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Effects of recirculation of seawater enriched in inorganic nitrogen on dissolved organic carbon processing in sandy seepage face sediments

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Abstract

Human activities are now the main source of bioavailable Nitrogen to Earth's ecosystems. Disruption of the N cycle can promote changes to the biogeochemical cycling of other elements in particular to the Oxygen and Carbon cycles. Nevertheless, information on how increasing Dissolved Inorganic Nitrogen (DIN) availability might affect the benthic processing of organic matter in coastal sandy sediments is currently unavailable. Here, we present a series of flow-through reactor experiments conducted with undisturbed sediment cores collected at an intertidal seepage face (Ria Formosa lagoon, SW Iberian Peninsula) in order to evaluate the effect of increasing DIN availability on benthic dissolved organic C (DOC) reactivity. Our results show that the metabolic activity of the benthic community can be a significant source of DOM to the porewater. Furthermore, in the absence of DIN, increasing porewater flow rates accelerated benthic DOM production and increased the recalcitrant nature of DOC transported across the sediment-water interface. In contrast, the co-occurrence of high DIN concentrations and high seepage velocities increased the microbial breakdown of organic material. Our results suggest that the availability of DIN in permeable seepage faces increases the net production of labile DOC, by enhancing the ability of the benthic microbial community to process refractory organic matter. Hence, we suggest that increasing DIN availability in coastal permeable sediments accelerates benthic organic C processing, promoting the release of more labile DOC to receiving water bodies.

Introduction

Coastal sediments are sites of intense processing of organic matter sourced from adjacent coastal seas or from land. Although most of the research on benthic organic matter reactivity has focused on organic-rich cohesive sediments, increasing evidence has shown coastal permeable sediments to be zones of intense organic matter processing and turnover (Boudreau et al. 2001; Rocha 2008). In permeable sediments, porewater advection induces the rapid interchange of solutes and particles between the overlying water and the benthic compartment, thus controlling the distribution and fate of important metabolites such as O_2 and organic matter (e.g. Huettel et al. 1996; Precht and Huettel 2003). The common low-organic matter content of coastal sands seems therefore to result from high benthic metabolic and solute exchange rates rather than result from the low reactivity of the organic C pool (Huettel and Rusch 2000; Beer et al. 2005).

When the availability of particulate organic matter (POM) is limited, Dissolved Organic Matter (DOM) may still play a crucial role in benthic metabolism since heterotrophic microorganisms can directly employ some of the DOM as an electron donor in redox reactions. However, little information is available on the chemical composition and reactivity of DOM in permeable sediment porewaters (e.g. Santos et al. 2009; Chipman et al. 2010; Kim et al. 2012) even though a substantial fraction of porewater DOM might be recalcitrant (Burdige 2001; Burdige et al. 2004). Benthic microbial degradation of either POM or DOM may be responsible for the production of this refractory material (Ogawa et al. 2001; Chipman et al. 2010), thus enhancing the sequestration of fixed carbon in the oceans (Hansell 2013).

Permeable sediments located at the land-ocean interface are particularly important as biogeochemical hotspots acting as reactive shunts intersecting different DOM delivery pathways. Indeed, local benthic metabolism may actively change the composition and reactivity of the

DOM in transit to the sea from land via Submarine Groundwater Discharge (SGD, i.e. any flow of water out across the sea floor; Burnett et al. 2003) and/or originating from local marine sources (Chipman et al. 2012; Kim et al. 2012). Elucidating the transfer and cycling of DOM in permeable sediments is hence important to ultimately understand the functional role of these sediments in the coastal and oceanic C cycle.

Due to the active processing of marine and terrestrial organic matter, sandy intertidal sediments are also sites of active Dissolved Inorganic N (DIN) regeneration (Beer et al. 2005; Santos et al. 2009). Other sources of DIN to the porewater can be linked to human activities in the coastal zone, such as wastewater disposal or the use of fertilizers. This anthropogenic N can enter the internal beach aquifer through direct disposal, recirculation of polluted seawater through the sediment or directly from land when a coastal aquifer is hydraulically connected to the sea (Slomp and Van Cappellen 2004). 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 N cycle can promote changes in the cycling of other elements, particularly O and C (Falkowski 1997; Mackenzie et al. 2002). Although substantial research has focused on understanding natural NO_3^- attenuation processes in coastal benthic systems (e.g. Bonin and Raymond 1990; Cornwell et al. 1999; Burgin and Hamilton 2007), to our knowledge, the impact of increased DIN availability on organic C transport and reactivity in coastal permeable sediments has not been investigated. Elucidation of the impact of DIN availability on organic matter processing in coastal permeable sediments could therefore offer valuable information on the resilience and plasticity of this coastal compartment to current and projected anthropogenic pressure.

The objectives of this study are therefore (1) to evaluate the role of intertidal permeable sediments in mediating DOM fluxes to the coastal sea and (2) to elucidate the effect of augmented DIN concentrations in the seawater circulating through the beach face on benthic dissolved organic C (DOC) processing. We performed a series of flow-through reactor (FTR) experiments to simulate active seepage through undisturbed sediment slices taken from an intertidal seepage face at the Ria Formosa coastal lagoon. The location has been subject to periodic DIN-rich SGD (Leote et al. 2008). The influence of porewater velocity and DIN concentrations over benthic DOM reactivity was experimentally evaluated by following the DOC levels at both the inlet and outlet of the experimental reactors and the makeup of the Chromophoric DOM (CDOM; the DOM fraction with measurable optical properties) pool.

