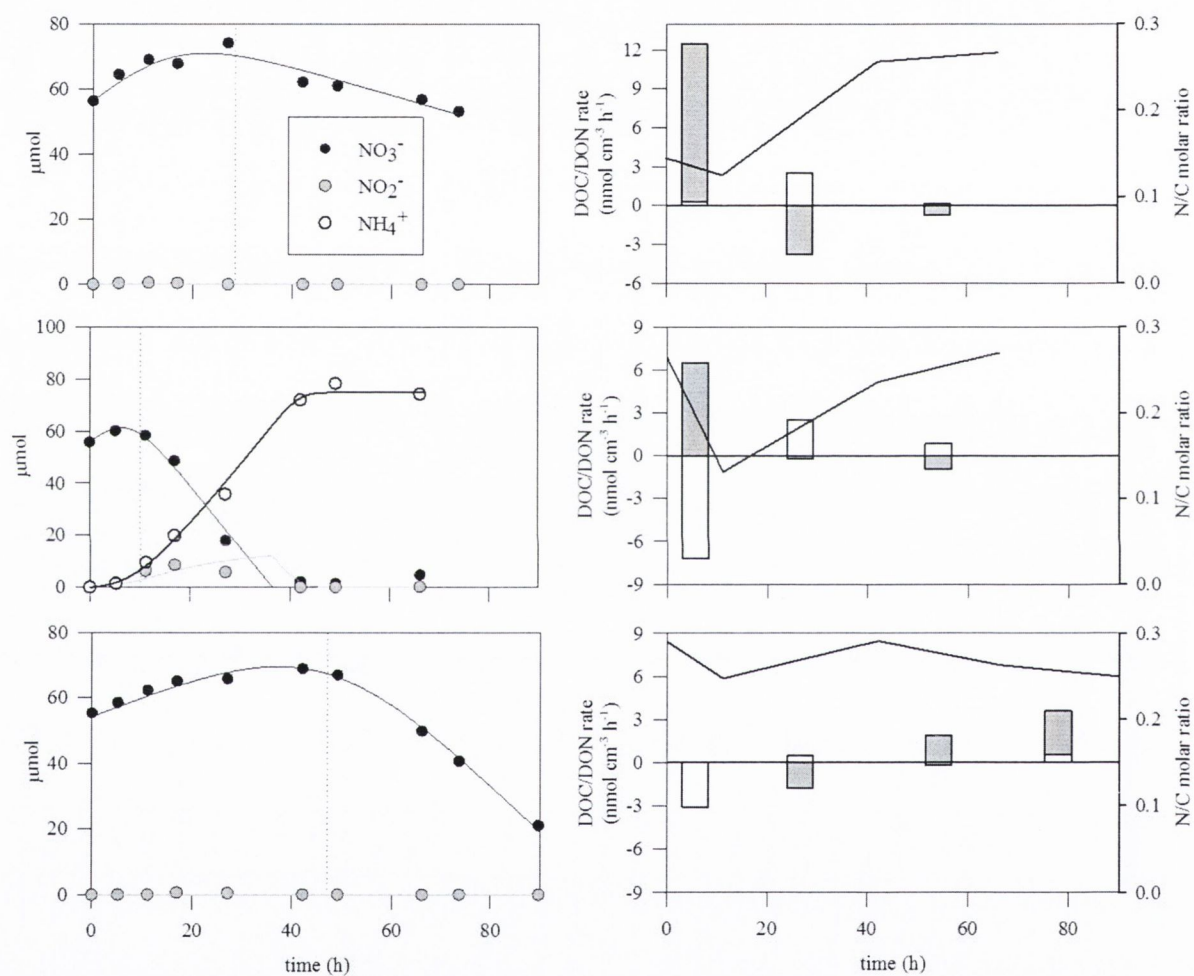


Figure B.9 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 9 (see Table VII.1 and VIII.1).

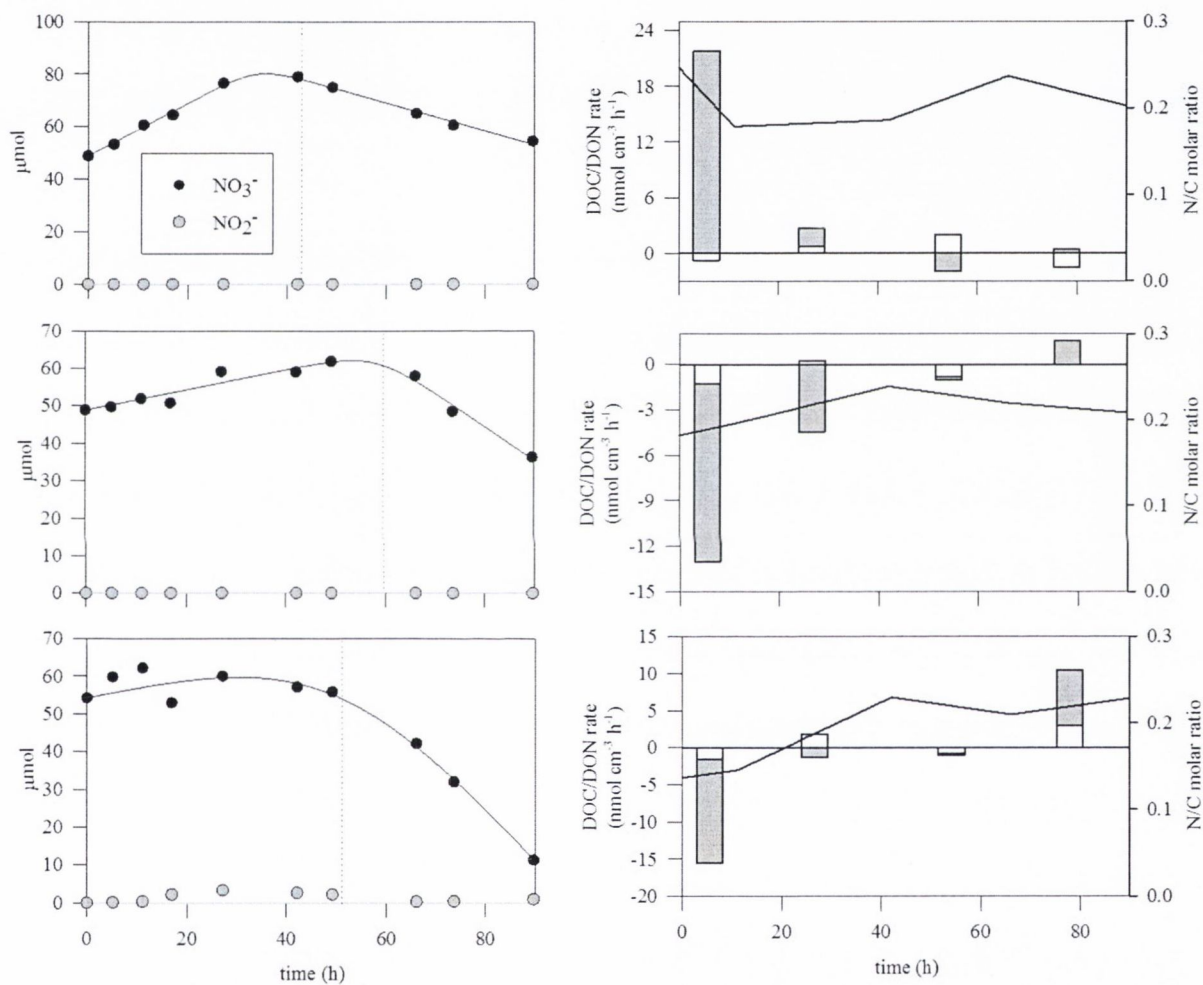


Figure B.10 Measured NO_3^- and NO_2^- temporal distribution for each FTR experiment plotted together with modeling results. Associated DOC and DON production/consumption rates are also shown. Experiment 10 (see Table VII.1 and VIII.1).