Materials and methods

Study Site

Ria Formosa (SW Iberian Peninsula; Fig. 1) is a 110 Km² coastal lagoon separated from the Atlantic Ocean by a multi-inlet sand barrier system. The lagoon is embedded within 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; Fig. 1) was designated a Nitrate Vulnerable Zone in 2001, and this was extended to the Campina da Luz aquifer system (NE Ria Formosa; not shown in Fig. 1) in 2005. Preliminary estimates of N loading through SGD suggest it to be a potentially important source of N to the lagoon (36.2 mol N year⁻¹ m⁻¹; Leote et al. 2008). NO₃⁻ and NH₄⁺ content of up to 187 µM and 40 µM respectively were measured in the seeping water at the sampling site

(Leote et al. 2008). The linear correlation of NO_3^- content with salinity in water samples taken from seepage meters extrapolates to a NO_3^- concentration of 422 μM at 0 salinity, indicating the potential maximum NO_3^- concentration at the site via groundwater input (Leote et al. 2008).

Flow-through benthic reactor experiments

Sediment FTR experiments were conducted in triplicate (with the exception of Experiment 1; see Table 1) from July 2010 to January 2011. The sediment used was taken from the permanently saturated intertidal area affected by periodic NO_3^- -rich SGD, on the inner part of Ancão Peninsula (37°00'04'' N, 7°88'57'' W; Fig. 1). Sediment at the sampling site is composed mainly of medium-coarse sand (average grain size 0.5 mm; silt+clay < 1.7% weight; Rocha et al. 2009), with high hydraulic conductivity ($1 \times 10^{-3} \text{ cm s}^{-1}$; Leote et al. 2008). Undisturbed, saturated sediment cores (40 cm length, 6.6 cm inner diameter) were collected with polycarbonate core liners in the permanently saturated lower intertidal area, close to the peak discharge area at the seepage face. These were collected manually by pushing the core liners into the sediment at low tide, digging them out and immediately transferring the cores to the laboratory. There, sediment cores were sliced into three different depth intervals (0-2 cm, 2-12 cm and 12 to 32 cm depth), according to the vertical distribution of the C:N ratio in the POM and benthic permeability gradient previously assessed at the site (Rocha et al. 2009). An adapted HTH core slicer (HTH Teknik, Luleå, Sweden; Renberg and Hansson 2008) was used to transfer selected undisturbed vertical sediment slices into Teflon FTR cells (2, 10 or 20 cm long, according to the length of each sediment slice). The adapted HTH core slicer included a reactor holder on top of the corer allowing the transfer of undisturbed sediment slices into the reactor cells with minimal disturbance of pore structure (Fig. 2A). The reactor cells were based on the

design described in Pallud et al. (2007) and include collimators and GF/F filters (Whatman, 0.7 μm average pore size) to ensure an even distribution of the water flow through the reactor's cross-section (Fig. 2C). The FTR cells containing saturated sediment were then sealed and submerged into a temperature-controlled water bath (Fig. 2B). Filtered seawater was then pumped through the reactor cells at a constant flow rate for at least 4 h to thermally and chemically equilibrate the system (i.e. to flush the ambient porewater and allow the sorption equilibrium of DOM with the solid phase) and adjust the interstitial flow rate before experiments started (Fig. 2B). After the acclimation period, experiments were started using filtered porewater collected in-situ with piezometers buried 40 cm into the seepage face and subsequently filtered through GF/F filters (Whatman, 0.7 μm average pore size) in order to remove particles. The porewater was then amended with known concentrations of KNO_3 and NH_4Cl (from <2 to 590 μM ; Table 1) and NaBr (used as flow tracer; ~ 1 mM final concentration). The range of NO_3^- and NH_4^+ concentrations employed in the experiments was chosen on the basis of the observed and potential maximum concentrations previously found at the site (Leote et al. 2008). The filtered seawater and porewater solutions used during the experiments were pumped from the bottom of the reactor cell toward the top, thus simulating the vertical flow during active seepage. Peak porewater velocities in excess of 30 cm h^{-1} were measured at our site during sediment exposure (Rocha et al. 2009). The imposed porewater velocity in the FTR experiments ranged from 3.7 ± 0.1 to $18.8 \pm 0.1 \text{ cm h}^{-1}$ (Table 1), a conservative representation of the local advective porewater flow measured during active seepage at our site. Porewater flow rate and temperature (ranging from 14.5 to 24.5 $^\circ\text{C}$; Table 1) were kept constant during both the acclimation period and the experimental run.

A sequence of different experiments was performed in order to a) study the reactivity of the benthic DOM in the absence of porewater DIN (Experiments 1 and 2; Table 1) and b) the influence of interstitial flow rate on benthic metabolic activity (Experiments 2 and 3). In a complementary experiment (2B) glucose was added ($\sim 20 \text{ mmol C L}^{-1}$ final concentration) to the porewater at the higher end of the range of imposed interstitial flow rates ($17.8 \pm 0.7 \text{ cm h}^{-1}$) to evaluate the influence of high labile DOC content in the circulating porewater over sediment-mediated DOM fluxes. Finally, another series of experiments (Experiments 4-7) was carried out with amendments of NO_3^- and NH_4^+ to the circulating porewater. O_2 concentration in the water seeping out at our site ranged from close to saturation to below 20% saturation as previously recorded at the site (Leote et al. 2008). The O_2 concentration in the circulating solution was established by purging the porewater mixture with helium and/or air prior to experimental runs. For the experiments run under suboxic conditions, the filtered porewater solution was isolated from the ambient atmosphere by a floating layer of liquid paraffin. In these cases, the solution was pumped from the bottom of the reservoir and paraffin was added to the solution just before the start of the experiments, thus minimizing paraffin dissolution and its carry-over into the pumping system. FTR experiments were performed for a period varying between 7 and 24 h while samples (20 mL) were taken from the outflow into sterile vacutainers at constant time intervals and stored at -20°C until analysis. DOC levels in the working solutions were those of the natural porewater at the sampling site (except Experiment 2B as explained, Table 1).

Flow regime in FTR experiments

The residence time of porewater within the FTR's was calculated as L/Q , where L is the length of the sediment column and Q the Darcy velocity of the solution (i.e. $v \theta$, where v is the

porewater velocity and θ the sediment porosity; e.g. Pallud et al. 2007). The non-dimensional hydrological Peclet (P_e) number was used to evaluate the relative contribution of advection and diffusion to solute transport in the reactors:

$$P_e = vl/D_{\text{molec}} \quad (1)$$

where D_{molec} is the diffusion coefficient of the target solute and l is the characteristic pore length, which can be approximated to the mean grain size in sandy sediments (Roychoudhury 2001). D_{molec} of Br^- (used as inert flow tracer) was used for the P_e calculation.

With the boundary conditions appropriate to an advection-dominated system, van Genuchten and Parker (1984) suggested the following truncated solution for the general advection-dispersion differential equation describing non-reactive solute transport in porous media:

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

where C_{out} and C_0 are the solute concentrations in the outflow and the inflow points respectively, D the dispersion coefficient and t the time. Br^- breakthrough curves obtained from the FTR experiments were modeled using eq. 2 in order to assess whether the reactors were operating properly (i.e. radial homogeneous distribution of the flow inside the reactor). The Br^- diffusion coefficient was calculated from Boudreau (1997).

Excitation-Emission-Matrix (EEM) Fluorescence of DOM and Parallel Factor (PARAFAC) analysis

Three-dimensional EEM spectra of CDOM in porewater samples from the FTR experiments were measured on a Cary Varian Eclipse spectrofluorometer. Prior to analysis, samples were kept in a temperature-controlled bath at 20 °C to avoid changes to spectral

intensities caused by temperature differences. Bandwidths were set at 10 nm for Emission and 5 nm for Excitation. Emission and excitation wavelengths ranged from 280 to 560 nm (2-nm intervals) and from 220 to 440 nm (5-nm intervals), respectively. Due to the low DOC levels found in our samples, inner filter effects were assumed to be negligible. Daily recorded Milli-Q water blanks were subtracted from the sample spectra to eliminate water Raman scatter peaks. EEMs were normalized to the integrated area of Milli-Q water Raman peaks as a calibration method for eventual instrument-dependent intensity factors (Lawaetz and Stedmon 2009). DOMFluor Toolbox for MATLAB was used to correct for first and second order Rayleigh scatter peaks and to perform the PARAFAC analysis of the samples (Stedmon and Bro 2008).

PARAFAC modeling of EEM fluorescence has been extensively described elsewhere (Andersen and Bro 2003; Stedmon and Bro 2008). Briefly, it allows the complex sample spectra to be decomposed into their individual fluorescence components (fluorophores) and thus permits subsequent quantification of scores obtained for each fluorophore present in each sample. These are directly proportional to the concentration of individual components. PARAFAC analysis was performed over a large dataset obtained from different FTR experiments carried out with sediments collected at the same location (1057 samples) and included the results presented here (346 samples). Split-half analysis validation of the model (Stedmon and Bro 2008) confirmed the adequacy and the uniqueness of the PARAFAC solution.

The five components identified through PARAFAC modeling could be separated into two groups according to their characteristic emission wavelength, i.e., the 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. 3). Emission at longer wavelengths is

associated with increased aromaticity or complexity of the target molecules (Coble et al. 1998). C3 and C4 showed spectra similar to humic components identified in previous studies (See Supplementary Materials Table 1). 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 seawaters derived from terrestrial sources (henceforth identified as Terrestrial Humic-like). The C4 peak corresponds to a humic fluorophore freshly produced in the marine environment by microbial metabolism (Parlanti et al. 2000; 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). These three components represent a labile, bioavailable DOM fraction (Hudson et al. 2007). C5 was a minor component of our dataset (<4% of the fluorescence scores) and was therefore excluded from the subsequent data analysis. C1 and C2 are referred herein as Tryptophan-like (Tryp) and Tyrosine-like (Tyr) components respectively.

Benthic DOC and CDOM transformation rates measured in FTR experiments

After complete turnover of the internal volume of the reactor and stabilization of the outflow CDOM concentration (i.e. steady state reached; see Fig. 4), DOC production-consumption within each reactor was calculated by simple mass balance between inflow and outflow concentrations. For this, the following expression was used (e.g. Pallud et al. 2007):

$$R = \frac{\Delta C Q}{V} \quad (3)$$

where R is the reaction rate, ΔC the change in solute concentration observed between the input and the output, Q the flow rate and V the reactor's volume. The final DOC transformation rate was obtained by integration of the discrete reaction rates obtained throughout the course of each experiment over time. Benthic metabolic activity, as expressed by the behavior of the four main

components of the local CDOM pool, was calculated as the ratio of change observed between the composition of inflow and outflow solutions. Finally, the non-dimensional first Damköhler (D_a) number was used to compare timescales of transport and reaction for the FTR experiments:

$$D_a = \frac{R L}{v C_0} \quad (4)$$

where R is the measured DOC production-consumption rate and L the length of the reactor cell.

Sediment properties

Sediment cores were collected within the permanently saturated zone by hand at the sampling site and subsequently frozen, then sliced into different depth-intervals (2 to 5 cm depth intervals, $n=6$) in order to evaluate relevant sediment properties. Since the sediment was composed mainly of medium-coarse sand, water content was measured on every sub-sample as a percentage of the weight loss after drying at 90 °C until a constant dry weight was reached (minimum 48 h). Since the sediment was saturated, porosity was calculated as:

$$\theta = \frac{V_{pw}}{V_{sand} + V_{pw}} \quad (5)$$

where V_{pw} is the volume of porewater and V_{sand} the volume of sediment at each sediment slice. V_{pw} was calculated from the mass difference between the wet and the dried sample divided by the density of freshwater. The density of the sediment (assumed 2.65 Kg L⁻¹; quartz sand) was used for V_{sand} determination, after correction for the mass of salts left in the dried sample (calculated from the salinities presented in Table 1). The Loss On Ignition (LOI; Dean 1974) was equated to the sum of the total organic matter and carbonate content of the sediment. The Total Organic Carbon (TOC) content was determined by combustion in a Vario EL Cube elemental analyzer after removal of inorganic C from bulk samples by acid (HCl) fumigation.