Appendix C

Flow-through reactor experiments. Temporal evolution of the CDOM (Chapter VIII)

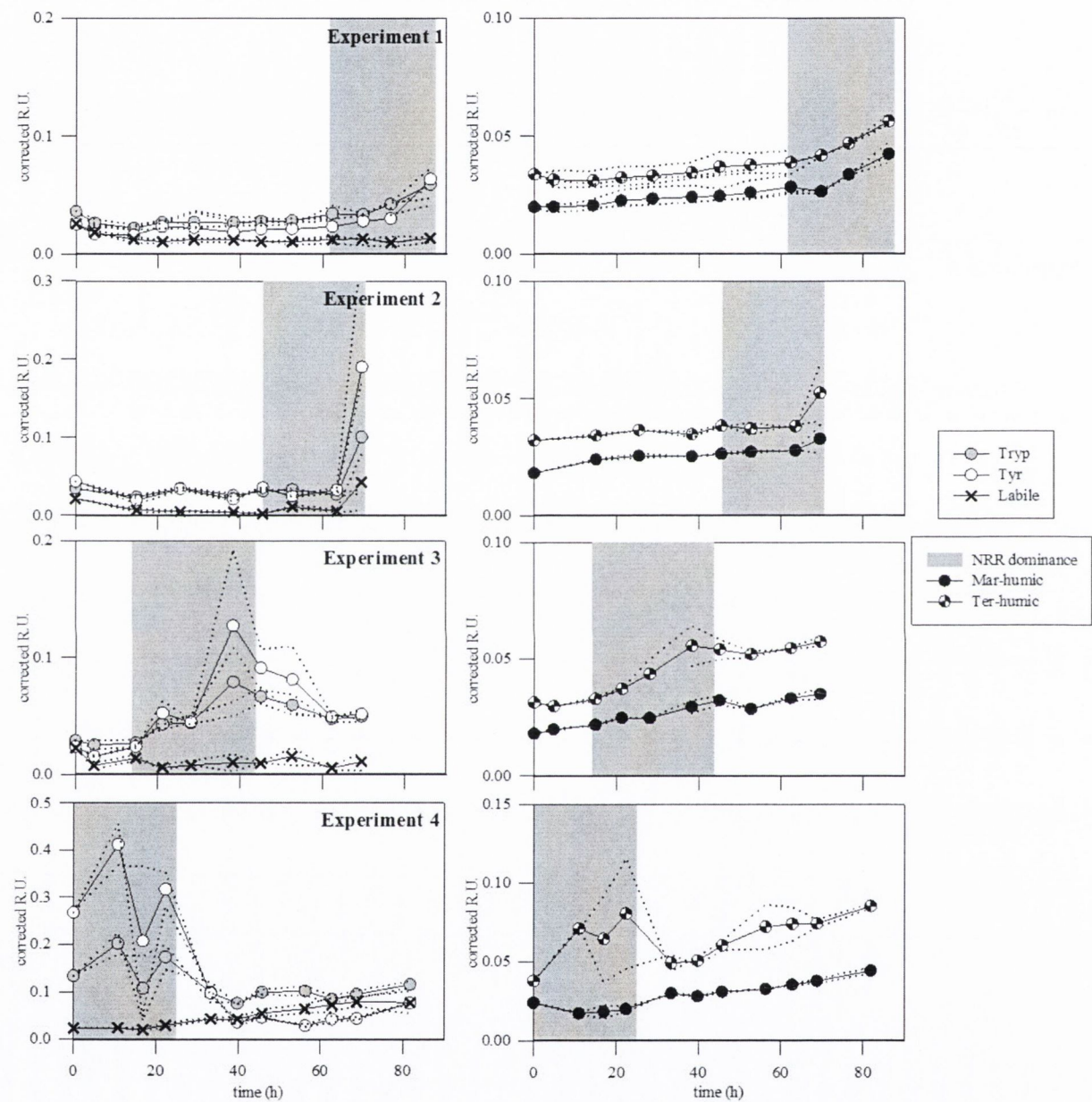


Figure C.1 Averaged temporal course of the five identified fluorophores in the CDOM pool through PARAFAC modeling of EEM's from the FTR experiments performed under recirculation of porewater amended with DIN. Experiments 1, 2, 3 and 4 are shown. Dotted lines correspond with each individual replicate. Identification of the averaged NO_3^- reduction area (period II) is also shown in dark background. See Table VIII.1 for initial conditions and Chapter VIII for discussion.