Porewater chemistry

Oxygen concentration in the circulating porewater solutions was measured following the standard Winkler titration (Grasshoff et al. 1983) adapted to low sample volumes (25 mL) and performed with a micrometer burette (2 mL volume; Gilmont Instruments, Inc., USA). 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 with a Lachat Quickchem 8500 Flow Injection Analysis system following standard colorimetric methods (Grasshoff et al. 1983). The methods used have a precision (MP) of 11% (NH_4^+) and 12% ($\text{NO}_3^- + \text{NO}_2^-$), with detection (DL) and quantification limits (QL) lower than 0.5 μM for both methods. Br^- concentration was determined with a Dionex ICS-1500 Ion Chromatography system coupled to a AS40 Automated Sampler in 12x sample dilutions to minimize the interference of Cl^- over the Br^- peak (MP: 6.20%, DL: 0.15 mM, QL: 0.21 mM). DOC was determined with a Vario TOC Cube elemental analyzer, after acidification (2M HCl) and purging of the solution to remove dissolved inorganic C (MP: 3.6%, DL: 33 $\mu\text{mol C L}^{-1}$, QL: 40 $\mu\text{mol C L}^{-1}$). Independent internal nutrient, Br^- and DOC reference solutions were used throughout each analysis as quality controls to ensure accuracy.

Statistical analysis

Data obtained for each experiment were compared using the Student t-test to evaluate the statistical significance of any differences found. Two-way ANOVA was performed on the data obtained at different porewater velocities to evaluate the significance of the effect of porewater advection on measured parameters. Pearson correlation and the F-Fisher test were used for linear regression, after normality, homogeneity and independence of the data were tested.

Results

Sediment and porewater properties

The largest variability of porosity values during the course of this study (July 2010-January 2011) was found in the top 2 cm of the sediment column (0.36 ± 0.01 , $n=6$; Fig. 5). Porosity remained unchanged in the deeper sediment layers (0.30 ± 0.01 from 2 to 32 cm depth). The LOI peaked in the top 2 cm and decreased with depth (from 0.56 ± 0.09 % at 0-2 cm to 0.36 ± 0.02 % at 12-32 cm; $n=6$; Fig. 5), whereas the TOC content remained almost unchanged with depth (0.036 ± 0.005 % at 0-2 cm; 0.036 ± 0.003 % at 2-12 cm; 0.033 ± 0.002 % at 12-32 cm depth; Fig. 5). The combustion of certain carbonates during the LOI determination of Total Carbon (TC) may explain the discrepancy between the TC and the TOC determinations (inorganic C was not determined in this study). Nevertheless, the results confirm low temporal variability and low standing stocks of organic matter within the sediment at the seepage face.

Porewater collected at the seepage face and used in the FTR experiments consisted mainly of seawater re-circulated through beach sands by the tide and characterized by high salinity and low DIN content (Table 1). The DOC content of the porewater was highly variable, with lower values found in Experiments 2 and 3 ($156\text{--}290 \mu\text{mol C L}^{-1}$), whereas, in the remaining experiments, DOC concentrations were within the range of $290\text{--}500 \mu\text{mol C L}^{-1}$ (Table 1). The composition of porewater CDOM was dominated by the labile components in terms of the scores obtained through PARAFAC analysis (Table 1). Significant linear correlation between all four components and DOC measured through our dataset was confirmed ($p < 0.05$; Tryp $R=0.6466$; Tyr $R=0.5847$; Ter humic-like $R=0.6387$; Mar humic-like $R=0.5459$; $n=111$).

Flow and reaction regime in the FTR experiments

Br⁻ breakthrough curves obtained from the FTR experiments were well described by equation 2, as the strong correlations for the fit indicate (Fig. 6). The magnitude of dispersion was amplified with increasing porewater velocity, and was within the same order of magnitude as figures calculated from the power law description of dispersion suggested by Bijeljic and Blunt (2006) (Fig. 6). The Br⁻ breakthrough curves did not indicate preferential flow inside the reactors.

Both the range of porewater velocities applied in the FTR experiments (from 3.7 ± 0.1 to 18.8 ± 0.1 cm h⁻¹; Table 1) and the different reactor cell lengths (from 2 to 20 cm) guaranteed the short residence time of porewater inside the reactors (from 0.4 ± 0.0 to 8.6 ± 0.2 h; Table 1). The duration of the experiments was at least three times longer than the porewater residence time. Even so, due to the heterogeneous pore structure of sandy sediments, residence time in smaller pores could be higher than the calculated residence time. Nevertheless, the duration of the experiments allowed for the reactor outflow composition to stabilize (i.e. steady state reached; Fig. 4). The calculated P_e ranged from 3.3 to 16.5, confirming advection as the dominant transport pathway. D_a calculated with DOC reaction rates ranged from 0.05 to 0.53 (Supplementary Materials Table 2) implying that for the domain under study, the timescale of advective transport were always higher than the reaction timescales. Thus, mass transfer of the metabolites was never a limiting factor on measured reaction rates in our experiments.

Benthic mediation of CDOM and DOC fluxes

Under oxic conditions and in the absence of porewater DIN (<10 μM; see Table 1 for initial experimental conditions) benthic metabolism exerted a significant effect on both

porewater CDOM and DOC fluxes (Experiments 1 and 2; Fig. 7; results are also shown in Supplementary Materials Table 2). At 18.5°C and fixed porewater velocity ($16.5 \pm 0.5 \text{ cm h}^{-1}$), the sediment served as a net sink for the labile CDOM components ($10.5 \pm 4.1\%$ Tryp-like and $22.7 \pm 13\%$ Tyr-like as integrated for the 32-cm sediment column; Fig. 7). Yet, no significant changes to the concentration of humic-like components of the CDOM pool were observed ($C/C_0 \sim 1$; $p > 0.2$). Measured net DOC production rates within the 2 to 12 cm depth layer were similar to those found between 12 and 32 cm depth. However, the surface layer (0-2 cm) was characterized by highly variable net DOC production/consumption rates, concomitant with the highest net consumption rates found for the labile fluorophores. Deeper sediment (2 to 32 cm depth) served as a net source of DOC at a depth-integrated production rate of $8.7 \pm 3.8 \text{ nmol C h}^{-1} \text{ cm}^{-3}$ wet sediment. Since the experimental conditions were similar when the three different sediment layers were tested (salinity, DOC, temperature, O_2 , porewater velocity; Table 1), the observed differences may be largely ascribed to the microbial activity and differential TOC content at each sediment depth.

Consumption of the labile fluorophores in the presence of low porewater DIN ($< 10 \text{ } \mu\text{M}$) was confirmed in the experiments performed at 14.5°C (Experiment 2, Table 1). A vertically-integrated net consumption of $25.6 \pm 2.6\%$ for Tryp-like and $14.4 \pm 4.7\%$ for Tyr-like CDOM was measured (Fig. 7). Depth-integrated net marine humic-like component consumption was also detected ($11.5 \pm 1.0\%$), but was mainly restricted to the 2-12 cm depth interval, while no significant changes to the porewater terrestrial humic-like fluorophore ($1.1 \pm 1.0\%$) was recorded. At 14.5°C, the higher rates of CDOM consumption and DOC production rates were restricted to the top 2 cm. In spite of the lower temperature relative to Experiment 1, integrated DOC

production rates measured during Experiment 2 at the 2 to 32 cm depth layer were higher (11 ± 4 nmol C h⁻¹ cm⁻³ wet sediment).

Due to the high variability of the measured DOC fluxes in the top 2 cm of the sediment horizon studied here (Fig. 7), and to discount the potential effect of the microphytobenthos over experimental DOM fluxes (e.g. Santos et al. 2009; Kim et al. 2012), subsequent experiments focused on the sediment originating from the 2 to 12 cm depth interval of the seepage face.

Influence of porewater advection rates on CDOM and DOC sediment production rates

At low porewater velocities (<10 cm h⁻¹), the 2 to 12 cm depth sediment layer served as a net source of the two humic-like CDOM components, whereas the labile components remained unaffected ($C/C_0 \sim 1$; $p > 0.5$; Fig. 8). In contrast, at the higher end of the imposed porewater velocities (Experiment 2; 17.8 ± 0.7 cm h⁻¹), the sediment served as a net sink for the labile fluorophores and one of the humic-like fluorophores. In addition, higher porewater velocities resulted in higher benthic DOC production rates (Fig. 8c; $p < 0.05$).

In the experiment performed at the highest porewater velocity (17.8 ± 0.7 cm h⁻¹), the addition of glucose to the circulating porewater promoted an increase in the net consumption of the labile components (Fig. 8a, b circled dots; $p < 0.05$). Under these conditions, the sediment also served as a net source of both CDOM humic components (Terrestrial: $36.5 \pm 6.6\%$; Marine: $25.2 \pm 11.9\%$) This contrasts with results in the absence of glucose, whereby the marine humic-like component was consumed and no change was observed to the terrestrial humic-like component content.

CDOM and DOC fluxes upon DIN addition to the porewater

The initial DOC concentration seemed to be one of the main factors determining the resulting net DOC reactivity after DIN was added to the porewater (Experiments 4 to 7; Fig. 9). In the experiments performed with porewater containing DOC in excess of 400 μM (Experiments 5B and 6B), the sediment served as a net sink for DOC whereas the opposite happened when $\text{DOC} < 400 \mu\text{M}$ (Fig. 9).

Under oxic conditions and in the presence of NO_3^- (500 μM final concentration; Experiment 4), the sediment column served as a net source of all four fluorophores ($C/C_0 > 1$). This contrasted with the net consumption or invariance of fluorophore concentrations observed for the same sediment depth interval in Experiment 1 (in the absence of DIN, but comparable porewater temperature and inflow DOM concentration; hereby termed as control; see Table 2 for Student t-test results). Net production of all four CDOM components in the presence of significant porewater NO_3^- was observed, in conjunction with a significant increase in the net sediment DOC production rate relative to the control ($24 \pm 3 \text{ nmol h}^{-1} \text{ cm}^{-3}$ wet sediment; Table 2).

Significant enhancement of CDOM net production rates under the presence of DIN was also observed under suboxic conditions ($< 50 \mu\text{M O}_2$; Experiments 5A and 6A, Table 1; Fig. 10 a, b) relative to the control (Table 2). Furthermore, comparison between the results of the experiments run under suboxic conditions and Experiment 4 (oxic, NO_3^- added) showed that only the fluxes of Tryp-like and marine humic-like fluorophores differed (Table 2). NO_3^- addition enhanced the net production of labile fluorophores by up to $49.0 \pm 4.5\%$ (Tryp-like) and benthic humic CDOM production by up to $20.9 \pm 1.8\%$ (Terrestrial humic-like). Addition of NO_3^- and NH_4^+ to the porewater (Experiment 6) resulted in a net enhancement of labile CDOM fluxes by up to $77.1 \pm 33.5\%$ (Tyr-like) while simultaneously enhancing humic-like CDOM release by up to

41.4±14.1% (Terrestrial humic-like). Net DOC production rates measured in both experiments under suboxic conditions were also higher than those measured in the control, but the difference was not significant ($p>0.15$). Both experiments were further extended using porewater with higher concentrations of naturally-occurring DOC, collected at the seepage face (Experiments 5B and 6B). Under this new set of conditions, the sediment reverted to being a net sink of DOC and the labile components of CDOM, while releasing humic-like CDOM (Fig. 9 and 10).