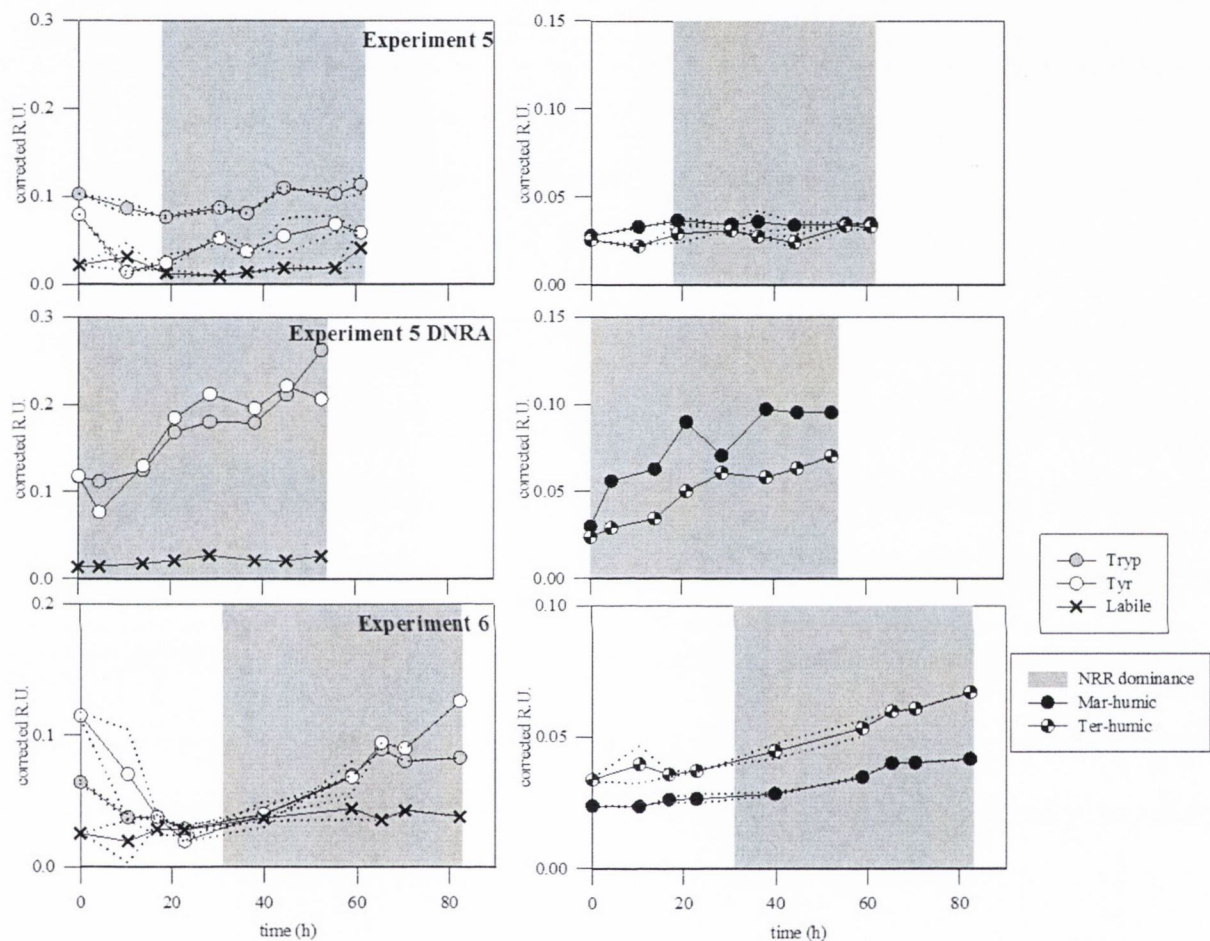


Figure C.2 Averaged temporal course of the five identified fluorophores in the CDOM pool through PARAFAC modeling of EEM's from the FTR experiments performed under recirculation of porewater amended with DIN. Experiments 5 and 6 are shown. Replicate from experiment 5 where DNRA was registered is shown separately for comparison. Dotted lines correspond with each individual replicate. Identification of the averaged NO_3^- reduction area (period II) is also shown in dark background. See Table VIII.1 for initial conditions and Chapter VIII for discussion.

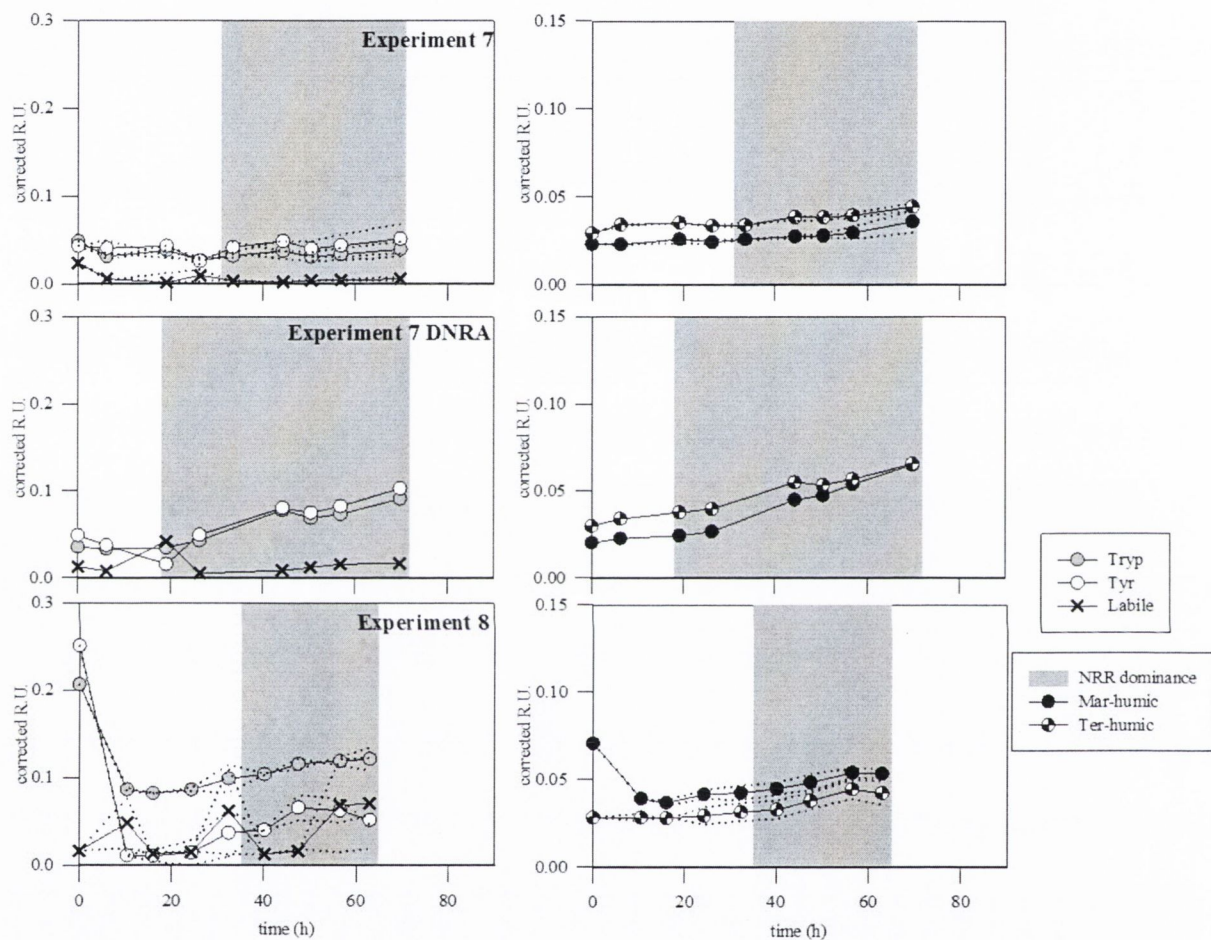


Figure C.3 Averaged temporal course of the five identified fluorophores in the CDOM pool through PARAFAC modeling of EEM's from the FTR experiments performed under recirculation of porewater amended with DIN. Experiments 7 and 8 are shown. Replicate from experiment 7 where DNRA was registered is shown separately for comparison. Dotted lines correspond with each individual replicate. Identification of the averaged NO_3^- reduction area (period II) is also shown in dark background. See Table VIII.1 for initial conditions and Chapter VIII for discussion.