At 24.5°C (Experiment 7), the results were consistent with those previously observed in the presence of DIN at a lower temperature (18.5°C) and comparable porewater O₂ concentration (Exp. 5A and 6A). Porewater with added NO₃⁻ (120 µM) significantly stimulated the production of all four CDOM fluorophores, in parallel with net DOC production (38±10 nmol h⁻¹ cm⁻³ wet sediment). At the second experimental stage (i.e., following NO₃⁻ and NH₄⁺ amendments), the previously observed enhancement of benthic CDOM fluxes was confirmed, with a significant increase in the labile CDOM net production relative to the first stage of the experiment (paired t-test: $p<0.05$). This was observed in parallel with the highest measured net DOC production rate (69±12 nmol h⁻¹ cm⁻³ wet sediment; $p<0.05$), in itself higher than those measured during the first stage of the experiment run with porewater containing NO₃⁻ only and with the exact same sediment slices ($p=0.07$).

Discussion

DOM processing at the seepage face

Despite the low benthic standing stock of bulk organic matter (<0.6% weight) and the lack of evaluation on the production of CO₂, benthic DOC production measured in the experiments under oxic conditions and absence of significant DIN concentrations suggests that

the microbial community was actively processing organic matter. Sorption of DOC onto particle surfaces could potentially influence the observed change to DOC fluxes (e.g. Rosenfeld 1979; Fitzsimons et al. 2006). Nevertheless, the stabilization of the reactors to constant temperature and homogeneous porewater flow prior the start of the experiments would allow sorption equilibrium to be established due to the fast sorption kinetics and low adsorption capacity of marine sandy sediments (e.g. Fitzsimons et al. 2006; Pitois et al. 2008). Furthermore, DOM reaction rates were only quantified after steady state was reached, i.e. after the adsorption equilibrium was established. Thus, the observed changes to DOM content as porewater flows through the sediment are essentially attributable to the activity of the local benthic microbial community.

Generally, porewater advection through the sediment enhanced DOC fluxes and, simultaneously, the consumption of labile CDOM components. The results suggest that, in the absence of significant DIN availability, the microbial community preferentially consumed the labile CDOM components, while the humic-like fraction remained essentially untouched. Even so, the 2 to 32-cm depth layer may still constitute an important source of organic C to the lagoon. Benthic DOC production during active seepage could contribute between 16 and 21 mmol C m⁻² d⁻¹ to the lagoon if we extrapolate the experimental DOC production rates to the timeframe of active seepage at our site (6h per day; Rocha et al. 2009). These figures suggest that microbial activity at the seepage face could act as an important source of DOC supporting heterotrophic metabolism in the lagoon's water column. Irrespective of the production of CO₂ through respiration of sedimentary organic C (not measured in this study), DOC production rates observed during our experiments indicates that the integrated TOC measured in the top 32cm of the seepage face could be released as DOC within 14.1-18.6 days. Thus, the results point toward

a local dependence of benthic heterotrophic metabolism on the periodic supply of allochthonous POM and DOM through seawater infiltration during high tide.

Benthic DOM processing controlled by porewater advection

With increasing porewater velocity, a significant enhancement of the benthic DOC production rate occurred (Fig. 8). Higher seepage rates seem, therefore, to accelerate organic matter decomposition. This is consistent with previous findings (e.g. Boudreau et al. 2001; Rocha 2008) showing that, in permeable marine sediments, the increased supply of metabolites and accelerated flushing of metabolic products brought by advection enhance benthic microbial metabolism. At the lower end of the imposed range of porewater velocities ($<10 \text{ cm h}^{-1}$; $P_e < 8.5$; residence time $>8\text{h}$), no significant change to the labile CDOM components was observed, in spite of the net production of both humic-like fluorophores. In contrast, the benthic community became a net consumer of labile CDOM components at the highest imposed porewater velocities ($\sim 17 \text{ cm h}^{-1}$; $P_e \sim 14.5$; residence time $<2\text{h}$), while the levels of humic CDOM were either not significantly altered or decreased. Microbial degradation of both DOM and POM in the marine environment has been linked with the concurrent production of humic substances (e.g. Ogawa et al. 2001). Thus, the change from production of humic-like CDOM at low porewater velocities to non-production or even consumption as the porewater flow velocity increases would suggest that benthic microbial metabolism is limited by both loading and composition of available organic matter. This hypothesis is further supported by the response of the benthic system to glucose amendment. The addition of easily microbially-degradable C (glucose) to the circulating porewater resulted in the production of humic-like fluorophores and an increased consumption of the labile fluorophores. These results are consistent with observations by Ogawa et al. (2001) in

laboratory studies with marine bacteria, and suggest that benthic heterotrophic metabolism and subsequent DOC production within coastal seepage sites could be dependent not only on the seepage rate but also on the quantity and quality of available organic matter (both particulate and dissolved). Furthermore, our results suggest that, under a purely advective regime ($P_e > 3$), the preferential consumption of the labile CDOM fractions by the benthic microbial community would increase the recalcitrant nature of the residual CDOM pool, thus increasing its persistence in the receiving water body.

DIN-driven change in the processing of DOM at the seepage face

In the experiments performed with DIN amendment, DOC content seemed to control benthic microbial activity. Higher DOC content (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 porewater DOC levels were lower, DIN induced a further shift in the way the benthos mediated CDOM fluxes by stimulating the production of the two labile components, under both oxic and suboxic conditions.

Yamashita and Tanoue (2003) linked protein-like CDOM components to the aromatic amino acid content in natural waters. Production of amino acids, peptides and exogenous proteins by the microbial community in the presence of DIN suggests that under organic-C limitation, N also becomes a limiting substrate for local microbial metabolism. Furthermore, the concomitant net production of labile CDOM and DOC after DIN enrichment (Fig. 10 c) suggests that DIN availability in the porewater enhanced benthic processing of organic matter. Previous research has shown that increased N availability enhances the processing of recalcitrant organic

matter in terrestrial ecosystems via the stimulation of hydrolase and oxidase enzyme synthesis and activity (Mack et al. 2004; Bragazza et al. 2006). This view is supported by observations of the way in which the sediment mediates the transfer of humic-like CDOM in our experiments. Whereas the relative abundance of the humic CDOM components did not change significantly in the absence of DIN, a net increase was measured after DIN amendment.