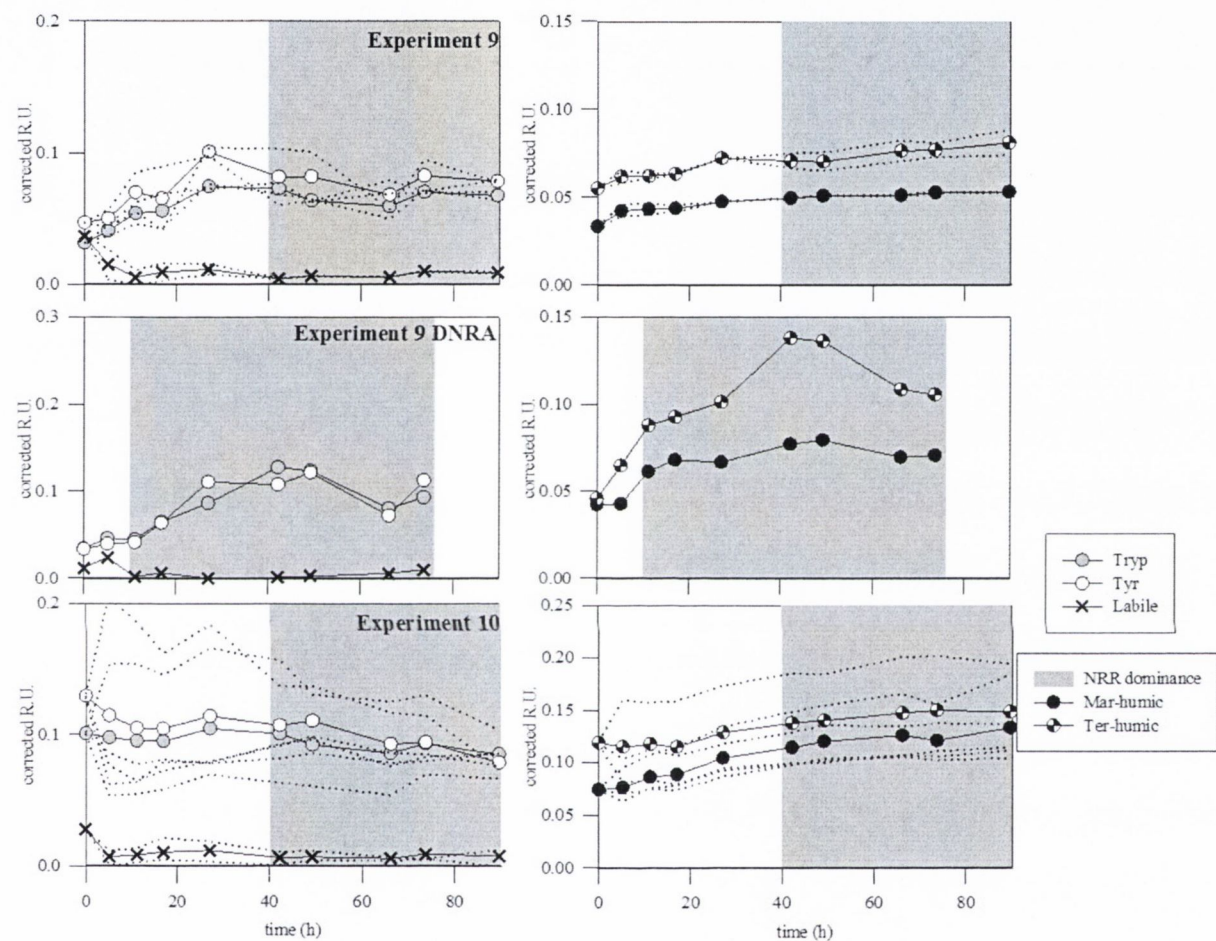


Figure C.4 Averaged temporal course of the five identified fluorophores in the CDOM pool through PARAFAC modeling of EEM's from the FTR experiments performed under recirculation of porewater amended with DIN. Experiments 9 and 10 are shown. Replicate from experiment 9 where DNRA was registered is shown separately for comparison. Dotted lines correspond with each individual replicate. Identification of the averaged NO_3^- reduction area (period II) is also shown in dark background. See Table VIII.1 for initial conditions and Chapter VIII for discussion.

Appendix D

Flow-through reactor experiments. DOC measurements (Chapter VI)

Appendix D

Experiment	Layer cm	Replicate	Time h	DOC $\mu\text{mol L}^{-1}$
1	0-2	all	0,0	292,9
		1	1,3	341,5
		1	4,2	319,6
		1	7,0	283,9
		1	9,6	280,3
		2	1,3	315,7
		2	4,2	286,3
		2	7,0	264,7
		2	9,6	194,5
	02-12	all	0,0	354,5
		1	4,2	380,6
		1	7,0	340,9
		2	4,2	370,5
		2	7,0	377,1
	12-32	1	0,0	354,5
		1	2,7	387,7
		1	4,2	358,6
		1	7,0	389,7
		1	9,6	364,2
		2	0,0	292,9
		2	2,7	402,2
		2	4,2	348,8
		2	7,0	329,2
		2	9,6	293,2

Table D.1 DOC determinations in Flow-Trough Reactor experiments used in Chapter VI. Experiment 1 (see Table VI.1 for initial conditions of the experiments and Table D.6 for individual advective velocities imposed at each replicate.

Flow-through reactor experiments. DOC measurements

Experiment	Layer cm	Replicate	Time h	DOC $\mu\text{mol L}^{-1}$
2	0-2	All	0,0	289,9
		2	2,0	324,5
		2	5,0	295,1
		3	2,0	311,5
		3	5,0	354,0
2	02-12	All	0,0	182,7
		1	4,5	272,3
		1	6,0	268,0
		1	7,5	263,4
		2	3,0	226,0
		2	6,0	-
		2	7,5	219,6
		3	3,0	195,1
		3	4,5	204,3
		3	6,0	187,0
2	12-32	All	0,0	188,3
		1	3,5	240,0
		1	6,5	192,1
		1	9,5	186,7
		2	3,5	219,5
		2	6,5	195,4
		2	9,5	178,5
		3	3,5	206,6
		3	6,5	189,4
		3	9,5	182,3

Table D.2 DOC determinations in Flow-Trough Reactor experiments used in Chapter VI. Experiment 2 (see Table VI.1 for initial conditions of the experiments and Table D.6 for individual advective velocities imposed at each replicate).

Appendix D

Experiment	ID	Replicate	Time h	DOC $\mu\text{mol L}^{-1}$
3	A	All	0,0	181,4
		1	7,0	219,1
		1	10,5	220,4
		1	17,0	290,0
		2	7,0	244,2
		2	10,5	263,8
		2	17,0	214,8
		3	7,0	251,2
		3	10,5	254,7
		3	17,0	265,7
3	B	All	0,0	155,6
		1	5,0	269,0
		1	9,0	230,9
		1	13,0	223,7
		2	5,0	259,3
		2	9,0	247,8
		2	13,0	245,8
		3	5,0	199,4
		3	9,0	238,3
		3	13,0	223,1

Table D.3 DOC determinations in Flow-Trough Reactor experiments used in Chapter VI. Experiment 3 (see Table VI.1 for initial conditions of the experiments and Table D.6 for individual advective velocities imposed at each replicate).