When compared with the marine humic-like component, the blue shift of the terrestrial humic-like component fluorescence wavelength suggests a more aromatic composition and, therefore, indicates that it originated from less bio-reactive organic matter (Coble et al. 1998; Burdige et al. 2004). The simultaneous benthic production of both humic-like fluorophores may suggest active microbial degradation of both fresh and older, more refractory, organic matter. Thus, our experiments suggest that the presence of DIN transported through the seepage face could stimulate organic matter decomposition, by increasing the ability of extant microbes to process refractory organic matter. Furthermore, the observed increase of the labile CDOM fluxes in the presence of DIN suggests that this process could constitute a net source of labile, short residence time organic matter to the adjacent coastal waters.

The presence of NH_4^+ seemed to further augment the NO_3^- -driven CDOM and DOC production described previously, as shown by the results of Experiment 7. Although NO_3^- is the most common reactive form of DIN in oxygenated permeable sediment porewaters, NH_4^+ may also be present at elevated concentrations as a result of incomplete or limited nitrification, land-derived waste inputs to the beach aquifers, DIN regeneration from organic matter mineralization, or as a result of microbial Dissimilatory Nitrate Reduction to Ammonium (DNRA). In spite of our relatively poor understanding of DNRA, studies in soils have suggested that DNRA gains importance relative to heterotrophic denitrification at high NO_3^- concentrations and/or when C:N

ratios in the available organic matter increase (Fazzolari et al. 1998; Tomaszek and Rokosz 2007). High temperatures also seem to favor DNRA over denitrification in marine sediments due to the greater standard free energy made available to ammonifiers than to denitrifiers (Dong et al. 2011). Evidence for the occurrence of DNRA has been presented for our sampling site (Rocha et al. 2009). The occurrence of DNRA in combination with DIN regeneration from organic matter mineralization has been put forward as an explanation for the seasonality of SGD-derived DIN fluxes (Ibáñez et al. 2013). According to our results, the potential occurrence of DNRA at the seepage face could further support the enhancement of NO_3^- -driven organic matter processing as identified in the FTR experiments.

Implications for C cycling at the seepage face

Organic matter oxidation is usually limited by C availability in permeable sediments, suggesting that both its quality and quantity are important rate-controlling factors (Hartog et al. 2004). Our results are consistent with these findings but they also suggest that N is a co-limiting factor for microbial activity at local seepage sites. These findings might also be generalized to other locations, providing the range of environmental characteristics is similar. Increasing N availability in coastal waters and porewaters may therefore enhance benthic cycling of organic C. On the basis of our results, the enhancement of microbial decomposition rates driven by DIN in combination with significant discharge rates of porewater across the seepage face could catalyze the microbial breakdown of refractory organic material. This would result in the acceleration of C turnover within the seepage face. A fraction of the recalcitrant organic C pool circulating within the sediment would be transferred to near-shore coastal waters in a more labile form through the SGD pathway. There, it would become available to further support heterotrophic

growth. The identified DIN-driven organic C processing enhancement could therefore have an important effect on the cycling of organic matter both locally and globally.

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Figure legends

Figure 1. Sampling location at Ancão Peninsula (Ria Formosa coastal lagoon, SW Iberian peninsula; top panel). Beach profile measured during the study along with the approximate area of the permanently-saturated seepage face is shown (bottom panel).

Figure 2. Schematic view of the experimental set-up: A. Sediment core-slicing procedure. B. Detailed reactor design. C. Flow-through reactor experimental setup.

Figure 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).

Figure 4. Example of the temporal evolution of the CDOM components in the outflow solution through the course of the experiments (Experiment 3a). The different colored symbols (black, grey and white dots) represent each of the triplicated analyses. The grey area identifies the period of time over which the integration of the outflow composition was performed in order to calculate sediment mediation over DOM fluxes, i.e. when the outflow composition became temporally stable (once steady state was reached).

Figure 5. Vertical distribution of relevant sediment properties (porosity, LOI and total organic C) measured during the experimental period (July 2010 to January 2011) at Ancão Peninsula, Ria Formosa. Sediment cores were collected within the permanently saturated intertidal area, close to the peak seepage. Dotted lines represent each of the replicates, whereas the black dots and lines correspond to the average of the measurements. Error bars represent SEM (n=6).

Figure 6. Br⁻ breakthrough curves obtained for the different sediment layers used in Experiment 2 at 14.5°C. Porewater velocities were $15.5 \pm 0.4 \text{ cm h}^{-1}$ for the 0-2 cm depth interval (squared dots), $17.8 \pm 0.8 \text{ cm h}^{-1}$ for the 2-12 cm depth interval (circled dots) and $17.6 \pm 0.2 \text{ cm h}^{-1}$ for the 12-32 cm depth interval (triangle dots). Lines represent best-fit solutions of equation (2) to the experimental data (symbols). Colored dots represent each of the individual triplicates. Calculated P_e and D (D_{calc}) and the D values obtained through fitting eq. 2 to the Br- breackthrough curves (D_{fit}) are also shown.

Figure 7. Vertical distribution of CDOM and DOC fluxes modulation by benthic activity measured at 18.5°C (upper panels) and 14.5°C (lower panels). 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 at 18.5°C and n=3 at 14.5°C).

Figure 8. Benthic reactivity over porewater CDOM and DOC under different advective regimes for the selected sediment layer (2-12cm depth). Circled dots correspond to the extension of the experiment following 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.

Figure 9. Benthic activity represented as porewater DOC production rate as a function of initial DOC concentration in the circulating porewater. Rates were calculated based on results of the FTR experiments performed with sediment slices from the 2 to 12 cm depth interval of the seepage face. Error bars represent SEM (n=3(2)).