Flow-through reactor experiments. DOC measurements

Experiment	Solution	Replicate	Time h	DOC $\mu\text{mol L}^{-1}$
4		All	0,0	313,7
		1	1,3	385,7
		1	4,2	388,1
		1	7,0	344,3
		1	8,3	321,3
		2	1,3	327,0
		2	4,2	360,9
		2	7,0	338,9
		2	8,3	360,4
		3	1,3	389,1
		3	4,2	329,6
		3	7,0	363,8
		3	8,3	383,7
5	A	All	0,0	301,2
		1	3,0	322,9
		1	6,0	322,5
		2	3,0	380,2
		2	6,0	368,6
		3	3,0	321,9
		3	6,0	329,4
	B	All	9,0	454,2
		1	10,0	-
		1	13,0	354,2
		2	10,0	311,7
		2	13,0	357,0
		3	10,0	321,1
		3	13,0	351,1

Table D.4 DOC determinations in Flow-Trough Reactor experiments used in Chapter VI. Experiments 4 and 5 (see Table VI.1 for initial conditions of the experiments and Table D.6 for individual advective velocities imposed at each replicate).

Appendix D

Experiment	Solution	Replicate	Time h	DOC $\mu\text{mol L}^{-1}$
6	A	All	0,0	370,8
		1	3,0	395,9
		1	7,5	389,9
		2	3,0	452,6
		2	7,5	464,2
		3	3,0	-
		3	7,5	363,8
	B	All	8,0	451,7
		1	10,5	371,4
		1	15,0	419,5
		2	10,5	386,7
		2	15,0	398,5
		3	10,5	371,7
7	A	All	0,0	348,5
		1	1,5	478,3
		1	2,8	415,9
		1	4,0	315,4
		1	5,3	406,9
		2	1,5	489,3
		2	2,8	413,5
		2	4,0	372,2
		2	5,3	421,9
		3	1,5	474,6
		3	2,8	454,2
		3	4,0	435,8
	B	All	0,0	329,0
		1	6,8	398,4
		1	8,1	446,0
		1	9,3	440,0
		2	6,8	457,8
		2	8,1	491,8
		2	9,3	474,0

Table D.5 DOC determinations in Flow-Trough Reactor experiments used in Chapter VI. Experiments 6 and 7 (see Table VI.1 for initial conditions of the experiments and Table D.6 for individual advective velocities imposed at each replicate).

Flow-through reactor experiments. DOC measurements

Experiment	Layer cm	Replicate	PW velocity cm h ⁻¹
1	0-2	1	15,2
	0-2	2	14,9
	2-12	1	17,6
	2-12	2	16,9
	12-32	1	17,1
	12-32	2	17,1
2	0-2	1	15,4
	0-2	2	16,1
	0-2	3	14,9
	2-12	1	17,0
	2-12	2	17,0
	2-12	3	19,3
	12-32	1	17,4
	12-32	2	18,1
	12-32	3	17,4
3a	2-12	1	3,9
	2-12	2	3,6
	2-12	3	3,7
3b	2-12	1	6,9
	2-12	2	6,6
	2-12	3	6,7
4	2-12	1	17,6
	2-12	2	17,8
	2-12	3	17,5
5	2-12	1	18,7
	2-12	2	18,9
	2-12	3	18,9
6	2-12	1	17,1
	2-12	2	19,3
	2-12	3	17,3
7	2-12	1	17,8
	2-12	2	17,4
	2-12	3	17,2

Table D.6 Imposed porewater velocity in the Flow-Trough Reactor experiments presented in Chapter VI.

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