Figure 10. Compilation of sediment-mediated CDOM and DOC fluxes measured in the flow-through experiments performed with DIN addition to the porewater (see Table 1 for initial conditions). Results from the control experiment are also shown for comparison. Note that the logarithmic scale in the two upper panels correspond to the results from the sediment CDOM mediation. Error bars represent SEM (n=3).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Table 1. List of relevant environmental and porewater conditions characterizing each of the FTR experiments discussed in this study. The column “Solution” indicates when more than one porewater solution was used with the same sediment slices (experiments 2, 5, 6 and 7), in which case each of the two different solutions used is indicated as A and B. Relative score percentage of each of the four relevant fluorophores identified in the DOM and the aim (Treatment) of each experiment is also shown for each of the input solutions. NO_3^- and NH_4^+ concentrations lower than $2 \mu\text{M}$ in the inflow solutions are shown as “<2”.

Experiment	Solution	Layer cm depth	n	T °C	Salinity	O_2 μM	Velocity cm h^{-1}	Duration (residence time) h	DOC $\mu\text{mol C L}^{-1}$	NO_3^- μM	NH_4^+ μM	CDOM composition (%)				Treatment
												Tryp	Tyr	Ter Humic	Mar Humic	
Exp. 1		0-2	2	18.5	36.1	225	15.1±0.2	9.6 (0.4±0.0)	290	< 2	< 2	24.7	45.6	11.1	18.6	Control
Exp. 1		2-12	2	18.5	36.1	225	17.3±0.3	9.6 (1.9±0.0)	350	< 2	< 2	24.5	46.9	10.1	18.4	Control
Exp. 1		12-32	2	18.5	36.1	225	17.1±0.0	10 (3.7±0.0)	320	< 2	< 2	24.6	46.3	10.6	18.5	Control
Exp. 2		0-2	3	14.5	35.4	249	15.5±0.4	8 (0.4±0.0)	290	< 2	< 2	30.6	31.0	14.4	24.0	Control
Exp. 2	A	2-12	3	14.5	35.5	252	17.8±0.8	8 (1.8±0.1)	183	9	< 2	23.1	39.4	18.0	19.6	Control, porewater velocity
	B				35.5	248		8 (1.8±0.1)	> 2x10 ⁴ *	8	< 2	29.9	54.1	5.3	10.7	Glucose addition
Exp. 2		12-32	3	14.5	35.5	245	17.6±0.2	10 (3.6±0.0)	180	< 2	< 2	26.4	43.4	11.1	19.2	Control
Exp. 3a		2-12	3	14.5	34.7	245	6.7±0.1	14 (4.8±0.1)	156	8	< 2	68.5	2.6	20.1	8.8	Porewater velocity
Exp. 3b		2-12	3	14.5	35.6	248	3.7±0.1	25 (8.6±0.2)	178	9	< 2	51.7	24.9	14.6	8.8	Porewater velocity
Exp. 4		2-12	3	18.5	36.4	225	17.6±0.1	8.3 (1.8±0.0)	310	500*	< 2	31.8	34.5	17.3	16.4	NO_3^- addition, oxic conditions
Exp. 5	A	2-12	3	18.5	36.4	37	18.8±0.1	7.5 (1.7±0.0)	300	100*	< 2	25.5	47.2	10.5	16.8	NO_3^- addition, suboxic conditions
	B				36.3	32		7.5 (1.7±0.0)	450	470*	< 2	33.0	40.4	13.2	13.4	NO_3^- addition, suboxic conditions
Exp. 6	A	2-12	3	18.5	35.7	29	17.9±0.7	7.5 (1.8±0.1)	350	580*	580*	30.5	27.0	21.3	21.3	$\text{NO}_3^- + \text{NH}_4^+$ addition, suboxic conditions
	B				35.7	44		7.5 (1.8±0.1)	430	590*	570*	35.4	41.9	13.0	9.6	$\text{NO}_3^- + \text{NH}_4^+$ addition, suboxic conditions
Exp. 7	A	2-12	3	24.5	36.9	78	17.5±0.2	7.5 (1.8±0.0)	350	120*	< 2	22.7	43.1	13.8	20.4	NO_3^- addition, suboxic conditions
	B				36.5	86		7.5	330	140*	110*	20.2	29.6	21.8	28.3	$\text{NO}_3^- + \text{NH}_4^+$ addition,

(1.8±0.0)

suboxic conditions

* Amended to the porewater collected in-situ

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Table 2. Student t-test results for comparison of DOM flux enhancement among relevant treatments imposed on FTR experiments.

Tryptophan-like (Tryp), Tyrosine-like (Tyr), Marine humic-like (Mar-humic) and Terrestrial humic-like (Terr-humic) fluorophores are compared, together with DOC fluxes.

	Oxic NO ₃ ⁻ added	Suboxic NO ₃ ⁻ added	Suboxic DIN added
Control			
Tryp	*	***	*
Tyr	***	****	*
Mar-humic	*	***	*
Terr-humic	*	*	*
DOC	*	0.16	0.31
Oxic NO₃⁻ added			
Tryp		****	*
Tyr		*	0.09
Mar-humic		***	*
Terr-humic		0.06	0.11
DOC		0.52	0.58
Suboxic NO₃⁻ added			
Tryp			0.32
Tyr			0.15
Mar-humic			0.11
Terr-humic			0.38
DOC			0.56

* p<0.05; ** p<0.01; *** p<0.005; **** p<0.001

Highlights

- > DOC production rates covaried with porewater flow rates in our experiments
- > Benthic organic matter decomposition increase the refractory nature of the resulting DOC fluxes in our experiments
- > High DIN concentrations and high seepage velocities act to catalyze the microbial breakdown of refractory organic material
- > The availability of DIN in the experiments increases the release of labile DOC
- > Our results suggest that increasing DIN availability in coastal permeable sediments enhances organic C processing within the sediment